THE LACTIC ACID BACTERIA

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WITH 51 PLATES

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INTRODUCTION

The development of dairy bacteriology is constantly clearing up more and more of the problems which confront the practical dairyman in his daily work. We know now that, in the great majority of cases, it is the true lactic acid bacteria which give dairy products their good qualities, while, the aerobic and anaerobic spore-formers (Duclaux' *Tyrothrix* species) which were formerly regarded as being of great importance in the ripening process of cheese, not to mention the pseudo lactic acid bacteria (the Coli-Aerogenes family) are the worst enemies of the dairy industry.

We are still, however, far from having arrived at a complete elucidation of all the questions involved. It is particularly difficult to understand how various sorts of hard cheese, apparently containing the same microflora, should each have its own characteristic taste and smell. There can hardly be any doubt that these sorts of cheese in reality contain different species of bacteria, only we are unable to distinguish them by the methods hitherto employed. The object of the present work is primarily to meet this want by describing the useful bacteria of the dairy industry, so thoroughly that it may be possible in the future to identify the strains encountered.

A point of particular interest to the Danish dairy industry is the study of the bacteria which occur in Danish "dairy cheese" (Mejeriost), and these have accordingly been subjected to particularly detailed treatment. The material used as a starting point consisted of prize-winning exhibition cheeses from both raw and pasteurised milk (noted in the tables as *R* and *P* respectively). And in order properly to investigate the influence of pasteurisation upon the microflora, we have in certain cases had cheeses made at the dairies from the same milk in raw and pasteurised state (in the tables, such cheeses are indicated by the same numbers, e. g. 8 *R* and 8 *P*).

On commencing the study of the lactic acid bacteria of milk and dairy produce, however, it will soon be realised that the work will become onesided unless it be extended to all lactic acid bacteria. For what we have to do is to ascertain whence the different species are derived, and how they can be found when wanted. It need hardly be said that the lactic acid bacteria of the dairy industry do not make up a complete whole in themselves. They are introduced into the milk to a great extent through the cowdung, the bacteria of which, again, are to an essential degree derived from the fodder, and it was therefore necessary to study the lactic acid bacteria both of the animals and the plants. We have therefore isolated and investigated the lactic acid bacteria most frequently met with in the excrements of cows and calves, as also those of human beings, adults and children,

and we have also studied the most important of the lactic acid bacteria occurring in soured beet slices, diffusion slices, potatoes and mash, as also in sour cabbage and sour dough. Some experiments made by the State Committee for Plant Culture, with souring of beets and potatoes, came in very conveniently for us in this respect.

The strains of bacteria which we were able to isolate from these various sources were further supplemented by related forms isolated by colleagues at home and abroad. For a number of pathogenic strains, for instance, we are indebted to the colleagues mentioned in the tables, and we hereby beg to express our thanks to the gentlemen in question for their kindness in providing us with the cultures.

Only 330 of the strains investigated will be mentioned in the work, as in the case of strains of the same origin, and which in the course of the investigation proved entirely identical, only one of them was included. On the other hand, we have included many apparently identical strains of different origin, which have been constantly subjected to parallel investigation in order to ascertain whether possible variations tended in the same direction or not.

The lactic acid bacteria of the dairy industry are, however, those which are most numerously represented and most thoroughly dealt with, inter alia because we have had them for observation from the very commencement of the work, whereas the lactic acid bacteria of other origin only came in gradually, and have therefore in many cases only been thoroughly tested a few times, which is not sufficient to determine the constancy of their qualities. It is obvious that only the most constant qualities can be used as species character, and in investigations of this kind, therefore, all the qualities in each individual strain must be tested again and again throughout a long period of years. The present work has also, for this reason, taken up fully ten years of my time; partly also that of my assistants, and I take this opportunity of thanking them — Miss Betzy Meyer and my wife, Mrs. Orla-Jensen — for the numerous chemical analyses, microscopical investigations and photographs to which they have devoted so much skill and care[1]).

We shall commence by describing the methods employed in cultivation and investigation, passing on then to description of the isolated species and their systematics, in connection with which, a key will be furnished for determination of the lactic acid bacteria, as also a survey showing where they are found. And finally, mention will be made of the experiments we have carried out with a view to utilising the isolated species.

For practical reasons, such of our microphotographs as it was found advisable to reproduce have been collected in a special album, and I beg to offer my best thanks to the Carlsberg Fund for having rendered it possible to publish the same. I also beg the Julius Skrike's Foundation to accept my best thanks for having defrayed the expenses of the English translation.

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[1]) I also beg to thank Mr. J. Lind, who has assisted me for a year, and Mr. Tholstrup-Pedersen, who in spite of having worked only a few months in the laboratory here has nevertheless contributed valuable assistance to the present work. The custodian of the laboratory, also, Mr. V. Jacobsen, I have to thank for the help he afforded us, as an experienced dairyman, in the cheese-making experiments mentioned at the close of the work.

Methods of Cultivation and Investigation.

a. Cultivation and Isolation.

Where many hundred strains of bacteria have to be kept under observation for years together, it requires no little work merely to keep their vitality in some measure unimpaired. At first, when we were unable to regulate the temperature during the summer months, this was a serious difficulty, and it was only by experience acquired in the course of years that we were able to master the situation. And even now, certain pathogenic streptococci, as well as some divergent rod forms, still cause trouble. The method of preservation is not the same for all species, but must be adapted to their particular demands.

As regards the lactic acid bacteria of the dairy industry, it might seem natural to propagate them by transference from milk to milk. Unless very low temperatures can be maintained, however, it will be necessary to repeat this process each week, as otherwise, the bacteria will be killed off by the acid formed. We have therefore only employed this method for the more specific milk bacteria. If chalk be added to the milk, and the cultures shaken regularly, the bacteria can retain their qualities unaltered for several months. This, however, necessitates the use of large flasks, as the chalk cannot be shaken up in ordinary test tubes, so that method is only practicable where but a few cultures have to be dealt with. The employment of large flasks, moreover, increases the danger of infection from the air during inoculation. We have used this method to preserve the power possessed by certain species of forming slime in milk; a quality which is very soon lost on solid substrates.

For preservation of the bacteria we have as a rule employed agar stab cultures in Freudenreich flasks. Such cultures present, in reality, the same advantages as milk mixed with chalk, as the acid, which is only formed in the stab, is thence diffused into the remaining agar mass. Although the true lactic acid bacteria only exceptionally grow in sugar-free broth, they will nevertheless all thrive in sugar-free agar[1]), which shows that the agar, after sterilisation, contains some soluble carbohydrates. The growth is furthered, however, by the addition of sugar, though in the case of keeping cultures, this should be restricted to ¼ % grape sugar, thereby preventing the formation of more than ¼ % acid. In order further to keep down the hydrogenion concentration, the substrate should be rich in buffers such as amino-acids and phosphates. Prior to sterilisation, the substrates were neutralised as exactly as possible to litmus paper. For nitrogen food, we used at first 2%

[1]) The growth of the sugar-loving thermobacteria is, however, extremely slight.

WITTE peptone (*W*) answering to abt. 0.3% *N*. As will subsequently be seen, this cannot compare with casein peptone (*C*) i. e. peptonised casein[1]). In certain cases, yeast extract (*Y*) i. e. autolysed press yeast[2]) proved the best source of nitrogen. It is somewhat dark in colour, however, and we therefore used, as far as possible, *C*-Agar — and this, it should be noted, with 0.5% *N*,i. e. with the same quantity of nitrogen as in milk. WITTE peptone, which evidently consists for the most part of albumoses, forms strong deposits with acid, so that the stab is no longer visible in old *W*-Agar cultures of powerful acid formers; the nitrogen sources I have suggested, on the other hand, are free from this disadvantage. They are, moreover, rich in phosphates. A further 2‰ dibasic potassium phosphate, and 1 ‰ magnium sulphate is, however, added. A slightly larges or smaller quantity, of common salt does not as a rule affect the growth of lactic acid bacteria; this point will be further referred to later on.

For preservation of the bacteria cultures, we have also tried cane sugar solution and other sugar solutions at various concentrations. Such solutions, which have been employed with great success for the preservation of yeast cells, are as a rule not suited to bacteria. An exception, however, is water with 2% soluble starch, which is gradually coagulated by the action of acid. To 10 cm³ starch mixture is added 1 cm³ of the precipitate from a broth culture. The starch solution, however, cannot as a rule compare with *C*-agar, as far as concerns the preservation of true lactic acid bacteria, but is on the other hand to be preferred to bacteria with surface growth (Coli-Aerogenes bacteria, micrococci, etc.) and in particular to strong ammonia-formers, which rapidly die off in the highly nitrogenous *C*-agar.

No less important than the composition of the nutritive substrate is the temperature at which the cultures are preserved. When kept on ice, however, the air is very liable to become so moist as to further the formation of mould[3]) and we therefore restricted ourselves to water cooling. As long as the temperature is kept below 18°, the great majority of bacteria can be preserved — without transference — for several years on the nutritive substrates mentioned. To make sure, however, we transferred the strains investigated every month, or every alternate month, according as they had proved more or less difficult to keep alive. Bacteria, it should be noted, are very tricky things to deal with, and it was found more than once that a bacteria strain suddenly weakened or died, while the same strain under apparently identical conditions remained unimpaired for a long time. Any

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[1]) 100 gr. (sugar-free) acid-casein was digested for a week at blood temperature with 1 liter water, containing 4,6 % *HCl* and 2 gr. pepsin. The solution formed contains, after neutralisation, sterilisation and filtration, abt. 1 % *N* and 1.2 % *NaCl*.

[2]) At 50°, the digestion is completed in 24 hours. The sugar disappears at the same time. The highly acid solution contains abt. 2 % *N*.

[3]) As this laboratory is also used for teaching purposes, and a great deal of work is done with mould fungi, we have at times had great difficulty in avoiding infection by mould. As the mould formation often proceeds from the labels, these were always moistened with sublimate solution, before being stuck on, and the FREUDENREICH flasks were of course sealed with sealing wax. When an agar culture exhibits mould on the surface, it may easily be cleaned by letting it stand a moment with spirit over. The surface, which is then no longer dusty, is peeled off, in a layer not too thin, and the inoculation can then be made from the lower part of the stab, which is always free from mould.

sudden rise of temperature may, it need hardly be said, prove fatal to the cultures, but we have also seen cases (e.g. with *Bacillus bulgaricus*) where a sudden fall in temperature, of only 5°, occasioned a serious weakening.

In order to determine whether the bacteria in the keeping cultures were still in possession of their full vitality, we compared the rate of growth with that in freshly reinoculated cultures. In the case of lactic acid bacteria growing in milk, all that is needed is to determine the length of time which elapses before the milk coagulates, and determine the quantity of acid formed when this has attained its maximum, which will always be the case after 14 days at 30°. The method, however, is not without its sources of error. Even where the sowing out is done throughout with the same needle, it is impossible to avoid sowing more cells at one time than at another, and what is worse, the cells themselves may exhibit marked individual differences. Consequently, as will be seen from Table I, we do not always find, as might be expected, an even decrease of vitality in course of time.

For producing pure cultures of lactic acid bacteria and, investigating the same, we likewise used substrates containing the mentioned nitrogen sources and salts. They differed from the keeping substrates only in containing more sugar. The sugar broth to be used for determination of the quantity of acid formed should thus contain at least 2% sugar as some of our strains can form over 1.5% lactic acid therein. It is also less easy to overlook any development of gas when the substrate is not too poor in sugar. A slight development of gas is best observed by close sowing in tall agar tubes (BURRI's tubes). By using certain species of sugar and litmus for the plates, we succeeded in isolating the species which could ferment the sugar species in question.

In order to isolate as far as possible all the lactic acid bacteria found in a starting material, it was sown out both in tall agar tubes and on gelatin plates, so that both aerobic and anaerobic forms might develop. Besides neutral, sugar-containing gelatin (S. G.), we also used alkaline sugar-free gelatin (A. G.) on which only certain species of lactic acid bacteria grow strongly. We also had recourse to various enrichment methods. In order to get at those which thrive at particularly high or low temperatures, the starting material was added to sterile milk, and left to stand for a time at the temperatures in question. The heat-resisting species of the milk we obtained by pasteurising it in various ways, and its acid-resisting species by adding greater or smaller quantities of acid (lactic acid or acetic acid) before placing to stand at the respective temperatures.

The raw cultures obtained at the first spreading were cleansed by continued spreading until all the colonies showed the same appearance and contained uniform cells. That the pure cultures thus obtained really were pure cultures was apparent from the work itself, which consisted in a constant checking of the isolated strains. As long as these remained uniform year after year, and in particular, constantly exhibited the same negative qualities, there can be no doubt as to their purity. Single cell cultures are only practicable where but a few species of bacteria have to be dealt with; not, as in the present case, where the object is precisely to study the greatest possible number of strains. As a matter of fact, we had really no practicable method of obtaining single-cell cultures when I commenced the work in 1907; it was not until 1914 that GERDA TROILI-PETERSSON published a modification of the BURRI Indian ink method, suitable for lactic acid bacteria[1]).

[1]) Centralblatt f. Bacteriologie, II. Abt. Bd. 42, p. 526.

Table I.

Table No.	Species of bacteria	No.	Preserved in	Immediately		After ½ year		After 1 year		After 1½ years		After 2 years		After 3 years	
				coagulated after number of days	‰ acid	coagulated after number of days	‰ acid	coagulated after number of days	‰ acid	coagulated after number of days	‰ acid	coagulated after number of days	‰ acid	coagulated after number of days	‰ acid
XIV	*Streptococcus lactis*	8	C-agar	2	5,4	2	5,9	2	5,2	2	5,4	2	6,1	3	5,6
	» »	14	»	1	8,8	2	7,4	1	7,4	1	7,7	1	7,7	1	6,6
XV	» *cremoris*	11	»	1	6,8	2	5,9	2	5,2	4	4,5	6	4,3		mouldy
	» »	18	»	1	7,0	2	6,3	2	6,8	2	5,9	2	7,0		»
	» »	»	Starch water	1	7,0	2	6,8	2½	5,9	2	6,1	2	7,0	3	4,5
XVII	» *thermophilus*	2	C-agar	1	8,1	5	7,2		dead						
	» »	»	Starch water	1	8,1	2	6,8	2½	5,9	2	6,1	3	7,0		mouldy
XX	» *fœcium*	8	C-agar	3	6,8	3	5,6	3½	5,6	3	5,6	4	5,6	3	4,7
XXIV	» *pyogenes*	10	»	5	4,7	6	4,5	6	4,3	6	4,3	8	3,8		mouldy
XXI	» *glycerinaceus*	4	»	4	5,0	5	4,5	3½	4,5	5	5,2	7	4,1	5	4,1
XXII	» *liquefaciens*	1	»	1	7,7	1	7,0	1	6,5	2	7,0	1	7,9	1	5,9
XXV	*Betacoccus arabinosaceus*	9	»	10	4,1		3,5		3,4		2,0		1,6		dead
	» »	»	Starch water	10	4,1	11	3,8		2,3		3,6		2,3		1,4
XXVII	*Tetracoccus*[1]) *casei*	5	C-agar	5	4,1	6	4,1	7	4,5	7	3,9		mouldy		
	» »	7	»	5	4,1		2,7	12	3,4		2,3		dead		
XXVII	» *liquefaciens*	10	»	3	3,8	2	3,2	3	3,8	2	2,7	4	1,8		dead
	» »	»	Starch water	3	3,8	3	2,7	3	2,9	2	2,7	4	2,0		mouldy
XXVII	» *pyogenes aureus*	13	C-agar	3	3,6	5	2,5	10	3,2	5	2,9		dead		
	» » »	»	Starch water	3	3,6	10	2,0	12	2,5	10	1,8		1,1	8	2,0
XXVII	» » *albus*	29	C-agar	6	4,1		2,7	10	3,2		2,7		2,0		2,0
	» » »	»	Starch water	6	4,1	10	3,2	11	3,2	14	3,2		2,3		2,0
XXVII	» *mycodermatus*	31	C-agar	10	2,7		1,8		2,0		0,9		0,7		dead
	» »	»	Starch water	10	2,7		1,6		2,0		1,6		1,4		mouldy
XXX	*Streptobacterium plantarum*	3	C-agar	13	3,4	11	4,1		2,3		2,0		1,1		2,5
	» »	7	»	9	5,0	10	4,5		3,2		2,5		2,3		2,5
	» »	12	»	10	6,0	11	5,6	7	6,3	11	4,3		2,7		mouldy
	» »	20	»	6	9,5	5	8,6	5	10,1	5	9,5	7	7,9	5	6,8
	» »	»	Starch water	6	9,5	8	6,1	10	5,6	8	6,3	8	7,2	10	4,5
	» »	30	C-agar	3	12,2	3	11,5	3½	11,5	3	12,4	4	12,4	5	11,3
XXIX	» *casei*	2	»	7	11,7	7	11,0	6	11,0	8	7,2	8	7,7	5	11,0
	» »	»	Starch water	7	11,7	14	8,3		3,8	9	9,5	6	7,9		4,1
	» »	4	C-agar	4	12,2	4	11,7	6	9,0	5	10,4	5	11,0		mouldy
	» »	11	»	4	14,0	6	12,4	3½	13,5	4	14,2	7	8,8		»
	» »	24	»	4	12,1	4	10,6	3½	12,2	3	11,9	4	10,4	4	11,3
	» »	28	»	5	10,3	6	8,5	9	7,2	7	7,4	9	7,7	5	10,6
	» »	34	»	2	16,0	4	15,8	3½	12,4	4	15,8	4	13,3		1,6
XXVIII	*Thermobacterium lactis*	9	»	1	12,2	1	10,8	2	9,9	2	10,8	6	7,4		dead

[1]) By Tetracoccus is meant acid-forming micrococci and sarcinæ.

b. Methods of Investigation.

In order to identify a micro-organism, the following points must be determined:

I. Its morphological and cultural features.
II. What sources of energy and nutritive matters it can utilise.
III. Its manner of utilising the same.
IV. Its attitude toward different temperatures.
V. Its agglutination and other possible specific qualities.

I. Morphological and Cultural Features.

Of the three principal morphological qualities of bacteria: the arrangement of the flagella, the shape of the cell, and the spore formation, the arrangement of the flagella is, as I have shown in „Hovedlinierne i det naturlige Bakteriesystem", of primary importance[1]). The shape of the cell, on the other hand, is merely a generic character, and we may, within one and the same family, encounter sphere, rod and screw forms, as is well known in the case of the red sulphur bacteria, and as we shall also find from an example among the lactic acid bacteria. As all these bacteria appear immotile, and do not form spores, we have, in reality, no morphological indication whatever to go upon when placing them in the bacteria system. On the other hand, their biological qualities are so pronounced, inasmuch as they require just as complicated nourishment as animals, that there can be no doubt as to their place, viz. among the order of *Peritrichinæ*.

A very important distinctive feature in lactic acid bacteria, and one which separates them sharply from the coli and aerogenes bacteria is that they are Gram positive. The first reaction therefore, to which we had recourse with the isolated acid formers was always to ascertain their behaviour in respect of the Gram staining process. Casein being Gram negative, the Gram staining method is excellently adapted to milk preparations. More frequently, however, the simpler staining with methylene blue is employed. This method is likewise used for demonstrating the presence of volutin grains which are frequently met with in several of the rod-shaped lactic acid bacteria. The grains are thereby stained dark blue as a rule, but at times also red. Fuchsin is not suitable for milk preparations, as it colours the casein as strongly as the bacteria. In demonstrating the formation of capsules in milk, however, this is an advantage in itself, as the unstained capsule then appears very distincly, and we have thus succeeded in showing that all lactic acid bacteria form capsules at their first stage of development. In some few strains, this capsule can attain very considerable size, but in most, it soon disappears without preliminary swelling or slime formation. The faculty of forming slime in milk is more frequent in some species than in others, but it is highly variable, and therefore absolutely inapplicable as a species character.

[1]) Die Hauptlinien des natürlichen Bakteriensystems. Centralblatt f. Bakt. II. Abt. 1909, XXII, No. 11—13. In this work it is pointed out that on the other hand the mere fact of a bacteria possessing flagella or not is of no systematic importance, and we also find closely related species (as for instance hay and anthrax bacille, coli and aerogenes bacteria) of which one is motile and the other immotile.

In staining lactic acid bacteria, their acid content cannot always be disregarded. Methylene blue, for instance, will not colour highly acid broth cultures at all. Instead of adding alkali to the staining material, which may easily overcolour the preparation, it is better to neutralise the culture employed. In making Indian ink preparations, which give the best microphotographs, such neutralisation is also necessary in order to prevent the colloid ink from flaking.

The illustrations in the album are, where not otherwise stated, invariably Indian ink preparations, magnified 1000 times; 500 times by means of the microscope, and twice again by the camera. We have therefore considered it superfluous to note the size of the lactic acid bacteria in the text; as a rule, they are from 0,7 to 1 μ think. A few small rod forms are exceptions; these we have called microbacteria (genus *Microbacterium*, Pl. XLIX—L) from their small size. It should be noted, however, that the apparent thickness of the bacteria is affected by the thickness of the layer of Indian ink, wherefore the latter should be applied as thinly as possible. In photographing and development also, the dimensions may be affected, so that unless the microphotographs are constantly subjected to careful control, they may turn out entirely misleading. When dealing with coloured preparations, it should be borne in mind that only the protoplasm, without the cell wall, is visible. Only capsule preparations give a thoroughly correct impression of the size of the bacteria. These preparations are generally photographed in water, while the other preparations are generally made in Canada balsam.

A more detailed description of the morpholigical features can only be given when dealing with the separate species. The variety of forms within the present group is in reality so great that nothing can generally be said beyond what has already been mentioned. A glance at the album will show us streptococci which divide in two directions (*Sc. cremoris* Nr. 20, Pl. VIII, *Bc. bovis* No. 46, Pl. XXIV) or form rods (*Sc. thermophilus* No. 2, Pl. XII, *Bc. bovis* No. 33, Pl. XXIII) and rods, which form streptococcus-like chains (*Sbm. casei* No. 9, Pl. XXXVII, No. 28, Pl. XXXVIII, *Sbm. plantarum* No. 44, Pl. XLV, *Bbm. breve* No. 3, Pl. XLVII) or more or less distinct screws (*Sbm. casei* No. 2, Pl. XXXV, No. 33, Pl. XXXIX, No. 34, Pl. XL. *Sbm. plantarum* No. 1, Pl. XLI and *Bbm. breve* No. 8, Pl. XLVII). These transition forms best show how futile it would be to take the shape of the cell as a basis for dividing the lactic acid bacteria into groups assignable to altogether different places in the bacteria system. The biological qualities will show that certain spherical and rod forms (e. g. streptococci and streptobacteria or betacocci and betabacteria) are at least as nearly related one to another as are the spherical forms or the rod forms respectively among themselves. The true lactic acid bacteria form altogether a family as natural as could be desired. But if this — like other natural bacteria families — cannot be broken to pieces because it contains species with more or less long, or more or less curved cells, as has hitherto been done in bacteriology, the old generic names, which are purely morphological terms, can likevise no longer be used without some supplementary prefix, or at any rate not without giving them a new meaning.

As regards the cultural features of the lactic acid bacteria, we can likewise be brief. With the exception of the tetracocci (the acid-forming micrococci and sarcinæ) and certain microbacteria, they thrive best without air, and have therefore, in stab cultures, no

marked surface growth, but grow evenly throughout the whole stab; some rod forms, indeed, grow more strongly deeper down. And in conformity with this, we find the streak cultures forming only a thin veil; the plate colonies are rarely more than 1 mm in diameter, and coagulation of milk cultures commences from the bottom. Only a single species, *Streptococcus liquefaciens*, liquefies gelatin, and only a couple of pathogenic streptococcus strains form colouring matter (red staining of the stab, but not of the surface). In contrast to this, the more aerobic tetracocci are generally distinguished by the power of liquefying gelatin, and form colouring matter (but always on the surface only). Some lactic acid bacteria, which we have called the betacocci (genus Betacoccus) form slime in cane sugar solutions.

II. and III. Sources of Energy, Nutritive Matters and Manners of Utilising the Same.

In dealing, as here, with heterotrophic organisms, sources of energy and nutritive matters must primarily be understood as meaning sources of carbon and nitrogen, and it will be correct to take each of these separately.

Carbon sources. The lactic acid bacteria utilise, like other organisms, a part of their carbon nutriment to build up their cells, especially the cell walls and other non-nitrogenous substances, the bulk of it, however, serves as a source of energy, going to form lacticacid. The organic acids are only poor sources of energy for the lactic acid bacteria[1]), many carbohydrates and higher alcohols, on the other hand, are particularly suitable. All our strains of bacteria were tested for the pentoses: Xylose and Arabinose, the methylpentose: Rhamnose; the hexoses: Lævulose, Dextrose, Mannose and Galactose, the disaccharides: Saccharose, Maltose and Lactose, the trisaccharide: Raffinose, the polysaccharides: Inulin, Dextrin, soluble Starch, Glycogen and Gum arabic; the alcohols: Glycerin (C_3), Erythrit (C_4), Adonite (C_5), Mannite, Sorbite and Dulcite (C_6); the cycloparafin: Inosit and the glycoside: Salicin[2]). All the sugars were d-forms with the exception of the pentoses, which were l-forms, and the dulcite, which was i-form.

The tables showing the qualities of the different species of bacteria do not include fermentation of glycogene, gum arabic, erythrite, adonite, dulcite and inosite. There are no lactic acid bacteria which ferment gum[3]), erythrite and adonite, and it is only extremely rarely that they ferment dulcite and inosite, the fermentation in all cases being only slight. In this respect, the true lactic acid bacteria differ from the pseudo ones, which generally ferment these substances as strongly as grape sugar. The emission of glycogene from the tables is due to the fact that this substance is fermented by exactly the same bacteria as starch. Animal and vegetable star-

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[1]) The most suitable is lactic acid, of which some species may form a little acetic acid and other products. See my work "Studien über die flüchtigen Säuren im Käse etc." 1904. XIII, 161.

[2]) Dextrine, starch, sorbite, and salicin, however, have not been included right from the commencement, consequently, the quantities of acid formed by these will not always be found in the tables. Rhamnose could not always be procured during the war, and the last strains dealt with have therefore not been tested with this sugar.

[3]) A single sarcina (Tetracoccus No. 11) was in freshly isolated state able to ferment a small amount of gum, but lost this power later on.

ches thus require precisely the same enzymes to produce any effect, and when we find that many pathogenic streptococci are capable of fermenting starch, and this very strongly, it is doubtless because they have became accustomed, in the host organism, to glycogene[1]).

Some experts may perhaps consider it quite superfluous to test the lactic acid bacteria with four hexoses which they are probably all able to ferment. We did not, however, restrict ourselves to ascertaining merely whether any fermentation took place at all, but determined in each particular case the exact quantity $^0/_{00}$ of acid formed, in order to find out what carbon sources were preferred. Thus employed, the four hexoses are often of as great importance for species determination as the carbon sources which are but rarely fermented. It is highly characteristic of several species, for instance, that they prefer lævulose to dextrose, or in the case of others, that they find it very difficult to attack mannose. As a rule, galactose is that of the four hexoses which is least femmented.

As with the milk, we also allowed the inoculated sugar broth tubes to stand for 14 days at the optimal temperatures of the respective bacteria before proceeding to titration. In order to calculate the quantity of acid formed, the original acid grade of the sugar broth tube was subtracted. Each tube was given exactly 10 cm.^{3} sugar broth. Many sugars are highly coloured by sterilisation in the nutritive liquids employed, which renders titration (with phenolphtalein as indicator) extremely difficult. The ones which colour most strongly are xylose and arabinose; then come galactose, lævulose, rhamnose, dextrose, mannose, maltose, lactose and dextrin, while the other (non-reducible) sugars are scarcely affected. The more strongly the sugars are browned on sterilisation, the higher will be the initial acidity of the nutritive substrate[2]). Even though this, owing to the buffers present, may not influence the hydrogen ion concentration, the supply of buffers is itself reduced thereby, and thus doubtless also the quantity of acid formed. As sterilisation is indispensable, it is consequently impossible to get an absolutely just comparison between the different sugars, even when they are all dissolved in precisely the same nutritive liquid — which of course we always did. It should be added, that the composition of the nutritive liquid greatly influences the transformation of the sugar on sterilisation. As our sugar-substrates contain no surplus of hydroxyl ions, it cannot be these which destroy the sugar; the transformation is, on the contrary, the more marked according as the substrates are richer in buffers. The reduction power of sugars is hardly impaired at all by sterilisation in pure water, whereas it is diminished abt. 20% in yeast extract, which is the richest of our substrates in respect of buffers.

It need hardly be said that we have employed only the purest sugars, from *Merck* and *Kahlbaum*, for our investigations, and we always made sure that new consignments

[1]) It is generally supposed that the primary division of raffinose (melitriose) into lævulose and melibiose is due to invertase, and it was also found that only saccharose-fermenting lacticacid bacteria were able to ferment raffinose, but it is by no means all saccharose-fermenting lactic acid bacteria which do ferment raffinose.

[2]) By way of example, the degree of acidity (cm.^{3} $\frac{n}{4}$ *NaOH* to 100 cm.^{3} nutritive liquid) for xylose, grape sugar and cane sugar, with casein pepton as nitrogen source, was respectively 23, 13 and 10, and with yeast extract as nitrogen source respectively 30, 20 and 15.

Table II a.

Table No.	Species of bacteria	No.	Source of nitrogen	‰ Acid formed from ‰ Dextrose						
				5	10	20	50	100	150	200
XIV	*Streptococcus lactis*	4	W	4,1	4,1	4,1	3,8	3,8	3,4	
	» »	6	»	3,6	3,4	3,4	3,4	3,4	2,7	
	» »	7	»	3,4	3,6	3,6	3,6	3,2	2,6	
	» »	8	»	3,8	3,6	3,6	3,6	3,6	2,7	
	» »	9	»	3,6	3,6	3,8	3,6	3,4	2,7	
	» »	12	»	3,6	3,6	3,6	3,4	3,2	2,7	
	» »	14	»	3,8	3,8	3,8	3,4	3,4	2,9	
	» »	17	»	4,1	4,1	4,1	3,4	3,4	2,7	
XX	» *fœcium*	7	»	1,0	2,0	2,7	3,4	0	0	
	» »	8	»	4,1	3,8	3,8	3,6	3,4	2,7	
	» »	14	»	4,1	3,8	3,6	3,4	3,4	2,7	
	» »	17	»	(4,3)	5,4	5,4	5,1	4,7	4,0	
	» »	»	C	(4,3)	6,3	6,7	5,6	5,2	4,1	3,4
	» »	18	W	(4,3)	5,0	5,0	5,0	5,0	5,0	
	» »	»	C	(4,3)	6,8	7,2	7,2	7,2	6,3	5,9
XXI	» *glycerinaceus*	1	W	3,8	3,8	3,6	3,4	3,4	2,7	
	» »	3	»	4,3	4,1	3,9	3,6	3,4	2,5	
	» »	4	»	4,1	3,8	3,8	3,8	3,6	2,9	
	» »	6	»	3,6	3,6	3,6	3,6	3,2	2,5	
XXII	» *liquefaciens*	1	»	4,3	4,5	4,3	3,8	3,7	3,2	
	» »	3	»	4,3	4,5	4,5	4,3	4,1	3,4	
XVII	» *thermophilus*	2	C	(4,5)	6,1	5,9	5,4	2,0	1,4	0
	» »	3	»	4,1	4,5	4,1	3,4	2,3	0	0
	» »	4	»	(4,5)	4,7	4,7	4,7	2,5	1,1	0
	» »	5	»	4,3	4,5	4,7	3,6	0,9	0	0
XVI	» *mastitldis*	2	W	3,2	3,4	3,4	2,7	1,7	0,7	
	» »	3	»	3,4	3,4	3,4	3,4	2,0	1,4	
XV	» *cremoris*	1	»	1,8	2,0	2,0	2,3	2,1	1,8	
	» »	2	»	2,9	2,9	2,9	3,2	3,2	2,3	
	» »	10	»	0	2,9	2,9	2,9	3,2	2,5	
	» »	11	»	2,5	2,5	2,6	2,7	2,6	1,6	
	» »	18	»	3,2	3,2	3,6	3,6	3,3	2,3	
	» »	19	»	2,0	2,0	2,0	2,7	2,8	1,8	
	» »	20	C	2,9	2,9	2,9	2,9	3,6	3,6	2,7
	» »	21	W	0,2	1,8	2,9	3,1	3,1	2,4	
	» »	«	C	3,6	3,6	3,6	3,6	3,8	3,6	2,5

Table II b.

Table No.	Species of bacteria	No.	Source of nitrogen	‰ Acid formed from ‰ Dextrose						
				5	10	20	50	100	150	200
XXV	*Betacoccus arabinosaceus* ...	6	W	1,6	2,0	2,3	2,7	2,6	2,5	
	» » ...	7	»	1,4	2,0	2,5	2,7	3,4	2,9	
	» » ...	8	»	1,4	2,0	2,0	2,3	2,5	2,5	
	» » ...	9	»	1,6	2,3	2,3	2,5	2,7	2,9	
	» » ...	12	C	2,7	4,1	4,5	4,5	4,5	3,8	3,8
XXVI	» *bovis*	33	W	1,6	1,6	1,6	1,8	2,3	2,0	
	» »	34	»	1,0	1,0	1,5	2,0	1,8	1,4	
	» »	35	»	1,8	1,8	2,0	2,0	2,5	2,4	
	» »	37	»	0,9	1,4	1,8	1,8	1,6	1,4	
	» »	40	»	1,1	1,6	2,5	2,9	3,8	2,7	
	» »	42	»	0,7	0,9	1,4	1,4	1,8	1,6	
XXVII	*Tetracoccus ?*	1	C	2,7	2,9	2,7	2,7	2,3	1,8	
	» »	2	»	4,5	5,4	5,4	5,4	5,2	4,5	
	» »	3	»	2,9	2,9	2,9	2,9	3,4	3,2	
XXVII	» *casei*	5	W	2,0	1,8	1,8	1,8	1,8	0,9	
	» »	6	»	2,7	2,3	2,3	2,3	2,3	1,1	
XXVII	» *liquefaciens*	9	»	1,1	1,4	1,5	1,1	0,8	0	
	» »	10	»	0,9	1,4	0,9	0,9	0,2	0	
XXVII	» *pyogenes aureus*	13	C	3,0	3,2	3,0	2,9	2,7	2,7	
XXVII	» » *albus* ..	29	»	2,9	2,7	2,5	2,5	2,5	0,9	
XXVII	» *mycodermatus* ..	30	»	2,9	2,9	3,2	2,9	2,9	2,9	
XXVIII	*Thermobacterium lactis*	6	W	0	0,2	1,1	1,4	1,4	1,4	
	» »	»	Y	(3,4)	6,8	9,7	11.0	11,0	10,4	5,4
	» »	7	»	(3,4)	6,8	8,6	7,7	5,9	3,4	0,9
	» »	8	«	(3,4)	6,3	13,7	15,3	12,4	8,1	5,4
	» »	9	W	0,9	0,9	1,1	1,1	1,1	0,5	
	» »	»	Y	(3,4)	(6,8)	13,7	13,7	10,4	5,4	0,7
	» »	11	W	4,1	4,1	4,1	3,8	3,6	3,6	
	» »	»	Y	(3,4)	(6,5)	12,4	11,3	7,4	2,9	0,7
XXVIII	» *helveticum*	12	W	0	0	0,8	0,7	0,7	0,2	
	» »	»	Y	(3,4)	(7,2)	13,3	12,8	8,1	4,1	0
XXVIII	» *Jugurt*	13	»	(3,4)	(6,1)	10,1	9,9	7,0	2,9	0,7
XXVIII	» *bulgaricum*	14	C	4,3	4,7	5,2	5,4	5,4	2,9	0
	» »	»	Y	(4,1)	6,8	7,7	7,9	4,3	0	0

Table II c.

Table No.	Species of bacteria	No.	Source of nitrogen	‰ Acid formed from ‰ Dextrose						
				5	10	20	50	100	150	200
XXIX	*Streptobacterium casei*	4	W	(4,5)	5,2	5,2	5,9	5,4	5,1	
	» »	5	»	(4,5)	5,4	5,5	5,6	5,9	5,4	
	» »	6	»	(4,3)	5,6	6,3	5,0	5,0	4,3	
	» »	»	Y	(3,4)	(6,8)	9,7	9,2	9,2	8,8	8,1
	» »	11	W	(4,3)	6,3	6,4	6,5	6,5	6,3	
	» »	»	Y	(3,4)	6,3	12,4	13,5	14,0	12,8	11,3
	» »	16	W	(4,3)	4,5	5,4	6,1	6,5	6,3	
	» »	32	»	(4,5)	7,2	8,3	7,9	7,0	6,2	
	» »	33	»	(4,3)	6,8	7,4	7,7	7,9	6,1	
	» »	»	Y	(3,4)	(7,0)	14,2	15,3	15,3	15,3	12,4
	» »	34	W	(4,5)	6,3	6,5	6,5	6,5	6,3	
	» »	»	Y	(3,4)	(6,5)	13,3	14,2	13,5	13,5	11,9
XXX	» *plantarum*	1	W	4,5	4,5	6,2	5,6	5,4	5,0	
	» »	3	»	0	2,5	5,4	4,5	4,5	3,6	
	» »	»	Y	(3,4)	(6,5)	11,7	10,3	10,1	8,6	3,8
	» »	7	W	(4,5)	5,6	7,2	7,2	6,1	4,1	
	» »	10	»	(4,5)	6,1	6,4	5,6	3,6	2,5	
	» »	»	Y	3,4	4,5	9,7	10,1	10,1	10,1	10,1
	» »	12	W	(4,5)	5,2	6,5	5,2	5,0	2,7	
	» »	20	»	(4,5)	5,0	5,6	5,9	5,9	5,4	
	» »	»	Y	(3,4)	(6,8)	10,6	11,7	10,1	7,7	6,8
XXXI	*Betabacterium breve*.....	4	W	1,4	1,8	4,5	5,0	4,5	4,1	
	» »	»	C	2,7	4,5	6,1	6,1	5,4	4,5	3,8
	» »	5	W	0,1	0,9	5,6	4,1	3,5	3,2	
	» »	»	C	2,3	3,6	7,7	7,0	6,3	6,3	5,6
	» »	6	W	0,7	0,8	2,3	2,5	2,7	2,5	
	» »	7	»	0,1	1,4	3,6	3,6	3,4	3,4	
	» »	10	»	0,6	1,3	2,9	2,9	2,8	4,1	
	» »	»	C	2,9	4,5	8,8	8,8	10,4	7,4	6,5
XXXI	» *longum*.....	32	»	1,6	3,2	4,5	3,6	2,9	2,9	2,5
XXXII	*Microbacterium lacticum*....	4	»	2,0	2,0	1,6	1,4	0,7	0	0
	» »	5	»	2,7	2,0	1,8	1,6	0,7	0	0
XXXII	» *flavum*.....	8	W	3,8	3,8	2,9	1,8	1,8	0,9	
XXXII	*Bacterium bifidum*	12	Y	(4,3)	(6,3)	12,4	11,9	9,7	1,4	1,1

exactly answered to the previous ones, by testing them both with bacteria which we knew fermented the substance in question and also with bacteria which we knew were unable to do so. Many of these sugars, however, are very expensive, and it was therefore ordered that as little as possible of them should be used. Owing to the strong fermentation of many of our strains, it is, as already mentioned, necessary to use at least 2% of the sugars. The question is, however, whether even this amount is sufficient in all cases — for when desiring to ascertain with certainty what sugars can be fermented, it is necessary that the test should be made under optimal conditions in every respect; also as regards the concentration of the sugar. Table II shows the acid production of several of our strains with various quantities of grape sugar. As we generally reckon the quantity of acid formed in $^0/_{00}$, the quantity of sugar employed is here also expressed in $^0/_{00}$. In order to see at all how the lower sugar concentrations behave, we have as a rule worked with an inferior source of nitrogen (*W* with 0.3% *N*); only where the bacteria did not thrive sufficiently on this we have used the better sources (*C* and *Y* with 0.5% *N*) but by this means, all the sugar is generally fermented in the lower concentrations[1]), so that the results here will be misleading.

It will be seen from Table IIa, that the Streptococci are very little affected by the concentration of sugar, not until 15% (150 $^0/_{00}$) is reached — more rarely 10% — does the effect become pronounced. Most frequently, the optimum is found to lie at ½, 1, or 2% sugar; only in the case of *Streptococcus cremoris* is it first reached at 5 or 10%. Table IIb, shows that the betacocci exhibit an even greater sugar concentration than the last-mentioned species, while the tetracocci (micrococci and sarcinæ) resemble the majority of streptococci in this respect. The sugar optimum of the thermobacteria lies at 2, 5 or 10% sugar, and according to Table IIc, most of the other rod-shaped lactic acid bacteria are much the same. An exception is formed by the microbacteria, which prefer the lowest concentrations[2]). The main result of the investigation, however, is that we can without hesitation employ 2% sugar, whether we wish to study one or another species of lactic acid bacteria, as in no case will the quantity of acid formed therewith differ essentially from the quantity formed at optimal sugar concentration.

The investigations mentioned apply only to grape sugar. In the case of the polysaccharides, the optimum of sugar concentration lies as a rule somewhat higher, though not so much as to be of any practical importance. The more or less complete exclusion of air may also affect the conditions here in question. Table IId. shows, by way of example, how *Thermobacterium cereale* (*Bacillus Delbrücki*) appeared under different experimental conditions. The nitrogen content in all tubes was 0.5%, and the quantity of acid formed is as usual expressed in $^0/_{00}$.

Even more important than knowledge of the sources of energy themselves is the knowledge of the manner in which they are utilised; it is this factor which determines

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[1]) Where this is the case, the quantity of acid formed is placed in parenthesis in the tables.

[2]) We have also investigated coli and aerogenes bacteria in their relation to the quantity of sugar, and found that they throve almost equally well with small or larger quantities. The aerogenes bacteria, however, form hardly any acid with $^1/_2$ or 1 $^0/_0$ sugar, as they are able to convert this slight amount of sugar almost entirely into gas.

Table IId.

No. of bacteria in Table XXVIII	3					4					5				
‰ sugar	10	20	50	100	200	10	20	50	100	200	10	20	50	100	200
Y + maltose (as malt extract) [1]	(9,5)	11,7	12,2	**13,5**	11,5	(10,4)	12,6	13,3	**16,2**	14,0	(9,5)	12,2	12,6	**13,5**	12,6
Y + dextrose	6,1	7,9	**9,2**	6,3	3,8	6,5	10,8	10,8	**12,0**	7,4	7,0	13,3	**14,9**	13,1	8,3
Y + dextrose covered with paraffin	8,7	9,5	**10,4**	6,3	5,0	6,0	13,3	**14,0**	13,1	11,0	5,4	**13,1**	12,2	11,5	11,5

whether we can reckon the strains investigated as belonging to the true lactic acid bacteria or not. The fermentation products formed by these bacteria consist as a rule, besides dextro-, lævo-, or inactive lactic acid only of a little succinic acid and volatile acids (acetic acid with traces of propionic and carbonic acid). Some few species can also form — chiefly from lævulose — a small quantity of mannite and hydrogen.

As I have already, in several previous works[2]), described in detail the methods employed for demonstration of the mentioned fermentation products, I shall not go into this again at length. It should merely be noted, that as the acid formed is often a combination of active and inactive acids, the zinc lactates (whose water content and rotary power are used to determine the modification of the lactic acid) should always be allowed to crystallise out in several fractions. The more heavily soluble inactive salts will then crystallise out before the more soluble active ones, and it will thus be possible to form an estimate of their respective values. It should also be mentioned, that mannite may very easily crystallise out from an alcoholic extract of the dessicated culture. If the lævulose has not been entirely fermented, the remains of this should be removed before drying, by boiling with lime milk. A decoloration with bone black may then be necessary. Jan Smit[3]), who has worked more particularly with mannite, points out that, where a quantitative determination is required after the lime treatment, oxalic acid should be added, and, for removing any possible surplus thereof, again some chalk, as otherwise part of the mannite will unite with the lime. If there should be too little mannite present, to form the crystals easily recognisable under the microscope, then it will be necessary to content oneself with demonstrating it by means of copper sulphate and caustic soda, with which, like other polyvalent alcohols, it gives a dark blue solution[4]).

For demonstration of the separate fermentation products, it is of course always an advantage to have them in not too weak concentrations; accordingly, the cultures are given at least 4% sugar and a quantity of chalk sufficient to neutralise all acid formed,

[1]) In the maltose, however, the grape sugar of the malt extract is included. As the malt extract also contains dextrine, which is to some extent fermented by the bacteria here investigated, it is quite possible to get a greater quantity of acid formed than corresponds to the maltose itself.

[2]) I may here refer especially to my "Studien über die flüchtigen Säuren im Käse etc." Centralblatt f. Bakt. II. Abt. 1904. XIII, 434.

[3]) Zeitschr. f. anal. Chemie. 1914. 53, p. 473.

[4]) To 50 cm³ liquid is added 25 cm.³ $4 \times nNaOH + 25$ cm.³ copper sulphate (as with Fehling's liquid).

thus permitting fermentation of the greatest quantity of sugar. The lactic acid can be quantitatively determined when it has been liberated with sulphuric acid and afterwards extracted with ether, but if it is only desired to accertain what quantity of the sugar fermented is turned into acid, it is better not to add chalk to the cultures, as the quantity of acid can then be simply determined by titration. In the case of the true lactic acid bacteria, the quantity of volatile acids is as a rule small, and that of succinic acid generally even less; we can therefore reckon all the acid formed as lactic acid, without risk of any essential error. The calculation is thus very simple, when dealing with a hexose. It is somewhat more complicated when, as in the case of milk, we have to start with a disaccharid lactose, which may not only have become more or less hydrolysed by the bacteria, but where also the two hydrolysis products, the grape sugar and the galactose, may have been fermented in unlike degree. If the milk sugar be hydrolysed completely (by heating to 115° for half an hour with 4% H_2SO_4,) then its power of reduction is increased some 40—43%[1]), and we can therefore, if such hydrolysis has been effected by the bacteria (see *Betacoccus bovis* No. 34, Table III) even find an increase of sugar during fermentation. It is consequently necessary to determine the degree of hydrolysis of the remaining sugar, and correct accordingly. All the experiments noted in Table III were made with the same milk, with 5.38% lactose, only in the last five experiments another milk was used, with 5.20% lactose ($C_{12}H_{22}O_{11}$, H_2O)

As will be seen, the remaining milk sugar is as a rule only slightly hydrolysed. In the present experiments, only a few thermobacteria (apart from the betacoccus already mentioned) were able to produce any considerable hydrolysis. As these formed over 1¼% lactic acid, it follows that lactase can act even in liquids with a considerable degree of acidity. In other experiments, where milk to which chalk had been added was used, it was found that not only had the majority of the betacocci (also those of the species *Bc. arabinosaceus*) strongly hydrolysed the milk sugar, but generally also the thermobacteria, betabacteria and strains of the species *Streptococcus thermophilus*, and *Streptobacterium plantarum*. The power of hydrolysing milk sugar can hardly be employed as species character, since it is met with, albeit not very frequently, in all species of lactic acid bacteria. The hydrolysis is only strong in old cultures, especially those in which the greater part of the bacteria cells are dead, (in the culture of *Betacoccus bovis* No. 34, for instance, all the cells were dead) which seems to suggest that the active lactase is in reality an endoenzyme which is only given off when the cells are weakened, and that consequently, hydrolysis of milk sugar outside the cell is not a normal process at all. Lactic acid bacteria behave in exactly the same way towards saccharose and maltose, so that in all probability, the disaccharides are taken in as such, and there is thus nothing to prevent their being even better sources of carbon than the monosaccharides of which they are composed.

As the differences shown in Table III between the quantity of sugar fermented and the amount of acid formed lie as a rule within the limits of possible error (especially considering that the quantity of sugar fermented cannot be corrected to entire accuracy) we must take it that true lactic acid bacteria transform nearly all the sugar to lactic acid. An exception, however, is *Betacoccus bovis*, and we shall shortly see that

[1]) In milk cultures, we reckon with roughly speaking 40%, as the more strongly reducing glucose is often fermented in greater quantity than the less reducing galactose.

Table III.

Table No.	Species of bacteria	No.	Increasing of the reduction power after hydrolysis ‰	Sugar not fermented ‰	Sugar fermented ‰	Sugar fermented corrected ‰	Lactic acid formed ‰
XIV	*Streptococcus lactis*	2	34	51,3	2,5	5,6	5,6
XV	» *cremoris*	18	37	48,7	5,1	6,6	5,6
XXII	» *liquefaciens*	3	37	48,8	5,0	6,5	6,3
XXV	*Betacoccus arabinosaceus*	4	36	49,9	3,9	5,9	4,1
XXVI	» *bovis*	33	39	43,3	10,5	10,9	5,6
	» »	34	0	59,4	÷ 5,6	18,2	5,6
	» »	36	33	47,1	6,7	10,0	3,8
XXVII	*Tetracoccus liquefaciens*	11	38	47,6	6,2	7,2	5,0
XXVII	» ?	12	34	47,7	6,1	9,0	5,2
XXVIII	*Thermobacterium lactis*	6	26	44,4	9,4	15,6	12,6
	» »	11	23	44,2	9,6	17,1	12,8
XXVIII	» *bulgaricum*	14	20	45,2	8,6	17,6	12,8
XXVIII	» *helveticum*	12	40	34,5	19,3	19,3	19,3
XXIX	*Streptobacterium casei*	2	35	41,3	12,5	14,6	13,1
	» »	4	38	41,6	12,2	13,0	12,8
	» »	5	37	42,8	11,0	12,3	11,5
	» »	6	37	42,9	10,9	12,2	10,1
	» »	13	29	42,8	11,0	15,7	13,7
	» »	18	35	41,6	12,2	14,3	13,3
	» »	32	37	36,3	17,5	18,6	16,9
	» »	34	37	37,8	16,0	17,1	16,7
XXX	» *plantarum*	1	34	45,0	8,8	11,5	10,4
	» »	3	37	45,7	8,1	9,4	7,7
	» »	8	37	48,2	5,6	7,0	6,5
	» »	13	36	43,2	10,6	12,3	10,1
	» »	15	37	45,2	8,6	10,0	7,7
	» »	20	38	42,6	11,2	12,1	11,9
XXXI	*Betabacterium breve*	3	39	50,6	3,2	3,7	2,0
	Bacterium coli A[1]	1	40	47,2	4,8	4,8	5,5
	» » B[1]	2	41	48,3	3,7	3,7	4,2
	» *prodigiosum*	1	41	42,5	9,5	9,5	3,6
	» *aërogenes*	1	19	2,3	49,7	50,0	4,1
	» »	2		0	52,0	52,0	2,8

Betacoccus arabinosaceus behaves in a similar way with other sugars than lactose. That we nevertheless reckon the betacocci as among the true lactic acid bacteria is due to the fact that they are allied to these in all other respects. The betabacteria also, can, in a freshly isolated state, transform a great quantity of the sugar to something other than lactic

[1]) A and B indicates the relation of the Coli-forms to sugars according to C. O. JENSEN. Both of them essentially form succinic acid, A besides some dextro-lactic acid, B some lævo-lactic acid. The quantity of volatile acids (1 part of propionic acid to 10 parts of acetic acid) makes up 30 per cent of the fermented sugar.

acid, but as a rule, they lose this tendency after continued cultivation in the laboratory, and will thus, in course of time, be found to differ in no essential degree from other lactic acid bacteria. In the system which we shall later set up for the lactic acid bacteria, we have nevertheless separated off the betabacteria and betacocci as a distinct sub-group.

At the close of Table III, we have shown, for purposes of comparison, the acid production of a few other bacteria. It is highly surprising to find that the two coli strains — despite abundant air formation — apparently form over 100% acid from the fermented sugar. The explanation, however, is that these bacteria only form a small quantity of lactic acid, but ferment the greater part of the sugar to succinic acid and acetic acid, thereby giving three equivalents of acid for every two obtained by lactic acid fermentation.

$$C_6H_{12}O_6 = 2\,C_2H_5OCOOH = C_2H_4(COOH)_2 + CH_3COOH + H_2$$

1 grape sugar = 2 lactic acid = 1 succinic acid + 1 acetic acid + 1 hydrogen.

By calculating the acid formed as lactic acid, then, we have thus put the yield of acid $^1/_3$ too high. *Bacterium prodigiosum* forms acids similar to those formed by the coli bacteria, but as it also transforms a quantity of the sugar completly into gas, there is a distinct wastage here. Transformation into gas is, however, most marked in the caseof the aerogenes bacteria, and especially No. 2, which has fermented all the sugar in the milk without coagulating it.

A point of no slight interest is the question whether a given lactic acid bacterium will under all circumstances form the same amount of by-products, especially volatile acids, and whether it always forms the same modification of lactic acid.

Here, above all, the quantity of sugar plays a part, for if there is not any more sugar than the occurring bacteria can casily ferment, part of the lactic acid formed will be further transformed. This is plainly seen from Table II where the quantity of acid formed is considerably less than the fermented quantity of sugar in the cases where all the sugar has been fermented, i. e. where the figures are put in parenthesis.

As regards the formation of acetic acid, KAYSER[1]) has already shown that this increases with the supply of air, and BARTHEL[2]) has pointed out that it is as a rule greater where the conditions of life (f. in. the temperature) are unfavourable. In accordance with this we have found a relatively far greater quantity of acetic acid in cultures without chalk than in those with. True, the quantity of acetic acid formed in milk cultures with and without chalk is about the same, but as a far greater quantity of sugar is fermented when chalk is added, it follows that from the sugar fermented more acetic acid is formed when chalk is omitted than when it is added. Some examples of this are shown in Table IV. We have reckoned one molecule hexose as giving three molecules of acetic acid.

The quantity of volatile acid formed by the lactic acid bacteria depends not only upon the conditions of life, but varies also in other ways. BARTHEL found, for instance, that *Sc. fæcium* No. 19, in a freshly isolated state, formed from the sugar fermented 39% volatile acid, whereas we, nine months later, under the same experimental conditions, found only 13% volatile acid. The bacterium in question exhibited no sign of weakening, but formed, indeed, more total acid in milk than it did in a freshly isolated state.

Sugars whose number of carbon atoms is divisible by six do not as a rule affect the

[1]) Contribution a l'étude de la fermentation lactique. Paris 1894.

[2]) Centralblatt f. Bakteriologie. II. Abt. 1900. Bd. IV, p. 420.

relation between lactic acid and by-products. In the case of the betacocci, however, it is otherwise, as they form from lævulose, and therefore also from saccharose, more acetic

Table IV.

Table No.	Species of bacteria	No.	Addition of chalk	Sugar fermented, corrected ‰	Acetic acid formed ‰	Acetic acid percentage of fermented sugar
XV	*Streptococcus cremoris*	20	+	31	1,0	3
			0	7	1,0	14
XXVIII	*Thermobacterium lactis*	11	+	52	0,5	1
			0	13	0,4	3
XXVIII	» *helveticum*	12	+	52	0,8	1
			0	19	1,2	6
XXIX	*Streptobacterium casei*	17	+	52	1,2	2
			0	12	1,0	8
XXIX	» »	18	+	52	0,5	1
			0	10	0,5	5
XXIX	» »	34	+	52	1,0	1
			0	18	1,0	6

acid and gas than from other hexoses. Table V shows the fermentation of lævulose and grape sugar in a strain of strong gas-developing character (*Betacoccus arabinosaceus* Nr. 12).

Table V.

Species of sugar	Sugar fermented ‰	Lactic acid formed ‰	Acetic acid formed ‰	Rest ‰
Lævulose	19,1	3,0	2,5	13,6
Dextrose	12,8	5,6	0,5	6,7

As will be seen, this coccus forms almost as much acetic acid as lactic acid from lævulose, but on the other hand, only $^1/_{10}$ as much acetic acid as lactic acid from grape sugar. The fermented sugar which is not transformed into acid (the remainder in the last column of the table) has not all been formed into gas (carbonic acid and hydrogen) at any rate not in the case of the lævulose culture, which gave a very distinct mannite reaction.

Now one molecule of hexose can by the lactic acid bacteria be simply broken up into two molecules of lactic acid, whereas sugars having a smaller number of carbon atoms, will of course be otherwise divided. It would seem reasonable beforehand to suppose that pentoses would be broken up into equivalent quantities of lactic acid and acetic acid, and as a matter of fact, there is really always a large quantity of acetic acid formed when pentoses are fermented by lactic acid bacteria. As a rule, however, we obtain considerably less acetic acid and more lactic acid than would answer to the equation

$$C_5H_{10}O_5 = C_3H_6O_3 + C_2H_4O_2.$$

It will be seen from Table VI, that we have found in the case of the betabacteria a division exactly answering to the equation

$$6\ C_5H_{10}O_5 = 8\ C_3H_6O_3 + 3\ C_2H_4O_2$$

6 arabinose = 8 lactic acid + 3 acetic acid.

The bacteria thus appear as true lactic acid bacteria, inasmuch as they form the greatest possible quantity of lactic acid from the source of energy given[1]). This is, however, on the supposition that chalk is added to the cultures; without chalk, we obtain here, as in other cases, proportionately more acetic acid.

Table VI.

Betabacterium breve No. 10	Arabinose fermented ‰	Lactic acid formed ‰	Acetic acid formed ‰	Lactic acid formed $cm^3 \frac{n}{4}$	Acetic acid formed $cm^3 \frac{n}{4}$	Ratio equivalent: Lactic acid / Acetic acid
Arabinose casein pepton broth with chalk.	35,2	28,0	7,2	125	48	8:3
» » » » without » .	17,8	13,1	4,7	58	31	2:1

Up to now, we have reckoned the entire quantity of volatile acids as acetic acid and this we may safely do, as there is at the outside $^1/_{10}$, more often only $^1/_{20}$ propionic acid, and a trace of formic acid mixed therewith.

In the case of certain betabacteria, we have already mentioned that more gas is formed with cultures in a freshly isolated state, and as regards the betacocci, the development of gas is undoubtedly in proportion to their well-being. Much would seem to suggest that the transformation of sugar in all cases takes place by way of lactic acid, and that it can only be further divided — i. e. made to yield more energy — by strains of particularly marked vitality. The majority of the true lactic acid bacteria, which do not develop any measurable quantity of gas, can, however, — likewise when in a state of particular vitality — produce in milk so much carbonic acid that fine stripes appear in the curd. The lactic acid bacteria, then, as mentioned, form most acetic acid under unfavourable conditions, whereas exactly the reverse is the case with the carbonic acid, of which most is formed under favourable conditions.

Experiences from our previous works warrant the supposition that the rotary power of the lactic acid formed constitutes an important specific character for the true lactic acid bacteria, and we have therefore determined, in the case of all strains examined, what sort of lactic acid they formed, not only in milk, but also in broth with different carbon and nitrogen sources. These investigations we have repeated from year to year. The new investigations have on the whole confirmed the correctness of our supposition. As a rule, neither the carbon sources nor the nitrogen sources affect the modification of the lactic acid. Those strains which in milk form pure dextro- or lævo-lactic acid will also in a nu-

[1]) This is the more surprising, as the ancestors of the strains used (*Bacillus γ.* in my thesis above quoted), formed much succinic acid, with abundant gas development.

tritive broth always form dextro- or lævo-lactic acid, whether the source of energy be alcohols, aldoses, ketoses, pentoses, hexoses or polysaccharides[1]). Those strains which in milk form purely inactive lactic acid — i. e. with like quantities of dextro- and lævo-acid — will as a rule also under other conditions maintain the equilibrium between the two acids, whereas strains which in milk form more of the one than of the other will under less favourable conditions generally only form the acid which they most easily produce. Indeed, we do not even need here to alter the nutritive substrate, as even in milk, these bacteria can in the course of years end by being only capable of producing the one acid. We have numerous examples, for instance, of cases where strains of the species *Streptobacterium casei*, which in a freshly isolated state forms, besides dextro-lactic acid, also smaller or larger quantities of lævo-lactic acid, have after a more or less considerable lapse of time been found capable only of forming dextro-lactic acid, and that without any decrease in the total production of acid.

These investigations, then, distinctly show that the modification of lactic acid is altogether independent of the stereochemical structure of the sugars, and depends entirely upon the species of bacteria. We must therefore presume that dextro- and lævo-lactic acid are formed each by its own independent enzyme. Strains which are equally supplied with both (as for instance *Thermobacterium helveticum*) will under all conditions form purely inactive lactic acid, whereas strains which can more easily produce one of the enzymes than the other, may often entirely lose the power of producing the latter, and thus the faculty of forming the corresponding lactic acid.

Nitrogen Sources. In contrast to the pseudo lactic acid bacteria, the true ones do not thrive with ammonia salts or single amino-acids as source of nitrogen. We have in this latter respect tested all our strains of bacteria with aspartic acid, but none of them showed any signs of growth. The true lactic acid bacteria demand just as complicated nitrogenous food as the animals, viz. genuine proteins or the entire complex of amino acids therein contained. Even incomplete proteins, such as gelatine (without addition of other nitrogenous nourishment) generally proves, as in the case of animals, an extremely bad nitrogenous food. As the lactic acid bacteria — save for the few species capable of liquefying gelatin — do not in a living state give off any proteolytic enzymes, the nitrogenous food must be given in a state of solution or in colloid form. Of all genuine proteins, the most suitable seems to be casein in the form in which it is found in milk. Even better, however, in many cases, is paracasein (or rather, perhaps, the peptones which rennet gradually forms from casein); this will be seen from Table VII, where we have noted the quantities of acid formed by various species of our lactic acid bacteria in the same milk with and without addition of rennet. The rennet employed was rendered germ-free by filtration through a sterilised CHAMBERLAND-filter[2]). The effect of the rennet

[1]) HERZOG and HÖRTH have, in their work "Zur Stereochemie der Milchsäuregärung" (Zeitschr. f. physiol. Chemie 1909. Bd. 60, p. 131) arrived at the same result.

[2]) As such filtration, which keeps back some nitrogenous matter, weakens the rennet very considerably, it is necessary to start with a very concentrated solution. We dissolved 10 HANSEN tablets in 200 cm.^{3} of water, and used 2 drops of the filtrate per 10 cm.^{3} milk. The nitrogen content of the

Table VII.

Table No.	Species of bacteria	No.	‰ Acid formed in milk	
			without rennet	with rennet
XIV	*Streptococcus lactis*	6	3,4	4,5
	» »	7	4,3	6,3
	» »	14	7,4	8,1
XV	» *cremoris*	1	4,7	6,5
	» »	2	4,5	6,1
	» »	10	5,6	6,5
	» »	21	6,3	7,2
XX	» *fœcium*	14	4,1	5,2
	» »	18	4,5	5,6
XXI	» *glycerinaceus*	4	4,5	5,6
	» »	6	2,5	4,7
XXII	« *liquefaciens*	1	7,1	7,1
XXV	*Betacoccus arabinosaceus*	4	5,6	6,8
	» »	7	5,2	5,4
	» »	8	3,8	5,2
XXVI	» *bovis*	34	3,2	3,8
	» »	40	0,5	1,1
XXVII	*Tetracoccus casei*	5	4,1	3,6
	» »	6	2,7	2,7
XXVII	» *liquefaciens*	9	2,5	2,7
	» »	11	0,7	0,7
XXVIII	*Thermobacterium lactis*	6	10,4	10,4
	» »	11	11,0	11,7
XXVIII	» *helveticum*	12	19,6	21,6
XXVIII	» *bulgaricum*	14	13,3	13,7

Table No.	Species of bacteria	No.	‰ Acid formed in milk	
			without rennet	with rennet
XXIX	*Streptobacterium casei*	1	6,1	6,1
	» »	2	9,9	11,3
	» »	4	11,3	12,8
	» »	9	10,4	10,6
	» »	10	11,9	13,3
	» »	11	14,2	14,4
	» »	14	9,7	11,5
	» »	15	13,1	13,1
	» »	23	11,0	11,9
	» »	25	12,8	12,8
	» »	27	13,1	15,8
	» »	32	14,6	16,0
	» »	34	16,0	17,1
XXX	» *plantarum*	1	5,9	8,8
	» »	2	2,9	5,4
	» »	3	3,2	7,0
	» »	7	3,8	9,2
	» »	9	8,3	10,4
	» »	11	3,6	5,6
	» »	12	5,0	8,8
	» »	14	9,5	11,0
	» »	15	4,1	7,4
XXXI	*Betabacterium breve*	4	1,8	1,6
	» »	5	0,9	0,9
	» »	10	1,8	2,0

is of course not perceptible if the bacteria themselves give off proteolytic enzymes, as is the case with *Streptococcus liquefaciens* and *Tetracoccus liquefaciens*. It is likewise unnoticed in the case of other tetracocci, certain thermobacteria, the betabacteria, and certain strains of the species *Streptobacterium casei*, but is quite extraordinarily distinct in the case of *Streptobacterium plantarum*. This species, for which casein is as a rule a relatively poor source of nitrogen, forms, when rennet is added to the milk, far greater quantities of acid than otherwise. This fact throws new light upon the ripening of rennet cheese, a process which is due to the action of the rennet and of the lactic acid bacteria. We have already previously shown that the action of the rennet is furthered to a high degree by

milk is thus not perceptibly affected, and the milk, being highly sterilised, does not coagulate. It does, however, coagulate, as soon as any trace of acid is formed, and after inoculation with lactic acid bacteria therefore, milk to which rennet has been added will coagulate far more rapidly than milk without rennet. If the bacteria themselves give off proteolytic enzymes, then there will of course be no difference, practically speaking, in the time required for coagulation.

the acid formed by the lactic acid bacteria[1]), and we have thus now shown, on the other hand, that the action of many lactic acid bacteria is furthered by the rennet. And in this connection it should be mentioned that BARTHEL has recently shown[2]) how considerably rennet increases the casein-splitting power in *Streptococcus lactic.* The various factors which give rise to the ripening of cheese thus mutually accelerate one another's action. Of other genuine proteins which may be utilised for the cultivation of lactic acid bacteria, we may mention gluten and legumin dissolved in the smallest possible quantity of sodium phosphate. This solvent may also well be employed where casein is to be used in connection with other sugars than milk sugar.

The most typical milk bacteria grow best in milk, and only with the greatest difficulty in peptone solutions; there are, however, lactic acid bacteria which, even though they ferment milk sugar in peptone solutions, thrive poorly in milk, or require at any rate to be accustomed to it, and which will in consequence rapidly lose the faculty of so doing if left for many generations without coming in contact with milk at all. Indeed, it seems possible to accustom the bacteria to largely dissimilar forms of nitrogenous nourishment, and it is doubtless in many cases here that the main difference lies between the parasites and the saprophytes most nearly related. The pathogenic forms have often so accustomed themselves to a certain particular nitrogenous food (that from which their toxins are also formed) that they can ill thrive without it. It has thus on the whole proved considerably more difficult to keep the pathogenic streptococci alive than the saprophytic. Many of the rod-shaped lactic acid bacteria of the digestive tract, also, were difficult to cultivate, owing to the fact that we had not succeeded in satisfying their particular requirements in respect of nitrogenous food.

When seeking to compare the values of different nitrogen sources, the only possible method is that employed in agricultural chemistry, to wit, by offering the organisms the same quantity of nitrogen in the different forms, but under conditions otherwise uniform. In Table VIII will be found noted the quantities of acid (calculated, in the usual way, as ‰ of the nutritive substrate) formed by some of our strains in horse serum (*S*) Liebig's meat extract (*L*), Cibil's do (*Ci*), Witte peptone (*W*)[3]), casein peptone (*C*) and yeast extract (*Y*), partly with the separate sources alone, and partly when used with the addition of a 2% Witte peptone solution. It was so arranged that the quantity of nitrogen was throughout 0.4%, 2 percent grape sugar was added, and the quantity of the different nutritive salts was also kept as far as possible uniform throughout, save that no potassium phosphate was added to the W_0. The last three columns of the table show the effect of this salt upon the casein pepton broth used by us, with 0.5% *N*.

We had expected that the pathogenic bacteria would have preferred blood serum and meat extract, and the milk bacteria casein peptone. This however, did not prove to be the case. The blood serum proved throughout a bad source of nitrogen, even for pathogenic bacteria, and this despite the fact that its bactericidal substances

[1]) Landwirtschaftliches Jahrbuch der Schweiz, 1904, p. 404, and 1907, p. 97.

[2]) Meddelande Nr. 171 från Centralanstalten för försöksväsendet på Jordbruksområdet. 1918.

[3]) The serum contained 1.13 % *N*, LIEBIG's Meat Extract 9.22 % *N*, and CIBIL's do. 3.11 % *N*. Equal quantities of nitrogen from the two meat extracts had very nearly the same effect upon most of the bacteria used.

Table VIII a.

Table No.	Species of bacteria	No.	All nutritive mediums with 2 % Dextrose and 0,4 % N										C. with 2 % Dextrose and 0,5 % N and ‰ K_2HPO_4:		
			S	*L*	W_0	*W*	*C*	*Y*	*W* + *S*	*W* + *L*	*W* + *Ci*	*W* + *Y*	0	2	4
XXIV	*Streptococcus pyogenes*	1	0,9	4,1	1,4	2,3	2,7	1,8	2,3	2,0	2,3	2,3	2,9	4,1	4,1
XXIV	» »	3	0,7	3,8	1,4	1,4	3,2	0,2	2,9	2,3	2,5	1,6	3,4	4,3	4,3
XXIV	» »	4	0,7	3,6	0,9	0,9	2,5	0,2	1,8	1,1	1,6	1,1	2,9	3,6	4,1
XXIV	» »	7	0,9	5,6	1,6	2,7	4,3	5,6	3,4	3,4	3,6	3,6	5,6	6,3	6,3
XXIV	» »	8	2,5	5,4	0,5	2,7	3,4	3,2	2,7	3,4	3,4	2,9	3,8	5,4	5,6
XXIV	» »	11	1,6	5,6	0,7	3,6	4,7	6,8	3,4	5,4	5,0	5,0	5,9	7,9	8,1
XX	» *fœcium*	8		7,0	0,7	3,8	5,2	6,8	3,6	4,5	4,7	5,0	5,4	7,2	7,7
XIV	» *lactis*	8	2,7	6,5	2,3	3,6	5,2	6,3	3,6	4,5	4,7	4,3	5,9	7,2	7,4
XV	» *cremoris*	1	0,2	4,7	1,6	2,5	2,9	2,9	2,7	2,3	2,3	2,9	4,5	4,7	4,7
XV	» »	18	1,1	5,2	1,8	3,2	5,4	3,6	3,8	3,6	3,6	3,6	5,2	6,5	7,0
XV	» »	19	0,2	3,4	0,9	2,5	4,3	4,3	4,1	3,6	4,5	3,4	4,3	5,6	5,9
XVI	» *mastitidis*	2	2,5	4,5	1,6	2,7	3,4	0,2	2,9	2,5	2,7	2,9	4,1	4,7	5,0
XVI	» »	3	2,0	5,0	1,4	2,7	3,4	2,9	3,2	2,9	2,9	2,3	4,5	5,4	5,6
XVII	» *thermophilus*	2	2,9	6,3	1,8	2,7	5,4	5,2	4,7	3,2	2,7	3,6	6,8	8,1	8,6
XIX	» *inulinaceus*	4	0,9	4,3	1,6	3,6	4,5	4,7	4,1	4,3	3,8	4,1	4,1	4,7	5,4
XXI	» *glycerinaceus*	4		6,3	2,5	3,6	4,3	5,4	3,4	3,8	4,5	5,0	5,9	7,7	8,1
XXI	» »	6	2,9	6,3	2,5	3,6	4,5	5,0	4,1	3,8	4,1	4,1	5,6	6,5	6,8
XXII	» *liquefaciens*	1	4,1	7,2	2,7	3,8	4,3	6,5	3,6	4,3	4,3	5,0	5,0	6,3	7,0
XXII	» »	5		6,3	2,5	3,2	4,5	5,6	2,5	3,6	4,1	3,4	5,4	7,0	7,4
XXV	*Betacoccus arabinosaceus*	4		0,7	0,9	2,9	3,4	6,8	2,9	3,8	3,6	4,5	4,3	5,2	5,4
XXV	» »	8	0,3	5,2	2,9	2,9	3,4	7,7	3,6	4,3	4,1	4,3	3,8	3,8	5,0
XXV	» »	12		5,2	2,7	2,7	3,8	8,1	2,5	3,6	3,6	4,1	4,5	5,4	5,4
XXVI	» *bovis*	34	0,5	5,4	2,0	2,7	4,5	7,0	3,2	4,3	4,3	4,3	5,9	6,1	6,1
XXVI	» »	41		7,0	0,9	3,2	3,4	7,2	2,7	4,3	4,5	4,5	5,0	5,0	6,1
XXVII	*Tetracoccus casei*	5		3,8	1,4	2,0	2,7	0,9	1,8	2,7	3,2	2,7	2,9	3,4	3,4
XXVII	» ?	8	2,0	2,5	0,9	0,9	1,8	0,9	1,4	0,9	1,4	0,7	1,6	2,3	2,3
XXVII	» *liquefaciens*	9		2,5	0,5	0,9	1,8	0,5	1,1	1,1	1,6	0,9	1,8	2,7	2,9
XXVII	» *pyogenes aureus*	13	2,0	4,5	2,0	2,3	2,5	3,6	2,3	2,3	2,9	2,0	3,4	4,3	4,5
XXVII	» » *albus*	29	1,1	3,2	0,7	0,9	2,0	1,8	1,4	1,4	2,0	3,2	1,8	2,9	3,2

had been inactivated by long standing; meat extract, on the other hand, was on the whole at least as good a source of nitrogen as casein peptone. Only *Microbacterium lacticum* decidedly prefers the latter. That we have not, in the present work, employed meat extract is due, besides its dark colour, to the variability of its composition. The meat extract now generally sold is no longer mainly composed of creatin and purin bases; far from it; the overwhelming majority of the nitrogenous matter it contains is furnished by albumoses and peptones. Yeast extract exhibits a more specific action, proving an extremely bad source of nitrogen for a number of pathogenic bacteria[1]), (*Sc. pyogenes* Nos. 3 and 4, and *Sc. mastitidis* No. 2), some few micrococci (tetracocci) and *Mbm. lacticum*, whereas it furthers to a surprising degree the develop-

[1]) The well-known therapeutic properties of yeast are doubtless connected with this fea ture.

Table VIII b.

Table No.	Species of bacteria	No.	All nutritive mediums with 2 % Dextrose and 0,4 % N										C. with 2 % Dextrose and 0,5 % N and ‰ K_2HPO_4:		
			S	L	W_0	W	C	Y	W + S	W + L	W + Ci	W + Y	0	2	4
XXVIII	*Thermobacterium lactis*	6	0	4,3	0,5	2,3	0,7	10,6	6,8	2,9	3,6	7,4	6,1	7,0	7,2
	» »	8	0	7,0	1,6	5,0	0,9	15,3	9,9	4,1	8,1	10,1	11,5	8,6	9,5
	» »	10	0	8,1	0,7	2,5	0,9	12,8	6,5	3,2	4,1	4,7	9,9	8,3	9,7
	» »	11	0	5,9	0,7	5,4	5,9	10,6	5,4	5,9	5,6	5,4	8,6	9,5	9,0
XXVIII	» *helveticum*	12	0	1,4	1,4	0,9	0,7	11,3	3,2	3,4	3,6	6,8	4,5	6,3	7,4
XXVIII	» *bulgaricum*	14	0	1,8	0,7	1,4	3,4	12,2	7,0	0,7	0,9	6,8	2,3	1,6	2,7
XXIX	*Streptobacterium casei*	1	0	7,7	2,7	3,2	3,4	15,8	3,8	3,8	4,1	5,4	2,3	3,6	3,5
	» »	2		8,6	4,0	4,0	7,0	17,6	2,3	5,4	3,8	7,2	6,5	8,3	8,8
	» »	11	0,7	9,7	3,6	6,1	8,3	18,0	8,1	9,0	8,1	11,3	10,6	10,8	11,5
	» »	16	0,5	8,1	3,2	5,2	6,8	17,8	7,0	7,4	7,4	7,7	9,9	10,4	11,5
	» »	34	3,8	11,7	8,3	8,3	11,0	17,6	9,0	8,8	8,3	8,6	13,7	14,2	14,4
XXX	» *plantarum*	1	0	8,6	2,0	6,3	6,3	17,6	8,6	6,3	7,2	11,0	7,2	6,8	4,5
	» »	3	0	8,1	1,8	5,4	7,3	14,4	7,7	6,8	6,1	6,8	8,8	10,4	10,6
	» »	8		8,1	1,6	6,8	8,3	12,4	5,2	7,7	8,1	9,7	11,0	12,4	13,1
	» »	11	3,4	9,0	3,6	3,6	8,3	16,2	6,1	6,5	7,0	8,0	9,5	10,8	11,3
	» »	12		8,6	0,7	6,8	7,2	16,7	5,0	7,4	7,4	9,9	7,3	10,1	11,3
	» »	21		8,6	3,4	5,9	9,5	17,3	5,6	6,8	4,5	7,4	10,1	11,9	12,4
XXXI	*Betabacterium breve*	3		6,1	3,4	5,2	5,2	6,1	2,5	4,7	4,5	5,9	5,2	6,3	6,8
	» »	4	1,8	6,8	2,3	4,5	3,6	7,0	4,1	2,0	1,6	7,0	4,7	5,0	6,1
	» »	10		5,0	2,5	2,5	5,2	8,6	2,7	2,0	2,0	7,2	6,1	6,5	7,9
XXXII	*Microbacterium lacticum*	2		2,4	1,4	1,4	2,5	0	1,6	1,4	2,0	2,4	2,9	3,6	3,8
	» »	3	2,3	0,9	1,4	1,8	2,9	0,2	2,5	1,8	2,3	1,4	3,2	4,5	4,5
	» »	4		1,8	1,6	1,6	2,9	0,2	1,8	2,0	2,3	1,6	2,9	4,1	4,5
	» »	6	1,8	1,1	1,4	2,0	3,2	0,5	2,3	2,0	2,3	1,4	3,2	4,3	4,3
XXXII	» *flavum*	8	0,5	5,6	2,5	3,2	5,6	6,5	1,6	5,6	5,2	6,5	5,4	6,5	7,4
	Bacterium coli A	1		4,0	2,3	2,3	3,6	3,8	2,5	2,9	3,4	2,9	3,8	5,4	5,6
	» *paratyphi*	6	2,7	4,5	2,3	2,3	3,6	3,8	2,3	2,3	2,7	2,3	3,8	5,2	5,2
	» *prodigiosum*	1		4,1	1,4	2,5	4,1	3,2	2,3	2,9	2,9	2,7	3,6	3,8	3,8
	» *aërogenes*	4	3,2	2,3	0,5	2,9	2,9	2,5	1,4	2,9	3,2	2,3	0,5	0,9	0
	» »	5	2,9	1,1	3,2	3,6	5,0	÷0,9	1,1	3,6	3,8	2,9	2,7	2,7	÷0,5
	» *acidi propionici*	3	0	9,9	0	0,5	3,6	13,7	0	0,9	0,7	11,5	8,1	10,4	10,6

ment, and even more the acid formation, of the genera *Thermobacterium*[1]) and *Streptobacterium*. For the genera *Betacoccus* and *Betabacterium*, also, yeast extract is fully as good a source of nitrogen as casein peptone, whereas the reverse is the case with

[1]) The results in the case of thermobacteria are often somewhat uncertain, as these bacteria are so sensitive to nitrogen sources, that they do not grow at all if there be the slightest hindrance. Once they have overcome the difficulties, however, they may then suddenly grow through very powerfully. Both meat extract and yeast extract for instance seem to contain substances which retard the development of *Tbm. bulgaricum*, but it generally overcomes the retarding effect of the yeast extract after the culture has remained at a standstill for a couple of days.

the ordinary saprophytic streptococci[1]). If several nitrogen sources be mixed together, giving a greater certainty of obtaining all the requisite building material for bacterium protein, then the specific qualities of the various sources in themselves will as a rule be effaced.

The favourable effect of phosphoric acid upon the lactic acid fermentation is seen most distinctly by comparing the W_0 and W; less markedly in the experiments with casein peptone and different quantities of phosphoric acid, as the casein pepton in itself contains some phosphoric acid. As a rule, it does not seem that anything essential is gained by adding more than $2‰ K_2HPO_4$, and a single strain (*Sbm. plantarum* No. 1) even appears to be quickly satiated with phosphoric acid[2]). We have also tried some outside species of bacteria, with different sources of nitrogen. Table VIII shows that the propionic acid bacteria exibit a great partiality for yeast extract, whereas the omnivorous coli bacteria of course exhibit a far slighter sensibility in regard to the species of the nitrogen source (as also, by the way in respect of temperature and many other factors) than the true lactic acid bacteria. As regards the aerogenes bacteria, the acid formation can here no longer be taken as any measure of the vital activity, as these bacteria turn more of the sugar into gas the better they thrive, and under favourable conditions, the inoculated tubes become even less acid than the control tubes.

Having investigated the question of which nitrogen sources the different species of bacteria prefer, it is then necessary to determine at what degree of concentration they should preferably be employed. As with sugars, the colour tone and price of the nutritive substrates are increased with increasing concentration, and it is therefore better to keep a little below the optimal limit than to exceed it. It is obvious, of course, that the amount of acid formed cannot be any thoroughly valid measure of the optimal concentration of nitrogen, as all organic nitrogenous nourishment acts as a buffer, and therefore a greater quantity of acid may thus possibly be formed — without exceeding the hydrogen ion concentration detrimental to the various species of bacteria — the more nitrogenous nourishment is given. Nevertheless, we have also in the present instance kept to this measure, not only because it gives a numerical expression of the vital activity of the lactic acid bacteria, but also because further study has shown that lactic acid bacteria really grow most rapidly, and are able to ferment most sugars (i. e. become most abundantly supplied with enzymes) in those nutritive substrates in which they form most acid.

For the experiments shown in Table IX, we used a 2% solution of grape sugar, with the necessary salts, and Witte peptone or casein peptone as source of nitrogen. Of Witte peptone, we used ½, 2, 5, 10 and 15%, answering to the quantities of nitrogen shown in the table. These are, in the case of the 10 and 15% Witte peptone broth, somewhat lower than they should be, as some ingredients of the Witte peptone are no longer fully soluble at these concentrations. As regards the casein peptone, we used partly the solution (with

[1]) In the case of these streptococci, yeast extract appears to be as good a source of nitrogen as casein peptone, judging merely from the quantity of acid formed; this is, however, due to the fact that the yeast extract has fully as great a buffer action as the casein peptone, a feature which we shall refer to more fully later on.

[2]) As the experiment was repeated several times, the figures given are not due to accidental circumstances.

1% *N*) obtained directly from digestion with pepsin, partly the same diluted to twice and four times the volume. The solutions were of course neutralised, and all contained the same quantity of nutritive salts.

The table shows distinctly enough that more acid is formed with increasing quantities of nitrogenous food. When the higher concentrations of nitrogen are reached, however, the increase does not always amount to anything worth mentioning, and in the case of the betacocci and betabacteria, the highest concentrations even appear to be detrimental. Despite the buffer action of the nitrogenous food, the quantity of acid cannot increase indefinitely with increasing quantity of nitrogenous nourishment, owing to the fact that, as van Dam has recently shown[1]), the lactic acid fermentation is not only checked by the hydrogen ions, but also by the lactate ions. It is also a well known fact that only the most powerful lactic acid formers are capable of fermenting all the milk sugar of the milk, even where chalk has been added, and the acid formed has been neutralised by constant shaking. These complications render it impossible to set up any hydrogen ion concentrations as the limit of fermentation for the different species of lactic acid bacteria. The better the buffer action of the nutritive substrate, the more the lactate ions will make their presence felt, and the final concentration of hydrogen ions will be lower in consequence. The lactate ions, however, are not nearly so dangerous to the life of the lactic acid bacteria as the hydrogen ions, and in cultivating lactic acid bacteria, therefore, care should always be taken to employ nutritive substrates with a good buffer action.

It will be seen from Table IX, that the broth with 0.5% *N*. in the form of casein pepton is a better nutritive substrate for lactic acid bacteria than broth with 0.7% *N* in the form of Witte peptone, and can in many cases even compare with broth having 1.35% *N*. in the form of Witte peptone. An das the casein peptone broth is also lighter in colour, than 5% or 10 % Witte peptone broth, and forms no deposit with acid, it will easily be understood that we preferred this for cultivation of lactic acid bacteria, and we use it with just 0.5% *N*. as any further increase of the concentration only exceptionally improves the action.

The peculiar behaviour of the aerogenes bacteria towards sugar, to which we have referred in the course of the explanation of Table VIII, is also apparent from Table IX. The quantity of acid rises, it is true, when the quantity of Witte peptone is increased from ½ to 2%, but when this point has been passed, we find that more and more of the acid formed is changed into gas, and as at the same time a certain protein decomposition

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[1]) Ueber den Einfluss der Milchsäure auf der Milchsäuregärung (Biochemischc Zeitschrift 1918, Bd. 87, p. 107). In this work, van Dam reproaches me with not having observed the influence of the hydrogen ion concentration upon the lactic acid bacteria, basing his accusation upon a brief statement made at a congress with regard to the present work, in which it was quite impossible to enter into any detailed explanation of the individual phenomena. I can, however, console my critic with the fact that Miss Jenny Hempel — three years before the date of van Dam's paper — had kindly investigated, at the Carlsberg Laboratory, the buffer action of the nutritive substrates which I was using, and found that it increased in the following order: Witte peptone, casein peptone, and yeast extract. Yeast extract broth with 0.5 % *N* has about the same buffer action as Witte peptone broth with 1.35 % *N* (i. e. with 10 % Witte peptone).

Table IX a.

Table No.	Species of bacteria	No.	% N as Witte peptone					% N as casein peptone		
			0,07	0,28	0,70	1,35	2,00	0,25	0,50	1,00
XIV	*Streptococcus lactis*	4	1,8	4,1	5,9	7,9			6,5	
	» »	6	1,6	3,4	5,2	6,3			6,3	
	» »	7	1,9	3,6	5,6	5,6			6,1	
	» »	8	1,9	3,6	5,9	6,8			6,5	
	» »	9	1,9	3,8	5,6	7,4			6,1	
	» »	12	1,7	3,6	6,1	7,2			7,0	
	» »	14	2,0	3,8	5,8	6,3			7,0	
	» »	16	1,3	3,8	5,0	7,2			7,4	
	» »	17	1,8	4,1	5,0	7,9	9,0	4,7	6,3	6,8
XX	» *fœcium*	8	1,8	3,8	5,0	6,1	7,7	4,5	6,2	6,5
	» »	14	1,6	3,6	5,4	6,3	8,3	5,2	6,8	6,8
	» »	17	2,6	5,6	7,2	7,4			9,2	
	» »	18	2,5	5,0	7,2	7,7			9,2	
XXI	» *glycerinaceus*	1	1,6	3,6	5,9	7,0			6,1	
	» »	3	1,6	4,0	5,0	7,2	9,2	3,8	5,6	5,2
	» »	4	1,4	3,8	5,6	6,8			6,5	
	» »	6	1,6	3,4	5,4	6,8			6,1	
XXII	» *liquefaciens*	1	1,4	4,3	6,0	6,8			5.9	
	» »	3	1,6	4,5	6,8	7,4			7,4	
XVI	» *mastitidis*	2	1,1	3,4	4,5	6,8			5,6	
	» »	3	1,3	3,4	4,5	6,1			5,0	
XVII	» *thermophilus*	6	0,5	2,5	5,2	6,5			4,1	
XV	» *cremoris*	1	1,1	2,0	4,1	5,0	5,6	2,7	4,5	6,8
	» »	2	1,4	2,9	4,7	5,9	7,7	3,6	6,1	7,2
	» »	10	1,1	2,9	4,1	7,2			6,3	
	» »	11	0,7	2,6	4,3	6,1			6,5	
	» »	18	0	3,6	5,0	8,1	8,3	4,1	5,6	7,7
	» »	19	1,8	2,0	3.4	5,2			4,7	
XXV	*Betacoccus arabinosaceus*	6	0,9	2,3	4,1	4,5			5,0	
	» »	7	0,9	2,5	4,3	5,9			5,4	
	» »	8	0,8	2,0	3.8	5,4			5,0	
	» »	9	0,9	2,3	4,1	5,0			5,2	
XXVI	» *bovis*	33	0,6	1,6	3,4	5,2			5,9	
	» »	34	0	1,5	2,9	4,3			5,9	
	» »	40	0,7	2,5	5,2	6,5	5,2	3,6	5,6	4,1
	» »	42	0,5	1,4	2,7	4,5	3,2	3,6	5,6	4,1

takes place, the final result is, that the nutritive substrate becomes alkaline[1]), which, as already mentioned, has a detrimental effect upon the bacteria. Nutritive substrates with good buffer action are therefore not suited to continued cultivation of aerogenes bacteria.

[1]) On the basis of this interesting feature, which I pointed out in a lecture at the international dairy congress at Bern in 1912 (later printed in Zeitschrift für Gaerungsphysiologie 1914, V, 10) Clark and Lubs have, in the Journal of Biological Chemistry, 1917, Vol. XXX, p. 209, worked out an easy method for the separating of coli and aerogenes bacteria.

Table IX b.

Table No.	Species of bacteria	No.	% N as Witte peptone					% N as casein peptone		
			0,07	0,28	0,70	1,35	2,00	0,25	0,50	1,00
XXVII	*Tetracoccus casei*	5	0,7	1,8	3,2	5,9	6,3	2,0	3,4	5,6
	» »	7	0,7	1,6	2,9	4,3	4,3	1,6	2,9	4,3
XXVII	» *liquefaciens*	9	0,5	1,5	2,5	3,2			3,2	
	» »	10	0,5	0,7	1,4	2,3			2,3	
XXVII	» ?	12	0,9	2,0	2,7	5,2	5,2	2,5	3,8	5,6
XXVII	» *mycodermatus* ..	31	0,7	1,7	2,7	3,6			3,4	
XXVIII	*Thermobacterium lactis*	6	0	1,1	2,5	4,7			1,6	
	» »	9	0	0,9	1,0	6,1			12,2	
	» »	11	1,3	4,1	7,7	7,7			10,8	
XXVIII	» *helveticum*	12	0	0,7	1,4	7,4	11,5	0	3,8	7,4
XXVIII	» *bulgaricum*	14	0	0,2	1,4	2,9	5,9	3,2	5,4	7,4
XXIX	*Streptobacterium casei*	4	2,8	4,5	8,3	9,7			10,6	
	» »	5	2,9	5,5	7,0	8,6			12,4	
	» »	6	3,4	6,3	6,8	9,7			10,4	
	» »	9	3,4	7,0	7,7	9,0			11,5	
	» »	11	1,8	6,3	7,2	13,3	12,4	7,2	12,8	
	» »	16	2,3	5,5	6,1	11,9			11,3	
	» »	32	3,6	8,3	9,9	12,2			13,3	
	» »	34	3,8	8,1	11,7	11,9			14,4	
XXX	» *plantarum*	3	0,7	5,4	7,4	9,5	11,5	7,7	11,0	
	» »	5	1,1	4,1	5,0	5,4	10,6	6,5	7,7	7,9
	» »	11	1,4	3,2	4,7	10,4	11,0	7,4	7,4	7,4
	» »	13	1,8	6,3	6,3	6,8	11,5	7,9	7,9	7,9
	» »	15	1,1	4,7	6,3	7,2	11,7	6,8	7,9	7,9
	» »	21	2,8	5,4	7,0	9,9			12,8	
XXXI	*Betabacterium breve*	3	0	3,8	6,1	5,9			5,2	
	» »	5	1,6	5,6	7,4	5,9			6,8	
	» »	7	0	3,6	3,8	6,1			7,7	
	» »	10	0	1,4	2,5	2,9	0,5	2,0	4,1	4,1
XXXII	*Microbacterium lacticum*	2	0	0,2	1,4	2,7			3,6	
	» »	3	1,1	2,2	2,5	4,1			3,8	
	» »	6	1,0	2,3	3,4	3,2			3,6	
XXXII	» *flavum*	8	1,8	2,9	5,4	8,3			6,8	
	Bacterium coli A	1	1,8	2,9	3,6	5,4			5,2	
	» » B	4	1,6	2,9	3,6	5,0			4,7	
	» *aërogenes*	1	1,1	2,9	0,6	÷			0,7	
	» »	2	1,1	3,0	0,7	÷			÷	

The same applies to the fluorescent bacteria, which, as typical water bacteria, are best without any over-abundance of nitrogenous food, with which they lose in the course of a few generations the power of forming colouring matter and then perish altogether.

As already mentioned, the lactic acid bacteria can thrive when there is only a trace of the requisite carbon sources present (they grow, for instance, in agar without added sugar) and many streptococci, and even some few rod forms, exhibit a certain growth

tn sugarless broth, when they have good sources of nitrogen at their disposal[1]). The lactic acid bacteria are far more difficult to satisfy in respect of nitrogenous nourishment, and it will be seen from Table IX, that several strains are unable to ferment grape sugar, if they have only ½% WITTE peptone available. Their demands in regard to nitrogenous food are further increased when dealing with sugars more difficult of fermentation, such as for instance inulin, which is often only affected when certain definite sources of nitrogen are present. Only when the nitrogenous nourishment is in all respects sufficient lactic acid bacteria are able to produce the numerous different enzymes (invertase, maltase, lactase, inulinase, etc.) which are required to decompose the di- and polysaccharides[2]). It is therefore absolutely necessary to know the best sources of nitrogen for the different bacteria, before proceeding to investigate which sugars they are able to ferment at all. In the various tables for the different species of bacteria it will be seen how greatly the source of nitrogen may affect the fermentation of the sugar. We will especially draw attention to *Streptococcus bovis* Nos. 1 and 2 (Table XVIII), *Betabacterium breve* No. 11 (Table XXXI), *Microbacterium lacticum* No. 2 (Tab. XXXII) and the many strains of *Streptobacterium plantarum* (Table XXX), which are particularly susceptible as regards nitrogenous food. In all our earlier investigations with the lactic acid bacteria, WITTE pepton was our sole source of nitrogen, but even after we had found other and better sources, we continued nevertheless to employ WITTE peptone in addition, as it is only by studying the fermentation of sugar as well with good as with bad sources of nitrogen that it is possible to obtain a proper impression as to which sugars the various strains prefer. The aerogenes bacteria are here, as in most other respects, found to take up a reverse position to the true lactic acid bacteria, as they will most easily render the nutritive substrate alkaline with the sugars which they find it most easy to ferment, and as a matter of fact, it is only with a slight quantity of nitrogenous nourishment that we can form any estimate as to which sugars they ferment, unless by measuring the quantitites of gas developed, which is the only rational method when dealing with these bacteria.

The lactic acid bacteria use their carbonic food chiefly as a source of energy, and consequently throw off therefrom a quantity of breaking down products (fermentation products); their nitrogenous nourishment, on the other hand, is employed principally as building material, so that they do not need to give off any considerable amount of breaking down products. As a rule, no perceptible decomposition of nitrogenous matters takes place at all, unless the bacteria are suffered to continue their vital activity for some length of time, and it can only be occasioned by gradual neutralisation of the acid as it is formed. Cultures in which it is required to study the decomposition of nitrogenous

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[1]) In this respect, however, there is a very essential difference between true and pseudo lactic acid bacteria, as the latter — at any rate under aerobic conditions — always thrive excellently in sugar-free peptone solutions.

[2]) It is not unlikely that the entire question of vitamins, of which I have recently given a survey in "Naturens Verden" 1917, also has some connection with the enzyme production of the organisms. JACOBY for instance, has shown (Bichem. Zeitschr. 1918, Bd. 86, p. 329) that the formation of urease in bacteria capable of splitting up urea is greatly furthered by the presence of a certain amino-acid, to wit, leucin.

substances must therefore have chalk added, and this is the more necessary, since the proteolytic enzymes of lactic acid bacteria can only exceptionally be active in acid liquids.

Only *Streptococcus liquefaciens*, and some few tetracocci (micrococci and sarcinæ) split up casein in sour milk, but they do so to a far greater degree when the acid is partly or entirely neutralised.

By way of measuring the degree to which the protein decomposition takes place in the milk, we have, as in previous works, determined the quantity of soluble nitrogen (*SN*) and decomposition nitrogen (*DN*) both expressed as % of the total nitrogen present. The former is simply the nitrogen in the filtrate, after the casein, albumin and globulin have been precipitated by boiling with a small quantity of acetic acid. As we work with sterilised milk, however, where the albumin and globulin are already coagulated, the boiling is generally unnecessary, and where the casein has been separated off by the lactic acid fermentation (the casein is precipitated despite the addition of the chalk) it is likewise superfluous to add the acid, so that as a rule, *SN* will mean the nitrogen in the filtrate of the culture. This filtrate is also used for determination of *DN*, by which is understood the nitrogen which is not precipitated by addition of sulphuric acid and phosphotungstic acid in certain definite proportions[1]). As with the determinations of acid content, where we always subtracted the degree of acidity in the control tubes, so also here we subtract the *SN* and *DN* of the original milk (sterilised with chalk). What we want to accertain is, of course, the quantities formed by the bacteria. These quantities may quite well be negative, provided the organisms in question consume more than they give off, and *DN* may also be slightly in excess of *SN*, in cases where any of the original *SN* in the milk has also become broken down. In peptone broth, where all the nitrogen is found in a state of solution, the determination of *SN* will no longer be required; we can, however, on the other hand determine the amount of formol-titratable nitrogen according to S. P. L. Sørensen's method[2]). It will nevertheless be necessary first to distill off the ammonia in the cultures with barium carbonate, and then remove the phosphoric acid with barium chloride and baryta. From the quantity of formol-titratable nitrogen found, we have throughout subtracted the amount of formol-titratable nitrogen in the non-inoculated broth, and by *FN*, therefore, we understand the formol-titratable nitrogen produced by the bacteria. In some cases, we have noted the first stage of the formol titration separately (i. e. prior to addition of formalin, from neutral point of litmus to faint red with phenolphtalein) and calculated the quantity of nitrogen corresponding thereto as a percentage of the total nitrogen content. According to Henriques and Gjaldbæk[3]), this stage will as a rule be the less, the farther the proteins have been decomposed. The figures thus found should then be inversely proportional to *DN*, which is also to some extent found to be the case (See Table X). In conformity with the fact that the true lactic acid bacteria are unable to live on ammonia salts or single amino acids, we find that they are also incapable of splitting up amino acids. They

[1]) 50 cm.^{3} of the milk filtrate + 30 cm.^{3} sulphuric acid of 25 % + 20 cm.^{3} phosphopungstic acid of 10 % are filled up to 250 cm^3 and left to stand for 12 hours before filtration. For determination of nitrogen by Kjeldahl's method, 50 cm.^{3} of the new filtrate is used, answering to 5 cm.^{3} milk.

[2]) Meddelelser fra Carlsberg Laboratoriet 1907, VII, p. 1.

[3]) Zeitschr. f. physiol. Chemie 1911, Bd. 75, p. 363.

Table X a.

Table No.	Species of bacteria	No.	Milk		Witte peptone broth		
			SN	*DN*	*DN*	*FN*	Stage I in % of formol titration figure
XIV	*Streptococcus lactis*	2	14,1	7,4	10,3	8,7	30
	» »	3	14,6	6,6	9,2	4,0	28
	» »	7	13,9	5,4	14,0	9,2	20
	» »	8	11,7	2,4	11,7	11,8	21
	« »	9	0	÷ 0,9	16,5	15,2	28
	» »	10	5,1	÷ 0,3	16,1	12,3	22
	» »	12	0	÷ 0,6	6,1	5,8	35
	» »	14	0,8	0,5	11,0	8,1	21
XV	» *cremoris*	1	5,0	1,6	14,1	8,5	26
	» »	2	8,5	2,4	7,0	9,0	
	» »	10	11,4	1,8	9,4	6,5	
	» »	15	20,4	8,0	8,2	8,1	33
	» »	18	6,7	0,9	15,2	6,5	
XVI	» *mastitidis*	3	2,8	1,9	7,1	7,8	35
XXIV	» *pyogenes*	2	No growth		33,6	19,2	
XX	» *fœcium*	8	4,1	0,6	15,7	8,3	
	» »	14	4,4	÷ 0,2	22,5	13,0	
XXI	» *glycerinaceus*	2	4,3	÷ 0,6	14,5	14,8	
	» »	3	0,3	0	7,7	8,9	32
	» »	4	3,4	0	19,6	13,3	23
	» »	6	0,8	0,8	7,2	10,9	
XXV	*Betacoccus arabinosaceus*	1	÷ 1,2	÷ 0,4	0,2	0,5	
	» »	5	2,8	0,6	1,0	3,8	45
	» »	9	3,7	1,6	0	2,7	
XXVI	» *bovis*	33	0,5	0,5	0,5	3,1	
	» »	34	12,2	3,2	1,7	1,6	
	» »	36	0	0,9	6,1	4,5	40
XXVII	*Tetracoccus casei*	5	1,3	1,3	13,9	13,9	21
	» »	6	0,5	0,9	29,6	20,3	15
XXVII	» *liquefaciens*	9	75	12	35,6	17,8	
	» »	10	71	16	25,1	19,9	21
	» »	11	71	38	13,7	8,1	
XXVII	» ?	12	48	16	11,8	10,2	25

cannot therefore, form more ammonia than is found as such in the proteins or peptones employed. As they generally only form 0—2% (in cultures from 6—9 months old 6% at the outside) NH_3 from the total amount of nitrogen, the quantity of ammonia formed is not shown in the tables.

Table X shows examples of the decomposition of protein occasioned by lactic acid bacteria. The cultures had, as mentioned, all been treated with chalk, and had been left to stand for a month prior to investigation at 30° (only the culture of *Thermobacterium lactis* at 40°).

Table X b.

Table No.	Species of bacteria	No.	Milk		Witte peptone broth		
			SN	*DN*	*DN*	*FN*	Stage I in % of formol titration figure
XXVIII	*Thermobacterium lactis*	11	18,8	18,9	34,8	21,1	
XXIX	*Streptobacterium casei*	2	9,9	10,7	15,6	14,9	25
	» »	3	17,1	19,4	15,3	12,3	22
	» »	5	12,8	9,8	21,0	15,2	
	» »	6	13,1	11,7	15,7	12,1	
	» »	7	9,5	8,0	32,4	26,9	20
	» »	10	11,3	14,5	19,0	15,8	
	» »	12	11,2	10,5	16,1	14,2	18
	» »	13	14,8	10,4	29,5	18,5	
	» »	14	9,8	10,2	14,7	12,3	
	» »	16	6,7	8,5	18,6	17,4	20
	» »	18	6,4	7,9	18,9	12,3	19
	» »	19	12,6	15,9	20,0	27,9	19
	» »	22	13,6	17,5	21,7	15,0	
	» »	24	10,7	13,8	14,0	14,1	
	» »	27	7,2	6,7	29,7	23,9	13
	» »	28	14,6	18,6	41,3	29,9	15
	» »	32	22,7	23,5	26,6	21,8	18
	» »	33	18,7	22,0	18,8	14,6	
XXX	» *plantarum*	1	0	0,1	8,0	4,9	30
	» »	5	÷ 0,8	0,5	0,8	2,4	53
	» »	8	÷ 0,3	÷ 1,9	11,4	10,3	27
	» »	11	1,2	1,1	1,9	5,6	
	» »	13	3,9	1,0	8,8	3,5	30
	» »	14	2,9	2,6	8,5	5,6	
	» »	15	1,1	÷ 0,6	3,2	4,7	44
	» «	20	16,3	18,7	31,2	33,9	
	» »	21	15,7	17,3	14,4	16,9	
XXXI	*Betabacterium breve*	3	0,8	÷ 0,5	1,5	1,1	
	» »	5	6,3	0,1	3,2	1,0	
	» »	6	2,4	0,6	2,7	1,8	
	» »	7	5,0	÷ 0,5	7,4	5,1	
	» »	10	÷ 3,8	0,3	2,8	÷ 0,7	
XXXII	*Microbacterium lacticum*	3	20,0	5,6	11,0	15,7	
XXXII	» *flavum*	8	0	0	8,7	4,2	

Only the gelatin-liquefying species — such as the tetracocci noted in the tables — show any strong solution of casein. *Tetracoccus liquefaciens* No. 11 even forms considerably more *DN* from casein than from Witte peptone, whereas most of the other strains find it easier to break down peptone than the proteins of milk. The casein splitting cocci always form more *SN* than *DN*; the thermobacteria and streptobacteria on the other hand, if capable of splitting up casein, form as much *SN* as *DN*. As it is chiefly monoamino-

acids which are not precipitated by phosphotungstic acid, it is thus likely that the rod forms in question simply peel off the monoamino acids from the casein molecules. That this really is so, we have shown in the case of a single thermobacterium (*Tbm. helveticum*), as the nitrogenous components not precipitated with phosphotungstic acid could not be further split up by boiling with hydrochloric acid. As they showed the same formol titration figure before and after boiling, they must consist of free amino acids. It is somewhat different with the nitrogenous decomposition products, formed by lactic acid bacteria from WITTE peptone; they must evidently contain a quantity of complex compounds (polypeptides) not precipitated by phosphotungstic acid, as otherwise, *DN* could not in the great majority of cases be greater than *FN*.

Disregarding the few species capable of liquefying gelatin, the proteolysis produced by lactic acid bacteria is most active in old cultures with many dead cells. There is no doubt that the hydrolysis of proteins — like that of the sugars — is due to endoenzymes, and does not therefore spread in the nutritive substrate until the cells have become weakened, or even autolysed. It is the proteolytic enzymes of the lactic acid bacteria which occasion the ripening of cheese, and this process only reaches distinct development long after the sugar in the cheese has been fermented, when the lactic acid bacteria have ceased their activity, being either dead, or in a latent state. The fact that lactic acid bacteria only decompose proteins to amino acids, but do not decompose the latter any further, makes the maturing of cheese equal to the process of digestion, and not to that of putrefaction, which is particularly characterised by a breaking down of amino acids.

We need not here go into the proteolytic qualities of the different species. They are least developed in the betacocci and betabacteria, but even among the betacocci, we may find a few strains, isolated from milk and dairy produce, able to split up casein to a perceptible degree. On the other hand, casein-splitting cocci may, when they have not for some time been cultivated in milk, lose their power of utilising casein as nitrogenous nourishment, and thus often the power to grow in milk at all, even where they are otherwise able to ferment milk sugar. The bacteria have, as mentioned, their own power of gradual adaptation to a new nitrogenous food, and great caution should therefore be observed in taking their proteolytic qualities as species character.

Relation to Common Salt. In order to be perfectly sure as to the most favourable conditions of nourishment for a bacterium, it is necessary not only to know how they behave with the various carbon and nitrogen sources, but also with different nutritive salts. We have, however, already referred to the attitude of lactic acid bacteria towards the most important one of these, viz. potassium phosphate, and will here restrict ourselves to a brief mention of sodium chloride, not because this salt is necessary to their development, but because lactic acid bacteria are often found in substrates such as cheese, sour cabbage, not to speak of anchovy pickle, in which common salt is abundant.

Table XI a and b shows the effect produced by greater or smaller quantities of sodium chloride upon the formation of acid. The casein peptone broth employed, which according

Table XI a.

Table No.	Species of bacteria	No.	Casein peptone broth with 2 % dextrose and % sodium chloride				
			0,5	2,5	5,5	10,5	15,5
XIV	*Streptococcus lactis*	3	4,3	4,2	1,6	0	0
	» »	8	4,9	3,8	1,3	0	0
	» »	13	4,1	3,6	0,9	0	0
	» »	16	4,3	4,6	1,1	0	0
XXIII	» ?	1	4,3	4,0	4,0	0	0
	» »	5	5,1	4,5	1,7	0	0
	» »	7	4,2	3,7	1,5	0	0
	» »	8	4,2	4,4	3,2	0	0
XX	» *fœcium*	6	5,3	5,6	3,5	0	0
	» »	8	4,2	4,6	4,0	0	0
	» »	12	5,1	4,7	3,4	0	0
	» »	14	3,5	5,1	2,9	0	0
	» »	17	4,7	5,3	2,8	0	0
	» »	18	6,2	6,1	4,1	0,1	0
XXIV	» *pyogenes*	10	5,1	5,3	3,5	0	0
	» »	3	1,6	1,5	0	0	0
	» »	7	3,5	2,8	0	0	0
	» »	9	2,7	2,2	0,6	0	0
XVI	» *mastitidis*	2	2,5	2,0	0,4	0	0
XV	» *cremoris*	18	5,0	3,6	0	0	0
	» »	20	4,5	3,8	0,8	0	0
XVII	» *thermophilus*	2	4,6	3,3	0	0	0
XVIII	» *bovis*	1	3,2	1,6	0	0	0
XIX	» *inulinaceus*	6	3,6	1,8	0	0	0
XXI	» *glycerinaceus*	1	4,5	4,4	3,1	0,7	0
	» »	3	4,4	4,4	3,3	0,7	0
	» »	6	4,3	4,4	3,1	0,7	0
XXII	» *liquefaciens*	1	4,5	4,2	2,8	0,5	0
	» »	5	4,5	4,0	3,1	0,5	0
XXV	*Betacoccus arabinosaceus*	1	3,4	2,8	1,3	0	0
	» »	11	4,5	4,0	2,5	0	0
	» »	12	4,5	4,2	2,8	0	0
	» »	20	4,6	4,5	3,5	0	0
XXVI	» *bovis*	29	2,9	2,5	1,3	0	0
	» »	34	3,8	3,7	0,5	0	0
	» »	46	3,8	2,0	0	0	0

to the manner of preparation contained ½% *NaCl*, was given a further quantity of 2, 5, 10 and 15% respectively of KAHLBAUM's purest sodium chloride. As will be seen, only a very few of our bacteria are affected by the presence of 2.5% salt. Only *Sc. cremoris*, *Sc. thermophilus*, *Sc. bovis*, and *Sc. inulinaceus* are somewhat retarded in their development thereby. On the other hand, *Sc. faecium*, certain tetracocci (Nos. 1 and 3) and streptobacteria (*Sbm. casei* Nos. 32 and 34 and *Sbm. plantarum* No. 3) grow better with 2.5% than with only ½% common salt. We find, however, that 5.5% salt is more or less harmful

Table XIb.

Table No.	Species of bacteria	No.	Casein peptone broth with 2 % dextrose and % sodium chloride				
			0,5	2,5	5,5	10,5	15,5
XXVII	*Tetracoccus ?*	1	2,7	3,6	3,6	2,9	1,8
	» »	3	2,9	3,2	2,9	2,3	1,6
	» »	15	2,3	2,3	2,3	1,6	0,9
	» »	23	3,2	2,8	2,7	2,2	1,1
	« *pyogenes aureus*	13	3,1	2.7	2,5	1,8	0,7
	» » *albus*	29	2,5	2,7	2,5	1,8	0,7
XXVIII	*Thermobacterium lactis*	11	3,2	0,4	0,2	0,2	0
XXIX	*Streptobacterium casei*	11	10,0	8,3	1,1	0	0
	» »	13	6,7	6,4	0	0	0
	» »	32	6,8	7,3	5,4	0	0
	» »	34	9,5	11,3	4,1	0	0
XXX	» *plantarum*	3	6,0	7,2	6,0	0	0
	» »	6	6,4	5,2	3,2	0	0
	» «	8	7,7	5,6	5,5	0	0
	» »	13	8,1	6,1	4,2	0	0
	» »	14	7,4	6,5	4,2	0	0
	» »	20	8,5	6,9	6,3	0	0
	» «	30	9,2	5,0	4,4	0	0
	» »	32	9,5	8,2	4,5	0	0
	» »	44	5,4	4,5	3,4	0	0
XXXI	*Betabacterium breve*	3	4,9	3,4	0	0	0
	» »	10	7,9	5,2	0	0	0
	» »	19	5,0	4,4	0	0	0
XXXI	» *longum*	32	2,6	2,2	0	0	0
XXXII	*Microbacterium lacticum*	3	2,5	2,5	0,1	0	0
	» »	6	2,7	2,7	0,1	0	0
XXXII	» *mesentericum*	7	1,9	1,7	0,4	0	0
XXXII	» *flavum*	8	4,6	5,4	4,5	2,2	0
	» »	9	6,8	5,1	3,3	0,7	0

to all lactic acid bacteria, and 10.5% salt stops the growth of most of them. An exception is formed by the tetracocci (micrococci and sarcinæ) which can as a rule stand 15.5% of salt. The good bacteria found in herring pickle[1]) are also chiefly tetracocci. *Tc.* No. 1, for instance, is from an anchovy pickle containing 15% *NaCl*. Possibly it may be the special shape of the tetracocci which makes them better able than other bacteria to stand the heavy osmotic pressure of strong salt solutions. *Bc. bovis* No. 46 however, is also a pronounced micrococcus, though it is not therefore able to endure greater quantities of salt.

[1]) The bacteria which render herrings rancid are, according to investigations which we have carried out for "A/S Dansk Fiskekonservering" liquefying rod forms.

IV. Attitude toward Different Temperatures.

Important characteristic feature in a bacterium are the minimal, optimal and maximal temperatures for its vital activity, and the death temperature.

The Minimal, Optimal and Maximal Temperatures for Vital Activity are determined simultaneously, by sowing the bacterium in a series of tubes with a good nutritive substrate, and placing them under observation at different temperatures. For the higher temperatures, thermostats with water jacket were employed; these could, thanks to the great heating capacity of the water, be regulated very accurately. For temperatures lower than that of the room, we used a *Panum's* thermostat, heated with gas to 20° at one end, and cooled with ice at the other. Unfortunately, the temperature in the different compartments varied from 1°—2°, according to the quantity of ice in the receptacle. All our strains were tested first at 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 degrees, and thereafter at certain intermediate temperatures if required, in order to determine the minimal and maximal temperatures more closely. Immediately after inoculation, the tubes must be heated in a water bath to the testing temperature, if this is over 40°, otherwise, some growth may take place before the air in the thermostat has communicated its temperature to the content of the tubes. At temperatures over 20°, the tubes were left to stand as usual for 14 days before titration; at 20° and under, on the other hand, they were left to stand for a month, as experience had shown that the maximum of acid is generally only reached after that time. When we are fortunate enough to strike the minimal or maximal temperature exactly, it will be possible to observe growth without acid formation, a state of things which is noted in Table XII by +.

It should be pointed out that the optimal temperature for growth of lactic acid bacteria cannot be determined by acid titration alone[1]), but only by daily observation, and where necessary by microscopic examination of the contents of the tubes. For the temperature at which the greatest quantity of acid is formed can lie somewhat below the temperature at which liveliest growth takes place, owing to the fact that the acid is more destructive in its effects at the higher than at the lower temperature. This was very distinctly seen in the case of *Sc. thermophilus* and the thermobacteria, which quite indisputably showed most rapid growth at 40°, but formed most acid at 30° and 35° respectively. And what is here said of the acid formation, applies in an even higher degree to the proteolytic action. I have previously shown that *Tetracoccus liquefaciens* (= *Micrococcus casei liquefaciens*)[2]) which grows most rapidly, and also ferments most sugar, at 30°, exhibits the strongest proteolysis at 20°, and Barthel has shown that streptococci[3]) whose optimal temperature is likewise about 30° can in the long run split up the casein in the milk most powerfully at indoor temperature. This question, however, is a more complicated one than that of the acid formation, since, as mentioned, the proteolysis occasioned by the lactic acid bacteria in the surrounding substrate is a pure enzyme action, which does not run parallel with the vital activity of the cells.

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[1]) It can even less be determined — as many writers have done — by the time required for curdling milk, since milk will curdle with smaller quantities of acid as the temperature is increased.

[2]) l. c. 1904, p. 32.

[3]) Meddelande Nr. 97 från Centralanstalten för försöksväsendet på jordbruksområdet 1914.

Table XII a.

Table No.	Species of bacteria	No.	Source of nitrogen	3°	5°—6°	7°—9°	9°—11°	11½°—14°	14°—16°	16°—18°	19°—21°	25°	30°	35°	37½°	40°	42½°	45°	47½°	50°	52½°
XIV	*Streptococcus lactis*	4	W		0		2,5		2,7		3,2	4,0	4,1	3,2	2,5	0		0		0	
	» »	6	»		0	0	2,7		3,2		3,4	3,6	3,6	3,2		0,5	0	0		0	
	» »	7	»		0	0,9	2,3		3,2		3,5	3,5	3,4	2,8	2,5	0		0		0	
	» »	8	»	0	1,1		2,9		3,2		3,8	3,8	3,5	2,9		1,1	0	0		0	
	» »	9	»		0	+	2,3		2,9		3,8	3,8	3,6	2,9		1,8	0	0		0	
	» »	12	»		0	0,5	2,7		3,4		3,4	3,5	3,5	2,9		0,5	0	0		0	
	» »	14	»		0	0	2,5		3,2		3,6	3,6	3,6	3,4		1,6		0,2	0	0	
	» »	16	»		0	0	2,7		2,7		3,2	3,2	3,2	3,2		2,3	1,6	0		0	
	» »	17	»		0	0	2,3		3,9		3,4	3,8	3,8	3,4	3,2	0		0		0	
XX	» *fœcium*	7	M[1])			0		0,7			2,5		4,1			3,8		1,4		1,1	0
	» »	8	W		0,2		2,5		2,9		3,6	3,6	3,6	2,7		2,7		2,7		1,8	0
	» »	14	»		0,5		1,8		2,5		3,3	3,5	3,5	3,3		2,3		2,0		0,2	0
	» »	17	»		0		2,7		3,3		4,3	5,6	5,6	4,7		2,9		2,9	0,8	0	
	» »	18	»		0,5		3,4		3,8		4,7	5,5	5,2	4,7		3,1		2,2	2,2	0	
XXIV	» *pyogenes*	10	C					5,9			7,7		8,8			7,9		6,8		5,6	
XXIII	» ?	7	W		0		2,5		2,6		2,9	3,7	3,7	3,2	2,8			1,6	0	0	
XIX	» *inulinaceus*	5	C		1,6		4,3		4,7		4,7		4,7			0,9		0,5	0	0	
XVIII	» *bovis*	1	M		0		0		0		0	1,1	2,7	3,4		0		0		0	
	» »	5	»		0		0		0		0	3,4	5,2	5,4		0,5		0		0	
XXI	» *glycerinaceus*	1	W		0	0	1,8		2,7		3,2	3,3	3,6	3,1		2,5		1,6	0	0	
	» »	2	»		0	0	1,8		2,9		3,2	3,2	3,4	3,2		2,5		1,6	0	0	
	» »	3	»		0		1,8		2,5		3,4	3,7	3,8	3,2		2,7		2,5	2,0	0	
	» »	4	»		0	0	2,3		2,7		3,4	3,4	3,8	3,6		3,2		2,2	1,6	0	
	» »	6	»		0	0	1,6		2,3		3,0	3,3	3,5	2,9		2,4		2,2		0,2	0
XXII	» *liquefaciens*	1	»		0	0	2,3		2,8		3,4	3,4	4,2	4,0		2,3		1,4	0,9	0	
	» »	3	»		0	0	2,3		2,8		3,8	4,1	4,5	3,6		3,2		3,1		0,1	0
XVII	» *thermophilus*	2	M		0		0		0		1,6		7,7	7,5		7,2		1,1		0,5	0
XXIV	» *pyogenes*	4	C		0		0		0		2,7		3,8			0,5		0		0	
	» »	9	»		0		0		0		4,5		5,2			2,7		0		0	
XVI	» *mastitidis*	2	W		0		0		0		1,4	2,6	3,4	2,6	2,6	+		0		0	
	» »	3	»		0		0		0		0,9	3,3	3,4	2,9	1,6	0		0		0	
XV	» *cremoris*	1	»		0	0	0,7		1,4		1,8	2,2	2,2	1,8	0	0		0		0	
	» »	2	»		0	0	0,5		2,3		2,5	2,9	2,9	2,6	2,3	0		0		0	
	» »	11	»		0	0	0,2		2,7		3,2	2,9	2,6	1,4	0	0		0		0	
	» »	18	»		0	0	2,0		2,5		3,3	3,6	3,6	2,5	0	0		0		0	
	» »	19	M	0	0,5		6,8		7,2		7,2	6,8	6,5	5,2	1,6	0		0		0	
	» »	21	»	+	0,9		6,3		6,5		7,2	7,0	6,5	5,4	0	0		0		0	

As it is hardly likely that the optimal temperature for the proteolytic enzymes should lie lower than that of the bacteria themselves[2]), the explanation must simply be that these enzymes are better preserved at a somewhat lower temperature.

[1]) M = milk.

[2]) It is very common, on the other hand, to find that the optimal temperature for the enzymes both of microorganisms and plants lies far above the optimal temperature for vital action of the organisms in question, which merely shows that these enzymes are particularly resistant to the effect of heating, since all enzyme action increases, in reality, with the temperature.

Table XII b.

Table No.	Species of bacteria	No.	Source of nitrogen	3°	5°—6°	7°—9°	9°—11°	11 03°—14°	14°—16°	16°—18°	19°—21°	25°	30°	35°	37½°	40°	42½°	45°	47½°	50°	52½°
XXV	*Betacoccus arabinosaceus* ...	1	W		0		0		0,2		1,3	1,7	2,3	1,6		0,7		0,5	0	0	
	» » ...	4	»	+	0,7		2,0		2,2		1,9	1,8	1,8	0,6	0	0		0		0	
	» » ...	5	»	+	1,5		1,6		2,0		2,7	2,0	2,0	1,4	0,1	0		0		0	
	» » ...	6	»	0	0,9		2,0		2,3		2,6	2,3	2,3	1,6	0,7	0		0		0	
	» » ...	8	»	0	0,5		2,0		2,3		2,5	2,4	2,0	0,9	0,7	0		0		0	
	» » ...	9	»	0	0,2		2,0		2,5		2,6	2,3	2,3	1,1	0,9	0		0		0	
XXVI	» *bovis*	33	»		0		0,2		0,7		1,7	1,6	1,6	0,7	0,1	0		0		0	
	» »	35	»		0	0,5	1,4		1,8		2,5	2,5	2,1	1,1	0	0		0		0	
	» »	37	»	+	1,1		1,4		2,0		2,3	2,0	1,8	0,9	0	0		0		0	
	» »	40	»		0	+	2,5		2,9		2,9	2,9	2,5	2,0	+	0		0		0	
	» »	42	»		0	+	1,1		1,8		1,8	1,9	1,6	0,4	0	0		0		0	
XXVII	*Tetracoccus casei*	5	»		0		+		0,2		1,6	1,9	2,0	1,6		0,1		0		0	
	» »	6	»		0		+		0,6		1,7	2,3	2 3	1,8	1,4	0		0		0	
	» »	7	»		0		+		0,7		1,6	1,9	2,0	1,6		0,1		0		0	
XXVII	» *liquefaciens*	11	»		0		+		0,7		1,8	1,8	1,9	1,8	0,2	0		0		0	
XXVII	» ?	12	»		0		0	+	0,5		1,4	1,6	2,2	2,0		1,8	1,8	0		0	
	» »	25	»		0		+	0,6	0,7		0,7	0,9	1,4	0,7	0	0		0		0	
XXVII	» *mycodermatus* ..	31	»		0		0,5		0,5		1,1	1,4	1,5	1,3	1,1	0		0		0	
XXVIII	*Thermobacterium lactis*	6	M		0		0		0		0	11,5	13,1	12,6		10,1		9,7		0,5	0
	» »	7	»		0		0		0		0		7,0			11,5		10,8		9,9	0
	» »	8	»		0		0		0		5,4		9,7			11,7		11,7		11,3	0
XXVIII	» *helveticum*	12	»		0		0		0		0	16,0	27,4	28,1		21,6		19,1		7,9	0
XXVIII	» *bulgaricum*	14	»		0		0		0		0	10,6	14,2	13,3		12,6		10,8		10,4	2,5
XXXI	*Betabacterium breve*	3	W		0		0		0,2		1,5	3,4	3,8	3,6	3,5	0		0		0	
	» »	4	»		0		0		0,5		1,1	2,6	3,6	3,4	3,3	0		0		0	
	» »	5	»		0		0		+		0,7	0,7	3,4	2,2	0,5	0		0		0	
	» »	6	»		0		0		0,2		0,7	2,3	3,2	3,2	0,6	0		0		0	
	» »	7	»		0		0		0,2		0,5	2,7	3,6	3,1	1,6	0		0		0	
	» »	10	»		0		0		0,2		0,8	0,9	1,5	2,3	1,5	0		0		0	
XXXII	*Microbacterium lacticum* ...	2	» [1]		0		0,2		1,4		1,6	1,9	1,1	0		0		0		0	
	» » ...	3	»		0		+		0,6		1,4	1,9	2,0	0,7	0	0		0		0	
	» " ...	6	»		0		0		+		1,4	1,9	2,3	1,8	0	0		0		0	
XXXII	» *flavum*	8	» [1]		0		0		+		0,2	1,9	2,0	0,7	0	0		0		0	
	» "	9	»		0		0		+		0,5	1,1	1,4	0,8	0	0		0		0	

In our earliest experiments with regard to influence of temperature upon the vital activity of lactic acid bacteria, where we were still unacquainted with the better sources of nitrogen, we employed exclusively WITTE peptone (W), or, where this proved altogether unsuitable, milk (M). In the later experiments, on the other hand, we used as a rule casein peptone (C). As, however, our first experiments included more intermediate temperatures

[1]) Later experiments with casein peptone as source of nitrogen did not change the minimum and maximum temperature.

Table XII c.

Table No.	Species of bacteria	No.	Source of nitrogen	3°	5°—6°	7°—9°	9°—11°	11½°—14°	14°—16°	16°—18°	19°—21°	25°	30°	35°	37½°	40°	42½°	45°	47½°	50°	52½°
XXIX	*Streptobacterium casei*	2	W		0		0		0		0,5	3,2	4,3	3,4	2,0	0		0		0	
	» »	4	»		0		0	0,5	0,5		2,3	5,4	5,4	4,5		0,7		0,2	0	0	
	» »	5	»		0		0,2		1,4		3,2	5,4	5,9	5,9	3,6	0		0		0	
	» »	9	»		0		0,2		0,9		3,4	4,5	6,8	6,8	3,2	0		0		0	
	» »	10	»		0		0,5		0,5		2,5	3,6	5,6	5,4	1,1	0		0		0	
	» »	11	»		0		0		+		2,7	5,5	6,4	5,8		3,1		0,4	0	0	
	» »	16	»		0		0,1		1,1		2,7	3,8	5,4	4,5		0,1	0	0		0	
	» »	17	»		0		0		0,5		2,9	4,0	5,6	5,6	5,2	0		0		0	
	» »	22	»		0		0,1		0,5		2,9	5,4	5,6	3,8	3,4	0		0		0	
	» »	24	»		0		0,1		0,9		3,4	4,1	5,0	4,7	3,6	0		0		0	
	» »	28	»		0		0,2		1,1		4,0	5,1	5,6	5,6		1,1	0	0		0	
	» »	32	»		0		0	+	0,7		2,6	5,6	8,1	7,4		4,3		3,2	0	0	
	» »	34	»		0		0		0,4		2,5	5,1	8,1	7,4		5,6		5,0	0	0	
XXX	» *plantarum*	1	»		0		0	0,2	0,5		2,3	5,6	6,1	4,7	1,1	0		0		0	
	» »	5	»		0		0		0,2		1,6	2,3	3,4	1,7	0,5	0		0		0	
	» »	7	»		0		0		0,2		5,9	8,2	7,3	6,1	3,6	0		0		0	
	» »	8	»		0		0,2		1,6		5,2	5,4	5,8	5,0	4,2	0		0		0	
	» »	10	»		0		0		+		1,8	5,9	5,9	3,6	2,3	0		0		0	
	» »	11	»		0		0		+		1,4	5,6	4,2	3,8	2,2	0		0		0	
	» »	12	»		0		0		0,2		1,7	6,1	6,1	1,8	1,6	0		0		0	
	» »	13	»		0		0	0,2	0,7		3,2	7,0	7,4	2,4		0,1	0	0		0	
	» »	14	»		0		0		+		1,1	5,9	5,6	4,7	1,9	0		0		0	
	» »	15	»		0		0		+	1,6	2,2	3,2	3,4	1,8	0,6	0		0		0	
	» »	20	»		0		0,4		1,4		3,2	3,7	5,6	5,4		1,8	0	0		0	
	» »	21	»		0		0	0,5	0,5		2,3	5,6	6,1	5,8	4,7	0		0		0	
	Bacterium coli A	1	»		0		2,0		2,0		2,3	2,5	2,7	2,5		2,2		2,0		0,2	0
	» » »	2	»		0		0,9		2,0		2,3	2,7	2,7	2,5		2,5		2,4	0	0	
	» » »	3	»		0		1,0		1,4		1,6	2,5	2,5	2,5		2,2		2,0	0	0	
	» » B	4	»		0		1,4		1,8		2,3	2,3	2,6	2,3		2,4		2,3	0,5	0	
	» *paracoli*	5	»		0		0		1,6		2,4	2,5	2,5	2,0		1,9		1,8	0	0	
	» *paratyphi*	6	»		0		1,0		1,6		2,3	2,5	2,5	2,3		2,0		1,8	0	0	
	» *aerogenes*	1	»		0,9		1,9		2,7		2,7	2,7	2,7	1,6		0,7		0,6	0	0	
	» »	2	»		0		0		1,8		2,3	2,7	2,7	2,3		1,7		1,6	0,5	0	
	» »	3	»		0		1,7		1,8		2,9	2,6	2,3	1,6		1,5		1,4	0	0	
	» »	4	»		0		0		1,8		2,0	2,6	2,7	1,8		1,6		1,5	0	0	

than those after tests made later on, Table XII refers chiefly to the first experiments. The source of energy employed in the broth was always 2% grape sugar, and the figures given are as usual for the quantity of acid formed expressed in ‰.

It was found that the optimal temperature was independent of the source of nitrogen, whereas the upper, and particularly the lower limit for vital activity may be somewhat altered by improving the source of nitrogen. With casein peptone as source of nitrogen, for instance, we succeeded in get-

ting the great majority of the streptobacteria to form appreciable quantities of acid at 10°, despite the fact that the growth at this temperature is as a rule not perceptible until after the lapse of 14 days. The maximal and minimal temperatures of the bacteria are affected far more by the vitality of the bacteria themselves than by conditions of nourishment, and weakened strains therefore exhibit much steeper temperature curves than those whose vitality is unimpaired.

Even though the attitude of the bacteria towards different temperatures may not be altogether constant, there is nevertheless hardly any other quality which better characterises the various species. It will be seen from Table XII a, b and c that *Sc. fæcium* and the thermobacteria thrive well at 47½—50°; some thermobacteria, indeed, at over 50°; that *Sc. glycerinaceus*, *Sc. liquefaciens*, and *Sc. thermophilus* as well as the coli and aerogenes bacteria grow well at 45°, that *Sc. bovis* and the thermobacteria as a rule do not grow at ordinary indoor temperature, and that a few strains of *Sc. cremoris* and of the betacocci can grow already at 3°.

As regards the optimal temperature, this lies, as already mentioned, in the case of *Sc. thermophilus* and the thermobacteria, at 40° or even a little higher; the same applies to *Betabacterium longum* (not shown in Table XII); for most pathogenic streptococci it is 35°—37°, whereas for all other lactic acid bacteria it is 30° or even lower. This last point cannot be too much emphasised, as many bacteriologists erroneously believe the optimal temperature of lactic acid bacteria to lie generally somewhere about blood heat, a temperature which on the contrary is detrimental to most of them. And in the fermentation test which is so important for the cheese maker, milk is placed just at this very critical temperature in order to determine whether good forms (i. e. true lactic acid bacteria) or bad (i. e. the pseudo lactic acid bacteria) predominate therein. For at low temperatures, the good bacteria, and at high temperatures the bad ones will too easily be able to get the upper hand (we have seen that the coli and aerogenes bacteria thrive well right up to 45°), even though they may by no means have been in the majority to begin with. The employment of different temperatures leads altogether to very active enrichment methods. Thus if milk be placed to stand at 45°—50°, then at first, *Sc. fæcium* and *Sc. thermophilus*, more rarely *Sc. glycerinaceus* will gain the mastery, to be subjugated later by the thermobacteria, which are far more powerful acid formers.

The Death Temperature. In order to determine the highest temperature to which a bacterium can be subjected without perishing, the capillary tube method is generally employed. It consists in drawing up a little broth culture of the particular bacterium into a series of capillary tubes, which are then fused up at the ends, and heated for a longer or shorter time in a water bath to different temperatures. Where the contents of the tubes exhibit no growth when sown out in a good nutritive substrate, the bacteria will have been killed.

This method is subject to two sources of error. In the first place, we work with highly varying hydrogen ion concentration, according as more or less powerful acid formers — or possibly alkali formers — are being treated. And it is well known that the effect of heating upon the bacteria is greatly augmented the more the surrounding

liquid diverges from the neutral point. In the second place, there is really no sharply marked limit at all which can be designated as the maximal temperature for the life of a bacterium, but experience has shown that most of the cells are killed off at a far lower temperature than is required to overcome the most resistant of the cells[1]). It is therefore impossible to obtain a thorough insight into the question without investigating what takes place at each of the temperatures tried.

In order to obtain accurate results, it will be necessary first of all to dilute the starting material before heating, to such a degree that its own concentration of hydrogen ions cannot produce any effect; secondly, to note the number of the cells sown out which survive the different temperatures. Both can easily be done by sowing out a couple of drops of the culture to be tested in agar tubes, and heating in the same. The surviving germs will grow out into colonies in the usual way, and can then be counted. We used BURRI tubes for this purpose, with a deep layer of casein peptone dextrose agar. It will of course be necessary to see that the agar has, prior to sowing out, reached the temperature of the water bath, and the germs must be distributed by rolling the tube to and fro in a vertical position, so that they do not settle higher in the tubes than the level of the water outside. When the heating is completed, the germs are thoroughly distributed by reversing the tube once or twice; the tube is then cooled as rapidly as possible, in cold water. When dealing with aerobic organisms — such as for instance the tetracocci — the agar is poured off into a petri dish after heating.

Table XIII a and b shows the results obtained with some of our strains after heating for a quarter of an hour to 60°, 65°, 70°, 75°, 80° and 85° degrees. A temperature of 60° suffices to kill off the pathogenic species such as *Sc. mastitidis* and *Sc. pyogenes*. *Sc. pyogenes* No. 10 is an exception; this bacterium is, however, as we shall see later on, not a true *Sc. pyogenes*, but a pathogenic variety of *Sc. fæcium*. At 65°, the betacocci are killed, and at 70°, the commonest lactic acid bacteria of milk, *Sc. lactis* and *Sc. cremoris*. Most of the cells of these will, however, have perished already at lower temperatures. Taking for instance *Sc. lactis* No. 2, we find from the table that only $^1/_{100}$ % of its cells have been able to endure heating to 60°. A greater power of resistance is exhibited by the remaining lactic acid bacteria, of which some few cells can stand heating to 70°—75°. The most resistant species is *Microbacterium lacticum*, which is not always killed off entirely even at 85°. In milk pasteurised at low temperatures, therefore, we encounter chiefly this and *Sc. thermophilus*[2]), besides certain tetracocci (micrococci); somewhat less frequently *Sc. fæcium* and *Sc. glycerinaceus*. Even though these streptococci only exceptionally survive heating to 75°, they have yet comparatively many cells which can stand 65°, and in practice, it is of far greater importance how the majority of the cells behave than what the most resistant individuals can stand, as these few cells will in any case be unable to make their influence felt in the natural competition.

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[1]) And even here we are not dealing with spore-formers, where the question is far more complicated.

[2]) In true low-pasteurised milk (heated only to 63°) *Mcm. lacticum* is, however, rare, at it is here unable to compete with the heat-resisting cocci. In milk heated to a somewhat higher temperature, on the other hand, we may find it almost as a pure culture.

Table XIII a.

Table No.	Species of bacteria	No.	Millions of bacteria pr. cm.³ in the not heated culture	Amount of bacteria pr. cm³ after 1/4 hour's heating to:					
				60°	65°	70°	75°	80°	85°
XVII	*Streptococcus thermophilus*	2	40	abundant	abundant	abundant	40	0	0
	» »	5	12	»	»	100	0	0	0
XX	» *fæcium*	6	46	»	»	2500	0	0	0
	» »	7	100	»	»	10000	40	0	0
	» »	8	50	»	30000	60	0	0	0
	» »	12	133	»	abundant	340	80	0	0
	» »	14	55	»	28000	1800	0	0	0
	» »	17	50	»	40000	5000	0	0	0
	» »	18	160	»	39000	11700	0	0	0
XXIV	» *pyogenes*	10	40	»	abundant	20	0	0	0
XXI	» *glycerinaceus*	1	not counted	»	»	1000	20	0	0
	» »	2	»	»	less than at 60°	400	0	0	0
	» »	4	50	»	20000	0	0	0	0
	» »	5	66	»	7500	740	200	0	0
	» »	6	27	»	15000	0	0	0	0
XXII	» *liquefaciens*	1	112	»	3000	0	0	0	0
	» »	5	20	»	less than at 60°	360	0	0	0
XIV	» *lactis*	2	90	9000	200	0	0	0	0
	» »	3	160	200	0	0	0	0	0
	» »	7	40	2400	120	0	0	0	0
	» »	9	100	9000	200	0	0	0	0
	» »	12	150	0	0	0	0	0	0
	» »	16	70	500	60	0	0	0	0
	» »	17	64	2000	20	0	0	0	0
XV	» *cremoris*	1	14	20000	200	0	0	0	0
	» »	2	36	2500	1200	0	0	0	0
	» »	11	16	2000	0	0	0	0	0
	» »	18	8	800	20	0	0	0	0
	» »	19	18	900	0	0	0	0	0
	» »	20	2	2000	2000	0	0	0	0
XVI	» *mastitidis*	1	98	0	0	0	0	0	0
	» »	2	2,5	0	0	0	0	0	0
XXIV	» *pyogenes*	3	22	0	0	0	0	0	0
	» »	6	1,5	0	0	0	0	0	0
	» »	8	40	0	0	0	0	0	0

As a rule, those lactic acid bacteria which grow at the highest temperatures can also stand the highest degree of heating[1]).

[1]) An exception is apparently formed by the highly heat-resisting *Mbm. lacticum*, which according to Table XII, does not grow at over 35°. As a fæces bacterium, however, it must in a state of nature be able to grow at any rate at blood heat, and the result of our experiment must thus doubtless be due to the fact that the sources of nitrogen employed did not altogether satisfy this bacterium, which is very particular in this respect.

Table XIII b.

Table No.	Species of bacteria	No.	Millions of bacteria pr. cm.³ in the not heated culture	Amount of bacteria pr. cm³ after 1/4 hour's heating to:					
				60°	65°	70°	75°	80°	85°
XXV	*Betacoccus arabinosaceus* ...	8	105	6000	0	0	0	0	0
	» » ...	11	100	6000	0	0	0	0	0
	» » ...	12	140	200	0	0	0	0	0
XXVI	» *bovis*	33	120	0	0	0	0	0	0
	» »	42	100	5000	0	0	0	0	0
XXVII	*Tetracoccus casei*.....	5	not counted	abundant	abundant	60	0	0	0
	» »	6	»	»	»	900	200	0	0
	» »	7	»	»	»	less than at 65°	1700	0	0
XXVII	» *liquefaciens*.....	9	1	»	80	0	0	0	0
	» »	11	6	»	800	360	0	0	0
XXVII	» *mycodermatus*...	31	6,5	»	40	20	0	0	0
XXXII	*Microbacterium lacticum*...	3	31	»	abundant	abundant	abundant	20	0
	» »	4	64	»	»	»	»	1200	0
	» » ...	5	23	»	»	»	»	less than at 75°	20
	» »	6	70	»	»	»	710	200	0
XXXII	» *mesentericum*	7	0,5	»	1400	0	0	0	0
XXXII	» *flavum*.....	8	1,6	»	abundant	17000	20	0	0
	» »	9	0,5	»	»	20000	5000	0	0
XXIX	*Streptobacterium casei*.....	2	58	»	600	60	0	0	0
	» »	4	40	»	abundant	80	0	0	0
	» »	8	15	»	160	180	0	0	0
	» »	11	21	»	abundant	300	0	0	0
	» »	13	20	»	0	0	0	0	0
	» »	23	21	»	5200	0	0	0	0
	» »	24	58	»	40	0	0	0	0
	» »	34	47	»	0	0	0	0	0
XXX	» *plantarum*	5	8	»	100	200	0	0	0
	» »	6	10	»	200	120	0	0	0
	» »	14	8	320	0	0	0	0	0
	» »	30	16	240	0	0	0	0	0
XXXI	*Betabacterium breve*.....	3	10	abundant	abundant	140	0	0	0
	» »	6	not counted	»	120	80	9	0	0
	» »	10	18	»	1500	300	0	0	0
XXXI	» *longum*.....	32	15	»	680	660	0	0	0

V. Other important Features.

The expert in fermentation physiology should, in the identification of micro-organisms, lay most stress upon the phenomena of fermentation; the medical bacteriologist, on the other hand, will attach more importance to questions of agglutination and immunity. The latter are of but slight interest to us here, but an investigation into the agglutination of the lactic acid bacteria would doubtless in many cases have supported the results of our research. BARTHEL, for instance, has succeeded in showing, with regard to the thermobacteria, that the species established by us, *Tbm. lactis*, *Tbm. helveticum* and *Tbm. bul-*

garicum, have each its own manner of agglutination[1]). We were unfortunately debarred from making experiments in this direction, as our laboratory is not equipped for experimental work with animals, and with the great quantity of strains here concerned, it was likewise impossible to carry out the work as guests at any other laboratory. The results obtained by such agglutination experiments are, however, not always of the same interest from the systematic point of view, since, as will appear from M. CHRISTIANSEN's work on the bacteria of the typhus-coli group[2]) the character of the agglutination need not always cover definite morphological, cultural or biological qualities. And this agrees excellently with the fact that, as we have mentioned, the bacteria are able to adapt themselves to new proteins. Among the most variable proteolytic qualities in bacteria are the hæmolytic, and, as we know, everything connected with pathogenity at any rate for the pathogenic bacteria coming into our sphere of work (certain strepto- and micrococci) is among the qualities soonest lost on cultivation in artificial substrates. On the other hand, the researches of C. O. JENSEN render it likely that non-virulent coli bacteria — and in analogy therewith, also other non-virulent bacteria — can under certain conditions become pathogenic. Consequently, the pathogenic qualities cannot be utilised at all as species character, but merely serve to indicate whether we are dealing with a pathogenic variety.

In LEHMANN and NEUMANN's »Bakteriologische Diagnostik" (5. Edition 1912) taurocholate of sodium is used to separate off *Sc. pyogenes* from the remaining streptococci. If 5—10% of this salt be added to broth cultures 24 hours old, of the various streptococci, then their cells should dissolve in the course of some few minutes, save in the case of cells of *Sc. pyogenes*. This seems most mysterious to begin with, and involves the unfortunate conclusion that no streptococci other than *Sc. pyogenes* could live in the intestinal canal of animals, or at any rate, in that of carnivores. We on our part have never succeeded in observing the slightest clearing in broth cultures of any of our strains on addition of taurocholate of sodium, far less any real dissolution of the cells perceptible under the microscope[3]).

Another reaction largely used for identification of streptococci is that based on their various power of reducing colouring matter. For this purpose, milk stained a pale blue with litmus[4]) is chiefly used. With like quantities sown out, and at like temperatures (which should preferably be somewhere near the optimal temperature for the bacteria in question), the power of reduction will be proportional to the time taken in decolo-

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[1]) Meddelande Nr. 68 från Centralanstalten för försöksväsendet på jordbruksområdet. 1912. *Tbm. Joghurt*, which is otherwise nearest to *Tbm. helveticum*, agglutinates, however, together with *Tbm. bulgaricum*.

[2]) Det kgl. danske Videnskabernes Selskabs Skrifter, naturvidenskab. og matematisk Afd. 1916, 8. Række, I, 3.

[3]) We made our experiments with a preparation from MERCK, and as this proved to contain at least as much sodium glycocholate as sodium taurocholate, we have ourselves prepared pure taurocholate of sodium from gall, by precipitating the sodium glycocholate from the mixture of gallic acids, with sugar of lead, and then removing the lead by means of sulphuretted hydrogen. Even the pure salt, however, gave no better result. It is only the cells of *Sc. lanceolatus* and *Sc. mucosus*, which are dissolved by taurocholate of sodium.

[4]) As milk, like other sugar-containing liquids, decolorises litmus during sterilisation, the litmus tincture must be sterilised separately, and dropped into each tube with a sterile pipette.

risation. Obviously, species which grow slowly in milk will also decolorise slowly. *Sc. pyogenes* and most of the betacocci do not decolorise at all. Both spherical and rod-shaped lactic acid bacteria can as a rule decolorise litmus milks more rapidly than they coagulate it, but there are also species — *Sc. thermophilus*, for instance — which do not decolorise the milk until long after it has coagulated. As the power of reduction is in the highest degree dependent upon the vitality of the bacteria at the moment, it is of very little value as a species character.

As has been shown by the present writer[1]) and by BEIJERINCK[2]), the true lactic-acid bacteria, in contrast to most other bacteria, are totally lacking in catalase, and the fact that broth cultures or surface colonies of the lactic acid bacteria do not develop oxygen with peroxide of hydrogen thus furnishes the principal test reaction for these bacteria. The tetracocci, however, are exceptions, which as a matter of fact do split up hydrogen peroxide to a very marked degree; the same applies to the microbacteria, which also in other respects behave somewhat differently.

Of the lactic acid bacteria, only some of those which do split up hydrogen peroxide are capable of reducing nitrate to nitrite, but when dealing with such, this feature should always be tested. The easiest method is to cultivate the bacteria in dextrose broth with 2% KNO_3; if nitrite is formed, then a small ladleful will form a dark blue spot in a mixture of zinc-iodine starch and sulphuric acid.

With the knowledge obtained, through the investigations here described, about the lactic acid bacteria, their further determination will not as a rule occasion any serious difficulty.

We always commenced, of course, with a microscopic examination of the isolated acid formers, in order to ascertain whether they were GRAM positive, and whether they were spherical or rod-shaped, but this is as far as it is possible to get by morphological investigation in the first instance. Not till we have learned the biological qualities of the strains, and grouped them accordingly, can we begin to consider the question of whether certain related strains may have certain morphological qualities in common, and this will also frequently be found — though by no means always — to be the case. The morphological differences between the different species of spherical forms, or between those of the rod forms, or even, indeed, between their various genera, are so slight that they cannot be determined until we know what strains are related together, as only the total impression of a great number of related strains can give a general idea of any value.

After the GRAM test, the next thing to try is the reaction with peroxide of hydrogen, whereby the tetracocci and most of the microbacterium are separated off. The attitude of the strains towards different temperatures is then noted, as also towards different sugars, and different sources of nitrogen. The acids formed, and also any splitting up of casein, are likewise further studied.

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[1]) Det kgl. danske Videnskabernes Selskabs Forhandlinger 1906, Nr. 5. In this work it is proved that also the butyric acid bacteria lack catalase.

[2]) Archives Néerlandaise des Sciences Exactes et Naturelles 1907, Série II, Tome XIII, p. 357.

None of these stages in the investigation can be dispensed with, and none of them can be taken as of decisive importance by itself, not even the reaction with a long series of different sugars, though this does give such a valuable insight into the biology of the bacteria. In the first place, it is necessary of course to know whether the acid formed is lactic acid at all, and in the second place, it is possible, as we shall see later on, for strains undoubtedly belonging to the same species to react very differently towards certain sugars. We cannot therefore lay too much stress upon the importance of avoiding the onesided classification according to reaction with sugars, which has been largely practised in particular by American writers. Only by taking equal note of all qualities can we arrive at a natural bacteria system.

Variability of Qualities.

Among the particularly variable qualities of bacteria are colour formation, and to some extent also the size and appearance of the surface growth; points which we shall deal with more closely when discussing the tetracocci. In this connection it will suffice to mention that comparatively anaerobic propionic acid bacteria transplanted from agar have in the course of years become slimy, and developed a marked surface growth, thus resembling in cultural respects the aerogenes bacteria.

As mentioned, the bacteria can, under particular circumstances, accustom themselves to new sources of nitrogen, but the reverse process — the loss of power to utilise a given source of nitrogen — is far more likely to occur, and on the whole, the variation which we have otherwise encountered in the lactic acid bacteria, can be explained as a further development of already existent tendencies, or more frequently as the results of weakness or degeneration. We have never, for instance, found any of our strains acquiring the power to ferment a new sugar, though we have occasionally found that its power of fermenting a certain sugar, originally but slight, may be increased, and we have often noticed that it has lost the power of fermenting certain sugars at all, those being chiefly those which it had always previously found difficult to utilise. As an indication of weakness also, we should regard the point already mentioned, that strains forming both dextro- and lævo-lactic acid may lose the power of forming that of the two which it formed in the lesser quantity, and that strains which besides lactic acid also formed abundant quantities of by-products lose altogether or in part the power of so doing, by which they will of course be utilising their source of energy to a less complete degree.

A highly variable quality is the power of forming slime in milk, but this is, as mentioned, only due to further development of a tendency possessed by all lactic acid bacteria in a youthful state.

It is interesting to note how closely-related strains are inclined to vary in the same way; or may, indeed, when cultivated under like conditions, often be seen to vary — and die off — at exactly the same time. All these apparent variations, therefore, are in reality no hindrance at all to species determination; on the contrary, the manner in which a bacterium is inclined to vary is often one of its most characteristic qualities.

The feature by which the alteration in vitality of lactic acid bacteria may be most directly observed, is the quantity of acid formed from a given sugar under uniform conditions, and even more so, the rate at which souring takes place; this can, in the case of milk cultures at like temperatures, be measured by the rate of coagulation. As the rate of souring (in contrast to the degree of acidity in the cultures after 14 days) is influenced to a high degree by the quantity sown out, we always inoculated the 10 cm^3 of milk employed with the same platinum loop, from a previous (just curdled) milk culture. Where greater fluctuations were observed in these respects, the extreme limits are noted in the tables. The highest amount of acid is shown first in the case of sugars whose fermentation has declined in course of years, and last in the case of sugars whose fermentation has grown stronger.

If the lactic acid bacteria are to be of practical use, and keep down the detrimental bacteria, then it is primarily necessary that they shall sour rapidly and strongly, and this is just where laboratory cultures are often liable to fall short. If their acidulating power has once declined, then it is as a rule very difficult to restore it completely, even when they are transferred daily from milk to milk. Surprising results may, however, often be obtained in this respect by using a larger quantity of inoculating material. We have had strains of *Streptococcus cremoris*, for instance, used for the souring of cream in making butter, which could not be brought to produce more than 4.7 $^0/_{00}$ lactic acid in twenty-four hours at 25°, however frequently we might transfer them with the platinum loop to a new 10 cm^3 of milk. When, on the other hand, we proceeded to a daily inoculation of 10 cm^3 culture in 200 cm^3 milk, we could then, after the lapse of two or three weeks, obtain in 20 hours the 7 $^0/_{00}$ lactic acid required for maintenance of activity in the pasteurised, though not therefore by any means germ-free, dairy cream. Next to unsuitable composition of the nutritive substrate and preservation at too high temperatures, the slight quantity of inoculating material generally used in laboratories is the main cause of the frequent degeneration in laboratory cultures[1]).

That an abundant quantity of inoculating material should give such favourable results is due to the fact that we then are more sure of transferring some of the strongest individuals in the culture. If a pure culture be spread on agar or gelatin, and a series of new strains isolated therefrom, then the latter will never behave altogether alike towards the different sugars, and all will as a rule—until they have been repeatedly transferred for some time — form a somewhat smaller quantity of acid than the original culture, as it would be a very fortunate chance to obtain on spreading, some of the most powerful individuals which stamp the culture as a whole. As a matter of fact, the majority of individuals in a bacteria culture are weakened, and have but slight power of resistance; this was very distinctly apparent from our heating experiments. This explains why pure cultures are never at first so powerfully effective in practice as the original culture previously employed, and shows, that it is by no means a matter of indifference whether a person becomes infected by a few cells of a disease bacteria or a great number of the same.

[1]) In the case of stab cultures of comparatively anaerobic lactic acid bacteria, as for instance the thermobacteria, it will be necessary to inoculate from the bottom of the stab, as the cells situated nearer to the surface will always be greatly weakened by the oxygen in the air.

Description of Species, and Systematism.

In discussing the separate lactic acid bacteria, it would be most natural to commence, as I have done in my "Dairy Bacteriology", with the rod forms, which are as a rule the strongest acid formers, proceeding then by way of the streptococci, which are all typical lactic acid bacteria, to the micrococci, where we find all possible transition forms between acid-forming and non-acid-forming bacteria. For practical reasons, however, I prefer in the present work to commence with the streptococci, as it seems easier here, in most cases, to define the separate species than is the case with the two other groups.

Streptococci.

By streptococci we understand, as is generally known, spherical bacteria dividing as a rule in one direction only. Distinction is made between the proper streptoccocus type, and the diplococcus type, according as the cells after division are inclined to remain hanging together in long chains, or to fall apart rapidly. As a rule, the streptococci stretch before division, so that the cells are then oval. After division has taken place, the daughter cells are often egg-shaped, the pointed ends turning outwards. Where the growth is lively, several of the long-chained forms will not have time to stretch before division, but form disc-like segments. Again, the double hemisphere form, with flat surface at the break, so typical among the micrococci, is one which we have encountered in some species (*Streptococcus fæcium*, *Streptococcus liquefaciens* and *Betacoccus bovis*). In broth, the long-chained strains form flakes which easily settle, so that the liquor above them rapidly clears, whereas the short-chained strains remain suspended for a long time, so that the liquid takes longer to clear.

These morhological differences have proved constant for all our strains of streptococci throughout the years during which we have had them under observation, and we cannot therefore refrain from considering them of some value as species characters, though it must be admitted that most strains in a weakened state form shorter and in particular far thinner chains than they did when at their full vitality. Here as with all other qualities in bacteria, it will be necessary to take into consideration the effect of temperature and of the nutritive substrate. Broth, for instance, increases the tendency to chain formation, whereas milk produces a reverse effect, and we have therefore only reckoned strains which also in milk grow in long chains as typical chain forms. In agar streak, and on gelatin plates, both types can produce rod forms, or other divergent forms. As regards

the influence of temperature, we generally find the most pronounced flake formation in broth near the maximum temperature.

According to their biological qualities, the streptococci fall into two main groups. The one forms, from all carbohydrates, pure dextro-lactic acid, with only a trace of by-products, whereas the other forms lævo-lactic acid, besides appreciable quantities of other fermentation products. The former group is by far the larger, and comprises not only the principal streptococci of milk, but also the pathogenic forms known under the collective term of *Streptococcus pyogenes*. They split up the sugars simply according to the formula $C_6H_{12}O_6 = 2C_3H_6O_3$, and the quantities of by-products, carbonic acid and acetic acid (acetic acid and propionic acid generally in the proportion of 20:1), which are formed, are so insignificant that they do not practically speaking diminish the theoretical yield of lactic acid. To the other group of streptococci belong the bacterium of sour cabbage, *Streptococcus brassicæ*, and the slime former so well known in sugar manufacture, *Streptococcus mesenteroides*, which forms slime from cane sugar and mannite from lævulose, and which can develop a considerable quantity of gas. With regard to nitrogenous nourishment also, there is a difference between the two groups, the former preferring casein peptone and the other yeast extract. The first group, again, generally prefers low sugar concentrations, the second high (5%—10% sugar).

These two groups differ altogether so widely in biological respects that they must be regarded as two distinct genera. And I therefore designated them originally as *Dextrococcus* and *Lævococcus*, but relinquished these names afterwards, on finding that some few bacteria belonging to the genus *Lævococcus* formed inactive lactic acid. For the first group, it would be tempting to employ the name *Lactococcus*, suggested by Beijerinck, if it were not for the fact that so many pathogenic bacteria were allied thereto. I have therefore preferred simply to reserve the generic name *Streptococcus* for the first group. The unavoidable alteration of names is thus reduced to the least possible; the only thing is, that we are obliged for the future to give the term a somewhat more restricted meaning, and only understand thereby such streptococci as form dextro-lactic acid. The second group, being chiefly met with on souring vegetable matter and particularly on beets, I have accordingly given the generic name of *Betacoccus* (Beta = beet).

Genus: Streptococcus. (Abbr. *Sc.*).

This genus I have divided into the following well-characterised species: *Streptococcus lactis*, *Sc. cremoris*, *Sc. mastitidis*, *Sc. thermophilus*, *Sc. bovis*, *Sc. inulinaceus*, *Sc. fæcium*, *Sc. glycerinaceus* and *Sc. liquefaciens*. In addition, there are also several strains — including pathogenic — which are allied to these, and cannot exactly be placed under the mentioned species, but we have not encountered them often enough to ascertain their entire range of variation, and I have not therefore ventured to establish them as separate species in themselves.

Streptococcus lactis (Table XIV). By this we understand the diplococcus which predominates in sour milk, and which is known in literature under the names of *Bacterium lactis* (Lister 1878), *Streptococcus acidi lactici* (Grotenfeld 1879), *Bacterium lactis acidi* (Leichmann 1894) *Bacterium Güntheri* (Lehmann & Neumann) and *Streptococcus lacticus*

Table XIV.

No.	Streptococcus lactis isolated from:	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN
1	Kefir 5	C	1,1	0,2	0	0	0,2	3,8	4,7	4,9	5,2	4,5	1.1	5,2	5,0	0,5	0,2	3,4	2,0	4,1	1	7,7	3,3	÷ 0,1
2	Dairy cheese 8 R 1 day	W	0,5	0,1	0,5 0	0	0	0,5 1,6	3,8	3,8	3,6	3,4	0,2	2,7	3,4	0	0	2,0			1	7,9 2,3	14,1	7,7
		C	0,2	0,2	0,2	0	0	5,2	8,3	8,1	7,7	7,4	0	5,4	5,4	0	0	3,6	0	5,4				
3	Milk (slightly acrid taste) WEIGMANN'S collection No. 15	W	0,7	0	0	0	0	1,8	3,8 3,0	3,4	3,8 3,3	2,7	0	3,2	3,2	0	0				1	7,2	14,6	6,6
		C	0,9	0,5	0,2	0	0,2	4,3	7,7	8,1	7,9	6,3	0	6,5	6,5	0,2	0	5,2	0	5,6				
4	Dutch butter 1	W	0	0,2	0,4	0	0,1	1,0	3,4	3,4	3,2	3,2	0	3,2	0,1	0	0				1	7,2	0	0
		C	0,5	0	0	0	0	3,2	6,5	8,1	7,9	6,3	0,5	6,5	0,7	0	0	3,4	0,2	4,1		0,7		
6	Milk 1 coagulated at 38°	W	0,2	0	0	0	0	1,0	3,6	3,8	4,0	3,2	0	4,1	3,2 1,8	0	0				1	4,5 2,9	14,7	7,0
		C	0,5	0	0	0	0	3,4	7,2	7,7	7,0	5,9	0,2	7,0	3,6	0	0	4,7	0,2	5,6				
7	Sourmilk[1]) 5	W	0	0	0	0	0	1,9	3,4	3,8	3,8	2,7	0,1	3,8	2,9	0	0	2,5			2	6,1	13,9	5,4
		C	0,2	0,5	1,4	0	0,5	3,8	4,1	7,0	6,5	5,0	0,2	6,1	5,2	0	0,2	5,2	0	4,3				
8	» 5	W	0	0	3,8 2,9	0	0	2,0	3,6	4,0	4,1	1,8 2,8	0	3,8	2,3	0	0	2,7			2	5,4	11,7	2,4
		C	0	0	6,8	0	0	4,7	7,2	7,4	7,0	5,9	0	6,1	5,0	0	0	5,4	0	5,2				
9	» 5	W	0	0	3,7 2,9	0	0	1,9	3,6	4,0	3,6	2,7	0	3,4	3,2	0	0	2,5			1	6,3	0	÷ 0,9
		C	0,2	0	5,4	0	0	3,8	6,8	7,4	7,4	5,6	0,2	7,2	6,3	0	0	5,0	0	5,0				
10	» 4	W	0	0	3,8 3,2	0	0	2,0	3,4	3,6	3,8	2,7	0,1	3,4	3,2	0	0	2,5			1	6,5	5,1	÷ 0,3
11	» 3	W	0	2,5	2,7	0	0	1,7	3,4	3,6	3,9	2,9	0,1	3,8	3,2	0,2	0	2,5			1	7,4	11,5	4,1
12	Dairy cheese 8 P	W	0 0,5	2,5	0,7 2,3	0	0	0	3,8	4,1	3,8	2,6 0,1	0	3,5	3,3 0,8	0	0				1	6,8 0,5	0	÷ 0,6
		C	0,7	3,4	0,5	0	0	0	7,2	7,2	7,2	0	0,5	5,9	1,6	0,2	0,2	3,4	0	6,5				
13	English ropy milk 1	W	0,5	2,7	2,7	0,2	0	0	2,5	3,2	3,1	0,9	0,7	2,7	2,7	0	0	2,5	0	2,0	1	6,1	0,8	0,5
		C	0,5	4,3	4,1 1,4	0	0,2	0,2	6,3	5,6	5,4	5,0	0	5,9	5,6	0,2	0	3,2	0	3,6				
14	Sourmilk 4	W	0	2,5 1,8	0,1	0	0	0	3.4	4,0	3,8	2,8	0,1	3,6	3,3	0	0	1,8			1	8,8	14,9	5,6
		C	0,5	5,2	1,4	0	0	4,5	7,0	7,7	7,2	6,3	0,7	6,8	7,0	0,5	0,7	3,6	0	6,3				
15	Kefir 1	W	0,2	1,6	0,4	0	0	0	3,2	3,6	3,6	2,9	0	3,4	2,9	0	0	2,1			1	7,2	4,4	1,8
16	Sourmilk 2	W	0,4	3,6 2,5	0	0	0	0	3,8	3,9	3,9 2,0	2,5	0	3,2	1,1	0,2	0	2,9			1	7,4 2,9	7,3	3,8
		C	0,7	2,7	0	0	0	0	6,8	7,0	6,8	5,2	0,5	6,3	2,5	0,2	0,2	3,2	0	6,5				
17	Dairy cheese 5 R	W	0,3	1,4 2,0	0,2	0	0	2,5	4,1	4,1	4,2	2,8	0,1	4,1	3,4	0	0				1	7,9	12,8	6,1
		C	0,5	1,6	0	0	0	3,6	7,9	8,1	8,1	6,1	0,5	7,4	5,2	0,2	0	4,7	0,5	6,5				

[1]) Sourmilk means milk, curdled spontaneously with acid.

(KRUSE). As will be seen, this bacterium has been regarded alternately as a rod form and as a spherical form, which is due to the fact that its cells are often — as are indeed those of most streptococci — somewhat longer than they are broad. As the earlier descriptions apply in reality to the entire genus *Streptococcus* (and largely also to that of *Betacoccus*), none of the names suggested can claim priority. I believe, however, that it will be in conformity with even the strictest requirements in this respect to use the name *Streptococcus lactis*, since the generic term *Bacterium* cannot be employed in the present instance.

Streptococcus lactis is killed at a temperature of 60°—70°. Its optimal temperature is 30°, but it can form just as much acid at 20°. It grows poorly as a rule below 10° or over 40°. In the case of several strains, the maximal temperature is reached already at 38°. No. 16 grows at 42½°, and Nos. 13 and 14 even at 45°.

Streptococcus lactis grows extremely fast under favourable conditions. When freshly isolated from milk, it will coagulate sterile milk in less than 24 hours at 30°, forming therein 7—8‰ lactic acid. In this state it is also generally capable of dissolving a small quantity of casein. This faculty is, however, in many strains, very soon lost, disappearing with surprising rapidity when they are cultivated on artificial substrates, and they are then but ill able to thrive at all in milk. The lost power can only rarely be restored by regular transference from milk to milk. This feature, and the fact that many strains prefer maltose to lactose, seems to suggest that milk is not the most natural substrate for the present species. Possibly it may be derived from cowdung. It gives milk and cream either a purely acid taste or an unpleasant flavour.

Streptococcus lactis is characterised by its lack of, or extremely slight power to ferment cane sugar. It is likewise incapable of fermenting raffinose, inulin, or (with the exception of No. 1) starch, but does ferment dextrin and salicin. Like most other lactic acid bacteria, it prefers lævulose, glucose and mannose to galactose. Of alcohols, it only ferments mannite, and not all strains can even ferment this. Its action with regard to pentoses varies. Some strains ferment neither arabinose nor xylose (*O*-forms); others ferment one of these pentoses, and others again both (*A* + *X*-forms). In *Sc. lactis*, as in most other lactic acid bacteria, the power of fermenting one or another pentose is generally impaired or altogether lost in the course of years; this faculty therefore is not suitable for further subdivision of the species; we must as a rule restrict ourselves to noting under each species *O*-forms, *A*-forms, *X*-forms and *A* + *X*-forms. In its relation to other sugars, *Sc. lactis* varies only very rarely, but may do so at times, and we may in this respect call attention to the interesting case of No. 12. This strain very soon lost the power of forming acid in milk. Later on, it also lost the power of fermenting galactose, and naturally enough, the fermentation of lactose in broth was reduced at the same time.

At 20°—30°, *Sc. lactis* (Pl. I—IV) appears as a diplococcus, or in very short chains. In milk, it is almost exclusively a diplococcus often slightly pointed. On agar streaks, it may be elongated and markedly pointed. On *AG* it now and again forms long chains. At 10°, it always forms long chains, both in broth and on agar streak. At maximal temperature, it either forms long chains of cells having the normal appearance, or short chains of irregularly swollen cells.

Streptococcus cremoris (Table XV). I have thus named the lactic acid bacteria first studied by STORCH, which, owing to its aroma formation, has become generally used

Table XV.

No.	Streptococcus cremoris isolated from:	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN
1	Buttermilk 1	W	0	0	0	0	0	0	2,0	2,3	1,4	2,3	0	2,3	2,5	0	0	0	0	1,0	1	5,2	5,0	1,6
		C	0,2	0	0	0	0	0	5,6	5,0	2,7	3,8	0,2	4,5	6,1	0	0	0	0	2,3				
2	Danish dairy cheese 3 R	C	0,5	0	1,1	0	0	0,5	5,6	6,1	5,6	3,6	0,9	2,7	6,3	0,2	0,5	1,4	0	3,2	4	5,0	8,5	2,4
3	Commercial Starter [1]) 1	C	0	0,6	0,6	0,2	0	0	3,6	4,5	4,6	3,8	0,7	2,7	4,2	0,1	0	1,4	0,2	2,5	3	5,2	0,4	0,4
4	» 2	C	0	0	0	0,5	0	0	5,4	4,5	4,5	1,4	0,2	1,1	4,7	0,2	0,5	0,7	0	0,9	3	5,2	1,5	0,4
5	Sourmilk 1	C	0	0	0	0	0	0	5,0	5,9	5,4	2,7	0	1,1	5,4	0	0	0,2	0	0	2	5,9		
6	Commercial Starter 3	C	0,2	0	0	0,4	0	0	3,2	3,8	3,8	2,7	0,2	0,7	4,5	0,7	0,5	0,5	0,2	3,6	2	6,5	3,1	0
7	Home Starter [1]) 1	C	0,2	0,4	0,5	0,2	0,2	0,1	6,0	3,0	2,9	2,7	0,8	1,3	3,2	0,5	0,2	0,5	0,2	0	2	4,7		
8	» 1	C	0,1	0,2	0,2	0,2	0,2	0,1	3,2	4,1	3,7	2,0	0,7	0,7	4,1	0	0	0	0	3,5	1	7,4		
9	Commercial Starter 4	W	0	0	0	0	0	0	2,9	3,4	3,2	1,8	0	0,2	2,9	0	0	0	0		1	7,0		
		C	0	0	0	0	0	0	6,5	6,8	6,3	5,2	0	0	6,3	0	0	0	0	3,4				
10	» 5	W	0	0	0	0	0	0	2,7	2,7	2,0	1,6	0	0,2	2,7	0	0	0	0		1	6,3	11,4	1,8
		C	0	0	0	0	0	0	6,8	6,8	6,5	2,0	0	0	5,9	0	0	0	0	3,4				
11	Buttermilk 2	W	0	0	0	0	0	0	2,7	3,2	2,9	2,3	0	0,1	2,6	0	0	0	0		1	6,8	15,4	5,6
		C	0	0	0	0	0	0	7,2	6,1	6,5	3,8	0,5	0	6,1	0	0	0	0	1,1				
12	Commercial Starter 6	W	0	0	0	0	0	0	3,6	3,4	3,6	2,7	0	0,5	2,9	0	0	0	0		2	6,3	8,5	1,2
13	Buttermilk 1	W	0	0	0,5	0	0	0	2,9	3,4	3,2	2,5	0	0,5	2,9	0	0	0	0		1	5,4		
14	Commercial Starter 7	W	0	0	0	0	0	0	3,6	3,6	3,6	2,7	0	0,2	2,9	0	0	0	0		1	5,9	10,8	2,8
15	Buttermilk 2	W	0	0	0,5	0	0	0	2,3	3,2	3,2	2,5	0	0,2	2,9	0	0	0	0		1	6,5	20,4	8,0
16	Commercial Starter 8	C	0,2	0	0,5	0,2	0,2	0,2	5,0	5,2	5,0	2,3	0,5	0,7	4,7	0,2	0,5	0,2	0,2	0,2	1	6,1		
17	Home Starter 1	C	0	0,1	0,1	0,2	0,1	0	3,2	4,4	4,3	2,0	0,5	0,2	4,7	0,1	0	0	0	0	1	7,2		
18	» 2	W	0	0	0	0	0	0	3,2	3,6	3,4	2,0	0	0,1	2,7	0	0	0	0		1	7,0	6,7	0,9
		C	0	0	0	0	0	0	7,2	6,8	7,0	5,4	0,5	0	6,5	0	0	0	0	0,5				
19	Swedish ropy milk 1	W	0	0	0	0	0	0	1,4	1,8	2,0	0,5	0	0,1	1,6	0	0	0	0	0,7	1	6,5	16,5	10,1
		C	0	0	0	0	0	0	6,8	5,2	6,3	4,7	0,2	0,5	4,5	0	0	0	0	2,9				
20	English ropy milk 1	C	0	0,2	0,9	0,2	0	0,2	6,1	5,4	5,9	5,0	0	0,5	5,6	0,2	0	0	0	0,2	1	6,8	11,4	4,0
21	Dutch ropy whey	W	0	0	0	0	0	0	1,6	3,4	2,5	1,6	0	0,1	2,0	0	0	0	0	0	1	6,1	10,1	7,9
		C	0,1	0,2	0,2	0,2	0	0,2	5,6	5,9	5,4	3,8	0	0,2	4,1	0,2	0	0	0	0				

for souring cream in the manufacture of butter. It is therefore found in most commercial starters and in buttermilk. When cultivated at 10°—18°, it often exhibits a tendency to render milk ropy, so that it can be drawn out into threads, and the well known bacterium

[1]) By commercial starter is meant the commercial culture of lactic acid bacteria, which is used or the ripening of cream. Home starter means such a culture cultivated for some time in the dairy.

of „Tätmjölk" *Bacterium lactis longi*, and the bacterium of the long whey *Streptococcus hollandicus*, are only slime-forming varieties of this species. When cultivated at higher temperatures on artificial substrates, they rapidly lose the power of forming slime. Most of the strains of *Sc. cremoris* showed only slight power of resistance, and died off in course of time; a few of the strains, however, were fairly resistant, and one (No. 18) has even kept alive in agar stab for over three years without re-inoculation. This same strain had previously distinguished itself in the butter manufacture, being found practically as a pure culture in a dairy starter which had not been renewed for many years.

Streptococcus cremoris is killed by heating to 65°—70°. Its optimal temperature lies below 30°, and the bacterium can, as is well known in practice, be trained to grow at fairly low temperatures, so that it sours rapidly even at 15°. Very powerful strains (especially those forming slime) may develop at 3°; others less strong will not grow at under 10°. It does not generally grow at blood heat. In most strains, the maximal temperature lies at 35°.

Streptococcus cremoris rarely forms much over 7 ‰ lactic acid in milk. It can develop such a quantity of carbonic acid that fine stripes appear in the curd (illustration of fermentation test g. 2). Most strains have a certain power of dissolving casein; No. 15 has even formed over 20% *SN*. It does not thrive so well on artificial substrates as *Sc. lactis*, and thus proves itself a more pronounced milk bacterium. In accordance with this, we also find that among disaccharides, it attacks chiefly lactose, and prefers this sugar at the concentration at which it is found in milk, or even higher. Saccharose is practically speaking not fermented at all, and maltose, and thus also dextrin, only exceptionally to any considerable extent. The best starters seem to have the least power of fermenting these sugars. Some strains ferment salicin, others not. The three monosaccharides, lævulose, glucose and mannose are on the whole fermented equally well. Alcohols and pentoses are as a rule not attacked at all (with good nitrogen sources, there may be a slight suggestion of fermentation of arabinose).

In morphological respects, *Sc. cremoris* (Pl. IV—IX) differs from *Sc. lactis* by forming chains, often of considerable length, at optimal temperature, both in milk and broth. The more powerful the bacteria, the thicker — and generally also longer — are the chains. Weakened strains can, in broth, form chains so twisted and clustered together that they may be mistaken for staphylococci (Pl. VI, No. 18). On agar streak, the cells as a rule assume a very abnormal appearance, becoming swollen, pointed, and often rod-shaped. The rods can, however, in many cases be resolved into discs on further observation, and then resemble woodlice or other articulata. On agar streak (at the optimal temperature), we also often find division in the longitudinal direction of the chains. (Pl. VIII, No. 20). At maximal temperature, chain formation ceases (Pl. IX, No. 2). In gelatin, the chains are often short, and the cells (especially on *AG*) irregular. As regards slime formation in milk, this is due to a slimy transformation of the highly swollen capsules, which may be found in all lactic acid bacteria in a young state, but is particularly frequent in this species. When the milk becomes so sour as to curdle, the slime formation ceases again. No. 19 (Pl. VII) shows this development distinctly. If a stained preparation of the bacteria in the capsule stage be exposed to pressure, the streptococcus chains will burst out from the capsules, appearing then as white worms.

Streptococcus mastitidis (Table XVI). This bacterium, which is also called *Sc. agalactiae*, produces mastitis in animals and man, but does not appear to be otherwise pathogenic[1]).

Table XVI.

No.	Streptococcus mastitidis isolated from:	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N SN	Milk: % of Total N DN
0	Fæces 1	C	0	0,5	0,5	0,5	0,5	0,5	4,1	3,8	3,6	2,5	3,6	3,6	3,6	0,7	0,9	1,6	0,2	3,8	2	5,6	2,6	0
1	Milk from woman with breast inflammation	W	1,1	0	0	0	0	0	2,7	2,5	2,7	0,9	2,3	2,0	2,5	0	0	1,1	0	2,3	4	5,0		
		C	2,3	0	0,2	0,5	0,2	0,5	5,9	6,3	5,0	3,2	5,2	5,4	5,2	0,7	0,5	2,5	2,9	4,1				
2	Milk from a cow with udder inflammation (Denmark)	W	0	0	0	0	0	0	2,0	1,4	1,6	0,7	0	1,8	1,1	0	0	1,1	0	0	2	5,4	1,6	1,9
		C	0,5	0	0,2	0	0	0	5,0	5,6	5,0	3,6	5,6	4,7	5,2	0	0	2,3	1,4	1,8				
3	Milk from a cow with udder inflammation (Hungary)	W	0	0	0	0	0	0	2,0	2,9	1,8	1,8	0,1	0,6	2,3	0	0	0	0	1,0	2	4,5	2,8	1,9
		C	0,5	0	0	0	0	0	7,0	6,8	5,6	3,2	6,3	5,0	6,5	0	0	0	0	2,3				

It is killed by a quarter of an hour's heating at 60°. Though thriving best at blood heat, it grows very slowly even at 40°. It develops both on *AG* and *SG* at ordinary indoor temperature, but growth ceases at 15°.

Sc. mastitidis, like *Sc. cremoris*, thrives especially well in milk. It requires, however, at least two days at 30° to curdle it, and rarely forms more than 5‰ lactic acid. Its power of attacking casein is perceptibly slighter than that of *Sc. cremoris*. It is best distinguished from the latter form by the fact that with a good source of nitrogen, it will ferment saccharose and maltose almost as powerfully as lactose. In a freshly isolated state it also ferments dextrin and starch, and — a very characteristic feature — forms an orange deposit in the starch tubes after about ten days. This feature was observed both in the *Sc. mastitidis* of woman (No. 1) and that of the cow (No. 2) which renders it likely that they are entirely identical. After long-continued re-inoculation in artificial substrates, *Sc. mastitidis* gradually loses the power of fermenting starch, and then the power of attacking dextrin; after a while, also, it becomes weaker in its action upon cane sugar and maltose, if indeed it has not died before reaching this stage, as it is difficult to keep alive. It can also altogether lose the power of souring milk. Strain No. 3 was already on the decline when we received it from Dr. Gratz, of Hungary. Gradually, as the power of fermenting

[1]) Strain 1, which I had isolated from milk taken from a woman suffering from inflammation of the mammary, appeared, from experiments kindly undertaken for me by Dr. Wilhelm Jensen, to be non-pathogenic, either for mice (subcutaneous and peritoneal inoculation) or rabbits (intravenous inoculation). Tbe bacterium had, however, produced a serious jaundice in the child, and could be demonstrated abundantly in the fæces, which were perfectly white, and highly acid. Though it was the right breast which was inflamed at the time, the bacterium was far more abundant in the left, which had been inflamed a month before.

a greater number of sugars is lost, it must be admitted that *Sc. mastitidis* markedly approaches the strains of *Sc. cremoris* which have greatest maltose and dextrin fermentation, and as we have, moreover, never found *Sc. cremoris* save in milk, and dairy products, there is much that might seem to suggest its derivation from the streptococcus of the udder. *Sc. mastitidis* can be introduced with the mother's milk (see footnote) into the digestive tract of children or young animals, but streptococci of this species are also now and again found in adults, as shown at the top of Table XVI.

In morphological respects, it entirely resembles *Sc. cremoris*. The cells are perhaps on the whole somewhat less liable to stretch before division, so that chains with disc-shaped links are more frequently met with. This feature (Pl. X) is however, far from reliable as a distinctive character. In broth, it generally forms flakes. No. 1, was however, so short-chained, that it rendered the broth cloudy. The GRAM staining process was not always equally successful. No. 1 rendered the dextrose broth slightly slimy after a considerable length of time. We have never, however, noticed that *Sc. mastitidis* was able to render milk slimy.

Streptococcus thermophilus (Table XVII) is the most frequently occurring streptococcus in low pasteurised milk and in milk which has been preserved at 40°—50°. It

Table XVII.

No.	**Streptococcus thermophilus** isolated from	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. S N	Milk: % of Total N. D N
1	Milk left to stand 24 hours at 45°	W	0	0	0	0	0	0	2,3	3,8	1,6	1,4	0	0,2	4,1	0	0	0	0	0	2	4,5	2,8	0,3
2	Milk 2 heated ½ hour at 70° and left to stand 24 hours at 6°—10°	» C	0 0	0 0	0 0	0 0	0 0	0 0	1,8 7,1	2,3 8,6	1,1 2,7	0,5 4,3	1,1 7,2	0 1,1	2,5 7,9	0 0	0 0	0 0	0 0	0 0,2	1	8,1	4,0	÷ 1,2
3	Milk 3 heated ½ hour at 70° left to stand 24 hours at 30°	W C	0 0	0 0	0 0	0 0	0 0	0 0	1,1 3,4	1,6 7,0	0,5 0,7	0 0,5	0 6,8	0 0	1,4 5,9	0 0	0 0	0 0	0 0	0 0	5	5,0	0,4	÷ 0,2
4	Milk 4 heated ½ hour at 70° and left to stand 24 hours at 40°	W C	0 0	0 0	0 0	0 0	0 0	0 0	0,9 7,0	2,3 8,3	0 4,3	0 0,7	0,7 5,4	0 0,2	2,0 6,8	0 0	0 0	0 0	0 0	0 0	1	7,0		
5	Milk 5 left to stand 24 hours at 50°	W C	0 0	0 0	0 0,7	0 0	0 0	0 0	0,7 5,9	1,8 7,4	0 2,3	0 3,2	0,9 5,4	0 0,7	2,0 6,5	0 0	0 0	0 0	0 0	0 0	3	6,8	0,4	0
6	Genuin Bulgarian Yoghourt 1	W C	0 0	0 0	0 0	0 0	0 0	0 0	0 2,9	3,2 5,0	0 0	0,9 1,6	0 6,1	0 0	3,2 6,3	0 0	0 0	0 0	0 0	0 0	1	7,0	1,5	÷ 0,3
7	Emmental cheese in the press	»	0,6	0	0	0	0,5	0,2	3,8	3,8	1,1	1,4	4,1	1,8	4,5	0,9	0,2	0,2	0,2	0,2	1	7,4		

also develops abundantly in freshly made Emmenthal cheese, where the temperature in the press only sinks slowly from 50°—35°.

In a state of good vitality, it is not certainly killed until 80°. It grows most rapidly at 40°—45°, and develops as a rule also at 50°. At indoor temperature, the growth is mostly slow, but we have met with strains which developed even at 5°.

Streptococcus thermophilus is almost as difficult to keep alive as *Sc. mastitidis*, unless a passage of milk be introduced between the inoculations from agar to agar. It grows best in milk, where it can as a matter of fact form more acid than *Sc. cremoris*. On artificial substrates, the power of souring milk is soon reduced, but may also easily be regenerated by frequent transference from milk to milk, always provided that it has not been altogether lost. Several strains can form thick capsules in milk, or even actual slime. We have never encountered any marked power of dissolving casein. As regards its attitude to the various sugars, *Sc. thermophilus* differs from *Sc. mastitidis* in its slight fermentation of maltose, and from *Sc. cremoris* in the strong fermentation of saccharose (when the nitrogenous nourishment is satisfactory). *Sc. thermophilus* is also characterised by a generally slight fermentation of mannose, and by the fact that it never attacks salicin.

A characteristic feature in *Sc. thermophilus* (Pl. X—XII) is the fact that it forms longer chains near the optimal temperature (at 45°) than at lower temperature (at 30°). In broth, therefore, it always forms typical flakes at 45°, but by no means always at 30°. In milk, it forms at 45° short chains, generally with irregular segments (disproportionately large spheres and woodlice); at 30°, many strains only appear as diplococci. On solid substrates, it is even more irregular than *Sc. cremoris*. It does not grow on *AG*. In stab cultures, it exhibits, like *Sc. cremoris* and *mastitidis*, no trace of surface growth, whereas *Sc. lactis* may under favourable conditions grow slightly about the stab.

Streptococcus bovis (Table XVIII) is the most common streptococcus in cowdung. It can as a rule stand heating to 60°, but not to 65°. It thrives best at 35°, and does not

Table XVIII.

No.	Streptococcus bovis isolated from:	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % af Total N. SN	Milk: % af Total N. DN
1	Bovine fæces 2	W	0	0	1,6	0	0	0	1,6	2,3	2,3	1,6	2,0	2,7	2,5	0	0	2,3	3,4	1,8	3	6,3	10,8	5,8
		C	0,2	0,2	2,7	0,5	0,2	0,2	3,2	3,6	3,4	2,5	3,4	3,6	3,6	3,8	3,8	3,6	4,3	3,6				
2	„ 4	W	0	0	0,7	0	0	0	2,0	1,8	2,0	1,4	1,8	2,0	2,3	0	0	2,0	2,5	1,6	3	5,9	11,7	5,8
		C	0,2	0,2	3,4	0,2	0,2	0,2	4,3	4,1	3,7	4,1	4,1	4,5	4,1	4,7	5,0	5,0	5,0	4,7				
3	„ 5	„	0,5	0	3,6	0,2	0,2	0,2	2,5	3,4	2,0	3,8	3,2	4,1	4,5	4,5	5,0	4,7	4,7	4,1	2	6,8	14,9	5,2
4	Calf fæces 2	„	0,4	0,2	2,5	0,4	0,2	0,2	3,2	2,9	2,7	2,3	2,3	2,7	3,4	4,7	5,0	4,5	4,5	3,2	4	6,1		
5	Fæces 2	W	0	0,1	0,2	0,1	0	0	0,5	0,9	1,0	0,6	1,0	0,6	0,6	0	1,4	0,5	0	1,1	6	5,0	15,5	8,6
		C	0,5	0,5	0,7	0,7	0,7	0,5	3,4	3,2	3,6	2,0	2,9	2,9	3,2	0,5	2,9	1,4	0,5	3,2				
6	Calf fæces 6	„	0,2	0,2	0,2	1,4	0,2	0,5	4,7	4,7	5,4	4,1	5,0	5,0	4,5	4,3	1,6	1,1	1,8	3,8	5	5,0		
7	„ 6	„	0	0	0,5	0,2	0,5	0,2	5,9	6,5	4,3	5,0	5,6	5,2	5,0	6,1	0,7	5,0	5,6	5,0	2	7,7	8,3	5,2

grow at 22° or under, and does not therefore normally develop in cow milk. In a freshly isolated state, all strains grew at 45°; later on, they would hardly grow at 40°. Its tempera-

ture interval is thus comparatively narrow. Two divergent forms from calves' dung (Nos. 6 and 7) did, however, grow both at the temperature of the room, and at 50°.

Sc. bovis grows well in milk, and attacks casein. It is a pronounced starch-fermenter, and will — with good sources of nitrogen — also ferment inulin and raffinose. An exception is formed by the strain derived from the human intestine (No. 5) which neither ferments starch nor raffinose. *Sc. bovis* ferments no alcohols. The typical strains from cowdung ferment arabinose.

In Gram-stained cowdung (Pl. XIV) *Sc. bovis* appears in the form of chains with comparatively broad segments. In milk, the chains are generally short, and surrounded by a thick capsule (Pl. XIII, No. 3). No. 5 formed in all substrates long thin chains, but has also capsule in milk (this is not apparent with the Gram process).

Streptococcus inulinaceus (Table XIX). This bacterium may be found in almost all sour milk when spread on inulin gelatin stained with litmus. It is killed by heating

Table XIX.

No.	Streptococcus inulinaceus isolated from:	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. D N
1	Bovine fæces 2	C	0,5	0.2	0,5	0,7	0	1,8	3,4	3,4	3,2	1,6	2,9	2,3	2,9	1,1	2,3	1,6	1,8	2,9	3	3,6		
2	» » 2	»	0,5	1,6	0,2	0,5	0,7	2,3	4,5	4,5	4,3	3,2	3,8	3,8	3,6	1,8	3,2	2,3	0,9	3,2	2	5,9	0,5	÷0,5
3	» » 2	»	0,5	1,8	0,5	0,5	2,0	2,5	5,0	4,3	3,8	2,9	4,3	3,8	3,8	2,9	2,3	3,4	2,0	3,2	2	4,7		
4	Sourmilk 7	W	0,2	0	0	0	0	0	2,5	2,3	2,7	1,4	1,6	2,5	1,4	1,6	1,6	1,8	1,1	1,8	2	5,6	1,0	÷0,7
		C	0,2	2,5	0	0	0	0,9	7,0	6,3	6,8	5,0	5,9	6,3	5,9	5,4	4,7	4,3	1,6	2,9				
5	» 6	W	0,5	0	0	0	0	1,1	2,3	2,0	3,3	1,1	2,0	2,0	2,0	1,8	2,0	1,6	0	1,6	2	5,6	0,8	0,1
		C	0,7	2,3	0	0	0	3,8	5,4	5,4	5,6	4,1	6,1	5,6	5,4	5,4	4,3	3,2	0	3,4				
6	» 4	W	0	0	0	0	0	1,8	2,5	2,3	2,9	2,3	1,8	2,4	2,4	1,8	2,1				2	5,2	0,7	÷0,3
7	» 2	»	0,8	1,7	0	0	0	2,0	2,7	2,5	2,7	2,3	2,5	2,5	2,4	2,0	2,3				2	5,9	0,1	÷0,3
8	Chinese hen's egg	C	2,5	2,5	2,5		0,7	3,2	4,5	4,7	4,5	4,0	4,1	5,0	4,5	2,9	2,7	2,7	1,1	4,1	2	4,7	4,7	0,6

to 60°. Its optimal temperature is 30°, and it thrives well at indoor temperature, may indeed grow at 5°. Nos. 2 and 3 do not grow at 40°, whereas the others do at 45°. It is difficult to keep alive.

Sc. inulinaceus differs from *Sc. bovis* in not attacking casein and in fermenting inulin and raffinose, even with *W* as source of nitrogen. It often ferments starch and mannite (No. 3 even sorbite). All strains with the exception of No. 1 will, with suitable nitrogenous food, ferment xylose. One strain (No. 8) which was found abundantly in some dried white of egg (hens egg) from China, was also found to ferment arabinose and glycerin[1]).

Sc. inulinaceus forms for the most part short chains on all substrates. It has no capsule in milk. On agar streak, the cells are often markedly elongated. In stab cultures, there is no indication of surface growth.

[1]) The East Asiatic Company, of Copenhagen, made some experiments in China with a view to the production of dried eggs. When concentrated in a vacuum at 30°—40° the white of egg turned to a slime, which held the water very strongly. In this slime, we found the above-mentioned inulin-fermenting coccus, together with a coli form. These bacteria do not appear, however, to be responsible, either separately or together, for the slime formation.

In their attitude toward the various sugars, *Sc. bovis* and *Sc. inulinaceus* are so closely related that they might be regarded as *A*- and *X*-forms of the sam especies. As, however, they also differ in so many other respects, I have considered it most correct to establish two distinct species, though I must admit that in the case of some intermediate forms (*0*-forms) it is difficult to say where they should be placed.

Streptococcus fæcium (Table XX) is the most frequently occurring streptococcus in the human fæces. It is also found in the fæces of other mammals, and in this respect, strains

Table XX.

No.	Streptococcus fæcium isolated from:	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN
1	Fæces 2	C	1,1	0,8	3,6	3,4	3,4	3,4	6,3	6,3	5,0	4,2	4,7 0,2	4,7	4,1	0,6	0	1,6	0,2	4,1	5	5,0	1,6	0,3
2	» 2	»	0,9	0,8	4,1	3,4	3,4	3,3	6,3	6,1	5,0	4,3	0,5	5,0	4,3	0,5	0	1,6	0,2	4,1	3	5,9	1,1	0,3
3	» 2	»	0,8	0,7	3,8	0,5	3,4	2,9	6,1	5,6	5,4	4,1	4,7	5,0	4,1	2,3	0,5	1,4	0,5	4,1	7	5,0	5,8	0,3
4	» 4	»	0,7	0,7	4,3	2,3	3,4	3,2	6,8	6,1	5,4	4,1	5,2	5,0	4,3	3,6	0	1,4	0,2	3,6	5	5,2	4,5	0,3
5	» 2	»	1,1	0,9	4,1	3,2	0,2	2,9	5,9	5,2	5,0	3,4	4,5	4,7	4,1	0,6	0	1,6	0,3	3,8	4	5,2	2,1	0
6	» 5	»	0,7	0	4,5	3,4	0,2	4,1	7,7	7,2	6,8	4,5	5,4	6,8	5,2	4,7	0	2,5	0,2	5,4	4	5,4		
7	Schwedish Yoghurt	»	0,2	0,2	5,6	1,8	0	3,8	8,3	7,4	6,8	5,0	4,5	7,4	5,4	0,9	0	2,5	0	5,9	6	4,5		
8	Milk left to stand 24 hours at 50°	W	0,8	0	2,7	0,5	0	2,0	4,4	4,3	3,8	1,9 2,7	3,4 4,1	4,3	3,2	0,9	0	1,6			3	6,3	4,1	0,6
		C	0,9	0	4,5	1,8	0,2	3,6	7,0	6,8	6,3	5,0	5,6	6,3	5,6	4,1	0,2	4,1	0	5,2				
9	Soured potatoes 1 I	»	0,7	0,5	4,0	2,1	0,5	2,7	5,4	3,0	4,1	3,8	4,5	5,2	4,1	3,0	0,1	1,4	0,2	4,2		3,6		
10	Soured potatoes 2 I	»	0,7	0,7	3,7	2,0	0,5	2,7	5,4	4,3	4,1	2,3	5,6	5,0	4,1	2,3	0,1	1,3	0,2	4,0		3,6		
11	Fæces 1	»	1,1	1,6	3,2	1,1	0,5	2,5	5,6	5,6	5,4	3,2	2,9	4,5	3,6	2,0	0,7	2,5	0,7	3,2	2	5,2	3,6	÷ 0,3
12	Fæces from bottle fed child I	»	0,9	1,6	3,6	0,2	0,2	3,4	6,5	6,8	6,5	4,1	4,7	6,1	5,2	4,3	0,2	3,2	0,2	4,7	9	4,3		
13	Fæces 2	»	0,7	0,9	4,1	0,7	0,5	3,2	6,8	5,9	5,9	4,3	5,2	5,0	4,1	3,4	0,5	1,4	0,5	3,4	4	6,8	4,5	0,4
14	Dairy-cheese 2 P 3 months	W	0,8	0,1	2,7	0	0	0,6 1,8	4,3	4,7	3,8	2,3	0	3,6	3,0	0	0				6	4,7	4,4	÷0,2
		C	0,5	0,2	5,2	0	0	3,4	7,0	7,0	7,0	4,5	0,9	6,8	5,4	0,5	0,2	2,0	0	5,9				
15	Fæces 2	»	0,7	0,9	4,1	0,4	0,2	3,2 0,5	6,3	6,5	6,1	4,3	0,5	4,7	4,3	0,5	0,5	1,4	0	3,4	2	6,3	4,2	0,3
16	Calf fæces 6	»	0,2	0,7	5,0	0	0,2	0,2	6,8	5,9	6,1	4,1	0,5	5,0	4,7	0,7	0,5	1,4	0,5	4,5	2	5,4		
17	Dutch butter 1	W	1,4	0,2	0,5	0	0	0	5,0	5,0	3,2	2,5	0,1	4,7	2,7	0,5	0	1,8			2	6,5	5,8	1,6
		C	0,9	0,2	1,1	0	0	0,2	9,0	9,2	8,8	5,9	0,5	8,6	6,5	3,2	0,2	3,4	0	6,5				
18	Mazun *Düggeli*	W	0,5	0	0,2	0	0	0	5,6	5,6	4,7	2,9	0,1	4,7	3,2	0,2	0				5	4,5	0,1	÷0,7
		C	0,9	0,2	1,1	0	0	0,5	9,5	9,2	8,6	6,1	0,7	8,3	5,4	1,1	0,7	2,7	0	7,4				
19	Fæces from arctic fox. North Greenland	»	1,6	3,4	4,7	2,3	3,6	3,8	6,1	5,6	4,7	4,3	5,9	4,7	4,7	1,6	0,4	2,3	0,5	4,5	2	4,7	5,6	0,2
20	Fæces from a seal. North Greenland	»	0,7	0,7	4,3	2,3	0,5	3,6	6,5	5,2	3,8	4,5	5,2	5,4	4,7	0,7	0,5	1,8	0,9	4,7		2,7	÷ 0,4	÷1,6

Nos. 19 and 20 are particularly interesting, having been isolated from fæces which were extracted with due precautions as to sterility from the rectum of a blue fox and a seal (*Phoca foetida*) in the extreme north of Greenland, at a spot where infection from without would be most unlikely to take place[1]). *Sc. fæcium* must therefore be one of the commonest intestinal bacteria among animals. It has a high power of resistance, and not a single one of the strains has died out under our hands during the years we have had them. It is also fairly omnivorous, and can grow at widely different temperatures, so that it should be able to thrive practically everywhere throughout the world.

It can stand heating to 70°—75°. All strains develop at 10°, and some even at 5°. It exhibits lively growth right up to 50°. Exceptions are the two strains (17 and 18) with slight fermentation of arabinose, which do not thrive over 47½°. Owing to its good growth at 40°—50°, it may be found in milk which has been kept at high temperatures, as with Nos. 7, 8 and 18. For the same reason, it is found in vegetable matter stored warmly in pits, as Nos. 9 and 10, which were found in boiled potatoes which had soured spontaneously.

Streptococcus fæcium grows rapidly in broth, but comparatively slowly in milk and never attacks casein to any considerable degree. It always ferments arabinose, but only exceptionally (Nos. 11, 12 and 19) any great quantity of xylose. It generally ferments mannite and saccharose, but in respect of this faculty, fluctuations may be observed even in one and the same strain. No. 1, for instance, on the first investigation, fermented saccharose, and No. 15 mannite, but by the very next investigation, they had permanently lost this power. In their relation to the sugars, the non-saccharose-fermenting strains resemble *Streptococcus lactis*. Nos. 1—4 ferment sorbite, and are thus nearer the species next following, *Sc. glycerinaceus*, *Sc. fæcium* often ferments rhamnose and raffinose, but in this respect, we may also find differences within one and the same strain. The present species is thus characterised by a comparatively wide range of variation, and it is therefore not remarkable that strains of the same origin (Nos. 2, 3, 5, 13 and 15) should exhibit such differences in respect of fermentation power as shown in the table.

Streptococcus fæcium is a pronounced diplococcus, (Pl. XV—XVI) which does not as a rule stretch before division, and may therefore — especially on solid substrates — present a micrococcus-like impression. Even in broth, it only forms very short chains. Some few strains (6 and 12) can form elongated cells on *AG*.

Streptococcus glycerinaceus (Table XXI). I have named it from its remarkably powerful fermentation of glycerin. The few strains which we have succeeded in isolating were all with one exception derived from cheese. As it grows, in contrast to most true lactic acid bacteria, equally well on *AG* and *SG*, its presence in a substance is most easily discerned by spreading a little on *AG*. In stab cultures, it exhibits a somewhat stronger tendency to surface growth than the other lactic acid bacteria. Like *Streptococcus fæcium*, it can often stand heating to 70°—75°. It grows between 10°—45°, and two of them (6 and 7) even developed at 50°.

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[1]) These strains arise from an expedition of Knud Rasmussen. The samples of fæces were taken by the Swedish botanist, the late Dr. Thorild Wulff, and sent to Prof. Barthel in Stockholm for a complete bacteriological investigation. The isolated lactic acid bacteria were further sent to me to be exactly identified.

Table XXI.

No.	Streptococcus glycerinaceus isolated from	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN
1	Dairy cheese 5 R 1 month	W	1,5	1,9 0,5	0,4		2,3	1,7	3,7	4,3	4,1	2,6	0,5	3,4	2,9	0,1	0				3	5,4	5,3	0,3
		C	2,5	2,7	0	1,8	4,3	4,7	6,5	8,3	6,5	5,4	0,5	6,5	5,9	0,2	0	2,7	0,2	5,9				
2	Dairy cheese 5 R 4 months	W	1,5	1,9 0,5	0,4		2,5	2,3	3,8	4,3	4,1	2,5	0,5	3,4	2,9	0,2	0				3	5,2	4,3	÷ 0,6
		C	2,5	2,7	0	2,3	4,5	4,7	6,5	7,7	7,0	5,2	0,7	6,1	5,6	0,2	0,2	3,4	0	5,9				
3	Dairy cheese 9 R 1 month	W	1,5	2,0 0,5	0,7 0		2,3	2,5	3,7	4,3	4,5	2,5	2,0	3,4	2,9	0,4	0,5				4	4,7	0,3	0
		C	2,5	1,1	0	2,0	4,3	5,0	7,7	7,9	7,2	3,6	7,0	6,5	5,4	0,7	0,5	2,5	0,2	5,9				
4	Dairy cheese 9 R 3 months	W	1,6	1,8 0,8	0,1		2,3	2,4	3,7	4,1	4,3	2,5	2,9	3,8	2,7	0,5	0				4	5,0	3,4	0
		C	2,5	2,7	0	2,0	4,5	5,0	7,7	7,9	7,7	5,4	7,0	6,5	5,9	0,5	0,5	2,7	0	6,1				
5	Fæces from bottle fed child I	C	2,3	0	0,5	1,4	3,8	3,8	6,3	6,5	7,8	4,1	5,2	6,1	5,2	0,9	0,2	2,9	0,5	5,6	11	4,7		
6	Dairy cheese 1 P 3 months	W	1,8	0,4 0	0,4 0		2,3	2,3	3,6	4,2	3,8	2,2	3,2	3,6	2,6	0,4	0				9	3,6	0,8	0,8
		C	2,7	0	0	1,1	3,4	4,5	7,9	7,4	7,7	3,8	6,3	7,0	5,4	0,7	0,5	2,7	0	5,0				
7	Surface of the Camembert cheese 4	C	2,4	0,7	1,4		3,2	3,4	5,5	5,4	5,2	3,4	3,2	4,7	3,7	0,8	0,6	2,5	1,0	4,6	6	4,3		

Sc. glycerinaceus always takes several days to coagulate milk at 30°, and it has likewise no pronounced power of attacking casein. It grows better in sugar broth, and grows not only, as mentioned, in sugar-free gelatin, but also in sugar-free broth, so that it is capable of developing in cheese after the lactose has been fermented. As a powerful fermenter of glycerin, its growth in cheese is furthered by the fat-splitting process which there takes place. It ferments not only glycerin, but also other alcohols, such as mannite and sorbite, and even shows some indication of fermenting dulcite. Still more remarkable is the fact that some strains (3 and 4) can form up to 2‰ lactic acid from inosite. It always ferments rhamnose, and often xylose. The bacteria coming under this head have shown a remarkably constant power of fermentation with all sugars, except the pentoses, a perceptible decline being here observed during the years we have had them under cultivation[1]); we are therefore also disinclined to attach great importance to the difference in power of fermenting xylose shown by the strains investigated. In contrast to the remaining strains, Nos. 1 and 2 have an extremely slight fermentation of saccharose. As regards the polysaccharides, dextrin is the only one which *Sc. glycerinaceus* ferments to any considerable degree.

[1]) The quantities of acid noted in the tables were in *W* derived from the freshly isolated cultures, wherefore there is sometimes a higher fermentatio of pentose in *W* than in *C*.

In this group (Pl. XVII—XVIII), we encounter both pronounced diplococci (1, 3, 4 and 7) and pronounced streptococci (2, 5 and 6); even strains which appear identical in biological respects (1 and 2, for instance, which were, moreover, isolated from the same cheese) may differ widely as regards their morphological features, the difference here being maintained throughout a period of years. No. 6 (Pl. XVIII) forms particularly long, tangled chains and thus makes a very typical flaked precipitate in broth. The diplococcus-like forms in streak cultures, and especially at maximal temperature, exhibit markedly pointed cells (No. 4, Pl. XVII).

Streptococcus liquefaciens (Table XXII) liquefies gelatine and peptonises milk strongly, especially when the acid formed therein is neutralised with chalk. Otherwise, in cultural and morphological respects, as well as in its relation to temperature and

Table XXII.

No.	Streptococcus liquefaciens isolated from	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curding	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN
1	*Micrococcus casei amari, Freudenreich*	W	2,0	0	0,6 0,1	0	2,5	2,3	4,2	4,3	4,3	2,4	3,4	3,8	2,9	0,5	0,1				1	7,7	82	34
		C	3,4	0	0	0	4,3	3,6	7,9	8,1	7,4	3,8	6,5	6,8	5,2	0,7	0,5	3,6	0,2	5,4				
2	Dairy cheese 1 P 3 months	W	1,7	0,1	0,1		2,5		4,1	4,4	4,3	1,9	2,3	3,7	3,1	0,2	0				1	6,8	74	25
		C	1,6	0,5	0,2	2,0	3,6	3,4	6,1	6,1	5,9	4,0	3,4	5,0	4,5	0,7	0,7	2,3	0,7	4,5				
3	Dairy cheese 5 P 1 months	W	1,7	0	0	0	2,9	2,5	4,1	4,4	4,5	2,4	2,7	3,8	2,9	0,2	0				1	7,2	81	28
		C	2,0	0,2	0,5	0,5	3,6	3,8	7,0	7,0	6,8	4,1	3,6	5,4	4,7	1,4	0,5	2,9	0,7	4,3				
4	Cheese 15 I	W	0,8	0,1	0,4	0	2,7	2,0	3,3	3,6	3,4	2,2	3,3	3,2	2,4	0,1	0	3,6	0		1	7,0	84	28
		C	2,7	0	0,5	0	5,2	5,6	5,6	6,5	6,1	4,5	7,2	6,3	5,6	0,9	0,2	3,6	0,5	5,2				
5	Fæces 6	C	2,7	0	1,6	0,9	4,7	5,0	6,1	6,3	5,4	4,3	4,7	6,5	5,2	2,0	1,4	3,4	1,6	4,5	1	7,0		

the sugars, it exhibits such complete resemblance to the foregoing species, that it should be regarded as a *Sc. glycerinaceus liquefaciens*. It is often found in the udders of cows[1]), and it is more than any other bacterium the cause of premature curdling of milk, or its becoming cheesy in the fermentation test. It curdles milk not only by its proteolytic enzyme, but also by strong acid formation. Owing to the peptonisation, it renders milk and cheese bitter to the taste, and has made itself remarked thereby. FREUDENREICH, who was the first to isolate it, also called it *Micrococcus casei amari*[2]).

We are in possession of these original cultures (1), which on Agar streak has a micrococcus-like appearance (Pl. XVIII). Strains subsequently isolated, on the other hand (2 and 3) showed elongated cells on Agar. A strain isolated from fæces (5) which was dis-

[1]) ROGERS and DAHLBERG, Journal of Agricultural Research 1914, p. 491. BURRI and HOHL, Schweizerische Milchzeitung 1916, Nos. 3—8.

[2]) Landwirtschaftliches Jahrbuch der Schweiz 1894, p. 136.

tinguished by being able to ferment some arabinose, raffinose, inulin and starch, was a pronounced streptococcus. No. 1 — like most of the gelatin-liquefying bacteria.— liquefies *AG* more strongly than *SG*, whereas the reverse is the case with Nos. 2 and 3; No. 2, indeed, does not liquefy *AG* at all. *Sc. liquefaciens* has shown no tendency to lose its peptonising power, and its power of fermenting sugars is no less constant than that of *Sc. glycerinaceus*, which is apparent, *inter alia*, from the fact that the No. 1 strain, which is now 25 years old, ferments the same sugars with the same degree of intensity as freshly isolated strains. Nos. 1 and 5 are powerful inosite fermenters.

Remaining Saprofytic Streptococci (Table XXIII). Of the streptococci which we have investigated, there are eight now left, of which no less than 6 are derived from cowdung (three of them, moreover, from the same cowdung) which cannot properly be placed under any of the foregoing species, and which on the other hand have not sufficient qualities in common to permit of their being taken together as a new species. In all

Table XXIII

No.	Streptococci not to be ranged in the before mentioned groups isolated from	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN
1	Bovine fæces 2	C	1,1	0,8	5,0	2,7	2,9	1,8	5,2	5,2	5,0	4,5	3,6	4,1	4,1	0,7	0,2	3,6	3,8	4,1	2	5,2	1,8	0
2	» » 2	C	1,4	0,9	4,7	2,9	2,5	2,5	3,4	3,4	3,2	2,9	2,7	4,3	3,6	0,7	0,2	1,8	0,9	3,2	3	5,6	0,4	÷ 0,3
3	» » 2	C	0,9	1,6	2,5	0,9	0,5	2,3	4,7	4,5	4,7	2,5	4,1	4,1	3,8	3,4	0,4	2,0	0,5	2,5	2	7,4	2,1	÷ 0,3
4	Sour cabbage 2 [1]	C	0,9	3,5	4,0	0,3	0,3	2,8	4,5	5,3	4,7	3,6	4,3	4,7	4,2	0,6	0,2	4,0	1,6	4,7	1	6,4	0	÷ 1,5
5	Bovine fæces 5	C	1,4	4,3	4,1	0,3	0,2	3,4	3,8	5,4	5,6	5,0	5,4	5,2	5,0	0,7	0,3	4,1	4,1	5,2	2	7,0		
6	» » 4	C	0,7	4,5	5,0	0,2	0	3,4	5,6	5,4	5,4	4,5	5,0	5,2	4,5	2,0	0,3	4,1	4,1	5,2	2	7,0	3,9	0
7	Kefir 2	W	0,3	1,6	0,6	1,0	0	1,8	3,6	3,2	2,3	2,7	3,4	2,6	3,2	0	0				1	7,7	14,1	5,7
		C	0	3,4	0,5	2,0	0,2	3,6	7,0	6,8	6,3	5,4	6,5	6,3	6,5	0,5	0,2	4,7	0	4,3				
8	Bovine fæces 6	C	0,9	5,9 0,3	0,5	0,5	0,3	0,2	5,0	5,3	5,0	4,3	5,4	5,0	4,5	2,9	0,5	2,0	0,7	4,3		2,3	1,7	2,2

[1]) German: Sauerkraut.

probability, we have here several new species but we know as yet too few strains of each to furnish material for an estimate as to which of their qualities are essential and which are not.

No. 3 grows best at indoor temperature, the remainder at 30°. With the exception of No. 7, which still grows well at 45°, none of these bacteria will develop at temperatures over 40°. In their relation to the sugars, and to common salt, the two first strains resemble the sorbite-fermenting strains of *Sc. fæcium*, but differ therefrom by the low maximal temperature. No. 1 further differs in its comparatively strong fermentation of starch, in which respect it resembles strains (4), 5 and 6. A transition form to these last is presented by No. 3, with its power of fermenting both xylose and arabinose. Of pentoses, No. 7 ferments only xylose (and, like Nos. 1 and 2, rhamnose) and No. 8 ferments neither pentoses nor alcohols, but, like Nos. 3 and 6, raffinose.

In morphological respects (Pl. XIX), all these bacteria resemble *Sc. lactis*, and it is not impossible that they should — with the exception of Nos. 1 and 2 — be regarded as saccharose-fermenting strains of this species. We have already seen that the power of fermenting saccharose is not one of the most constant qualities, and we find accordingly, in several species (e. g. *Sc. fæcium* and *Sc. glycerinaceus*) both saccharose-fermenting and non-saccharose-fermenting strains. In any case, No. 7, with its not inconsiderable power of attacking casein, is closely allied to *Sc. lactis*.

Remaining Patogenic Streptococci (Table XXIV, Pl. XX). For purposes of compari-

Table XXIV.

No.	Examples of pathogenic Streptococci	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	
1	*Sc. pyogenes Kral*	C	0	0	0,2	0	0	0	3,6	4,1	3,4	2,7	3,8	4,1	0	0	0	2,7	3,4	3,2		0,2	
2	Strangles *C. O. Jensen*	»	0	0	0	0	0	0	4,1	5,0	4,5	2,3	5,2	4,1	0	0	0	4,5	5,2	3,2		0,2	
3	Epizootic Pneumonia of the horse *C. O. Jensen*	»	0	0	0,5	0	4,1	0	4,5	5,4	4,7	3,8	5,4	4,5	4,5	0	0	5,0	5,6	3,4		1,6	
4	Petechial fever of the horse *C. O. Jensen*	»	0	0	0	0	4,3	0	4,3	5,0	4,5	3,6	5,2	4,7	4,3	0	0	5,0	5,6	3,6		1,6	
5	Navel and joint evil of foals *C. O. Jensen*	»	0	0	0	0	4,1	0	4,3	5,0	4,5	3,4	5,4	5,0	4,3	0	0	4,7	5,6	3,8		1,6	
	Sc. glycerinaceus Nr. 5	»	2,3	0	0,5	1,4	3,8	3,8	6,3	6,5	6,8	4,1	5,2	6,1	5,2	0,9	0,2	2,9	0,5	5,6	11	4,7	
6	Facial erysipelas *Oluf Thomsen*	»	0,2	0	0	0	0	3,8	3,8	4,7	4,1	2,7	5,0	4,3	3,2	0	0	2,7	0,7	3,4		1,1	
7	Sc. mouse *Vilh. Jensen*	»	0	0	0,5	0	0	2,9	5,6	5,6	5,2	4,3	5,4	4,3	0,2	0	0	2,7	0	5,2		1,8	
	Sour cabbage 1	»	0,2	0,2	0,2	0	0	0,9	5,6	5,4	5,3	3,4	5,2	3,4	0,6	0	0	2,3	0	5,3		1,6	
8	Sc. rabbit *Vilh. Jensen*	»	0,9	0,2	0,5	0	0	0,2	4,5	4,5	4,3	3,2	4,7	5,2	0	0,5	0	2,7	0	2,9		0,7	Red. in Agar stab.
9	Sc. pus *Vilh. Jensen*	»	0,9	0,2	0,5	0	0	0,2	5,0	4,3	4,1	3,2	5,0	5,0	0	0,5	0	2,7	0	2,9		0,7	»
	Sc. fæcium No. 12	C	0,9	1,6	3,6	0,2	0,2	3,4	6,5	6,8	6,5	4,1	4,7	6,1	5,2	4,3	0,2	3,2	0,2	4,7	9	4,3	
10	Sc. new *Vilh. Jensen*	»	0,5	0,2	5,4	0	0,2	3,6	7,7	7,2	7,0	4,5	7,0	6,5	5,6	3,8	0	2,3	0	5,9	5	4,7	
11	Sc. Fredericia *Vilh. Jensen*	»	0,5	0,2	5,6	0	0,2	4,1	7,9	7,4	7,0	4,5	7,0	6,8	5,0	4,5	0	2,5	0	5,9	6	4,3	
	Sc. fæcium No. 6	»	0,7	0	4,5	3,4	0,2	4,1	7,7	7,2	6,8	4,5	5,4	6,8	5,2	4,7	0	2,5	0	5,4	4	5,4	

son with our saprophytic streptococci, we have investigated, by the same methods, some few pathogenic streptococci, kindly furnished by Professor, Dr. C. O. JENSEN, Dr. WILHELM JENSEN and Dr. OLUF THOMSEN. All of them form dextro-lactic acid without any considerable quantity of by-products. Nos. 1—9, which appear in all nutritive substrates as shorter or longer chains, will not stand heating beyond 60°, and thrive very poorly below 15° and over 40°. Nos. 10 and 11, on the other hand, which in all nutritive substrates appear chiefly as diplococci, are not killed until 75°, and grow well at temperatures from 12° to 50°. They are thus not affected even by the highest fever temperatures. Nos. 8—11 were not difficult to keep alive with the nutritive substrates employed, whereas most of the other pathogenic streptococci died off in course of time.

As regards the chain-forming strains, it would be natural to consider them related to *Sc. mastitidis*, which forms the proper connecting link between the streptococci of milk and the true pathogenic streptococci; they resemble this form also in growing particularly badly in yeast extract. Some of them also ferment starch (without attacking raffinose and inulin), which was, as we have seen, a particular characteristic in the freshly isolated strains of *Sc. mastitidis*. They form no colouring matter from it, however, and differ perceptibly from *Sc. mastitidis* by growing poorly in milk; several of them, indeed, do not ferment lactose at all. Like the other long-chained forms, *Sc. mastitidis*, *Sc. cremoris*, *Sc. thermophilus* and *Sc. glycerinaceus* No. 6, they do not ferment pentoses. The strains which produce epizootic pneumonia, as well as petechial fever of the horse and navel and joint evil of foals are distinguished by fermenting sorbite, without being able to attack mannite, which is otherwise far easier to ferment. No. 8, and especially No. 9, are red in the stab in casein peptone agar, a feature which we have not observed in other lactic-acid bacteria, but which is said to be characteristic of *Sc. lanceolatus* and *Sc. mucosus* and which is very common in the propionic acid bacteria. There can hardly be any doubt that the present group contains several new species. In the table, we have shown the fermentation figures for those of our saprophytic streptococci which most nearly resemble them. The one isolated from sour cabbage is a long-chained streptococcus which does not grow at temperatures over 40°.

As regards Nos. 11 and 12 these should, from their appearance, their relation to temperature, and their fermentation of sugars, simply be regarded as pathogenic strains of *Sc. fæcium*.

It follows then, that the name *Streptococcus pyogenes* — like most of the bacteria names hitherto employed — is a collective term, embracing several species. WINSLOW[1]) sets up no less than six species, three of which do not resemble any of our strains.

With regard to *Sc .lanceolatus*, of which we have also investigated one or two strains, and *Sc. mucosus*, these form no considerable quantity of lactic acid at all. Judging from their shape, we may just as well reckon them among the micrococci, and WINSLOW also places them in the genus *Diplococcus*, which comprises the most pathogenic micrococci.

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[1]) Systematic Relationships of the Coccaceae. New York 1908.

Genus Betacoccus (Abbr. *Bc*).

As mentioned in the introduction to the streptococci, the betacocci are found in green vegetable matter and juicy roots. They are as widely distributed in beets as the saccharomyces on sweet and juicy fruits. They are introduced with vegetable food into the intestinal canal of animals, and pass thence into the milk. In the retting process we always encounter arabinose-fermenting betacocci, which might be connected with the fact that pectin substances always contain an arabinose group[1]). As these bacteria can make vegetable matter tender, e. g. sour cabbage, it is possible that they play some part in the retting process itself; this point, however, we have not yet been able to elucidate thoroughly. As the betacocci are far more variable in all respects than the streptococci, it is very difficult to divide them up into clearly defined species, and I therefore prefer to treat the genus *Betacoccus* under one head, and merely note in conclusion what features might seem to justify our uniting certain strains into independent species. In Tables XXV a and b and XXVI, they are arranged principally according to their relation to the pentoses.

The betacocci can as a rule stand heating to 60°, but rarely to 65°. In a slimy state, however, they can stand higher temperatures, as the slime protects them, and it has been observed at sugar factories that thin syrup which had been heated to 80°—85°, and could not possibly have become infected afterwards, has grown slimy (No. 11 has formed zoogloea masses under such conditions). The optimal temperature lies at about 30° or under, a single strain (No. 14) was even found to grow best at indoor temperature, and this temperature is, as in the case of *Sc. cremoris*, the most favourable one for slime formation. The maximal temperature is 35°—37° (rarely 40°) and the minimal 5°—7°. Some few strains (Nos. 1, 45, 46 and 47) grow, however, at 45°, but on the other hand thrive but poorly below 15°.

The betacocci always form lævo-lactic acid, more rarely also equivalent quantities of dextro-lactic acid, so that we find inactive lactic acid (Nos. 43—47). As certain strains (Nos. 6, 41 and 42) in a freshly isolated state formed inactive lactic acid, and later only lævo-lactic acid, this is evidently a variable quality, which cannot be used by itself as a species character. The betacocci also as a rule develop gas, (carbonic acid with more or less hydrogen). The gas development is strongest in lævulose solutions, and next in cane

[1]) Felix Ehrlich: Chem. Zeitung 1917, 41, p. 197.

Table XXV a

No.	Betacoccus A-and A + X forms isolated from	Rotatory power of the lactic acid.	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid.	Milk: % of Total N. SN	Milk: % of Total N. DN	Appearance in saccharose gelatin
1	Emmental cheese	1	W	0	0,7	3,2	0	0	0,5	2,9	2,5	2,5	2,5	2,3	1,4	2,3	0	0	0	0	0					
			C	0	0,9	9,9	0	0	0,9	6,5	6,3	5,0	4,7	1,4	1,4	6,1	0	0,2	0,2	0	0,5	6	5,4	÷1,2	÷0,4	0
			Y	0	0,9	6,8	0	0	0,9	5,6	5,6	4,7	4,1	2,3	2,9	6,1	0	0	0,2	0	0					
2	Bovine fæces 1	1	C	0,5	0	4,3	0,7	0,2	0,5	3,6	2,9	2,7	1,6	1,6	2,7	2,9	2,5	0,2	0,5	0,5	0,2		0,7			0
3	» » 2	1	C	0,2	0,7	3,6	0,5	0	0,2	2,7	1,8	2,3	0,9	2,3	1,8	1,1	1,1	0	0,5	0,5	0		0,7			0
4	Dairy cheese 8 R 3 months	1	W	0	0	4,7 1,8	0	0	0	1,1	2,0	1,8	1,4	0 1,1	1,1	1,1	1,1	0	0	0						
			C	0,2	0	7,7	0	0	0	5,0	5,2	5,4	3,2	1,6	2,0	4,3	1,1	0	0	0	1,8	6	5,5	÷1,6	0	m
			Y	0	0	5,2	0	0	0		6,8		4,1	5,6	6,5		6,3	0	0	0	1,8					
5	Dairy cheese 7 R 4 months	1	W	0	1,4	3,8	0	0	0	2,7	2,7	2,7	2,7	2,3	2,7	2,3	1,4 0,2	0	0	0		2	7,2			
			C	0	0,9	7,9	0	0	0	6,5	6,3	5,4	5,2	1,6	4,1	4,3	1,1	0,2	0,2	0	4,5	9	5,4	2,8	0,6	m
6	Dairy cheese 9 R 4 weeks	i	W	0	0,9	5,4	0	0	0,7	4,3	2,7	1,8	2,3	3,4	1,6	1,6	0,2	0	0	0						
		1	C	0	7,2	0 6,8	0	0	1,6	6,8	6,8	7,0	0	5,6	2,0	0,7	0	0	0	0	4,1	4	5,4 2,0	÷2,8	÷0,6	Ml
			Y	0	1,1	4,5	0	0	1,6		6,1		3,6	5,0	6,1	5,4	5,6	0	0	0	4,1					
7	Dairy cheese 9 P 1 week	1	W	0	0,9	5,4 0	0	0	0	3,2	2,9	1,8	2,0	2,7	1,4	2,0	0	0	0	0	0					
			C	0,2	0 6,5	0	0	0	0	7,0	5,4	7,0	3,2	6,8	8,1	5,0	0,2	0	0	0	0	3 8	10,4 3,4	0,7	0	Ml
			Y	0	6,1 1,1	0	0	0	0		6,1			5,2			0,7	0	0	0	0					
8	»	1	W	0	0,9	5,4 0	0	0	0 0,7	4,3	2,7	2,0	2,3	2,9	1,6	1,9	0,8 0,1	0	0	0	0					
			C	0	0	0	0	0	0	6,8	5,0	4,5	2,7	6,1	4,7	4,7	0,2	0,2	0	0	0,5	2 10	6,8 3,8	÷0,1	÷1,0	Ml
			Y	0	1,8	0	0	0	1,4	5,9	5,9	6,5	3,6	6,3	5,0	7,9	0	0	0	0	0					
9	»	1	W	0	0,9	5,0 3,2	0	0	0 0,9	4,3	2,9	2,0	2,3	3,6	2,0	2,3 1,4	0,8 0,1	0	0	0	2,5	3	6,2	3,7	1,6	Ml
			C	0	0	5,4	0	0	0	7,2	5,9	2,7	1,6	2,5	4,7	1,1	0,2	0,2	0	0	4,5		2,7			
			Y	0	1,8	3,6	0	0	0,9		6,1	5,6	3,8	5,6		2,9	2,3	0	0	0	4,5					
10	Kefir 2	1	C	0	3,6	3,2	0,2	0	0	5,2	1,6	2,0	0	4,3	1,6	0	0,2	0	0	0	0		2,5			Ml
			Y	0	5,0	2,9	0	0	0		4,1	4,1	3,4	5,0	4,7	5,0	0	0	1,6	0	0					
11	Slimy thin sirup from NakskovSugar Factory. The sirup was heated 3/4 hour at 80° – 86°	1	W	0	3,4	4,1	0	0	0	3,4	2,5	1,1	1,1	2,9	2,3	2,5	1,1	0	0,2	0	1,6		2,0			Ml
			C	0	7,9	9,0	0	0	0	5,9	6,3	4,1	1,1	6,1	4,1	1,8	0,5	0	0	0	4,1		2,3			
			Y	0	4,5	3,4	0	0	9,0	2,7	5,9	1,8	0,5	5,0	2,9	1,8	0,7	0	0	0	4,1					
12	Slimy raw juice from NakskovSugar Factory		W	0	2,7	1,8	0	0	0	5,9	2,0	6,8	4,5	2,5	5,4	1,6	2,7	0	0,5	0	2,0					M
			C	0	8,6	7,9	0	0	0		7,0			6,8		4,5	6,5	0	0	0	5,0					Ml
			Y	0	9,0	1,8	0	0	0,9		7,2			6,3			5,2	0	0,2	0	5,0					

Table XXV b

No.	Betacoccus A- and A + X forms isolated from	Rotatory power of the lactic acid	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN	Appearance in saccharose gelatin
13	Rotten swede II	l	W	0	0,8	2,3	0	0	1,1	5,2	2,5	2,7	2,0	2,7	1,8	2,0	2,3	0	0	0	2,3		2,0			M
			C	0,2	8,8	10,4	0	0	0,5	6,3	6,8	5,0	3,6	6,1	1,6	4,1	5,6	0	0	0	3,4					
14	Rotten swede IV	l	W	0	2,5	4,7	0	0	0,7	5,0	2,0	1,1	0,7	3,6	1,1	0,9	1,6	0	1,1	0	0		4,1			Ml
			C	0	7,4	8,3	0	0,2	0,5	5,6	4,5	5,2	2,7	5,4	2,7	3,8	5,2	0,2	1,4	0,5	2,9					
15	Rotten swede III	l	W	0	0,5	6,3	0	0	0	5,4	2,5	2,3	2,5	2,9	2,5	2,3	2,0	0	0	0	2,0	12	3,4			Ml
			C	0	7,2	10,6	0	0	0,5	6,3	6,5	4,1	3,2	5,6	3,8	4,3	5,2	0	0	0	4,1					
16	Rotten swede I	l	W	0	0,5	2,9	0	0	0,9	5,0	2,5	2,7	2,3	2,9	2,0	1,6	1,8	0	0,5	0	2,0	8	3,4			ml
			C	0	6,1	10,4	0	0	0,5	6,3	6,1	4,5	4,3	6,1	3,4	4,1	5,0	0	0,2	0	4,1					
17	Soured potatoes 1 I	l	C	0	7,5	5,5	0,1	0,1	0,9	6,1	5,4	3,5	3,3	4,5	4,8	2,8	3,8	0,1	0,6	0,1	3,3		1,6			Ml
18	Soured potatoes 2 II	l	C	0	10,3	10,1	0,3	0,1	0,3	6,3	4,7	2,0	3,4	5,3	3,3	3,6	5,1	0,2	0,6	0,1	3,2	12	4,3			Ml
19	Sour cabbage 1	l	C	0,1	3,4	4,5	0,2	0,2	0	5,4	5,2	5,0	3,2	5,4	4,7	3,8	5,0	0,7	0	0	1,6		1,6			ml
20	» » 2	l	C	0,1	6,9	7,9	0,1	1,6	0,7	5,9	4,8	4,5	3,5	5,6	3,6	3,8	4,5	0,1	0,5	0	2,8		0,9			ml
21	» » 2	l	C	0,1	5,2	9,0	0,1	0,5	0,2	5,9	2,9	3,6	2,7	5,6	4,3	4,3	5,0	0,2	0,5	0	2,5		1,6			M
22	» » 1	l	C	0,1	6,8	7,9	0,2	0,5	0,5	6,1	5,0	4,3	3,6	5,2	5,0	4,5	5,0	0,7	0,1	0	3,4		1,6			M
23	» » 2	l	C	0,1	7,1	7,7	0,1	0,2	0,3	6,1	5,4	5,0	3,6	5,4	5,4	4,5	5,2	0,2	0,6	0	2,9		1,8			M
24	» » 1	l	C	0,1	3,7	5,4	0,2	0,3	1,1	5,9	4,5	4,1	2,9	4,7	3,6	1,6	4,5	0,7	0,1	0	3,6		0,9			M
25	» » 1	l	C	0,1	4,7	5,9	0,1	0,2	1,5	6,0	4,1	4,1	2,5	5,4	4,5	0,5	0,2	0,7	0	0	3,4		0,5			M
26	» » 2	l	C	0,1	5,0	8,8	0,1	0,2	0,3	5,9	5,2	4,3	3,8	5,4	5,2	3,6	4,7	0,2	0,3	0	3,8		1,6			Ml
27	» » 2	l	C	0,1	8,3	8,1	0,1	0,2	0,2	6,1	5,0	4,5	3,8	5,6	5,0	4,3	5,2	0,2	0,6	0,3	0,7		2,0			Ml

sugar solutions. Under these conditions, we find a considerably greater quantity of acetic acid formed than in dextrose solutions. In lævulose solutions, the strongest gas-developers (*A* + *X*-forms) also produce some mannite. In most strains, however, the gas development is so slight that it can only be observed by sowing out strongly in tall sugar agar tubes, and a few strains (Nos. 1, 2, 3, 28, 36, 37 and 43—47) do not seem to develop gas at all. Several of these last grow chiefly in the upper part of the agar tube, though they are not otherwise obligatorily aerobic. In stab cultures, for instance, they do not show real surface growth, any more than the other betacocci. The gas-developing strains all form more (*M*) or less (m) slime (*mucosus*) from cane sugar. These which do not develop gas on the other hand form (except No. 28) no (0) slime; some of them indeed (as most of those forming inactive lactic acid) hardly attack cane sugar at all. In accordance with the investigations of ZETTNOW[1]), we have found that the slime is only formed from cane sugar (though some strains show an indication of slime formation with raffinose), and

[1]) Zeitschrift für Hygiene 1907, p. 154.

Tabel XXVI.

No.	Betacoccus X - and O - forms isolated from	Rotatory power of the lactic acid	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN	Appearance in saccharose gelatin
28	Sour cabbage 2	l	C	0	3,5	0,1	0	0	0	5,9	3,6	3,6	2,1	5,6	3,9	3,3	4.2	0	0,3	0	3,4		2,0			m
29	Bovine fæces4	l	C	0,2	5,2	0	0,2	0	0	5,9	3,4	3,6	2,3	5,6	3,2	3,2	3,8	0,5	2,3	0,5	2,5	2	8,3	7,9	1,2	m
30	» » 4	l	C	0,2	6,3	0	0,2	0	0	5,9	2,9	3,6	2,3	5,6	2,0	2,7	4,1	0,2	1,6	0,5	1,8		1,1			m
31	» » 5	l	C	0,5	4,7	0	0,5	0	0	5,6	3,4	3,4	2,5	5,0	3,4	3,2	4,5	0,5	1,8	0,5	3,2	9	7,4	0,7	0	m
32	Calf fæces 2	l	C	0	4,5	0,5	0,5	0,2	0	4,3	2,9	2,5	1,4	3,4	2,3	2,5	3,4	0,5	1,8	0,5	2,0	2	8,6			m
33	Milk Weigmann's collection No. 31	l	W	0	0,2	0	0	0	0	3,6	2,9	3,2 1,4	1,8	3,8	0,9 1,8	0,9	2,0	0	0	0	0		2,9	0,5	0,5	m
			C	0	5,0	0,9	0	0	0	5,9	5,0	5,0	4,7	5,6	4,5	0,5	5,2	0	0,5	0	0		0,5			
			Y	0	2,0	0	0	0	0	6,1	4,7	0	0	0	4,7	0,7	0	0	2,0	0	0					
34	Dairy cheese 9 R 3 months	l	W	0	2,0	0	0	0	0	3,8	1,8	1,4	1,4	3,2	0,9	0,7	1,8	0	0	0	0		2,3	12,2	3,2	m
			C	0	7,4	0,9	0	0	0	6,1	5,9	4,3	5,2	7,2	5,6	5,2	5,4	0	0,9	0	0,5	7	7,7			
			Y	0	2,3	0	0	0	0	5,9	6,3	6,8	1,1	6,8	5,6	0,5	7,4	0	0,1	0	0					
35	Dairy cheese 5 P 4 months	l	W	0	0,6	0,1	0	0	0	2,0	2,5	2,0	2,0	1,7	2,4	1,6	0	0	0	0	0					
			C	0	5,4 2,9	0,9 0	0	0	0,2	5,9	5,6	4,3	3,6	4,7	4,5	2,5	0	0	0,2	0	0	2	7,2 2,3	0,6	1,4	m
			Y	0	0	0	0	0	0	6,8	5,4	5,6	3,6	0	5,9	0	0	0	0	0	0					
36	Sourmilk 3	l	W	0	0	0	0	0	0	2,0	2,1	2,8 1,8	1,7 1,0	2,3	2,3	2,3	0	0	0	0	0					
			C	0	0	0	0	0	0	5,2	6,3	5,2	2,0	6,1	5,4	5,0	0	0	0	0	0	4	5,0	0,9	0	0
37	» 3	l	W	0	0	0	0	0	0	1,1	1,8	1,1	1,1	1,4	1,4 0,5	1,1 0,2	0	0	0	0	0					
			C	0	0	0	0	0	0	4,3	5,4	1,8	2,0	1,6	4,3	0,5	0	0	0	0	0		2,0 0,5	1,2	0	0
			Y	0	0	0	0	0	0		5,6	5,0	3,8	5,0		0,9	0	0	0	0	0					
38	Soured potatoes 1 I	l	C	0	0,1	0,1	0,1	0	1,4	6,1	5,0	4,4	0,1	4,7	4,4	0	0,1	0	0,2	0	0,1		0,2			M
39	»	l	C	0	0	0,1	0,1	0	1,4	5,9	4,6	4,3	0,1	5,2	4,3	0	0,2	0	0,2	0	2,4 0,1		0,2			MI
40	Dairy cheese 8 P 3 months	l	W	0	0	0	0	0	0	2,3	2,5	1,8	2,0	0,7	0,2	0,1	0	0	0	0	0					
			C	0	0	0	0	0	0	5,2	5,2	5,2	3,2	2,5	0,5	0,2	0,2	0	0	0	0	2	6,3 0,5	0	0	M
			Y	0	0	0	0	0	0	7,2	5,2	6,5	5,6	6,8	0	0	0	0	0	0	0					
41	do. 1 week	i	W	0	0	0	0	0	0	2,0	2,0	2,0	1,8	1,1	0,1	1,4 0,1	0	0	0	0	0	10	7,4 0,5	0	0	M
		l	Y	0	0	0	0	0	0	5,2	6,5	6,1	3,6	3,2	0	0,2	0	0	0	0	0,2					
42	Dairy cheese 8 R 1 week	i	W	0	0	0	0	0	0	1,8	2,5	1,6	1,8	2,3 0,9	0,2	1,4 0,1	0	0	0	0	0					
		l	C	0,2	0	0	0	0	0	4,7	4,5	2,7	3,8	5,0	0,2	0,5	0	0,2	0	0	0		2,7 0,7	1,0	0	M
			Y	0	0	0	0	0	0		6,1	4,5		6,1	0	0,5	0	0	0	0	0					
43	Bovine fæces2	i	C	0,7	0,5	0,2	0,5	0	2,7	4,1	3,8	3,4	2,0	0,5	3,2	0,5	0,2	0,2	0,7	0,5	0		0,7			0
44	» » 2	i	C	0,5	0,2	0,2	0,5	0	0	4,3	3,4	3,6	3,4	0,9	4,5	2,9	0	0	1,1	0,5	3,4		0,7			0
45	Calf fæces 6	i	C	0	4,3	0,2	0	0	0	2,5	2,7	3,2	2,0	1,5	2,7	0,9	0,2	0	0	0	0,9		1,4			0
46	Sourdough	i	C	0,1	5,9	0,9	0	0,1	0	5,3	5,4	4,1	3,7	0,2	0,2	0,1	0,1	0	0	0	5,3		0,5			0
47	»	i	C	0,2	5,9	1,0	0	0	0	5,7	5,4	5,2	3,8	4,2 0,2	0,2	0,1	0	0	0	0	5,1		0,4			0

consequently not from dextrose, as has been asserted by LIESENBERG and ZOFF[1]). Neither do they form any trace of slime with equal parts of the two components of saccharose, dextrose and lævulose. Acccording to BEIJERINCK[2]), the slime consists of dextran. As shown in ZETTNOW's illustrations (Pl. XXIV), it proceeds from the cell wall. It is formed more rapidly with 5—10% cane sugar than with 2%. In liquids with less than 2 % cane sugar, the slime formation is only slight. Cane sugar broth becomes at first slimy all through, and some few strains keep at this stage for several months (chiefly *X*- and *O*- forms), whereas in the case of other strains, the slime soon contracts, so that zoogloea masses are formed at the bottom of the flask. On cane sugar agar, the slime develops but poorly, but appears in a very characteristic manner on cane sugar gelatin. Large colonies, clear as water, appear on the plates, resembling the colonies of certain aerogenes species (the slimy aerogenes forms produce, however, slime from all the sugars which they ferment) and in stabs, we get very characteristic pictures, as shown in the photographs on Pl. XXV. Though these bacteria do not liquefy ordinary gelatin, and are not provided with other proteolytic qualities, several of them can, after some length of time, liquefy cane sugar gelatin, which figure is indicated in the tables by *l* (*liquare*)[3]).

As the genus *Betacoccus* contains all possible degrees of sliminess and liquefying power, we cannot attach too much importance to these characteristics, and we may find cases where of two strains, otherwise entirely alike (as Nos. 38 and 39, which were, moreover, found in the same sample of material), one will liquefy and the other not.

When isolated from vegetable matter, the betacocci thrive as a rule but poorly in milk; when isolated from milk, on the other hand, or from dairy products, and sometimes from dung, they can form comparatively large quantities of acid in milk, and even dissolve some casein (Nos. 29 and 34). The power of souring milk, however, is comparatively soon lost, but can be regenerated by continued transference from milk to milk. The bacteria are often abundantly supplied with lactase, and it may happen that nearly all the lactose of the milk is hydrolysed without any considerable quantity of it being fermented, which shows that the proteins of the milk are a poor source of nitrogen for them. In contrast to the streptococci, they thrive at least as well with yeast extract as with casein peptone as source of nitrogen. When isolated from beets, they prefer beet juice to casein peptone (Nos. 11 and 12, Pl. XXV).

The betacocci exhibit a certain preference for pentoses. Strains isolated from vegetable matter for the most part ferment both xylose and arabinose, whereas those isolated from dung, milk, or dairy products, will as a rule ferment only one of the two, or sometimes no pentoses at all. Of the hexoses, they often prefer lævulose, and of the disaccharides, often saccharose. They frequently ferment raffinose, but of true polysaccharides, only a little dextrin at the outside. With regard to salicin, the different strains vary con-

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[1]) Beiträge zur Physiologie und Morphologie niederer Organismen. Leipzig 1893, Heft 1.

[2]) Folia microbiologica 1912, I, Heft 4.

[3]) ZETTNOW distinguishes between two types of *Sc. mesenteroides*, *Opalanitza* and *Aller*, which, though it is not stated, undoubtledly correspond to the non-liquefying and liquefying betacocci respectively.

siderably. When they do not ferment raffinose, then in most cases they will not ferment salicin either. The betacocci do not as a rule attack alcohols to any perceptible degree; only a few strains ferment a little mannite (No. 20 even a little sorbite).

No other bacteria have proved so variable with regard to the sugars as the betacocci. The power of fermentation generally declines somewhat in course of time, not only as regards lactose, but also for maltose and raffinose; even, indeed for their favourite foods, such as saccharose and pentoses, and the source of nitrogen employed often exerts a quite remarkable influence. Yeast extract, for instance furthers the fermentation of raffinose in Nos. 4, 6, and 9, but retards it in No. 33. Similarly, it furthers the fermentation of cane sugar in No. 37, but retards it in Nos. 33 and 35. The betacocci which in a natural state (i. e. freshly isolated from vegetable matter) ferment both pentoses as a rule, however, prefer arabinose, which is best seen when using an inferior source of nitrogen (*W*); examples of this are Nos. 13, 14, 15, and 16. In accordance with this, they are more easily liable to lose the power of fermenting xylose than arabinose, and the *A*-forms are therefore nearly related to the *A* + *X*-forms. It may happen that the *A*-forms also lose the power of fermenting arabinose, and of the three strains from the same cheese (Nos. 7, 8, and 9) which were at first entirely alike with regard to their action upon the pentoses, two simultaneously lost the power of fermenting arabinose, while the third, which differs from the others in fermenting salicin, has still retained this power. One of those which lost the power of fermenting arabinose, on the other hand developed, its power of fermenting xylose, so that it has really become transformed from an *A*-form to an *X*-form. The explanation is doubtless this that these bacteria in reality possess the power of fermenting both pentoses, and it is more or less a matter of chance which of them they are better able to deal with at the moment (cf. also Nos. 6 and 12).

For the same reason, also, it is difficult to draw any sharp limit between the true *X*-forms (which ferment xylose, but never any considerable quantity of arabinose) and the foregoing. The *X*-forms have only slight slimy growth in cane sugar gelatin, and never liquefy it. They may, however, be found to lose the power of fermenting xylose, becoming at the same time strong slime-formers. By continued re-inoculation of No. 33 in cane sugar gelatin, we succeeded, for instance, in producing a variety which resembled No. 40 in nearly all respects. From this we may conclude that the *O*-forms are closely related to the *X*-forms.

Without cane sugar, the betacocci form small colonies, and are in all cultural respects indistinguishable from the streptococci. Morphologically also, they resemble the latter (Pl. XXI—XXIV), though the faculty of dividing in two directions is often more developed. The *A*- and *A* + *X*-forms, when cultivated in broth or milk, make shorter or longer chains, and closely related strains may differ in this respect. No. 11 (Pl. XXII), for instance, forms long chains, whereas No. 12 (Pl. XXI) appears chiefly as a diplococcus. When the betacocci are cultivated on gelatin, they form as a rule only short chains. The *X*- and *O*-forms will often appear as rods on agar streak (Nos. 33 and 36, Pl. XXIII), or as micrococci (No. 35, Pl. XXIII)[1]). In fluid substrates also, they can assume this irregular appear-

[1]) On agar streak, the one (No. 33) of two related strains can form rod-shaped cells, and the other (No. 35) cells of the micrococcus type. We noticed the same thing in the case of *Sc. liquefaciens*.

ance, and Nos. 46 (Pl. XXIV) and 47 are, from a purely morphological point of view, more like micrococci than many of the true micrococci we have investigated.

The above-mentioned morphological differences between the *A*- and *A* + *X*-forms on the one hand, and the *X*- and *O*-forms on the other, render it likely that we have here to deal with two distinct species. As the former always (at any rate unless in a weakened state) ferment arabinose, we will term them *Betacoccus arabinosaceus*, and as the latter (especially the typical *X*-forms) can be isolated from most cowdung after enrichment in acid sugar broth, we will call them *Betacoccus bovis*. As the betacocci are for the most part known under the name of *Streptococcus mesenteroides*, it would have been reasonable to use the name *Betacoccus mesenteroides* for one of the species, had it not been that both comprise strains which do not form slime, and consequently also no mesentery. It is possible that the betacocci should be divided into more than the two mentioned species. With all experiments in this direction, however, I have felt that I was working on treacherous ground, as these bacteria exhibit such great variability in almost all respects.

Appendix to Streptococci,

Not all chainforming cocci are true lactic acid bacteria, as e. g. the bacterium described by *Boekhout*, which in his opinion contributes to the aroma formation in the creamsouring[1]). Cultivated in milk it inverts a part of the lactose and forms a trace of acetic acid and perhaps also a little non-volatile acid. It is killed at a temperature of 57°. Its optimal temperature is 20°, and it already thrives badly at 31°.

We succeeded in isolating the same bacterium from commercial starters. It forms no appreciable quantity of acid in milk and does not attack the casein. It is a pronounced milk bacterium and grows badly on artificial substrates. In stab culture on whey agar with 1 % *Witte* peptone it shows distinct growth after one day at 20° but first after 2—3 days at 30°. The chains in youthful state have thick capsules. They can be very long but easily break in pieces. The bacterium is *Gram* positive and does not develop oxygen with peroxide of hydrogen, which makes it likely, that — in spite of its lack of acid formation — it is related to the lactic acid bacteria.

Perhaps it is a variety of *Sc. cremoris*, that has lost the power to form acid but on the other hand has got the power to form aroma increased.

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[1]) Vereeniging tot Exploitatie eener Proefzuivelboerderij te Hoorn. Verslag over det jaar 1917. Already some years before his death *V. Storch* has found the same as *Bockhout*, but his work has not yet been published.

Micrococci and Sarcinæ.

As already mentioned, there are certain streptococci and betacocci which can appear as typical micrococci or divide in several directions. And again, several of the micrococci can stretch prior to division, or form short chains, thus resembling the foregoing families. We cannot therefore, on morphological grounds alone, distinguish the micrococci from these. In cultural respects, however, the micrococci and the Sarcinæ are both, as a rule, easily distinguishable by the fact that on gelatin plates, they form larger — often coloured — colonies, and in agar stab, growth on the surface. This quality also differs in the different species, and in the accompanying table (XXVII a and b), we have arranged our strains with particular regard to their more or less pronounced need of air. The four first (Nos. 1—4), it will be noticed, exhibit no surface growth at all, and thus present no difference in cultural respects from the streptococci and betacocci, whereas the last (Nos. 30—31) form a thin wrinkled, mycoderma-like membrane. In contrast to the truer lactic acid bacteria, however, the micrococci and sarcinæ are furnished with catalase, so that their broth cultures give a development of oxygen with peroxide of hydrogen, — almost always of the nature of an explosion — and this reaction is therefore a reliable character by which to distinguish them from the other cocci, and especially from the micrococcus-like betacocci.

As the micrococci on the one hand, in morphological respects pass over by gradual transition into the streptococci and betacocci, so, on the other hand, we find no sharply distinct line of demarcation between the micrococci and sarcinæ. From our investigations, we have reason to suppose that both these groups can divide in all three directions, but the typical pachet only arises where there is a strong cohesion between the cells after division. In the micrococci, this cohesion is as a rule but slight, and they therefore appear most frequently as diplococci or in a grape-cluster form (as staphylococci) without any pronounced direction for division; in the sarcinæ, on the other hand, it is generally stronger, and we find these, accordingly, far more often than the micrococci, forming tetrads, and now and again even distinct pachets. As micrococci and sarcinæ exhibit no differences in biological respects, it is altogether unjustifiable to set them up as two distinct genera; the difference between them is not greater than between short- and long-chained streptococci; we therefore suggest the generic name *Tetracoccus* for both groups. Micrococcus ti in any case meaningless as a generic name, since the micrococci are by no means smaller shan the other spherical bacteria.

In the present work, we have only devoted attention to the sugar-fermenting strains. Whether those which do not ferment sugar form another genus, we are not able to deter-

mine with certainty, as the tetracocci are on the whole but weak acid formers, and their power of forming acid is easily weakened, so that we have all possible transitions between acid formers and non-acid-formers. There is one point, however, which seems to suggest that the two groups should be regarded as two distinct genera, viz. that all acid formers are without exception more or less GRAM-positive, whereas non-acid-formers as a rule are GRAM-negative. WINSLOW even goes so far as to divide all spherical bacteria into two sub-families; the acid formers, or *Paracoccaceæ*, including my streptococci, betacocci and tetracocci, and the non-acid-formers, or *Metacoccaceæ*[1]).

Genus: Tetracoccus (Abbr. *Tc.*).

In this genus (Table XXVII) I include all sugar-fermenting micrococci and sarcinæ. From the sugar,they form, besides lactic acid, smaller or greater quantities of acetic acid. The quantity of lactic acid was in many cases so small that we were not able to determine with certainty of what sort it was. As they thus stand at the limit of what we will term lactic acid bacteria, we have not sought for them systematically, as for the cocci already described, and thus make no claim to have found, even approximately, representatives of all species belonging thereto, but merely of some of those most frequently met with in the dairy.

As already mentioned, our strains have been arranged according to their need of air (see last column of the table), which I consider to be one of the best characters, even though the surface growth of the stab cultures do not always assume quite the same dimensions, and though a strain which at first exhibits no surface growth at all may in course of years develop some surface growth, as happened in the case of No. 4. The strains which spread most markedly on the surface, however, are found on the other hand to grow poorly deeper down. In shaking cultures, the most aerobic forms grow only on or close under the surface — and this even when the substrate contains sugar. Like the coli and aerogenes bacteria, as a rule they spread more on the surface of sugar-free substrates (*AG*) than on those containing sugar (*SG*). A small amount of sugar (but not more than ½%) on the other hand will strengthen the colouring. This is, by the way, not a little variable, and it is therefore very unfortunate that most of the bacteria coming under this head have been named thereafter. There are, it is true, pure white forms, which never exhibit any trace of colour, and the typical *Aureus*-forms also are fairly constant in respect of colouring, though the first generation may be a good deal paler, if sowing be done from the bottom of the stab instead of from the upper part. In most strains, however, the colour fluctuates, from one inoculation to another, between white, greenish, yellowish and brownish. Slight alterations in the composition of the nutritive substrate will at once affect the chromogenic power. Quite fresh cultures are often white, only becoming tinged after *a* few days, or even weeks. Some strains remain white for years together, and only in a single generation exhibit a slight shading; others may be coloured, and then become suddenly white. At times, the surface culture consists of white and coloured rings, or a coloured star may form in the centre. On sowing out from the white and the coloured

[1]) Systematic relationships of the Coccaceæ. New York 1908.

portions separately, the first generation will be white and coloured respectively, but in later generations, the two will as a rule again grow more alike. A *Citreus*-form from *Kral*, which, like most of the other pure yellow micrococci, does not ferment sugar, was one day found to exhibit yellow centre and white margin; the forms isolated from centre and margin respectively retained their individual colours, pure yellow and pure white, for four years, but were in all other respects entirely alike. In the fifth year, the white variety turned pink.

The size of tetracocci is highly variable. Strains which appeared gigantic on isolation had in the course of a few generations come down to the usual size.

Spores were not observed in any of our strains. Nor did we succeed, by direct observation, in discerning any motility. Nevertheless, we must suppose that the most aerobic forms (the *Albus*-forms, the mycodermalike as well as those which do not ferment sugar) are capable of moving from a spot as, in old agar stab cultures, we constantly find isolated colonies under the surface or at several mm. distance from the stab.

None of our strains could stand any great quantity of ammonia, and they are therefore not able to effect any considerable fermentation of urea. The greatest transformation of urea was effected by the *Aureus*- and *Albus*-forms, and by the intermediate dung bacteria. On the other hand, most of our sugar-fermenting strains can reduce nitrate to nitrite, (the non-sugar-fermenting *Citreus*-forms lacked this power) thus differing from the strepto- and betacocci. Though nitrate reduction appears to be a constant quality, it is nevertheless not always to be used as a species character. True, it agrees very nicely when we find that neither of the two closely related strains, Nos. 1 and 2, nor either of the two identical strains, Nos. 30 and 31, are able to reduce nitrate, but the agreement is no longer maintained when we come to consider the two strains, Nos. 3 and 4, which are entirely alike save for the single fact that one of them reduces nitrate and the other not. It would be unnatural to count them as distinct species on that account..

The tetracocci usually liquefy *AG*, though as a rule but slowly, often, indeed, only after several weeks at 20°. The rapidly liquefying forms also liquefy *SG*, albeit somewhat more slowly. Nos. 30 and 31, which are otherwise slightly liquefying, liquefy *SG* a little faster than *AG*. Only strains which liquefy *SG* strongly (Nos. 9—13) attack the casein of milk to any considerable degree. Exceptions are, however, Nos. 3 and 4, but they are not able to break down the dissolved casein any further.

The pathogenic tetracocci (the two *Aureus*- and *Albus*-forms) are killed already at 65°; most of our other strains, however, can stand a considerable length of time at 70° or even 75°. The pathogenic forms grow at 45°; but the maximal temperature for the remaining forms is rarely much above 40°, and often only 37½°. The optimal temperature is 30°, and the minimal rarely much below 15°, though the growth may still be rapid at ordinary indoor temperature.

The tetracocci can stand high concentrations of sugar and salt, though as a rule they thrive better in weaker concentrations. No. 1, which was isolated from anchovy pickle with 15% *NaCl*, grows most rapidly with 5% *NaCl*. The power possessed by the tetracocci of forming acid in sugar solutions with 10—15% common salt (See Table XI b) can as a matter of fact be used as a specific character for them. As the tetracocci can stand both heat, and high concentrations in the nutritive substrate, it is not strange that they should

Table XXVII a.

No.	Tetracoccus isolated from:	Rotatory power of the lactic acid	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % af Total N. S N	Milk: % af Total N. D N	Liquefying: A G	Liquefying: S G	Reduction of nitrate	Growth on the surface of C-Agar stab culture
1	Anchovy pickle	d	C	0,3	0,2	1,4	0	0,6	1,5	1,9	2,5	2,1	1,8	0,8	1,9	0,1	0,5	0,5	0,7	0,2	1,8		0,3			0	0	0	0
2	Condensed yeast extract	d	C	1,1	0,5	3,6	÷	0	3,2	5,6	5,1	5,2	3,4	4,7	4,5	4,3	1,4	0	1,1	0	3,6	5	5,4	5,2	0	0	0	0	0
3	Milk from women with mastitis	d	C	1,6	÷	÷	÷	÷	0	4,1	3,4	2,7	3,4	4,1	4,3	4,1	0,2	0	1,6	0	0	4	4,5	34	0	+	0	0	0
4	»	d	C	2,3	0	0	÷	0	0	4,5	3,6	3,2	4,3	4,3	3,8	5,0	0,2	0	2,3	0	0	10	3,4	34	1,9	+	0	+	0
5	Dairy cheese 8 P 1 week	d	W	0	1,6 0,3	1,9	0	0	0	2,1	2,5	1,6	1,8	1,8	1,4	2,5	1,7	0	0	0		5	4,1	1,3	1,3	0	0	+	white, yellowish green, light yellow
			C	1,1	1,8 0	2,7	0	0	0	5,2	5,2	3,5	3,6	4,3	2,7	5,2	4,3	0	0,5	0	3,8								
6	Dairy cheese 8 R 3 months	d	W	0	1,5 0	2,3	0	0	0	2,1	2,1	2,1	2,0	0 1,8	1,6	1,9	1,6	0	0	0		10	3,8 2,7	0,5	0,9	0	0	+	»
			C	1,4	0,7	2,7	0	0	0	6,1	5,4	4,5	4,5	4,3	5,0	5.2	3,8	0	0,5	0	4,1								
7	» 24 hours	d	W	0,2	1,0 0	1,4	0	0	0	2,0	2,0	1,8	2,0	0,7 0,1	1,1	2,0	0,3 1,4	0	0	0		14	3,4	0	0	0	0	+	»
			C	1,6	0	2,0	0	0	0	6,1	4,3	4,3	4,3	0,7	2,9	3,4	3,8	0	0	0	2,9								
8	Dairy cheese 8 P 1 week	?	W	0,2	0	0	0	0	0,2	0,9	0,9	0,8	0,8	0,9	1,0	1,2	0,2	0	0	0	0		2,9	19	10	+ 0	0	+	brownish thick and knotty.
			C	0,9	0,5	0	0	0,2	2,0	3,6	3,6	1,8	2,5	3,4	2,9	3,2	0,5	0,2	0,7	0,5	0,7								
9	*Micrococcus casei liquefaciens, Orla-Jensen*	d	W	0,5	0	0	0	0	0	1,0	1,4	0,7	0,9	0,2	1,4	1,4	0	0	0	0	0	3	3,8	75	12	+	+	+	white
			C	1,8	0	0	0	0 0,5	0 0,7	2,9	3,2	0,9	1,6	1,8	3,8	3,6	0,7	0,7	0,8	0,2	0,5	4	2,5						
10	Dairy cheese 8 R quite fresh.	d	W	0,1	0	0	0	0	0	0,8	0,8	0,5	0,7	0,5	1,0	1,0	0	0	0	0	0	3	3,8	71	16	+	+	+	white, light yellow, light brown.
			C	1,1	0	0	0	0	0	2,9	2,9	2,3	2,0	1,4	3,2	3,4	0	0	0,7	0	0								
11	Butter 8 cheesesour[1])	d	W	0	0	0	0	0	0	2,3	2,3	1,6 0	2,0	0,1	0,3 0,1	2,3 0	0,1	0	0	0	0	4	3,4	71	31	+	+	+	yellow (pink)
			C	1,4 0	0	0	0	0	0,7 0	4,7 2,7	3,6 2,7	0,7	3,6 2,0	1,1 0	0,9 0,2	0,7 0	0,7 0,2	0,9 0	0,5 0	0,2 0	÷	4	0,7						
12	Dairy cheese 8 R 24 hours	i	W	0	0	0	0	÷	1,1	2,3	2,3	1,7	1,6	2,3	1,9	1,8	0	÷	1,0	÷	0	3	4,1	48	16	+	+	+	orange
			C	3,8	0	1,4	0	÷	3,8	6,3	4,7	3,2	3,2	3,8	4,3	3,8	÷	÷	1,8	÷	0,2	8	2,3						
13	*Mc. pyogenes aureus, Kral*	l	C	2,0	÷	÷	÷	÷	2,0	4,5	4,1	2,9	1,6	4,5	4,1	4,5	÷	÷	2,5	÷	0	3	3,6	30	5	+	+	+	»
14	*Mc. pyogenes aureus. OlufThomsen*	i	C	2,0	÷	÷	÷	÷	1.8	4,3	3,4	2,3	2,5	3,8	4,3	3,6	÷	÷	2.3	÷	÷	7	2,5	13,4	0,5	+	0	+	orange

[1]) Cheesesour is a butterfault.

Table XXVII b.

No.	Tetracoccus isolated from:	Rotatory power of the lactic acid	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % af Total N. S N	Milk: % af Total N. D N	Liquefying A G	Liquefying S G	Reduction of nitrate	Growth on the surface of C-Agar stab culture
15	Condensed Milk 1	l	C	0,2 1,6	0	0	0	0	0	3,6	2,5	0,9	1,6	3,4	2,7	2,9	0,7	0	0,2	0	0	3	2,5	16,7	5,3	+	0	0	orange, yellow, white.
16	Yellow spot in experiment cheese 15	i	C	2,9	1,1	÷	÷	÷	1,4 ÷	4,1	2,9	3,6	1,8	3,6	2,7	1,6	÷	÷	÷	÷	0 ÷	4	2,0	8,4	0,2	0	0	+	»
17	Fæces 3	?	C	0,2	0,5	0	0	0	0,5	1,6	6,1	1,6	0	0,5	1,4	0,9	0	0	0	0	0,5		2,0			0	0	+	orange
18	Calf fæces 2	?	C	1,4	÷	0,7	0	0,9	1,2	1,8	1,1	1,1	0,5	1,6	1,6	1,8	1,1	0	0	0	1,6		1,8			+	0	+	brownish
19	Bovine fæces 1	?	C	0,5	0,2	0,2	0,2	0,7	0,7	1,6	4,0	1,1	0,2	1,4	2,3	1,1	0,5	0	0,5	0	1,4		1,1			+	0	+	white
20	» » 1	?	C	0,7	0	0,2	0	0,2	0,2	1,4	1,4	1,4	0	1,8	1,1	0,9	0,5	0	0,2	0	0		0,9			+ 0	0	0	brownish, white
21	» » 3	?	C	1,1	0	0,2	0,2	0,7	0,7	1,8	1,4	1,6	0,2	0,7	1,4	1,6	1,4	÷	0	÷	1,1		1,8	2,6	0	+	0	+	white
22	» » 3	?	C	0,3	0,2	0,1	0,1	÷	0,9	1,3	1,3	0,9	0,8	0,8	1,1	1,2	0,1	0	0,1	0	1,1	13	2,5	3,1	0	0	0	+	»
23	Condensed Milk 2	i	C	1,4	÷	÷	÷	÷	2,5	2,7	3,2	2,5	1,1	0,2	2,5	2,3	0	÷	0,2	0	0		1,6	0	0	0	0	+	white, light brown.
24	Condensed Milk 2	i	C	1,8	0	0,7	÷	2,3	2,3	3,2	3,2	2,5	1,1	2,5	2,5	2,5	÷	÷	0,5	÷	1,8		1,6	0	0	0	0	+	yellow, light brown
25	Dairy cheese 9 R 4 weeks	i	W	0,7	0,5	0,6	0,1	1,4	1,4	1,4	1,4	1,1	1,4 0,7	1,6	1,4	1,4	1,1	÷	0,2	0			1,4	3,9	0,9	+	0	+	white and very extending
			C	1,4	1,1	1,8	0,7	1,6	2,0	4,1	4,1	3,2	1,6	4,7	3,6	2,3	2,3	0	0,7	0	2,5								
26	Vegetable margarine not keeping	?	C	1,1	0,2	0	÷	1,8	1,6	1,3	1,3	1,4	0,9	1,8	1,8	1,3	0,7	0	0,2	0	0		1,8	0	0	÷	0	+	»
27	Surface of Cammembert cheese 2	?	C	0,2	0,5	0,8	0	1,4	1,6	2,3	1,8	1,4	0,9	2,0	1,8	÷	÷	÷	÷	÷	0,3		0	0	0	0	0	+	»
28	*Mc pyogenes albus Olaf Thomsen*	?	C	1,1	0,2	0,9	0	1,1	0,9	2,7	2,3	2,0	0,9	2,7	2,9	1,6	2,3	÷	1,1	0,7	0		2,0			+	0	+	»
29	*Mc pyogenes albus Kral*	?	C	2,0	0,9	1,1	2,7	3,4	3,2	3,2	2,5	2,5	1,4	3,6	2,7	2,5	2,5	0	÷	÷	2,5	6	4,1	9,6	1,6	+	0	+	»
30	Surface of Cammembert cheese	d	C	3,0	0,9	1,1	÷	2,0	1,1	1,9	3,2	1,4	0	1,8	1,1	0,9	÷	÷	÷	÷	÷		3,4	6,6	÷0,7	+	+	0	brownish yellow myco-derma like
31	Experiment cheese 27	d	C	3,2	1,1	1,8	÷	2,7	1,8	3,8	4,5	2,9	0,9	4,1	2,3	3,2	0,9	÷	÷	÷	0,7 0	10	2,7	3,7	0,1	+	+	0	»

frequently be met with in condensed milk[1]) and other extracts concentrated in vacuum (Nos. 15, 23, 24 and 2).

As seen from Table XXVII, the less aerobic, as also the mycoderma-like tetracocci form dextro-lactic acid, whereas the lactic acid in *Aureus*- and *Albus*-forms, as far as they produce any noticeable quantity at all, is inactive or lævorotatory. The by-products are as a rule acetic acid. Those bacteria which formed too small a quantity of lactic acid for identification will possibly burn a part of the sugar entirely, to carbonic acid and water. They are, at any rate, like so many other aerobic organisms, capable of burning a quantity of organic acids, as for instance that formed by sterilisation of the sugars, and the degree of acidity will therefore often decrease gradually in cultures with sugars which are not fermented; this is indicated in the tables by ÷. The decomposition of proteins, however, also contributed in some degree to this diminution of the acid.

As the limit between slight fermentation of sugar and none at all is somewhat vague in the case of the tetracocci, the relation of these bacteria to the different sugars has not quite the same value as in the case of the other lactic acid bacteria. Some points are, however, fairly characteristic. The two least aerobic strains, for instance (Nos. 1 and 2), ferment arabinose, mannite and salicin, whereas the two mastitis bacteria (Nos. 3 and 4) do not ferment any of these sugars. Nos. 5, 6 and 7, which do not liquefy gelatin, have all three shown a decrease, in the course of years, of their power to ferment xylose, but all still ferment arabinose, raffinose and salicin. Nos. 9, 10 and 11, which are powerful liquefiers of gelatin, do not, on the other hand, ferment the mentioned sugars to any considerable degree. The typical *Albus*-forms (Nos. 28 and 29) are distinguished from the typical *Aureus*-forms (Nos. 13 and 14) by fermenting sorbite, and often also pentoses, raffinose and salicin. The mycoderma forms (Nos. 30 and 31) likewise ferment pentoses and sorbite, and have a relatively high fermentation of glycerin. They therefore thrive well on the surface of cheese where a splitting up of fat takes place.

As regards the morphology of the tetracocci, we have little to add to what has already been said. Of the strains investigated by us, only No. 8 (Pl. XXVII) appeared throughout unaltered as a typical sarcina in all respects. And in accordance with this, it has a knotty, or at times even mesenteric surface growth, and forms in broth compact small clumps which at once sink to the bottom. No. 3 (Pl. XXVII) has, in broth, a slight tendency to sarcina form. No. 11 (Pl. XXVI), which in milk originally formed pachets, lost this quality through regular re-inoculation, and then appeared as a diplococcus. After long periods of rest (it could be preserved for three years in starch water without re-inoculation), it however regained the sarcina form for a time. This feature, together with the strong yellow (even at times orange or pink) markedly spreading surface growth, distinguishes No. 11 from the related strains Nos. 9 and 10. As I have previously called these important cheese bacteria *Micrococcus casei liquefaciens*[2]), they should, according to the altered nomenclature, be called *Tetracoccus casei liquefaciens*; I prefer, however, simply to call them *Tetracoccus liquefaciens*. Nos. 5, 6 and 7, which are also of frequent occurrence in cheese, can reasonably be called *Tetracoccus casei*. No. 5 is distinguished from the two

[1]) We are here concerned, of course, with sugared milk; the unsugared, it need hardly be said is sterilised.

[2]) Orla-Jensen, Doktordisputats 1904.

others by forming large capsules in milk (Pl. XXVI) which do not disappear again (turn into slime). Nos. 3 and 4 we can call *Tc. mastitidis*, with the reservation, however, that it may be found to be the same species which produces the so-called staphylococcus-mastitis in cows; a point which I have not myself had any opportunity of investigating. The table shows, as distinctly as could be wished, that there are many other points of difference between (*Tc.*) *Mc. pyogenes aureus* and (*Tc.*) *Mc. pyogenes albus* than the colour alone, and we are therefore perfectly justified in regarding them as two distinct species[1]). The tetracocci which we have isolated from condensed milk and dung appear to be intermediate links between these extreme forms. Nos. 30 and 31 can suitably be called *Tetracoccus mycodermatus*. The true lactic acid bacteria as a rule coagulate milk in the deeper layers first; this species, on the contrary, coagulates milk from the surface downwards.

[1]) The only pyogenic *Citreus*-form I have investigated is, as mentioned, non-sugar-fermenting, and Gram-negative, and thus belongs to quite another genus. It liquefies *AG* extremely slowly, and is not able to reduce nitrate to nitrite. A number of saprophytic yellow micrococci behaved in the same way.

Rod Forms.

The rod-shaped lactic acid bacteria are on the whole stronger acid-formers than the spherical, and this is most distinctly apparent when yeast extract is used as source of nitrogen. If they grow at all on gelatin plates, the colonies are as a rule not visible until after the lapse of 5—6 days. They fall into three well characterised genera; *Thermobacterium*, *Streptobacterium* and *Betabacterium*. Of these, the thermobacteria, which do not grow at ordinary temperature are more anaerobic than the other lactic acid bacteria, whereas the streptobacteria and the betabacteria behave towards the oxygen of the air — as indeed towards most other things — like the corresponding streptococci and betacocci. The streptobacteria do not develop gas, and always ferment salicin; the betabacteria, on the other hand, develop more or less gas, and never ferment salicin. Finally, to the rod-shaped lactic acid bacteria should be added some small rods, the genus *Microbacterium*, which, however, differs quite as much from the true lactic acid bacteria as the tetracocci. Among the microbacteria should possibly be reckoned the *Bacillus acidophilus* often mentioned in medical works. And in conclusion, we should mention *Bacillus bifidus*, as being — albeit without further justification — generally reckoned as belonging to the rod-shaped lactic acid bacteria.

Genus: Thermobacterium (Abbr. *Tbm.*).

The thermobacteria are as a rule not killed by heating until a temperature of over 75° has been reached, they also require, as their name implies, a high temperature for their development. With the exception of No. 8 (Table XVIII) which grows at 18°, none of the strains investigated develop at anything below 20°—22°, and even at 30° the growth is very slow. Therefore they will not grow in gelatin plates. The optimal temperature lies about 40°, and here the growth proceeds at a furious rate, as long as the strains are not weakened from any cause. The maximal temperature is as a rule 50°; *Tmb. bulgaricum* (No. 14) grows at $52^1/_2$°, and the mash bacteria at even higher temperatures, as is known from the distilleries. The method of enriching thermobacteria in milk or mash is therefore to let the liquids stand at temperatures between 47° and 54°. They may likewise be forced in fresh cheese mass which is strongly heated (cooked).

These bacteria are quite extraordinarily particular in respect of the nutritive substrate they require, and we have not succeeded in producing artificial substrates which

Table XXVIII.

No.	Thermobacterium isolated from:	Rotatory power of the lactic acid	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. S N	Milk: % of Total N. D N
1	Sour mash 1	l	Y	0	0	0	0	0	0	6,5	6,3	0	0	0	8,1	0	0	0	0	0	0		0		
2	» » 1	l	Y	0	0	0	0	0	0	7,4	4,3	2,9	0	0	3,6	0	0	0	0	0	0		0		
3	» » 2	l	Y	0	0	0	0	0	0	14,4	14,4	10,1	0	11,0	10,6	0	0	0	3,4	0,2	0		0		
4	» » 2	l	Y	0	0	0	0	0	0	14,5	14,2	13,1	0	16,0	10,4	0	0	0	2,3	2,5	0		0		
5	» » 3	l	C	0	0,3	0	1,2	0	1,2	2,7	3,6	5,0	0,5		7,5	2,7		0	1,6	1,2	0,3		0		
			Y	0	0	0	0,9	0	1,1	12,4	13,1	12,4	0,9	14,9	13,1	7,4	10,8	0	3,8	2,0	0				
6	Milk left to stand 24 hours at 47°	l	W	0	0 0,5	0	0	0	0	3,2 0,9	3,2 1,6	2,2 1,6	1,1 0,2	0 0,5	0,2 0,7	2,6 1,1	0	0	0	0	0				
			C	0	0	0	0	0	0	7,0	7,0	6,1	0,2	0,7	8,3	5,0	5,2	0,7	1,5	0	2,0 6,8	1 4	14,0 10,4	8,4	10,4
			Y	0	0	0	0	0	6,8	11,3	9,7	9,2	0,7	10,8	12,4	7,2	10,1	1,6 4,1	5,2	1,1	9,5				
7	Milk (Mjölk I) *Chr. Barthel*	l	C	0	0	0	0	0	0	11,5	9,0	11,5	5,6	11,0	7,9	9,0	0,7	0	3,2	1,1	1,1	1	14,6	27,5	23,3
			Y	0	0	0	0	0	0	12,2	11,3	10,6	4,1	10,8	13,5	8,1	0,5	0	4,1	2,9	1,1				
8	Milk heated ½ hour at 70° and left to stand 24 hours at 45°	l	C	0	0	0	0	0	0	0,5	10,1	0,7	1,4	0	2,3	0	0	0			1,1	1	16,2	18,5	16,3
			Y	0	0	0	0	0	0	16,2	15,1	13,5	7,9	14,9	11,9	9,0	0,7	0	3,2	0,5	6,3	2	11,7		
9	Emmental cheese 1 *Burri*	l	C	0	0	0,5	0	0	0	6,3	11,3	11,0	9,5	0,5	5,2	2,5	0,9	0			1,1	1	14,4	19,6	21,8
			Y	0	0	0	0	0	0		12,2	11,3		11,7			3,4	0,2	3,4	1,1	10,1				
10	Emmental cheese 2 *Burri*	l	C	0	0	0	0	0	0	0,9	9,7	0,9	7,9	0	0,5	0,5					0,7	1	14,0	32,1	26,4
			Y	0	0	0	0	0	0	9,7	10,6	10,8		8,1	9,7	7,4	2,9	0,5	2,9	0,9	7,4			22,4	18,1
11	Emmental cheese 3 *Freudenreich*	l	W	0	0	0	0	0	0	5,4 2,4	3,8	5,2 0	2,3	4,1 0	1,1 3,2	4,5 2,9	0	0	0	0	0	1	13,3	18,8	18,9
			C	0	0	0	0	0	0	9,5	10,8	0	7,9	10,6	10,1	8,8	0,2	0	1,1	0	0				
12	Emmental cheese *Bacterium casei* ε	i	C	0	0	0	0	0	0	0,7	6,3	7,7	8,3	0	9,9	0,9	0	0	0,5	0	0	1	27,5	36,1	34,6
			Y	0	0	0	0	0	0	3,8	10,1	10,1	8,8	0	10,3	11,9	0	0	2,3	0	0	1	19,6	16,9	20,0
13	English Yoghourt (Lactigen)	i	C	0	0	0	0	0	0	1,1	5,4	2,5	4,7	0	0	6,8	0	0	0	0	0	1	27,0	34,6	36,7
			Y	0	0	0	0	0	0	0	10,6	5,2	8,3	0	0	0	0	0	0	0	0	1	21,6		
14	True Bulgarian Yoghourt 1	l	W	0	0	0	0	0	0	1,7	1,4 2,7	0,1 1,0	1,0 0,2	0	0,1	0,1 1,1	0	0	0	0	0				
			C	0	0	0,7	0	0	0	4,7	6,5	2,5	1,1	0	0,1	7,9	0	0	0	0	0	1	16,4	27,0 19,9	27,3 16,7
			Y	0	0	0	0	0	0	2,7	3,2	1,8	0	0	0	0	0	0	0	0	0				

can altogether replace the natural milk or (non-sterilised) mash. For the milk bacteria, whey-yeast-extract-agar was found best. With the exception of No. 6, (which in contrast to the other thermobacteria grows well with WITTE peptone as nitrogen food, and which we have also kept alive in casein peptone for a couple of years without re-inoculation) they cannot be transferred every time from agar to agar, but often require a passage of milk, or they will be weakened. Nos. 12, 13 and 14 should best be constantly transferred from milk to milk. If chalk be added to the milk and the mixture be shaken up now and again, the bacteria will retain their vitality unimpaired for several months, but when using milk without chalk, it will be best to re-inoculate each week. No. 12 will however, even under these conditions, keep unimpaired for several months at 15° and remain alive for up to two months at 20°, though it will after a time be perceptibly weakened. No. 13, which is probably identical with the yoghurt bacterium first described by BERTRAND and WEISSWEILER[1]), since it forms similar pennete colonies, I did not succeed at all in transferring from one artificial substrate to another. It only takes on artificial substrates when coming directly from the milk[2]). The lactose-fermenting thermobacteria of mash does not form acid in milk; the maltose-fermenting thermobacteria of milk, on the other hand, thrive well enough in mash. For mash bacteria, a sterilised malt extract solution is not nearly as good a nutritive substrate as might be supposed, owing to the fact that an essential quantity of the nitrogenous substances therein contained are precipitated on sterilisation. An addition of yeast extract renders it more suitable, and we have found that a solution of the trade malt extract (with abt. 50 % maltose and 0,5 % *N*) to 7 parts of yeast extract (with 0,5 % *N*) gives a good nutritive substrate, with the sugar concentration most favourable for these bacteria (see Table II d). Stab cultures in high layers of an agar thus prepared are a good form for preservation.

The lactic acid formed by thermobacteria is as a rule lævo-lactic, more rarely (Nos. 12 and 13) inactive. In addition to lactic acid, they form some acetic acid, and, as BARTHEL first showed, a trace of succinic acid[3]). In powerful milk cultures of the thermobacteria of milk, there is often so much gas produced that the curd exhibits fine stripes, and the quantity of lactic acid rises to 1½ %; for inactive acid indeed, even up to 2¾ %, which is far in excess of the amount of lactic acid formed by other lactic acid bacteria.

The thermobacteria of milk can under certain circumstances easily become slime-formers. BURRI and THÖNI have shown, for instance, that No. 12 as a rule becomes slimy when it has been cultivated for any length of time together with a certain mycoderma species[4]). The slimy varieties are just as powerful acid formers as the non-slimy ones. We have a slimy variety of No. 13 (presented by Mr. BLICHFELDT, manager of the laboratory of Mønsted's Margarine factory, in England), which, in contrast to the streptococci, so strongly retains its power of forming slime that even after long cultivation at high temperatures we did not succeed in transferring it to a non-slimy variety. The thermobac-

[1]) Annales de l'Institut Pasteur 1906, Bd. 20, p. 977.

[2]) We have not, however, tried the malt germ decoction suggested by BERTRAND and DUCHACEK (Biochemische Zeitschrift 1909, 20. Bd, p. 102).

[3]) Meddelande Nr. 69 från Centralanstalten för Jordbruksförsök. Stockholm 1912.

[4]) Landwirtschaftliches Jahrbuch der Schweiz, 1909, p. 271.

teria of milk attack all casein strongly, with direct formation of mono-amino-acids, and they are therefore of importance in the ripening of the cooked sorts of cheese, which are not till 24 hours after cooled below the temperatures most favourable for development of thermobacteria. No. 12 in particular is of the greatest importance in the ripening of Swiss cheese (Emmental cheese)[1]).

The thermobacteria do not as a rule care for pentoses and alcohols. Only No. 6, which is further distinguished by being able to ferment inulin, exhibits any considerable fermentation of mannite. Most of the mash bacteria do not ferment galactose and lactose, and the two yoghurt bacteria do not ferment maltose. Saccharose is not fermented by Nos. 1, 2, 12, 13 and 14. The maltose-fermenting thermobacteria as a rule also ferment some dextrin, and often also a small quantity of starch, besides raffinose and salicin. Yeast extract will at first retard the growth of the yoghurt bacteria, and can thereby prevent them altogether from attaining development in certain sugars (lactose, for instance). There is altogether something capricious in the attitude of the thermobacteria towards the sugars, due to the fact that our artificial substrates did not entirely satisfy their needs[2]).

The thermobacteria (Pl. XXVIII—XXXIV) are pronounced long-rod forms, with a tendency to grow out into threads, often strangely curling. They consist, however, of several segments, which is not to be seen in the water preparation, but is distinctly apparent when the preparation is laid in Canada balsam (See Pl. XXXII). In a young and vigourous state, they occur for the most part singly, or two and two. As they are seriously affected by the oxygen in the air, they assume irregular shapes in streak cultures. Here, they always form an extremely thin layer, and some few (as No. 14, for instance) do not grow on the surface at all. When stained with methylene blue, they generally prove to contain volutin grains. No. 13 does not appear to form grains, and No. 12 but rarely. The granular formation is most marked in No. 14 (Pl. XXXIII), which is also in German often called Körnchenbazillus. With some strains, most grains are found in the quite young rods (No. 14 for instance); in other strains, again, in rods 2—3 days old (as No. 6 Pl. XXIX) and the size and shape of the grains depends to a high degree upon the nutritive substrate. In the case of No. 14, the mere fact that the milk used had been heated some few degrees more or less is sufficient to alter the picture entirely. Normally, this yoghurt rod, when stained with methylene blue, has round, dark-blue grains, and no capsule; when cultivated in pasteurised milk, on the other hand (and especially in milk heated for half an hour to 80°) it has oblong red grains and distinct capsule (Pl. XXXIII). According to GRAM, Nos. 12 (Pl. XXXI) and 13 (Pl. XXXII) are not stained completely, but exhibit a quantity of irregular granules. Something similar may be observed in the case of other thermobacteria, if very old for instance, or if stained in a too acid state. As these bacteria are such strong acid-formers, it is altogether best first to neutralise the cultures to be used for colour preparations. The thermobacteria are often over 1 μ thick. In old cultures, they can develop greatly swollen or otherwise involved forms.

[1]) See further in my Dairy Bacteriology 1916, p. 112.

[2]) This explains certain points of difference with other writers. BARTHEL (l. c.), for instance, has found that the identical strain No. 12, which we have worked with, ferments mannite and a small quantity of saccharose, which we have never been able to observe.

The bacteria of mash are noted in the literature under the little characteristic names of *Bacillus Delbrücki* and *Bacillus acidificans longissimus*. As they are derived from the mashed grain, I propose to call them *Thermobacterium cereale*. There may possibly be several species (as for instance lactose-fermenting and non-lactose-fermenting), and I would in this connection refer to HENNEBERG[1]), who has made a very thorough study of the lactic acid bacteria of mash. The common thermobacteria of milk (Nos. 6—11) should of course be termed *Thermobacterium lactis*. Here also it is possible that there may be several species (the three first, for instance, which are isolated from milk, grow more strongly in agar than the three last, which were isolated from Emmental cheese). They have all, however, one point in common: like the mash bacteria, they form lævo-lactic acid. This is also the case with No. 14, which is an even more pronounced milk-rod than the foregoing, and therefore does not ferment maltose. As we have always found this rod in genuine Bulgarian yoghurt, whether obtained through Professor METSCHNIKOFF or directly from Professor PRANTSCHOFF of Sofia, I consider myself justified in calling it *Thermobacterium bulgaricum*. The bacterium of Swiss cheese, No. 12, which we have formerly called *Bacterium casei* ε, I now propose to call *Thermobacterium helveticum*. Related to this bacterium is No. 13, from the fact of its forming inactive lactic acid, from the strong acid formation in milk, and from its morphological features. It differs, however, in the fact that like No. 14, it does not ferment maltose, and in the far greater difficulty it finds in growing in artificial substrates, as also by its markedly radiating colonies. We consider therefore, that it should be established as a separate species, and as it is said to occur in yoghurt, we can call it *Thermobacterium Jugurt*[2]).

Appendix to Thermobacteria.

We may here mention an interesting lactic acid bacterium which we have come across during our controlling work with commercial starters for creamsouring. Even where these have proved pure by direct investigation they may nevertheless contain a trace of mould, yeast, or rod-shaped lactic acid bacteria, which can gradually develop in the dairy. We therefore always make the additional test of leaving an unopened jar of the culture to stand for a week at 25°, which is the highest temperature used in the souring of the dairy starter or the cream. The detrimental contamination will thus accumulate, while the good acid bacteria (*Sc. cremoris*) perish in their own acid. It may then chance, that the starter, instead of becoming sterile by this treatment, as it properly should, becomes transformed to a pure culture of a long-rod form, which in milk forms over 2 % inactive lactic acid, and which also attacks casein (12,3 % *SN* and 13,5 % *DN*). Even at the optimal temperature, 30°, it does not curdle milk until after 2—3 days, and is inclined to render it slimy. It is such a pronounced milk bacteria that in artificial substrates, it ferments practically no sugars at all. In older cultures, it is no longer so markedly a long-rod form, but breaks up into chains of short segments, which can be over 1 μ thick. It

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[1]) W. HENNEBERG: Gärungsbakteriologisches Praktikum. Berlin 1900.

[2]) In Bulgarian, Yoghurt is called simply „sour milk" (kisselo mleko) but in Turkish, Jugurt, and it is this word which has passed over, under various forms, into the other languages.

(Pl. XXXIV) is incompletely stained by the GRAM process, in the same way as thermobacteria Nos. 12 and 13. It thrives well at ordinary temperature, but does not grow at over 38°. It is thus not a thermobacterium, but in spite of this, it appears most nearly related to those thermobacteria which form inactive lactic acid. As we are not quite certain as to the systematic position of this bacterium, we must refrain from giving it any name at present. On plate XXXIV it is designated as *Thermobacterium* No. 15.

Genus: **Streptobacterium** (Abbr. *Sbm.*).

These rod forms we have called streptobacteria, from their tendency to chain formation. As they grow, even at their optimal temperature, more slowly than the lactic acid cocci, they do not make themselves apparent in spontaneously souring milk until after the milk has curdled, but they will then, on the other hand, gradually supersede the lactic acid cocci, being able to stand, and capable of forming, up to twice as much acid as the latter. They therefore abound in butter and cheese after more or less time has elapsed. They are likewise always found where vegetable matter is left to sour. There are probably a great number of species, which are difficult to distingusih one from another, owing to the many gradual transitions between. I will here content myself with making distinction between the two extreme groups, the typical cheese bacterium, *Streptobacterium casei*, represented by the *Bacterium casei α* which I have formerly described, and the typical vegetable bacterium, *Streptobacterium plantarum*. The former we have never met with in vegetable matter, but the latter is of frequent occurrence in milk and dairy products; it cannot escape being introduced into the same from the food and bedding (grass or straw) of the cows.

The streptobacteria are always killed by heating to 75°, many strains already at 70°, and some few even at 65°. The optimal temperature is 30°, and the maximal as a rule from 37½°—40°. Only a few strains can grow more or less well at 45° (*Sbm. casei* Nos. 4, 26, 31, 32, 33 and 34, and *Sbm. plantarum* Nos. 28, 29, 30, 32 and 34). The minimal temperature lies probably in most cases at about 10°, but the growth here is, even with the most favourable source of nitrogen (*Y*), so slow that it will often be impossible to discern anything at all until after 14 days. The growth on gelatin plates, also, at ordinary temperature, is very slow, and the colonies are still considerably smaller than those of the cocci. They grow better, of course, on *SG* than on *AG*.

Streptobacterium casei (Table XXIX) forms either pure dextro-lactic acid or more or less considerable quantities of lævo-lactic acid in addition, so that we also find inactive lactic acid in the cultures. The power of forming dextro-lactic acid is, however, by far the most constant, and many strains which at first formed almost exclusively inactive lactic acid have yet in the course of years ended by forming pure dextro-lactic acid[1]). As men-

[1]) We have consequently been led to investigate considerably more strains than we otherwise should have done, for of course we could not know that two bacteria, otherwise alike — even though isolated from the same place — were identical, despite the fact of their forming different lactic acids. These parallel investigations of identical strains have, however, further increased our knowledge of the same, and it is interesting to see that they often exhibit exactly the same mutations at the same time. They may, however, also be found to differ suddenly in their relation to one or other of the sugars.

Table XXIX a.

No.	Streptobacterium casei isolated from:	Rotatory power of the lactic acid	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN
1	Mazun *Düggeli*	d	W	0	0	0,2	0	0	0	2,5	2,5	2,6	1,8	0	1,1 0,2	2,0 1,0	0	0	0	0	0	7 10	7,2 4,3	9,2	8,8
			C	0	0,2	1,4	0	0	0	3,2	4,5	4,1	4,5	0	0,5	1,4	0	0	0	0	0,7				
			Y	0	0	0	0	0	2,7 0,9	7,7	7,9	7,4	4,5	0	3,6	5,6	0	0	0	0	0				
2	Butter 3 cheese sour	i (i) d	W	0	0,1	0,5	0	0	0,2	4,1	4,2	2,7	4,1	0,1	0,2	2,9	0	0	0	0		7 13	11,7 7,0	9,9	10,7
			C	0	0,5	1,4	0	0,2	1,1	10,1	11,9	9,2	9,2	0,9	0,9	7,0	0,5	0,5	0,2	0	1,6				
			Y	0					6,1		14,4			4,1			0	0	0	0	2,3				
3	»	i d	W	0	0	0,5	0	0	1,6 0,1	3,8	4,5	3,8	3,4	0,2	3,6	4,5	0	0	0	0		3 11	12,2 9,7	17,1	19,4
			C	0	0	0,9	0	0	0,2	11,5	12,4	13,7	10,4	0,2	6,5	10,8	0,5	0,2	0,2	0	7,0				
			Y	0					5,9		14,0			2,7			0	0	1,8	0	10,6				
4	Butter 5 cheese-sour	i (Galactose) d	W	0 0,5	0,2	0,5	0	0	1,9	5,0	5,4	5,2	4,6	0,2	3,2	4,1	0	0	0	0		4	12,2	19,8	20,5
			C	0,5	0,9	1,4	0,2	0,2	3,4	11,0	11,0	11,0	10,1	1,1	4,7	8,8	0,5	0,7	0,7	0	5,0				
			Y	0					6,3		14,0			0			0	0	2,0	0	9,2				
5	Dairy cheese 3 R 3 months	i i d d	W	0,4	0,2	0,5	0	0	2,5	5,6	6,1	2,9	4,1	0,5	6,1	3,8 0,5	0	0	0	0		3 10	9,9 7,4	12,8	9,8
			C	0,5	0	0,5	0	0	5,4	12,8	13,3	7,0	10,6	0,9	12,6	0,5	0	0	0,2	0	7,4				
			Y	1,8					5,6		12,6			4,7	12,6	0	0	0	1,6	0	8,3				
6	Dairy cheese 5 R 2 months	i i d d (i)	W	0,2	0	0,2	0	0	1,6	6,3	5,6	4,1	4,5	0,3	3,6	3,6 1,6	0	0	0	0		6 10	12,6 7,4	13,1	11,7
			C	0,5	0,2	2,5	0	0	2,3	11,7	10,4	8,6	10,4	0,7	5,9	2,3	0,7	0,7	0,2	0	6,5				
7	Dairy cheese 9 R 3 months	d	W	0	0	0	0	0	0,9 0	4,5	4,4	4,5	4,1	0,1	0,2	4,1	0	0	0	0		3	11,3	9,5	8,0
			C	0	0	0	0	0	1,8	10,1	12,8	10,1	10,1	0,7	0,9	9,2	0,2	0	0	0	6,1				
			Y	0	0	0	0	0	5,2		14,6			0,5	0,7		0	0	0	0	8,6				
8	»	d	W	0,2	0	0	0	0	1,8	4,3	4,3	4,3	3,8	0,5	2,2	4,3	0	0	0	0		11 3	7,4 12,2	11,7	13,0
			C	0	0	0	0	0	2,3	11,3	11,7	11,9	9,9	0,2	5,6	9,9	0,2	0,2	0,2	0,2	5,6				
9	Dairy cheese 9 P 3 months	d i d	W	0	0	0	0	0	2,1 0,7	5,9	5,6	6,1	5,0	1,5	4,7	4,3 6,3	0	0	0	0		7	10,4	8,4	3,4
			C	0,7	0,2	0,9	0,2	0,2	2,9	12,8	12,2	12,6	10,6	5,0	8,3	11,3	0,5	0,7	0,5	0	9,2				
10	Dairy cheese 6 P 4 months	d	W	0	0	0	0	0	2,0	6,1	6,3	5,2	4,7 3,2	0,2	3,4	5,9	0	0	0	0	1,6	5	11,5	11,3	14,5
			C	0,1	0	0	0	0	2,3	8,3	10,1	7,7	10,8	0,6	5,6	9,9	0	0	0	0	6,1				
			Y	0	0	0	0	0	5,9		14,6			0			0	0	0	0	6,1				
11	Emmental cheese (*Bacterium casei a*)	d	W	0,2	0	0,5 0	0	0	1,1	5,2	6,8	5,4	3,6	0,2	1,1	5,0	0	0	0	0		3 7	15,5 11,5	24,7	26,3
			C	0,8	0	0	0	0	2,5	11,7	13,7	13,5	12,2	10,1 2,5	12,4	12,5	0	0	0,2	0	10,8				
			Y	0,7					7,1	15,8	15,1	14,6	12,8	0,2	0,7	15,3	0	0	0	0	12,8				

Table XXIX b.

No.	Streptobacterium casei isolated from:	Rotatory power of the lactic acid	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. S N	Milk: % of Total N. D N
12	Milk *Weigmann's* collection No. 32	d	W	0,1	0	0	0	0	1,0 0	6,3	6,2	7,0	5,9	0,2	3,9	5,4	0	0	0	0		5	11,3	11,2	10,5
			C	0,2	0	0	0	0	2,9	13,5	13,7	14,2	12,4	0,9	7,2	13,7	0	0	0	0	3,4				
			Y	0,2					6,8		15,1			4,5			0	0	0	0	11,0				
13	Dairy cheese 1 P 3 months	d	W	0	0,2	0,2	0	0	1,4 0	5,2	6,1	4,2	5,0	0	0,6	2,7 3,6	0	0	0,2	0	2,5	7	9,5	14,8	10,4
			C	0	0	0	0	0	0	7,9	10,6	9,0	7,9	0	0,5	2,9	0	0	0,2	0	8,0	4	11,7		
			Y	0					5,6		13,7			2	3,8	8,3	0	0	0,2	0	9,0				
14	»	d i d	W	0,1	0,1	0,2	0	0	2,3 1,1	5,2	5,6	4,7	4,3	1,4	4,5	4,5	0	0	0	0		3	11,3	9,8	10,2
			C	0,7	0,2	1,6	0,2	0	3,8	11,0	10,1	9,2	9,5	3,1	7,2	10,4	0	0,9	0,2	0	1,4	6	8,8		
15	Dairy cheese 8 R 1 week	i i d d	W	0	0	0,5 0	0	0	1,8 0,5	5,0	5,2	5,0	5,0	3,8 0,5	6,3 1,4	5,4	0,1	0	0	0		10	9,0	5,8	7,4
			C	0,7	0	0	0	0	3,6	11,3	11,7	9,2	9,9	3,8	3,6	9,2	0,2	0,2	0,5	0	8,3	4	13,1		
			Y	0,7					8,3		14,6			8,3	5,9		0	0	0	0	8,3				
16	»	i d i d	W	0	0	0,1	0	0	0,2	4,5	6,1	5,9	4,5	0,6	2,9	4,3	0,1	3,6 0,5	0	0	2,3	3	14,2	10,6	9,3
			C	0	0	0	0	0	3,6	11,0	11,5	10,4	11,9	9,5 0,9	8,3	11,5	0,2	6,8 0,5	0	0	5,9	5	11,3		
			Y	0	0	0	0	0	6,1		14,6			1,6			0	0	0	0	9,2				
17	Dairy cheese 8 R 3 months	i d i d	W	0,5	0,5	0	0	0	1,8	5,6	5,0	5,0	4,1	6,1 2,5	6,3	4,7	0	0	0	0		3	12,8	6,7	8,5
			C	0	0	0	0	0	2,9	14,4	13,1	14,2	11,9	1,4	8,3	9,2	0	0	0,2	0	7,4	5	9,7		
			Y	0	0	0	0,2	0	6,1		14,9			7,7			0	0,2	0	0	11,9				
18	»	d i d	W	0	0	0	0	0	0	3,8	3,6	4,1	3,6	0,6 0,1	5,2	3,4	0	0	0	0		6	11,3	6,4	7,9
			C	0	0	0	0,2	0	0,9	7,9	9,0	9,2	8,1	0,5	9,9	7,9	0	0	0	0	4,7				
			Y	0	0	0	0	0	5,9		11,9			7,4					0,2		8,6				
19	Dairy cheese 8 P 3 months	d	W	0,3	0	0	0	0	0,2	6,8	6,5	6,8	5,0	0,6	3,2	5,0	0	0	0	0		3	14,2	12,6	15,9
			C	0,5	0	0	0	0	3,4	12,4	13,5	13,5	11,3	0,9	6,8	11,7	0,5	0,5	0,2	0,2	7,4	5	9,7		
			Y	0,9	0	0	0	0	6,8		14			3,2			0	0	0,2	0	9,5				
20	»	d	W	0	0	0	0	0	1,4 0,5	4,1	3,8	4,3	4,3 1,9	0,5	4,5	2,9	0	0	0	0		3	12,8	15,6	19,4
			C	0	0	0	0	0	0,5	10,6	11,0	11,0	8,3	0,2	11,7	9,5	0,2	0,5	0,2	0	6,1	4	10,1		
21	Kefir 2	d	C	0,2	0	0,7	0,7	0,7	3,6	9,0	8,6	6,8	7,0	0,9	2,7	8,1	0,7	0,2	0,7	0	4,7	4	11,7	10,0	9,0
			Y	0					5,9		12,8			0	3,4	10,8	0	0	0	0	8,3	5	9,0		
22	Dairy cheese 5 P 2 months	d	W	0,2	0,2	0,2	0	0	2,3	7,0	6,3	6,8	5,2	1,2	4,3	4,7	0	0	0	0		4	12,2	13,6	17,5
			C	0,9	0,5	1,4	0,2	0,2	5,0	12,4	12,2	12,4	11,0	3,8	6,5	8,1	0,7	0,7	0,7	0	7,7				
23	»	d	W	0	0,2	0,3	0	0	2,0	4,7	5,4	5,4	3,6	4,7	4,1	5,2 0,5	0,2	0	0	0		6	11,0	12,2	9,7
			C	0,5	0	0	0	0	2,5	10,2	9,7	7,9	9,0	9,0	8,6	1,9	0,2	0,2	0	0	9,2	9	8,8		

Table XXIX c.

No.	Streptobacterium casei isolated from:	Rotatory power of the lactic acid	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. S N	Milk: % of Total N. D N
24	Dairy cheese 7 R 4 months	d	W	0	0,2	0,3	0	0	2,0 0	5,4	5,4	5,4	5,0	0,9	5,4	4,3	0	0	0	0		3 6	15,1 10,1	10,7	13,8
			C	0,7	0	0	0	0	2,3	10,8	11,0	11,3	11,5	3,6	9,2	8,1	0,5	0,2	0	0	6,5				
25	»	d	W	0,7 0	0	0,5	0	0	1,4 0	5,2	5,9	5,2	4,1	1,6 0,5	3,6	4,6 1,6	0	0	0	0		2 4	14,4 12,2	5,4	6,5
			C	0,9	0,5	1,6	0,2	0,2	3,4	12,4	11,9	12,4	10,6	4,7	6,5	11,0	1,1	0,7	0,7	0	9,5				
26	Swedish manor-seat cheese *Bacterium curvatum*	d	C	0,9	0,7	0,7	0,5	0,5	4,3	10,4	11,0	12,2	8,1	3,4	3,2	10,6	0,5	0,9	0,7	0,2	3,4	5	10,4	5,1	3,2
			Y	1,8	0	0	0	0	7,0		14,9	14,9	13,1	8,8	4,5	12,6	0,2	0,2	0,2	0,2	3,8				
27	Dairy cheese 9 R 4 weeks	i d i d	W	0,5	0,7	0,2	0	1,6 0,2	1,8	5,9	5,9	5,0	4,1	5,6 3,4	5,6	4,5	0,1	0				4	12,2	7,2	6,7
			C	0,7	1,1	0	1,4	0,7	3,4	13,1	12,4	12,2	12,4	4,1	10,1	10,8	0,2	0	2,5	0	7,2				
28	» 3 months	d	W	0	0,7	0	0	2,3	2,3	5,0	4,8	5,2	5,0	1,8	5,0	3,4	0	0				2	12,4	14,6	18,6
			C	0,2	1,4	0	0	4,5	5,0	12,2	14,0	12,6	11,5	4,1	9,9	11,5	0,5	0,5	2,3	0,2	9,5	12	6,8		
			Y	0,2	1,1	0	0	5,9	5,9		15,3			5,0			0	0	2,0	0	11,0				
29	Milk left to stand at 30° with 1,4 % lactic acid	d	C	0,2	0,9	0	0	3,4	3,2	10,1	7,7	11,0	10,8	1,8	2,9	9,0	0,2	0	0	0	7,2	3	13,1	8,3	5,4
			Y					3,8	6,1		11,7			3,8	6,1		0	0	0,2	0,2	9,9				
30	Fæces 2	d	C	0,7	0,9	0,7	0,9	3,2	2,7	11,3	10,4	9,2	7,4	3,8	4,3	9,0	0,7	0,7	0,9	0,5	8,8	3	15,3	4,8	4,7
31	» 3	d	C	0,9	0,5	0,9	4,1	3,4	3,6	10,4	9,7	9,9	8,1	2,3	2,3	8,6	0,7	0,7	1,1	0,5	6,3	3	14,4	7,7	10,3
32	Butter 1 fine	d	W	0,5	0,5	1,6	1,4	2,0	2,5	7,0	7,0	7,0	6,1	2,3	0,3	6,5	0	0				2	16,0	22,7	23,5
			C	0,7	0,9	2,7	5,2	3,0	3,8	14,4	14,0	14,2	13,3	4,7	1,8	13,1	0,2	0,7	0,5	0	7,9	3	14,6		
33	Emmental cheese from „Alnarp“	d	W	0,5	0,1	0,5		2,3	2,3	6,8	7,2	7,0	6,3	2,7	3,6	6,3	0	0				5	15,3	18,7	22,0
			C	1,1	1,1	2,5	5,0	1,0 2,3	4,5	14,0	15,3	14,4	13,5	0,2 2,9	4,7	13,5	0,5	0,9	0,9	0	8,1				
34	Cheese culture from „Alnarp“ *Rosengren*	d	W					3,8									0,5								
			C	1,1	0,9	1,6	5,0	4,1	5,9	16,4	14,4	16,0	13,7	6,8	6,1	14,9	1,4	0,7	0,9	0	8,1	2	16,0	22,0	19,7
			Y	0,7	1,1	0,5	4,5	6,8	7,2		15,1			6,5	4,7	12,2	0	0	3	0	7,9				

tioned in the introduction, both the source of carbon and the source of nitrogen can, in this species, affect the relation between the different lactic acids. And we may note, as a curious fact, that Nos. 33 and 34, which only form dextro-lactic acid of lactose and all monosaccharides, also form inactive lactic acid from saccharose and maltose.

Streptobacterium casei thrives well in milk, and can, like *Thermobacterium lactis*, form abt. 1½ % lactic acid therein. In accordance with its slower growth, however, it takes

at least a couple of days, and as a rule from 3—5, or indeed often longer, to coagulate the milk. It always attacks casein, though with varying strength, in the same manner as the thermobacteria of milk, and is therefore of the greatest importance in the ripening of cheese. Some few strains can for a time, though for no perceptible reason, render the milk more or less slimy. One such slimy strain, of No. 34 (Pl. XL), lost the power of fermenting cane sugar, though its power remained quite unimpaired as regards all other sugars. Later, when its power of forming slime had disappeared, it at once regained the faculty of fermenting cane sugar.

Streptobacterium casei for the most part ferments galactose nearly as strongly as the other monosaccharides, and lactose more strongly than the other disaccharides. Nevertheless we may find some few strains altmost or entirely losing the power of fermenting lactose in artificial substrates (Nos. 5 and 23). Yeast extract will in many cases increase the power of fermenting saccharose (Nos. 2, 3, 5, 12, 15, 17, 18, 19 and 26). A similar effect is produced by this extract with regard to mannite, which is fermented — albeit often only to a slight degree — by all streptobacteria. Nos. 27—34 ferment sorbite and most of them also rhamnose, a little inosite and dulcite. As they, moreover, form only dextro-lactic acid, and exhibit lively growth at 45°, they resemble *Streptococcus glycerinaceus* in almost all respects. *Sbm. casei* has no marked power of fermenting pentoses or polysaccharides. Now and again we may discern a slight tendency to fermentation of arabinose, more rarely xylose. No. 16, in the first years, fermented some inulin, but lost this power after a time (its power of fermenting cane sugar decreasing at the same time).

Streptobacterium casei (Pl. XXXV—XL) forms, in broth, chains of short rods, with the ends as a rule cut off straight. The chains have often sharp breaks, are as a rule very long, and tangled, and then flake off, while the surrounding liquid is clear. The rods can be so short that if rounded, they may resemble streptococci (No. 9, Pl. XXXVII and No. 28, Pl. XXXVIII). They may, however, also grow out into longer rods, often curved. In milk, the chains are as a rule shorter than in broth, and on solid substrates as a rule even shorter still, but on the other hand, we often find longer rods here. On agar (No. 34, Pl. XL nethermost) and at times also in broth (No. 2, Pl. XXXV) screwed chains are often discerned; these arise by bending of the rods themselves. Even quite short rods can be curved (Nos. 33, Pl. XXXIX, as here the layer of indian ink is too thick the bacteria appear too thin) and unite two and two with the concave side inwards, forming rings or shapes of a deceptive likeness to that of the micrococci. These irregular shapes are most frequently met with in the rhamnose and sorbite-fermenting strains which, besides the qualities already mentioned (growth at 45° for instance) also differ from the remaining strains by exhibiting livelier growth with 2 % common salt than with only a trace of the same. There is thus much to advocate separation of these strains as a distinct species, which might suitably be called *Streptobacterium curvatum*[1]), if it were

[1]) Miss Troili Petersson (now Mrs. Almquist) has, in her "Studien über die Mikroorganismen des Schwedischen Güterkäses" (C. f. Bakt II. Abt. 1904, Bd. XI, p. 137) already established a certain species of lactic acid bacteria, *Bacterium curvatum*, distinguished by just these above-mentioned morphological qualities. One strain of this (No. 26) kindly furnished by the writer in question, exhibited lively growth at 45°, but did not otherwise agree with my typical strains of *Streptobacterium curvatum*. The extremely slight splitting up of casein (only 3.2 % *DN*) might, however, seem to suggest that it has degenerated on the whole during the long time of preservation.

Table XXX a.

No.	Streptobacterium plantarum isolated from:	Rotatory power of the lactic acid.	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid.	Milk: % of Total N. S N	Milk: % of Total N. D N
1	Butter 2, fine	d	W	0	0,1	2,5	0	0	0	3,8	5,2	4,7	3,8	5,4 0,5	5,6	5,0	5,0	0				7	5,9 3,4	0,0	0,1
			C	0,5	0	10,1 4,5	0,5	0,7	0,7	9,7	10,1	10,1	7,7	10,6	9,9	7,2	10,1	0,5	4,3	1,4	9,9				
			Y	0	0	3,8	0	0	0	15,5	13,5	14,2	10,1	15,8	14,6	14,9	15,8	0	4,3	1,4	9,9				
2	Milk *Weigmann's* collection No. 3	i	W	0	0,5	0	0	0	1,8 0	4,1	3,6	3,6	2,5	1,8 0	4,7	4,7 0	0	0	0	0		13	3,6 0,8	0,7	0,3
			C	0	1,8	2,3	0	0	2,3	11,0	10,6	12,2	7,9	7,2	10,6	7,7	0	0	0,2	0	8,8				
3	Butter 7 cheese-sour	i	W	0	0,5	1,6	0	0	0	4,1	5,2	4,1	2,3	0	4,5	4,1	0	0	0,2	0		13	3,4 2,5	÷4,8	÷1,5
			C	0,7	1,4	6,1	0	0	2,3	9,9	11,0	10,8	7,7	7,4	10,4	9,0	0,5	0,7	0,9	0	8,8				
4	Butter 4 cheese-sour	i	W	0	0,2	1,6	0	0	0	4,1	5,2	3,6	2,3	0	4,8	3,6	0	0	0,3	0		13	3,4 2,0	2,1	1,6
			C	0	0,2	6,1	0,2	0	1,1	9,5	11,7	11,5	8,1	9,9	10,8	9,5	0	0	1,1	0	8,3				
5	Dairy cheese 8 R 4 weeks	i	W	0,2	0,2	1,2	0	0	0,8	3,4	3,4	2,9	2 3	0,1	4,5	4,3	0	0,1	0,2	0		4	11,3 3,2	÷0,8	0,5
			C	0,9	1,6	7,4	0	0	3,4	10,6	9,7	10,6	9,5	0,7	10,1	10,8	0,2	0,7	0,9	0	8,8				
6	Dairy cheese 8 P 1 week	i	W	0,2	0	0,7	0	0	1,1	3,2	3,4	2,9	3,2	0,1	4,3	3,8	0	0	0,2	0		4	9,0	3,4	3,1
			C	0,7	0,5	8,3	0	0	1,8	12.2	12,8	12,2	10,1	0	11,7	11,0	0	0,2	0,9	0	8,3				
7	» 4 weeks	d	W	0	0,1	0,1	0	0	2,0 0	6,3	6,3	4,7	4,1	5,9 0	3,2	5,0	0	0				4	7,2 4,1	÷0,9	0,3
			C	0,9	0,2	0,9	0,2	3,6	4,1	11,9	11,7	10,8	8,8	11,9	10,4	9,5	0,9	10,6 7,9	0,9	0,2	9,9				
8	»	i d d i d	W	0	0	0,1	0	0	2,3 0	6,5	7,4	4,7	3,6	7,7 0	2,7	5,2	0,1	0						2,9	÷0,3	÷1,9
			C	0,9	0,9	1,4	0,2	2,5	3,8	12,2	11,5	11,7	7,7	11,7	4,5	9,7	0,5	10,4 0	0,7	0	9,2				
			Y	0,7	0	0	0	5,6	5,6		9,0			9,2				0	0	0	8,1				
9	»	i	W	0	0,2	0,2	0	0	1,4	5,0	5,2	4,1	3,6	4,1	5,9	4,1	0,2	0								
			C	0,7	1,1	1,4	0	0	2,9	11,3	11,0	11,0	9,7	9,9	11,0	10,8	8,6	0,5	0,7	0,5	9,0	4	9,0	2,2	0,3
			Y	0,9	0	0	0	0	7,2		12,2			10,8			10,4		1,1	1,1	9,7				
10	» 3 month	i	W	0,5	0,5	0	0	0	0,9	4,5	4,1	4,3	2,5	7,0 0,7	5,6	3,8	0,2	0	0,9	0	2,5	5	9,7 3,2	2,1	2,8
			C	0,9	1,8	0,9	0	0	3,4	10,8	11,5	11,9	8,8	10,1	11,0	10,4	0,5	0,7	1,1	0	9,0				
11	Dairy cheese 2 P 8 months	i	W	0,2	0	0	0	0	1,6	4,7	4,2	5,0	2,0	0,2 3,2	4,5	4,1	0,2	0				14	3,6 1,8	1,2	1,1
			C	0,7	0,9	1,1	0	0	2,9	10,8	11,0	11,3	7,3	8,6	10,6	8,8	2,3	0,7	0,9	0	8,6				
12	Milk *Weigmann's* collection No. a	i	W	0	0,7	0	0	0	0,3	3,6	3,6	3,8	2,5	1,6 0,2	3,4	3,8	0,2	0				10	6,0 4,1	1,2	0,8
			C	0,7	2,3	2,0	0	0	3,4	10,8	11,0	11,5	9,0	7,7	11,9	10,4	9,5	0,5	0,9	0	9,0				

Table XXX b.

No.	Streptobacterium plantarum isolated from:	Rotatory power of the lactic acid.	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN
13	Whey, *Weigmann's* collection No. 27	i	W	0	0,7	0	0	0,2	1,1 0	5,0	4,7	3,8	2,0	4,3 0	6,5	2,9	2,5	0				4	11,3	3,9	1,0
			C	0,9	2,7	1,6	0	1,4	3,2	13,1	13,5	13,3	9,9	9,7	12,4	9,7	10,6	0,5	0,5	0	10,8	8	8,1		
			Y	0,5	0	0	0	6,5	7,2		13,1			9,9			11,7		1,1	0	10,8				
14	Dairy cheese 7 R 4 months	i	W	0	0,5	0,1		1,8	1,6 0,1	4,1	4,1	3,8	4,1	1,8 4,5	5,2	4,5	0,5	0				4	10,6	2,9	2,6
			C	1,1	1,4	1,1	2,9	1,8	3,2	11,9	11,5	12,4	9,9	11,0	11,7	10,6	10,4	0,5	0,9	0	8,8				
15	Dairy cheese 9 R 3 months	i	W	0,2	0,5	2,5	0,2	1,6 0	1,8 0,5	4,1	4,1	4,3	2,3	0,5	4,7	3,0	0,5	0	0,7	0	1,8	10	4,1	1,1	÷ 0,6
			C	1,1	1,8	10,6	3,4	1,1	2,9	11,7	10,4	11,9	7,9	7,4	11,3	5,3	6,3	0,7	0,9	0	9,0				
16	Kefir 6	i	C	0,9	0,9	8,8	3,4	2,9	3,4	10,4	8,3	8,6	6,1	6,8	9,1	7,5	8,6	0,9	2,7	0,7	7,4	2	10,4	1,0	2,4
17	Calf fæces 6	i	C	0,9	0,7	8,8	0,5	2,5	2,7	9,7	5,9	8,6	5,6	6,5	8,1	5,6	7,0	0,7	1,8	0,5	6,3	10	5,2	3,3	÷ 0,1
18	»	i	C	0,9	0,7	0,5	1,1	3,4	3,6	9,9	8,1	9,5	6,8	8,6	9,5	7,7	8,3	0,5	1,8	0,5	5,9	5	8,6	3,9	2,0
19	Camembert-cheese 1	i	W	0,1	0,1	1,1	0,1	0	0	2,5	0,7	0,5	0,5	0,2	2,9	1,3	0,1	0,1	0,4	0	0,6		2,9	÷ 1,0	÷ 1,0
			C	1,5	0,5	9,0	0,2	3,4	3,6	7,4	7,4	7,7	6,3	3,6	8,1	7,2	5,6	0,5	0,5	0,5	6,1				
20	Dairy cheese 6 P 4 months	d i	W	0,2	0,2	0,5		1,8	2,0	4,5	5,0	4,6	3,8	5,4	2,9	3,6	0,1	5,2				3	13,3	16,3	18,7
		d	C	0,9	0,5	1,1	5,0	3,8	3,8	11,5	11,9	13,1	10,6	12,6	5,9	9,0	4,1	12,6	0,5	0	6,8	6	9,5		
21	Dairy cheese 5 R 4 months	d	W	0,1	0	0,5		1,1	1,6	4,7	5,6	4,3	4,7	5,2	3,8	4,7	0	2,9 5,4				3 7	14,6 10,8	15,7	17,3
			C	0,5	0,5	1,1	0,2	2,7	3,4	12,8	12,8	11,9	11,9	13,3	7,7	11,3	0,5	13,7	0,7	0	7,7				
22	Soured potatoes 1 II	d i	W	0,1	0,2	0,5	0,1	1,4	1,5	5,3	6,0	5,5	3,6	0,7	5,6	1,8	0,1	5,5	2,0	0	3,5	3	15,3	4,4	5,9
			C	0,8	6,6	0,3	0,5	4,2	3,1	11,4	12,9	12,7	10,1	12,7	11,0	11,9	6,7	12,6	3,0	0,5	8,3				
23	» 1 II	d(i)	W	0,1	0,7	1,0	0,1	1,6	1,8	6,1	4,2	4,7	3,6	4,3	4,1	3,5	0,5	5,4	1,8	0	2,2	2	15,5	5,1	5,9
			C	0,8	6,8	10,5	0,4	3,6	7,1	12,5	12,2	12,1	10,9	9,8	9,9	10,9	9,9	13,5	2,5	0,2	7,7				
24	» 1 I	i	W	0,1	2,5	2,8	0,1	0	0,7	2,6	1,9	1,1	1,0	2,2	6,5	1,8	0,1	0	1,1	0	2,3		2,9	0	0
			C	0,5	15,1	8,3	0,2	0,2	2,9	8,9	7,8	6,9	5,6	6,2	7,4	6,5	6,4	0	1,9	0	5,1				
25	» 1 II	d(i)	W	0,4	0,5	0,7	0,1	1,7	1,7	5,4	4,2	5,2	1,8	1,5	2,9	3,8	2,7	0,1	1,6	0	0,9	3	13,3	2,3	2,4
			C	0,7	7,3	0,4	0,4	2,7	3,3	11,7	11,9	11,9	9,7	8,1	11,1	9,0	9,9	0,2	7,4	0,6	7,5				
26	» 2 II	d(i)	W	0,2	0,5	0,6	0,1	0	1,1	5,8	5,5	3,6	3,4	0,2	4,5	3,5	2,5	0,2	2,0	0	1,1	3	15,1	5,3	5,4
			C	0,7	6,4	0,3	0,2	0,3	2,7	9,6	12,2	11,6	9,0	7,0	10,1	9,0	6,3	0,5	2,7	0,4	6,5				
27	» 2 II	d(i)	C	0,3	6,1	0,3	0,3	0,3	4,3	10,6	9,8	10,4	8,2	9,2	8,3	7,4	1,0	0,7	2,5	0,3	6,0	3	13,3	2,8	1,1
28	» 2 II	d(i)	C	0,7	0,7	0,5	0,7	0,3	3,5	12,2	11,9	11,9	9,7	2,9	9,7	10,4	0,4	0,5	2,3	0,4	9,7	3	14,2	2,1	1,2
29	» 1 II	d i	C	0,7	0,9	0,7	0,7	4,2	3,5	10,8	11,1	10,6	10,5	3,6	9,3	7,0	0,6	0,5	2,0	0,4	3,6	5	10,8	3,0	4,0

Table XXX c.

No.	Streptobacterium plantarum isolated from:	Rotatory power of the lactic acid	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN
30	Soured diffusion slices	i	W					1,1	1,4					0,2			0	5,4							
			C	0,7	2,0	1,8	0,2	1,1	3,4	11,3				13,3			0,7	13,7	0,5	0	6,8	3	12,2	5,6	4,8
			Y	0,9	1,1	0,5	0	6,1	6,8	13,1	14,0	14,0	11,9	14,6	5,2	9,2	0	15,8	0	0	9,0				
31	Soured beet slices I	d	W	0,4	0,6	0,5	0,2	1,3	1,5	5,6	4,5	5,2	3,4	3,6	5,4	3,2	0,1	5,1	2,2	0	4,6				
			C	0,6	1,0	0,5	0,6	1,6	2,9	10,1	9,0	8,8	9,5	9,3	7,7	8,8	0,5	9,7	2,5	0,5	4,5	2	13,3	5,0	4,4
32	» I	d	W	0,3	0,1	0,5	0,1	1,3	1,4	5,2	5,0	5,1	3,6	3,7	4,7	0	0,1	4,7	1,4	0	3,3				
			C	0,5	0,7	0,7	0,4	2,3	2,3	10,4	10,8	9,9	7,9	10,4	8,6	0,3	0,2	11,0	1,8	0,2	3,8		0,5		
33	» I	i	W	0,2	0,5	0,6	0,1	1,6	1,9	5,8	4,0	4,6	2,7	3,2	4,7	2,0	0,5	0,1	1,4	0	2,4				
			C	0,7	1,1	0,6	0,5	3,2	3,6	11,7	11,3	11,6	8,2	7,7	9,2	6,5	5,9	10,6	2,6	0,5	4,5	4	7,4		
34	» I	i	W	0,1	0,1	0,2	0	0,8	0,7	4,2	2,3	2,0	0,7	1,0	6,0	1,7	0,2	0,2	0,4	0	0,9				
			C	0,7	0,9	0,6	0,4	2,9	3,4	8,1	8,3	8,0	4,6	5,3	8,4	7,3	7,2	0,2	2,0	0,3	6,8		2,0		
35	» I	i	W	0,2	0,1	0,5	0	0	0,5	4,1	1,0		0,9	1,6	0,7	2,0	1,4	0	0,8	0	0,7				
			C	0,8	0,9	0,5	0,4	0,2	2,4	7,3	6,6	4,7	4,5	4,7	6,3	5,7	5,9	0,1	1,9	0,2	3,9		2,0		
36	» II		C	0,4	0,7	0,1	3,6	1,5	1.0	6,8	7,5	6,5	5,2	6,0	6,3	0,2	0,5	0,1	0,5	0	5,4		0,5		
37	Pickled cabbages 2	i	W	0	0,2	0,5	0	0,5	0,7	0,7	1,0	0,6	0,5	0,7	0,6	1,0	0,1	0,1	0,2	0	1,1				
			C	1,4	0,7	9,2	0,2	3,6	3,6	8,1	8,6	9,9	4,3	8,8	8,1	6,5	8,1	7,7	2,0	0,7	8,1		2,7		
38	» 2	i	W	0,1	0,4	1,3	0,1	1,4	1,1	0,7	1,6	0,8	0,7	1,6	0,7	1,5	0,5	0,1	0,2	0	1,1				
			C	1,1	0,5	8,6	0,2	3,2	3,4	9,2	7,9	8,6	1,1	8,3	8,6	7,0	8,3	8,1	1,6	0,5	7,9		2,0		
39	» 2	i	W	0,4	0,1	0,9	0,1	1,6	1,7	4,0	2,4	1,8	1,7	1,8	3,8	1,4	0,1	0,1	0,4	0	2,0				
			C	1,4	0,5	9,0	3,2	4,3	3,6	8,6	8,5	8,3	8,1	7,7	9,9	8,6	7,9	0,5	1,8	0,7	8,1		1,6		
40	» 2	i	W	0,1	0,5	0,3	0,4	0	1,1	4,2	1,4	1,8	0,6	0,9	4,6	1,8	0,1	0,1	0,4	0	2,3				
			C	1,1	0,5	0,7	3,3	0,7	3,4	8,3	7,7	8,3	6,1	7,9	8,8	8,6	5,5	0,5	2,0	0,5	6,8	14	4,0		
41	» 2	i	W	0,4	0,2	0	0	0	0,4	2,8	0,8	0,7	0,9	0,4	0,6	0,6	0,1	0,1	0,4	0	0,7				
			C	0,9	0,5	0	0,4	0,5	2,9	8,1	7,0	8,8	5,2	6,8	8,5	6,5	5,9	0,2	1,8	0,5	8,1	11	6,1		
42	Sour dough D	i	W	0,1	0,5	0,4	0	2,0	0,6	5,3	3,7	2,5	1,7	1,3	3,5	3,5	5,2	0,1	0,4	0	0,8				
			C	0,8	0,9	0,4	0,2	2,5	2,7	10,7	10,6	10,5	6,5	7,2	12,4	8,3	9,2	0,2	2,9	0,2	9,2	14	3,8		
43	» D	i	W	0,1	0,1	0,7	0,1	1,4	1,5	4,3	1,6	1,7	1,1	0,7	4,0	4,1	0,1	0,1	0,2	0	0,7				
			C	0,8	0,7	8,9	4,0	2,4	2,6	10,7	10,7	10,5	7,7	8,9	9,9	8,2	7,6	0,2	2,5	0,2	8,3	14	5,6		
44	» B	i(d)	C	0,1	0,2	0	0	0,1	2,0	9,5	10,1	9,6	0	7,4	9,7	0	0,1	0	1,9	0	9,5		0		

not that we find exactly similar curved rods in the species *Sbm. plantarum* (No. 1, *SG*-plate Pl. XLI). On *SG*, the cells of *Sbm. casei* are most frequently short, and on *AG*, they are always short, and even rounded, so their chains resemble streptococci (No. 4, Pl. XXXV, No. 9, Pl. XXXVII and No. 32, Pl. XXXIX). When stained with methylene blue, some few rods (especially in older milk cultures) can at times exhibit dark grains similar to those noted in the thermobacteria.

Streptobacterium plantarum (Table XXX a, b and c) most frequently forms pure inactive lactic acid, but can also form dextro-lactic acid; in some few cases, indeed (Nos. 1[1]), 21, 31 and 32) even exclusively dextro-lactic acid. Though, like most strains isolated from potatoes, it forms much acid in milk, a powerful splitting of casein is nevertheless exceptional, and has only been demonstrated in the two inulin-fermenting strains (Nos. 20 and 21) which were isolated from cheese[2]). Nos. 2, 3 and 4 are slimy (ropy) in sugar agar, but not in milk. Nos. 7 and 8 have at times shown some slime formation, especially in cane sugar agar. Radiations from the agar stab are now and then seen, always in the case of No. 11.

As regards its relation to the sugars, *Streptobacterium plantarum* is affected to an extremely high degree by the source of nitrogen employed. It is common, for instance to find the fermentation of raffinose and inulin — and even of saccharose, mannite and pentoses — fail with *W* as source of nitrogen. There are indeed some strains which can hardly ferment monosaccharides (Nos. 24, 35 and the sour cabbage bacteria) unless coaxed to a certain degree with their favourite dishes. With *C* as source of nitrogen it prefers as a rule maltose and cane sugar to lactose, and often ferments raffinose, some strains also inulin. The fermentation of sorbite and rhamnose is far more frequent than in *Sbm. casei*[3]). A number of strains show a comparatively powerful fermentation of arabinose, and a few (as for instance most of those isolated from potatoes) of xylose. No. 1 can ferment a little starch. Briefly then, these bacteria can on the whole — as was to be expected of plant bacteria — utilise a far greater number of carbon sources than bacteria living normally in milk, where there is no other source of carbon beyond lactose.

From a morphological point of view, it will hardly be possible in all cases to decide whether we are dealing with a strain of *Sbm. casei* or of *Sbm. plantarum* (Pl. XLI—XLV). In broth, *Sbm. plantarum* as a rule forms shorter chains, or even isolated long rods. If they exceptionally form chains of small segments, then these latter are rounded, and consequently much like streptococci in appearance (No. 44, Pl. XLV). On solid substrates, isolated rods, or a very few together will most frequently be found.

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[1]) No. 1 has, however, formed a little inactive lactic acid from lævulose and saccharose.

[2]) We have therefore doubted, if we should reckon these two strains to *Sbm. casei* or to *Sbm. plantarum*, and only their powerful saccharose- and inulin-fermentation decided us for the latter.

[3]) Several sorbite-fermenting strains of *Sbm. plantarum* (as for instance Nos. 19, 20 and 33) also ferment some inosite, but only a trace of dulcite.

Genus: **Betabacterium** (Abbr. *Bbm.*).

The betabacteria (Table XXXI) are in most respects so closely allied to the betacocci that they may be regarded as the analogous rod forms, and they can, like the betacocci, be divided into those which ferment arabinose (Nos. 1—20) and those which do not (Nos. 21—33). The *Bacterium casei* γ and δ[1]) formerly described by me are typical representatives of these groups, and as they also differ in morphological respects, — the arabinose-fermenting rods being generally shorter than the others — we consider ourselves justified in establishing the species *Betabacterium breve* and *Betabacterium longum*. Besides occurring in vegetable matter, they are also found in cheese, fæces and kefir grains. The rods forming the tissue of the kefir grains (Nos. 1 and 2) are, however, so different from the remaining betabacteria that they must undoubtedly be reckoned as a distinct species which we will call *Betabacterium caucasicum*, as the kefir rods are now for the most part known under the name of *Bacterium caucasicum*. This last name is also erroneously used for streptobacteria, which are likewise also found in kefir (though not as a rule in the grains themselves), and which are considerably easier to obtain in pure cultures.

Most of the betabacteria are not altogether killed by heating until 75°. They form inactive lactic acid, at times with a surplus of dextro-lactic acid. The great majority of strains develop gas (carbonic acid and more or less hydrogen). When the development of gas is strong, succinic acid is also formed. The power to form any considerable quantity of gas, and thus succinic acid, is, however, soon lost in artificial nutritive substrates, and in most of our strains, gas development could only be observed by sowing out closely in sugar agar tubes. From lævulose, some few strains can form a small amount of mannite, and as the mannite-forming mash bacterium *Lactobacillus fermentatum*[2]), which has been closely studied by Jan Smit, has the greatest resemblance to the betabacterium (judging from its fermentation of sugar, with *Sbm. longum*) it should doubtless be reckoned under this head. Possibly the mannite bacteria[3]) so dreaded in the making of wine also belong here. Some of these form, like the betacocci, slime from cane sugar, which the betabacteria investigated by us never do.

The betabacteria grow poorly in milk, and do not as a rule attack casein at all. When cultivated at optimal temperature, however, some few strains, in a freshly isolated state, were able to curdle milk, and even with a slight development of gas, but this power was soon lost. The frequent occurrence of these bacteria in cheese is due to the fact that they are better able to utilise lactate of lime as a source of carbon than are most other lactic acid bacteria.

A characteristic feature in the betabacteria is their lack of ability to ferment salicin and alcohols, and, with the exception of the *Bbm. longum* forms, their preference for

[1]) Studien über die flüchtigen Fettsäuren im Käse etc. Centralblatt f. Bakteriologie II. Abt. 1904, XIII, p. 604.

[2]) Zeitschrift für Gärungsphysiologie 1915, Bd. V, p. 273.

[3]) In Müller-Thurgan and Osterwalder's big work: "Die Bakterien im Wein und Obstwein und die dadurch verursachten Veränderungen" (Centralblatt f. Bakt. II. Abt. 1913, Bd. 36) there is also a list of works on this subject.

Table XXXI a.

No.	Betabacterium isolated from	Rotatory power of the lactic acid	Production of gas	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN
1	Kefir 5		0	C	0	0	4,2	0	0	0	0,5	0	0	0	0	0	0	0	0	0	0	0		0		
2	Kefir 3	i	0	C Y	0 0	0 0,2 0	11,7 10,4 14,2	0 0	0 0	0 0	0 2,0 3,8	0,7 5,0 7,0	0 0,5 0	0 5,0 7,7	0 0	0 0 0	0 0 0	0 0 0	0 0	0 0	0 0	0 0		0		
3	Dairy cheese 9 R 1 week	i	+	W C	0 0	0 0	7,9 13,7	0 0	0 0	0 0	6,1 5,9	4,3 4,5	0,2 0,2	4,3 3,8	0 0 3,4	0 2,3 3,8	0,7 3,4	0 0 2,5	0 0	0 0	0 0	0 0		0,7 1,4	0,8	÷0,5
4	Dairy cheese 7 R 4 months	i	+	W C	0 0,2	0 0	3,6 7,2 10,1	0 0	0 0	0 0	4,7 6,3	4,5 6,8	0 0,2	2,3 2,3	0,9 0 0,2	3,4 0,2 0,9	3,4 0,5 0,9	0 0,5	0 0,2	0 0	0 0	0 0		1,8 0,7		
5	Dairy cheese 5 P 4 months	i (d) i	+	W C Y	0 0,1 0	0 0 0	5,6 11,0 7,4	0 0 0	0 0 0	0 0 0	1,1 3,2 4,1	4,1 5,9 4,5	0,3 0,1 0	4,1 5,9 4,7	0 0,2 0	5,0 2,7 1,6 5,2	4,7 0,2 0,5 1,1	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0		0,7	6,3	0,1
6	Dairy cheese 5 R 4 months	i (d)	+	W C	0 0	0 0,5	2,5 8,3 16,4	0 0	0 0	0 0	2,0 3,4	4,3 6,5	4,1 0,2 0,5	3,2 7,4	0 0,5	4,1 3,2 2,5	3,6 1,8 1,1	0 0	0 0	0 0,2	0 0	0 0		3,6 0,7	2,4	0,6
7	Dairy cheese 6 P 4 months	i	+	W C	0 0	0 0,5	4,3 8,8 16,7	0 0	0 0	0 0,9	3,8 6,5	3,8 7,7	2,5 0 0,5	4,1 8,8	0 0,2	3,2 0,9 4,3	2,9 0,9 3,8	1,8 0 0,5	0 0	0 0	0 0	0 0		1,6 0,2	5,0	÷0,5
8	»	i (d)	+	W C Y	0 0 0	0,7 7,4 6,8	1,4 8,6 7,9	0 0 0	0 0 0	0 0 0	0,5 0,9 1,6	1,6 5,0 4,7	0 2,0 1,1	1,4 3,4 3,8	0 0 0	1,4 0 3,6 5,2	1,4 0 0,5 3,2	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0		0,7 0,2		
9	Calf fæces 6	i	+	C	0,5	0,2	2,3	0,2	0,2	0	0,2	5,9	0	1,8	6,1	5,4	4,7	5,2	0,5	0,5	0,2	0,2		0,7		
10	Emmental cheese (*Bacterium casei γ*)	i	+	W C Y	0 0,2 0	3,4 13,5 6,8	2,9 16,2 9,0	0 0 0	0 0 0	0 0 0	1,6 7,9 6,8	3,6 8,6 6,1	0 1,1 0,2	2,5 7,4 6,1	0 0,2 0	2,7 5,9 6,3	2,3 0,9 1,4 8,6	0,5 0 3,6 0	0 0,2 0	0 0 0,5	0 0 0	0 0 0		1,8 0,5	÷3,8	0,3
11	Sour dough E		0	C	0,1	0,5	8,6	0,2	0,2	0,1	5,6	4,3	3,4	4,7	0,3	4,5	3,8	4,5	0,2	0,3	0	0		1,7		
12	» E	d	0	C	0,1	0.2	0,2	0,2	0,2	0,2	5,4	4,3	2,7	5,4	0,5	4,5	4,7	5,9	0,1	0,2	0	0,1		1,1		
13	Fæces 3		+	C	0,1	0,9	3,8	0,2	0,5	0,9	6,4	0,9	0,8	0,9	0	0,9	0,3	0,6	0	0,2	0	0				
14	» 3	i	0	C	0	0,2	2,0 5,0	0,5	0,2	0,7	6,8	0,7	0,6	0,8	0,5	0,9	0	0,2	0	0,2	0	0		0	1,5	÷0.6
15	» 3	i	0	C	0	1,1	1,8	0,5	0	0,5	5,2	1,1	0,5	0,9	0,5	1,6	0,2	0	0	0	0	0		0,5		
16	Soured potatoes 1 l	i	0	C	0	6,5	7,7	0,3	0,2	0,8	5,6	2,9	0,8	2,1	5,9	5,1	3,4	0,3	0	0,3	0,1	0,3		0,9		

Table XXXI b.

No.	Betabacterium isolated from	Rotatory power of the lactic acid	Production of gas	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. SN	Milk: % of Total N. DN
17	Soured potatoes 1 I	i	0	C	0	7,9	8,6	0,2	0,1	0,9	5,9	1,7	0,5	2,5	4,4	5,6	0,2	0,1	0	0,2	0,1	0,3		0,5		
18	Soured beet slices I		+	C	0,2	8,0	6,5	0,2	0,2	0,9	5,6	4,5	0,5	3,4	3,0	4,3	3,8	0,3	0,2	0,3	0,3	0,5		1,6		
19	Soured potatoes 1 II		0	C	0	12,2	5,6	0,3	0	0,3	6,3	5,5	0,6	3,5	1,1	3,9	0,2	0,2	0,2	0,1	0	0,1				
20	» 2 II		0	C	0,1	12,8	7,4	0,5	0	0,6	7,4	5,6	0,8	3,6	1,0	2,8	2,6	0,5	0,3	0,3	0,1	0,1		2,3		
21	» 2 II	i	+	C	0	6,1	0,3	0,5	0,1	0	6,1	3,8	0,9	4,1	3,8	1,7	1,0	1,4	0,2	0,5	0,1	0,1		0,5		
22	» 2 I		+	C	0	4,1	0,3	0,1	0	0	7,4	5,4	2,9	3,4	7,2	7,2	6,5	7,3	0	1,6	1,4	0,7		2,3		
23	» 2 I	i	+	C	0	3,8	0,3	0,2	0,1	0,1	5,9	3,7	0,9	2,9	4,0	4,0	1,8	4,2	0,1	0,5	0,2	0,1		0		
24	» 1 II	i	0	C	0,1	0,1	0	0,1	0,1	0,1	5,4	4,3	2,0	4,7	5,4	4,3	4,4	5,2	0,2	0,8	0,2	0,1		2,2		
25	» 1 I	i	+	C	0	0,5	0,5	0,3	0,2	0,2	6,0	3,3	0,9	2,1	2,5	4,4	2,3	3,3	0,1	0,7	0,5	0,1		1,4		
26	Soured beet slices II		0	C	0,2	5,4	0,2	0,1	0,1	0,1	5,4	4,1	2,6	5,0	4,5	3,6	4,5	4,2	0,1	0,2	0,1	0		0,6		
27	» II		0	C	0,1	0,1	0,2	0,1	0,1	0,1	5,3	4,1	2,5	3,9	3,7	3,6	1,2	4,2	0,2	0,2	0	0		0,7		
28	» II		0	C	0,2	0,2	0,1	0,3	0,2	0,2	5,3	5,5	3,2	5,3	5,3	4,1	4,8	5,0	0,1	0,5	0,3	0		2,5		
29	Fæces 3	i	+	C	0,2	1,6	0,5	0,5	0,2	0,2	6,8	3,8	2,0	2,0	3,8	4,1	6,3	5,4	0,2	0,9	0,9	0		2,3		
30	» 3	i	+	C	0,1	0,5	0,2	0,5	0,5	0,5	5,0	3,8	2,3	4,1	3,8	4,7	4,5	4,1	0	0,9	0,7	0		1,1		
31	Emmental cheese 1 *Burri*	i	+	C	0	0	0,5	0	0	0	5,6	5,4	2,0	5,6	6,3	5,9	5,0	0,7	0	0	0	0		1,4		
32	» 2 (*Bacterium casei δ*)	i	+	W C Y	0 0 0	0 0 0	0 0,2 0	0 0 0	0 0 0	0 0 0	1,6 5,2 7,4	1,8 5,2 6,5	0 2,5 3,2	1,1 5,6 5,4	0 5,4 9,0	1,1 5,6 6,3	0,7 5,4 6,5	1,6 4,3 7,7	0 0 0	0 0 0	0 0 0	0 0 0		 0,7 		
33	» 3	i	+	C Y	0 0	0 0	0,2 0	0 0	0 0	0 0	5,9 6,1	5,6 7,0	1,4 4,7	3,2 6,5	4,1 7,9	1,4 6,3	4,3 7,2	7,9 7,2	0,2 0	0,2 0	0,2 0	0 0		 0,5		

pentoses. The betabacteria have also always a slight fermentation of mannose. Some of the strains (as for instance Nos. 6 and 7) which in a freshly isolated state fermented mannose comparatively strongly, lost this power later on. As *Streptococcus thermophilus*, which likewise does not ferment salicin, has also a comparatively slight fermentation of mannose, there seems to be a certain relation between the power of fermenting salicin and that of fermenting mannose. As we shall see later on, this remarkable feature is repeated in *Bacillus bifidus*.

Betabacterium caucasicum has its optimal temperature at under 30°, and still grows well at 10°. In milk richly inoculated with kefir grains, it can even at less than 20° rapidly form 1.5 % inactive lactic acid. The sugar is not hydrolysed prior to fermentation, as some investigators have asserted. If the kefir grains be sifted off, however, the degree

of acidity will rise very slowly in the remaining kefir, and in pure cultures, the kefir rods form no acid in milk. *Bbm. caucasicum* grows altogether slowly as a pure culture. It thrives best in yeast extract, and its powerful growth in kefir grains must therefore be due to a symbiontic relation to the yeast cells therein. After cultivation for many years in casein peptone, however, it can become so accustomed to this source of nitrogen, that it will ferment the same sugars with this as with yeast extract (see No. 2). Of all the betabacteria, *Bbm. caucasicum* is the most pronounced *A*-form, as with less suitable nitrogen sources it will only ferment arabinose at all, and if tested therefore, under these circumstances, with all other sugars than arabinose in particular, one must necessarily conclude that it does not belong to the lactic acid bacteria.

Bbm. caucasicum forms shorter or longer rods, which are apt to clump together, in broth and milk, to miniature kefir grains (Pl. XLVI). Large kefir grains, however, I have never succeeded in producing with pure cultures. In the true grains, the rods are often very long and curved, and tangled together. In highly acid cultures the kefir rods can, after treatment with methylene blue, exhibit unstained parts, which have previously been regarded as spores (*Dispora*).

Betabacterium breve, has its optimal temperature at 30°. It thrives very badly below 15° and above 37½°. It grows somewhat better than the streptobacteria on sugar gelatin.

Bbm. breve has not only difficulty in fermenting mannose, but some few strains (Nos. 8 and 9, and with bad sources of nitrogen also Nos. 5, 6 and 10) have a relatively low fermentation of lævulose, and, which is still more remarkable, other strains (Nos. 13, 14, 15, 16 and 17) even find it difficult to utilise dextrose. The fermentation of galactose is as a rule on a level with that of dextrose, and we can therefore — in contrast to what we have otherwise seen in the case of the lactic acid bacteria — find strains which ferment galactose considerably more strongly than lævulose. Only few strains ferment saccharose or raffinose. As with the betacocci, the power of fermenting maltose and lactose has in many strains perceptibly decreased during the time we have had them under cultivation. We find altogether something of the same variability as in the betacocci, which renders the line of demarcation between *Bbm. breve* and *Bbm. longum* somewhat vague.

Bbm. breve (Pl. XLVI-XLVIII) occurs most frequently in the form of regular, isolated rods with rounded ends. The most common size is 0.7—1 × 2—4 μ. Chains of short segments are, however, found, especially in broth at the first stage of development (No. 3, Pl. XLVII). Later on, the segments become longer, and fall away from one another. Only a single strain (No. 8, Pl. XLVII) forms rings similar to those often seen in certain streptobacteria. When stained with methylene blue, several strains exhibit the granulation so characteristic of the thermobacteria.

Betabacterium longum grows most rapidly at a couple of degrees below its maximal temperature, which lies about 45°. It does not develop at anything under 18°. A number of strains ferment xylose, but none arabinose. As a rule, it ferments saccharose and raffinose. It often forms long rods (No. 33, Pl. XLVIII) which can resemble those of the thermobacteria. Generally, however, they exhibit no granulation, when stained with methylene blue. In a few strains (see photos of No. 22) the rods are inclined to develop irregular swellings.

Genus: **Microbacterium** (Abbr. *Mbm.*).

Of the other GRAM-positive, rod-shaped lactic acid bacteria (Table XXXII) which we have encountered in our investigations, the majority (Nos. 3—10) are considerably smaller than the rod forms hitherto described, and have so many peculiar qualities in common that it will be only natural to collect them in a genus, which may suitably be named *Microbacterium.*

The microbacteria do not, for the most part, curdle milk, and are on the whole weak acid formers, and produce dextro-lactic acid, with the single exception of No. 10, which forms inactive lactic acid. When sown out in high agar tubes, they grow only in the upper part, and in stab cultures, they exhibit more or less pronounced surface growth. No. 7 even forms a highly curled surface layer. Nos. 3—6 on the other hand, give only surface growth with favourable sources of nitrogen, and even then not always to any perceptible degree. The best nitrogen source for these bacteria is casein peptone; yeast extract, on the other hand, is as a rule very unfavourable. With the exception of No. 1, they split up hydrogen peroxide, and reduce nitrate to nitrite. In biological respects, the microbacteria thus greatly resemble the tetracocci, and there is also a gradual transition to forms which are no longer acid formers, but which liquefy gelatin to a slight degree, and can break down amino-acids. The true microbacteria never ferment pentoses, and of alcohols, at the outside a little mannite.

The microbacteria fall again into several well distinguished species. Nos. 3, 4, 5 and 6, for instance, are closely allied forms, and as they very often occur in milk, we will call them *Microbacterium lacticum.* No. 7 we will call *Microbacterium mesentericum*, from its very characteristic surface growth, and Nos. 8 and 9, which have constantly exhibited a powerful yellow surface growth, *Microbacterium flavum.* No. 10 probably also constitutes a distinct species.

Microbacterium lacticum can endure heating to 80°—85°. It can therefore be obtained — sometines as a pure culture — by sowing out freshly pasteurised milk. It grows extremely poorly at anything over 35°, and hardly under 10°. Some strains develop better on *AG* than on *SG.* Some few strains produce a fairly powerful solvent effect upon casein, without, however, splitting it up very deeply. *Mbm. lacticum* may lack the power of fermenting cane sugar; it never ferments raffinose and inulin, but often starch. It occurs mostly in the form of single small rods, 0,3—1 μ (Nr. 5, Pl. XLIX) but can also be swollen, having then a more coccus-like appearance (No. 4, Pl. XLIX). When stained with methy-

Table XXXII.

No.	*Remaining rod shaped lactic acid bacteria* isolated from	Rotatory power of the lactic acid	Source of nitrogen	Glycerin	Xylose	Arabinose	Rhamnose	Sorbite	Mannite	Lævulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin	Milk: Time of curdling	Milk: Amount of acid	Milk: % of Total N. S N	Milk: % of Total N. D N
1	Fæces 3		C	0,2	0,5	0,7	0,7	0,5	0,5	2,7	2,7	3,2	1,4	2,3	3,4	2,5	1,4	0,7	1,1	1,1	2,0	7	2,9	0,5	1,2
2	Milk *Weigmann*		W	0	0	1,4 0	0	0	0	1,6	1,4	1,7	0,9	0,1	0,5	0,6	0	0	0	0			1,4		
			C	0,7	0	2,3	0	0,9	0,7	3,8	3,6	3,4	2,0	1,4	2,0	2,5	2,3	2,3	2,3	1,8	1,8				
3	**Microbacterium** Dairy cheese 6 P 4 Months	d	W	0	0	0	0	0	0	1,8	2,0	2,0	1,5	1,1 0	1,6	1,4	0	0					1,8	20,0	5,6
			C	0	0	0	0	0	0,5	5,2	5,2	5,0	2,7	3,6	4,3	3,4	0	0	3,8	4,5	2,0				
4	Cheese-sour butter 6	d	W	0	0	0	0	0	0	2,0	2,0	1,9	1,5	0	1,1	1,8	0	0	1,4	0,5	1,1		4,1	5,6	1,3
			C	0	0	0	0	0	1,1	5,2	5,2	4,7	1,8	2,9	2,9	3,6	0	0	2,0	1,8	2,9		1,8		
5	Milk heated 1/2 hour to 80° in an agar tube		W	0	0	0	0	0	0,9	1,8	1,8	1,8	0	0	0,2	1,4	0	0	0	0	1,1				
			C	0	0	0	0	0	1,6	4,7	5,2	5,2	1,8	0	3,4	4,3	0	0	2,0	2,0	2,0	5	4,3	8,0	0,9
6	*Bac. acidophilus Kral*	d	W	0	0	0	0	0	0	1,8	1,8	1,8	1,5	0,1	1,6	0,5	0	0							
			C	0	0	0	0	0	0	4,5	4,7	4,7	1,8	2,9	2,9	0	0	0	3,4 1,4	0	2,9		0,7		
7	Rotten red beet 4		C	0,5	0	÷	÷	0	0,2	2,3	3,2	1,6	0	2,9	1,8	0,7	1,8	0	1,6	1,8	3,2		0		
8	Dairy cheese 6 P 4 months	d	W	0,1	0	÷	0	0	1,0 0	4,3 1,8	5,0 2,0	4,5 2,3	0,9 0,2	0	0	0	0	0	0	0	0		0,7		
			C	0,8	0	0	0	0	0,5	10,4	8,3	7,0	0,2	0,2	0,1	0	0	0	0	0	0				
9	Good butter 1		W	0	0	÷	0	0	0,8 0	4,5	3,4 2,0	4,1 1,1	1,8	0	0	0	0	0	0	0	0		0,7		
			C	0,3	0	0	0	0	0,5	9,7	6,1	0	2,0	0	0	0	0	0	0	0	÷				
10	Calf fæces 6	i	C	÷	0	÷ 0	÷ 1,1	÷ 0	0 0,7	1,6	1,6	1,1	0	1,6	0	÷ 0	0	÷ 0,2	÷ 0,2	÷ 0	÷ 1,8		0		
11	**Bacterium bifidum** Infant fæces III	d	Y	0	6,1	7,9	0	0	0	5,2	14,6	1,1	11,5	0	11,3	16,2	0	0	0,9	0	0		0		
12	» » I		Y	0	0	0	0,7	0	7,0	8,6	13,5	0	9,5	11,0	10,6	11,7	15,1	4,1	2,0	0,9	0	10	7,0		
13	**Propionic acid bacteria** Swedish manor-seat cheese *Troili Petersson*		C	1,1	0,2	15,3	0	0	0,2	6,3	5,4	3,6	9,9	7,2	7,4	0,9	2,3	0	0	0		13	9,7	4,6	÷0,8
14	Emmental cheese *Bacterium acidi propionici x*		C	8,4	0	7,9	8,6	8,8	11,5	12,8	10,4	9,7	10,6	12,8	9,1	12,2	1,0	0	1,4	3,6		13	8,3	3,8	÷3,7

lene blue, it sometimes appears highly granulated, and thus resembles an extremely small streptococcus.

Closely related to *Mbm. lacticum* are apparently the rods Nos. 1 and 2, though they are about normal thickness, and altogether without surface growth[1]). In streak cultures, No. 1 (Pl. XLIX) is often swollen up to a club shape, and partly GRAM negative. They do not reduce nitrate to nitrite, but No. 2 splits up peroxide of hydrogen.

Of the strains of *Mbm. lacticum* above mentioned, No. 6 should be a typical representative of the acidophile intestinal bacterium *Bacillus acidophilus*, discovered by MORO[2]) and FINKELSTEIN[3]). None of the many writers who have studied this GRAM-positive rod have interested themselves in its biology, but have devoted all the more attention to its morphological features, and it has been described, now as a thin form, now as a thick one, now as isolated long rods and again as chains of short segments. As some investigators have observed that it can be very irregular (compare with our No. 1), they go so far as to declare it identical with *Bacillus bifidus*. This is an excellent instance of what can be attained by morphology alone in bacteriology. As it is quite impossible to determine, from extant literature, what GRAM-positive intestinal bacterium is really meant by *Bacillus acidophilus*, there is certainly no reason to retain the name. When endeavouring to discover the acidophile fæces bacteria, by enrichment prior to sowing, in sugar broth with ½—1 % acetic or lactic acid, we have either encountered betabacteria (strains 13, 14, 15, 29 and 30 were isolated in this way) or microbacteria most nearly approaching strain No. 1.

Microbacterium mesentericum. This form is, as mentioned, easily recognisable by its enormous mesentery. It is killed by heating to 70°. It is only a weak acid former, and in its relation to the sugars most resembles *Mbm. lacticum*. It forms long, thin, often highly granulated rods, which, as they lie in slime, appear much thicker in Indian ink preparations than in colour preparations. On *AG*, where it thrives better than on *SG*, it looks like a streptococcus (Pl. L).

Mibrobacterium flavum is nearly as resistant to heating as *Mbm. lacticum*, and like the latter, thrives poorly at anything over 35°. It also grows very slowly below 20°, and has thus only a slight temperature interval for its development. In contrast to the other microbacteria, it will still thrive in a sugar solution containing 10 % common salt. It can form up to 1 % lactic acid from lævulose, but it is only with monosaccharides that it forms any considerable quantity of acid at all. It forms a finely flaked precipitate in broth, and is even more aerobic than *Mbm. lacticum*. The yellow colour is more strongly apparent on *SG* than *AG*. *Mbm. flavum* (Pl. L) forms clumsy rods, measuring for the most part 0.5 × 1—2 μ, though they can also grow out into threads 10 μ long. When coloured with methylene blue, it is distinctly granulated.

Rods such as strain No. 10 (Pl. L) are frequent in calves' dung. Treatment with methylene blue will as a rule only stain the poles, and they are thus easily confused with small micrococci. Although the surface growth is more often white than yellow, and they are only weak acid formers, they are doubtless closely allied to *Mbm. flavum*.

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[1]) No. 2, however, has now, after 10 years' cultivation, begun to exhibit a slight yellowish-green surface growth, like Nos. 3—6.

[2]) Wiener Klin. Wochenschrift 1900, No. 5.

[3]) Deutsche med. Wochenschr. 1900, p. 263.

In milk, and especially in cheese, we encounter some small GRAM-positive rods, which entirely resemble *Mbm. lacticum*, and can, like the latter, stand heating to 80°, and only grow in the upper part of the agar tube. In stab cultures, the surface growth is a faint yellowish-green, which it can also be, at times, in *Mbm. lacticum*. They form, however, too little acid to be included among the lactic acid bacteria. They curdle milk in the course of a week without altering the reaction to any considerable degree, and they dissolve the casein gradually. In milk to which chalk has been added, some few strains can form up to 80 % *SN*, 54 % *DN* and 6 % *AN*. They therefore also liquefy gelatin, especially that without sugar (*AG*). Dextrose gelatin on the other hand, will not liquefy if closely sown, as the acid formed will impede the action of the proteolytic enzymes. They split up hydrogen peroxide, but do not reduce nitrates. We will for the present call this bacterium *Microbacterium liquefaciens* (Pl. L). It is possible, however, that it may belong to quite another place in the system.

Bacterium bifidum *(Bacillus bifidus)*.

At the commencement of the century, TISSIER[1]) made the discovery that the overwhelming majority of the bacteria in the fæces of breast children did not consist, as with adults, of GRAM-negative coli bacteria, but of a GRAM-positive, irregular rod form, which could be club-shaped, or even forked, wherefore he termed it *Bacillus bifidus* (Pl. LI). As the bacterium does not form spores, it will be correct to alter the name to *Bacterium bifidum*. It is immotile, obligatorily anaerobic, and requires sugar for its development. It forms an acid not precisely defined, but no gas. Casein and gall appear, strangely enough, to impede its growth. It grows for the most part so slowly, that even at the optimal temperature, 37°, it may often take a whole week before the colonies become visible. It is killed by heating to 60°, can still grow at ordinary indoor temperature, and at 40°, albeit extremely slowly. According to TISSIER's investigations, it is said not to be quite so predominant in the fæces of infants reared from the bottle.

We have also investigated a great number of fæces samples both from healthy breast-reared children and healthy bottle-fed infants, the material being procured, partly from private acquaintances, partly through the courtesy of Professor LEOPOLD MEYER, from the Lying-in Hospital. The direct microscopic examination revealed, besides a quantity of more or less GRAM-positive cocci[2]), on the whole not more GRAM-positive than GRAM-negative rods. No constant difference in the flora of fæces from breast childern and fæces of bottle-fed children could be discerned.

It was very difficult to isolate the GRAM-positive rods, and when we finally succeeded, they perished so rapidly that as a rule we had not time to investigate them further. We were never able to keep a strain alive for more than a year. Strains which could be further inoculated from one anaerobic agar tube (high tubes, from which the oxygen of the air was excluded by means of pyrogallate of potassium, according to STRIBOLT's method) to another, often refused to grow in freshly sterilised liquids covered with fluid paraffin

[1]) Recherches sur la flore intestinale des nourrisons. Paris 1900.

[2]) In a single instance, we found only streptococci in the fæces of a breast child. It was stated that both nurse and child were healthy.

(as in the case of the club-shaped strain shown in the photo). Yeast extract is undoubtledly the best source of nitrogen, and with this, we were able, in the case of the two strains shown in the table, to study its relations to the different sugars. Of these strains, No. 11 (the strain from infant III, Pl. LI) is highly forked whereas No. 12 is only very slightly irregular. They behave towards the sugars (Table XXXII) as *Bbm. breve* and *Bbm. longum* respectively, and thus probably represent different species. *Bacterium bifidum* has, however, probably no connection with the betabacteria, and if it were not that we had previously met with strains which, at any rate in a freshly isolated state, formed great quantities of by-products, we should hardly have reckoned *Bact. bifidum* among the true lactic acid bacteria, as, though it does form some dextro-lactic acid, 30—40 % of the sugar fermented is turned into acetic acid. Unfortunately, we did not succeed in preserving any of the isolated strains long enough to enable us to observe whether the quantity of by-products gradually decreased.

The different species of *Bact. bifidum* doubtless constitute a separate genus, possibly forming a connecting link between the lactic acid bacteria and the propionic acid bacteria first isolated by FREUDENREICH and myself[1]). The rod forms coming under this head can, as will be seen from the accompanying photographs (Pl. LI), also be more or less forked. We cannot here enter further into the qualities of the propionic acid bacteria, and I must therefore refer to my Dairy Bacteriology. In Table XXXII will be found, by way of example, details of two strains in their relation to the sugars, I would merely add here, that these strains are also very strong fermenters of inosite.

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[1]) Landwirtschaftliches Jahrbuch der Schweiz. 1906, p. 320.

Key to Identification of

The lactic acid bacteria ferment carbohydrates and higher alcohols to lactic acid. They do not grow with acids therein contained. They are Gram-positive, immotile, sporeless rod- or sphere-forms. We cannot here repeat all nearest related forms.

Rod Forms.

A. Without catalase, reduction

a. Produce only a trace of by-

GENUS: **Thermobacterium.** Form laevo- or inactive lactic acid. Except *Tbm. cereale* they strongly break down casein and thrive well in yeast extract. They never ferment pentoses and frequently not salicin. Long-rods, which grow at 50° or more, but do not on the other hand grow at lower than 22°.

Tbm. helveticum. Inactive lactic acid. Ferments maltose, but not saccharose. Able to form more than 2,7 % lactic acid in milk.

» *Jugurt.* Inactive lactic acid. Does not ferment either saccharose or maltose. Able to form more than 2,7 % lactic acid in milk.

» *bulgaricum.* Laevo-lactic acid. Does not ferment either saccharose or maltose. Forms at most 1,7 % lactic acid in milk.

» *lactis.* Laevo-lactic acid. Ferments both saccharose and maltose. Forms at most 1,7 % lactic acid in milk.

» *cereale.* Laevo-lactic acid. Ferments as a rule both saccharose and maltose. Does not curdle milk.

GENUS: **Streptobacterium.** Form inactive or dextro-lactic acid. Thrive well in yeast extract and as a rule also in milk. Always ferment maltose and salicin and only exceptionally not lactose. Shorter or longer chains of shorter or longer links. Maximum temperature as a rule 35°—40°.

Sbm. plantarum. Mostly inactive lactic acid and no breaking down of casein. As a rule prefers maltose and saccharose to lactose and frequently ferments raffinose, inulin and pentoses.

Sbm. casei. Mostly dextro-lactic acid and breaking down of casein. Prefers as a rule lactose (especially in milk) to saccharose and maltose and only exceptionally ferments raffinose, inulin and pentoses.

b. As a rule produce perceivable amounts of

GENUS: **Betabacterium.** Almost always form inactive lactic acid. Thrive well in yeast extract, but as a rule badly in milk. Never ferment considerable amounts of mannite, inulin, dextrin, starch or salicin. Have a comparatively small mannose fermentation.

Bbm. caucasicum. Prefers arabinose. Ferments no disaccharides out of the Kefir grain.

» *breve.* Ferments arabinose with predilection, frequently xylose too. Maximum temperature 38°.

» *longum.* Never ferments arabinose, but frequently xylose, and as a rule raffinose. Maximum temperature 45°.

B. As a rule catalase, reduction

GENUS: **Microbacterium.** The undermentioned strains form dextro-lactic acid. Thrive badly in yeast extract. Very small rods, only exceptionally more than 0,5 μ thick.

Mbm. lacticum. Ferments maltose and frequently some starch. Slight, often greenish yellow surface growth.

» *mesentericum.* Ferments maltose, raffinose and starch. Strongly curled, white surface growth.

» *flavum.* Does not ferment any di- or polysaccharides. Vigorous yellow surface growth.

the Lactic Acid Bacteria.

ammonia salts or single amino acids, but like the animals require complete proteins or the entire complex of amino that caracterises the separate species, but only note the qualities by which they are best distinguished from the

Sphere Forms.

of nitrate and surface growth.

products besides the lactic acid.

GENUS: **Streptococcus.** Always form dextro-lactic acid and thrive well in milk but not as well or even badly in yeast extract. *Sc. pyogenes,* some strains of which do not generally ferment lactose, however thrives badly in milk. As a rule they only divide in one plane.

α. Mostly shorter or longer chains. Never pentose fermentation.

Sc. thermophilus. Ferments saccharose, but not maltose, dextrin and salicin. Does not break down casein Maximum temperature 45°—50°.

» *cremoris.* Does not ferment saccharose, maltose or dextrin, frequently not salicin. As a rule it breaks down casein. Maximum temperature 35°—38°.

» *mastitidis.* Ferments saccharose, maltose, dextrin, starch and salicin. Does not break down casein.

» *pyogenes.* Ferments saccharose, maltose, dextrin and salicin, frequently starch too. Does not curdle milk.

β. Diplococci as well as longer chains. Mostly pentose fermentation. Always ferment maltose, dextrin and salicin, as a rule also saccharose. Maximum temperature 45°.

Sc. liquefaciens. Ferments sorbite and glycerin. Breaks down casein and liquefies gelatin.

» *glycerinaceus.* Ferments sorbite and glycerin. Does not break down casein nor liquefy gelatin.

» *inulinaceus.* Ferments raffinose and inulin, frequently starch and xylose. Does not break down casein.

» *bovis.* As a rule ferments raffinose, inulin, starch and arabinose. Breaks down casein. Does not grow at lower than 22°.

γ. Mostly diplococci. Always ferment maltose, dextrin and salicin, mostly pentoses too.

Sc. fœcium. Always ferments arabinose, as a rule saccharose too, and frequently raffinose and rhamnose. Does not break down casein. Maximum temperature 50°.

» *lactis.* Never ferments saccharose, raffinose nor rhamnose. Frequently breaks down casein. Maximum temperature as a rule 38°—40°.

gas and other by-products besides the lactic acid.

GENUS: **Betacoccus.** Form laevo-lactic acid or exceptionally inactive lactic acid. Mostly form slime from saccharose. Thrive well in yeast extract, but only now and then well in milk. Never ferment inulin and starch, exceptionally dextrin. On the other hand frequently ferment raffinose. If raffinose fermentation fails, salicin fermentation as a rule fails too. Low optimum temperature.

Bc. arabinosaceus. Ferments arabinose with predilection, frequently xylose too. Diplococci or chains.

» *bovis.* Never ferments arabinose, but frequently xylose. Often irregular forms dividing in two planes.

of nitrate and surface growth.

GENUS: **Tetracoccus.** The undermentioned strains form dextro-lactic acid. Division in two or three planes.

Tc. casei. Ferments arabinose, raffinose and salicin. Does not break down casein nor liquefy gelatin.

» *liquefaciens.* Does not ferment arabinose, raffinose and salicin. Breaks down casein and liquefies gelatin.

» *mycodermatus.* Ferments arabinose, glycerin, sorbite and mannite, but not raffinose and salicin. Does not break down casein, but liquefies gelatin. Mycoderma resembling surface growth.

The Lactic Acid Bacteria, Arranged According to Their Habitat.

The species are arranged after the frequency of their occurrence.

1. Mash, at temp. over 50°.

Tbm. cereale.

2. Sour Dough.

Sbm. plantarum, *Bbm. breve* and *Bc. bovis* (dividing in two directions).

3. Souring Potatoes.[1])

At first, chiefly *Bc. bovis*, *Bc. arabinosaceus*, *Sc. fæcium*, *Bbm. breve* and *Bbm. longum*.
Later, chiefly *Sbm. plantarum* (xylose-fermenting) and betabacteria.

4. Sour Cabbage.

At first, chiefly *Bc. arabinosaceus*, more rarely *Bc. bovis*.
Later, chiefly *Sbm. plantarum*.

5. Souring Beets and Diffusion Slices.

Bc. arabinosaceus, *Sbm. plantarum*, *Bbm. longum* (and *Mbm. mesentericum*).
(In rotten beets we find, besides mould fungi and pectin-fermenting plectridia, aerogenes bacteria and *Bc. arabinosaceus*).

6. Human Fæces.

Microbacteria, *Bact. bifidum*, *Sc. fæcium*, betabacteria, *Sc. glycerinaceus* (and *Sc. liquefaciens*).

The great bulk of the intestinal flora of sucking infants consists of true lactic acid bacteria, whereas in adult human beings, as in adult animals, it is, as we know, composed of coli and butyric acid bacteria.

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[1]) The mass investigated was a mixture of raw and boiled potatoes which had been stored in cold (1) or warm (2) state. The investigation was made partly a couple of months after storage (I), and partly when it was taken out of the pits in March (II). The figures quoted are used in the tables under the finding place of the separate strains.

7. Cowdung.

Tetracocci, *Sc. bovis*, the streptococci not fully identified in Table XXIII, *Bc. bovis*, *Sc. inulinaceus*, microbacteria, *Bc. arabinosaceus* and betabacteria.

8. Calves' Dung.

Microbacteria, *Sbm. plantarum*, *Bc. bovis* and *Sc. fæcium.*

In cowdung, tetracocci of various colours predominate; in calves' dung, on the other hand, rod-shaped lactic acid bacteria predominate.

9. Cows' Milk.

Fresh: Tetracocci (besides cocci not fermenting sugar) and *Sc. liquefaciens.*

Standing at ordinary temperature:

At commencement of souring: *Sc. lactis*, *Sc. cremoris*, *Sc. inulinaceus* and *Bc. bovis.*

Later: *Sbm. casei* and *Sbm. plantarum.*

Standing at 40°—50°:

At commencement of souring: *Sc. thermophilus* and *Sc. fæcium.*

Later: *Tbm. lactis*, rarely other thermobacteria.

Pasteurised at low temperature: *Sc. thermophilus*, *Sc. fæcium* and *Mbm. lacticum.*

Pasteurised at 70°—85°: Chiefly *Mbm. lacticum* (besides spore-formers).

Yoghurt: *Tbm. bulgaricum*, *Sc. thermophilus*, *Sc. fæcium* and *Tbm. Jugurt.*

Kefir-Grains: *Bbm. caucasicum* (besides yeast).

In Kefir also *Sc. lactis*, *Bc. arabinosaceus*, *Sbm. casei* and *Sbm. plantarum.*

Condensed milk and sugar-containing salt pickle: Tetracocci.

10. Butter from Soured Cream.

Fresh: *Sc. cremoris* and *Sc. lactis.*

Later: *Sbm. casei*, *Sbm. plantarum*, Tetracocci (yeast and mould).

11. Hard Rennet Cheeses.

Not highly heated (as for instance Danish dairy cheese).

Fresh: *Sc. lactis*, *Sc. cremoris*, *Tc. liquefaciens*, betacocci and *Sc. liquefaciens.*

Later: *Sbm. casei*, *Sbm. plantarum*, *Bbm. breve*, *Sc. glycerinaceus*, *Tc. casei* and microbacteria also gelatin-liquefying forms), more rarely the species occuring in fresh cheese.

Highly heated cheese (e. g. Emmental cheese).

Fresh: *Sc. thermophilus*, *Tc. liquefaciens* and *Tbm. helveticum.*

Later: Thermobacteria, *Sbm. casei*, betabacteria, streptococci and tetracocci.

On surface of cheese: *Sc. glycerinaceus*, *Tc. mycodermatus*, besides other tetracocci and cocci not fermenting sugar (also *Bact. limburgensis*, mould, yeast, etc.).

In the case of Danish dairy cheese, no considerable difference was found between the flora in cheese from pasteurised and cheese from raw milk, which is simply explained by the fact that these cheeses are given so much buttermilk that it is the bacteria of the latter which chiefly dominate there.

Experiments with a view to utilising the isolated species.

It would be natural, of course, to test our lactic acid bacteria in practice at the places from where they were isolated. There is no doubt, for instance, that the loss of dry matter in souring of potatoes, beets and other fodder could be reduced considerably if the gas developing bacteria there abounding could be at once subdued by rich inoculation with such lactic acid bacteria as thrive well in the vegetable matter concerned. Experiments on a large scale in this direction were also planned by me in co-operation with the State Plant Cultivation Committee („Planteavlsudvalg") but were relinquished for the time being owing to scarcity of fodder.

We have attained some extremely favourable results with sour cabbage, at the *Ama* preserving factory. Cabbage inoculated with *Bc. arabinosaceus*. No. 19 and *Sbm. plantarum* No. 37 developed a much better aroma and was more tender than cabbage which had soured spontaneously.

Under the heading of *Sc. cremoris* I have given several hints which will be of value in the production of starters for souring of cream.

Particularly interesting are the cheese bacteria, and it was indeed the desire to study these further which led to the undertaking of the present work. Simultaneously with our bacteriological investigations therefore, we have carried out a great number of cheese-making experiments, in order to ascertain the effect produced in cheese by the bacteria found in cheese — and especially in Danish dairy cheese.

The experiments were carried out with sterile apparatus and with so called iced milk from "The Copenhagen Milk Supply". The milk was previously partly freed from germs, either by heating for half an hour at 70°, in the cheese vat itself, or by twentyfour hours' treatment with 1.5 % hydrogen peroxide at 50° in an acid demijohn. The surplus of hydrogen peroxide was removed, immediately before adding the rennet, by means of HEPIN, an extract of ox liver, containing catalase, placed on the market by the BEHRING works, at Marburg. By this latter method, previously employed by GERDA TROILI-PETERSSON[1]), the power of the milk to coagulate with rennet is less impaired, but the milk

[1]) Centralblatt f. Bakteriologie. II. Abt. 1909, Bd. 24, p. 343. According to our investigations, nearly 1 ‰ H_2O_2 must be added to the milk in order constantly to prevent the development of yeast and bacteria. The hydrogen peroxide does not, however, impede the action of the proteolytic enzymes in the milk, and milk to which hydrogen peroxide has been added will therefore curdle in the course of 2—3 weeks, and then again dissolve. After six months, milk with 2 ‰ H_2O_2 was found to contain 79 % *SN* and 10.5 % *DN*.

is not rendered more free from germs than by heating to 70°. The bacteria of putrefaction were subdued in the experimental cheeses inoculated with lactic acid bacteria; whereas in the control cheese not inoculated, they developed so abundantly that these latter often contained more *SN* than the former. We were therefore obliged to disregard the results of the chemical investigations, and be content with a purely practical estimate, from taste and structure alone. If an experimental dairy should ever be established in Denmark, we hope to be able to resume the experiments with milk containing fewer bacteria. For the rennet, we used HANSEN's tablets, these being practically free from germs, which is by no means the case with the usual rennet extracts.

As our experimental cheeses constantly proved better than our control cheeses, we can only say that all the lactic acid bacteria thus tested produce a favourable effect upon cheese, if only by subduing the putrefaction bacteria and furthering the proteolytic action of the rennet. An exception, however, is found in *Sc. liquefaciens*, which, as FREUDENREICH has already shown, renders the cheese bitter and makes it run. In cheeses with pure cultures of this streptococcus, we found up to 80 % *SN*. *Tc. liquefaciens* also can make the cheeses rather soft, but can also give them, as we have noted before, a flavour resembling that of "Danish Swiss cheese", or "Russian Steppe cheese". The best effects are obtained with *Sbm. casei*, which is a cheese ripening bacterium par excellence, but also the casein-splitting strains of *Sc. lactis* and *Sc. cremoris* gave an extremely good cheese, so that we can willingly agree with BARTHEL[1]) that a greater importance should be ascribed to such streptococci in the ripening of cheese than has hitherto been conceded. Even *Sc. glycerinaceus*, which does not break down casein, had a favourable effect on the ripening of the cheese. *Sc. fæcium*, *Sc. thermophilus*, *Sc. inulinaceus*, *Sbm. plantarum*, betacocci and betabacteria do not as a rule affect the flavour. Vigorous strains of the two last-mentioned groups can occasion some formation of holes. The great importance attaching to certain thermobacteria, especially *Tbm. helveticum*, in the ripening of strong heated cheeses, is a point we need not here discuss further. In non-cooked cheeses, where cooling takes place rapidly, they cannot of course produce any affect.

It will be seen then, that many different species of lactic acid bacteria contribute to the ripening of cheese, but there is hardly any doubt that a cheese — as has been proved in the case of Emmenthal cheese — only obtains the proper character when certain definite species are predominant. The bacteria to be used for the ripening of cheese must, like those used for souring of cream, be freshened up for a time in milk, and it will be necessary to make sure that the casein-splitting strains have not lost this power.

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[1]) Meddelande Nr. 97 från Centralanstalten för försöksväsendet på Jordbruksområdet. 1914. do No. 171. 1918. As regards the ripening process of cheese otherwise, I can refer to my Dairy Bacteriology 1916.

Summary.

1. The present work is generally concerned with the true lactic acid bacteria, and more particularly with those which are of importance to the dairy industry. The strains described number 330 in all, most of these having been under observation for many years in order to ascertain the constancy of their qualities.

2. The true lactic acid bacteria form a great natural group of immotile, sporeless, Gram-positive cocci and rods, which in fermenting sugar form chiefly lactid acid. In a freshly isolated state however, some few species approach the pseudo lactic acid bacteria, the coli and aerogenes bacteria, by forming fairly considerable quantities of by-products. These latter consist principally of acetic acid and carbonic acid, at times also succinic acid; more rarely, mannite and hydrogen. That we nevertheless reckon such species among the lactic acid bacteria is due to the fact that they resemble them in all other respects, and gradually lose, partially or entirely, the power of forming by-products, thus ending by becoming true lactic acid bacteria.

3. As the sources of energy are utilised more completely where the quantity of by-products formed increases, the formation of these is a sign of vitality. An exception, however, is the formation of acetic acid, which is proportionately greatest under unfavourable conditions, as for instance where air and acid abound, and at too high temperatures.

4. The lactic acid formed can be either dextro-rotary or lævo-rotary. Where equal quantities of these two acids are formed, we obtain pure inactive lactic acid. Strains of bacteria which in milk form pure dextro-, or pure lævo-lactic acid, will also in any nutritive broth likewise form respectively dextro- and lævo-lactic acid, whether the source of energy be alcohols, aldoses, ketoses, pentoses, hexoses or polysaccharides. Strains which in milk form equal quantities of dextro- and lævo-lactic acid will as a rule also under other conditions maintain the equilibrium between the two acids, whereas strains which in milk form more of the one than of the other will under unfavourable conditions generally form only the one which they more easily produce. Even in milk, indeed, such bacteria may end by forming only the one acid, and it may happen without reducing their total production of acid.

5. As the modification of the lactic acid is entirely independent of the stereochemical structure of the sugars, and determined solely by the species of bacteria, we must suppose that dextro- and lævo-lactic acid are formed each by their own particular enzyme.

6. In the pure lactic acid fermentation, the hexoses are simply split up into two molecules of lactic acid; the pentoses, on the other hand, are not, as might be expected, split up into one molecule of lactic acid and one molecule of acetic acid, but as a rule according to the formula:

$$6\ C_5H_{10}O_5 = 8\ C_3H_6O_3 + 3\ C_2H_4O_2$$
$$6 \text{ pentoses} = 8 \text{ lactic acid} + 3 \text{ acetic acid.}$$

which thus gives proportionately more lactic acid.

7. The enzymes which hydrolyse the disaccharides appear to be endo-enzymes, and we must therefore suppose that these sugars are taken in as such. There is consequently nothing to prevent the disaccharides from being better nutriment than the monosaccharides of which they are composed, and as regards the betacocci in particular, they can form slime from cane sugar, but neither from dextrose nor from lævulose, just as various other lactic acid bacteria likewise form slime from lactose but neither from dextrose nor from galactose. In the latter case, however, the nitrogenous nourishment is also of importance, as the slime formation only occurs in milk.

8. Of the four hexoses: lævulose, glucose, mannose and galactose, the last is as a rule that which the lactic acid bacteria find most difficulty in fermenting. Some few species however are altogether incapable of fermenting mannose, and will then often not attack salicin either. In the case of some few species, the power of fermenting mannose and that of fermenting arabinose are inversely proportional.

9. Only saccharose-fermenting bacteria are able to ferment raffinose. Bacteria which ferment starch can also ferment glycogen, and vice versa. The same enzymes therefore, are required to affect vegetable and animal starch.

10. The true lactic acid bacteria never ferment gum arabic, erythrite and adonite, and only rarely dulcite and inosite. The fermentation of the two last is in any case only slight.

11. In studying a bacteria, it is not sufficient merely to investigate which sugars it ferments at all, but the quantity of acid formed must be estimated accurately in order to determine in what order the different sugars are preferred. The order of preference of the sources of energy is our most important means of identification but may however, like any other character, lead to serious errors if employed alone.

12. Only few lactic acid bacteria can thrive altogether without sugar, but all of them grow as long as there is only a trace of sugar present. In the case of the streptococci, and the tetracocci, the sugar optimum lies between ½—2 %. An exception, however, is formed by *Streptococcus cremoris*, which, like the betacocci, prefers 5—10 % sugar. The sugar optimum of the rod forms lies between 2—5 %. A sugar concentration of 2 % is, however in all cases extremely favourable.

13. The lactic acid bacteria are as a rule but poorly supplied with proteolytic enzymes. Some strains have no effect at all worth mentioning either upon peptones or casein (the majority of betacocci, betabacteria and partly also of the species *Streptobacterium plantarum*); others affect peptones, but not casein (most of the streptococci, and all strains of the species *Tetracoccus casei*); others again both peptones and casein (most of the tetracocci and of the species *Sc. lactis*, *Sc. cremoris*, *Sc. bovis* and *Sbm. casei*). The active enzymes are in all these cases endo-enzymes (probably erepsin), which act only in nearly

neutral liquid. Only *Sc. liquefaciens* and some few tetracocci give off proteolytic enzymes in a living state. These liquefy gelatin and can act in a slightly acid liquid, but are most powerful, however, with neutral reaction.

14. The cocci which split up casein decompose it gradually through peptones to amino-acids; the casein-splitting rod forms, however, peel off the mono-amino-acids from the casein molecule without previous formation of peptone. From the peptones, the lactic acid bacteria appear to form a quantity of polypeptides, which are not precipitated by phosphotungstic acid.

15. The true lactic acid bacteria are incapable of breaking down amino-acids, and they, therefore, in splitting up proteins, do not form more ammonia than is present in the protein molecule as such.

16. In accordance with this, the true lactic acid bacteria are, in contrast to the pseudo lactic acid bacteria, unable to thrive with single amino-acids or ammonia salts as source of nitrogen, but demand a nitrogenous nourishment as complicated as do the animals, viz. genuine proteins or the entire complex of amino-acids therein contained.

17. As the lactic acid bacteria are as a rule not provided with ectoenzymes, the proteins must be given in a state of solution or in colloid form. Casein is particularly suitable in the finely divided form in which it occurs in milk. The digestion is, however, rendered easier as a rule by the addition of rennet. Among other genuine proteins, gluten and legumin (dissolved in sodium phosphate) can be used. Gelatin, on the other hand, is a very bad source of nitrogen. Many lactic acid bacteria will comparatively rapidly lose the power of utilising casein, just as on the other hand they can gradually accustom themselves to other sources of nitrogen.

18. Inactivised blood serum is, even with the addition of potassium phosphate, a poor source of nitrogen even for pathogenic streptococci. LIEBIG's extract of meat, and casein pepton, on the other hand, are excellent sources of nitrogen for all lactic acid bacteria. The microbacteria, however, do not thrive so well with the meat extract. Yeast extract had a very specific action, being an extremely bad source of nitrogen for pathogenic streptococci, but by far the best nitrogen source for the thermobacteria and streptobacteria. Nevertheless, casein peptone is generally to be preferred, on account of its light colour. WITTE peptone is a far poorer source of nitrogen than casein peptone, and as it gives abundant deposits with acid, it is ill-suited to solid substrates intended for cultivation of strong acid formers. In agar stab cultures, for instance, the stab is rendered altogether invisible.

19. Many lactic acid bacteria do not grow at all with only ½ % *Witte* peptone, answering to 0.07 % *N*. All thrive the better, the greater the quantity of assimilable nitrogen at their disposal. The betacocci and betabacteria, however, are impeded by concentrations of 2 % *N*. In the case of casein peptone or yeast extract, the effect is not as a rule increased to any essential degree by using more than corresponds to 0.5 % *N*. For our nutritive substrates therefore, we always use the last mentioned sources of nitrogen at these concentrations.

20. That the lactic acid bacteria thrive better with increasing concentration of nitrogen, is essentially due to the fact that the organic nitrogenous nourishment acts as a buffer, and the fermentation of sugar is therefore increased with increasing quantity of

nitrogen in the nutritive substrate. That *Witte* peptone is inferior as a source of nitrogen to casein peptone and this latter — in the case of the strongest acid-formers — again poorer than yeast extract, lies to some extent in the fact that the buffer action of these sources of nitrogen rises in the order given.

21. That the difference between the mentioned sources of nitrogen is not exclusively due to their different buffer action, however, is distinctly evident from the relation of the lactic acid bacteria to the sugars. Only a nitrogenous nourishment absolutely sufficient in all respects will enable the lactic acid bacteria to produce the many heterogeneous enzymes (invertase, maltase, lactase, inulinase, etc.) which are required to hydrolyse di- and polysaccharides. It is therefore necessary to know which are the best sources of nitrogen for the different bacteria before we can judge which sugars they are able to ferment at all.

22. Sugar fermentation should not, however, be tested only with a good source of nitrogen, but also with a poorer one; this will give a more complete impression as to which sugars are preferred.

23. The lactic acid fermentation is impeded not only by the hydrogen ions, but also by the lactate ions. The better the buffer action of the nutritive substrate, the more will these latter make themselves felt. As, however, the lactate ions are not nearly so dangerous to the life of the lactic acid bacteria as the hydrogen ions, nutritive substrates with good buffer action should be used for preservation cultures. In agar with only ¼ % dextrose and ½% nitrogen in the form of casein peptone, we have succeeded in preserving lactic acid bacteria unweakened for over three years, and this, be it noted, without re-inoculation.

24. As the aerogenes bacteria will, with a good nitrogenous nourishment, turn all the sugar into gas, and render the substrate alkaline, they are better preserved in substrates with less abundant nitrogenous food. The same applies to the fluorescent bacteria, strong alkali formers, and the more aerobic micrococci. Many of these can be well preserved in water with 2 % soluble starch.

25. Like most other bacteria, the lactic acid bacteria are also able to grow with hardly perceptible quantities of inorganic salts. Potassium phosphate is the most important nutritive salt, and furthers the development of the lactic acid bacteria in increasing quantities up to 2 ‰ or even more (this, however, with a single exception). The favourable effect of this salt, however, depends like that of the nutritive substrate, partly upon buffer action. This is very distinctly seen in the case of the aerogenes bacteria, which turn all the sugar into gas, with great quantities of potassium phosphate.

26. There is considerable difference in the amounts of common salt which the different species of lactic acid bacteria can stand. As a rule, 2.5 % will produce no detrimental effect; certain species are slightly impeded, others advanced in their development thereby. With 5.5 %, all are impeded, and 10.5 % will in most cases stop the growth entirely. An exception, however, is formed by the tetracocci, which can as a rule stand 15.5 % commom salt.

27. Important characters in a bacterium are the minimal, optimal and maximal temperatures for its vital activity, as also the maximal temperature at which it can live at all.

28. The optimal temperature for the acid formation, as also for the proteolytic action often lies slightly below the optimal temperatures for growth.

29. The optimal temperature is not affected by the conditions of nourishment, or by the vitality of the bacteria. The minimal and maximal temperatures, on the other hand, are to a certain degree affected, and weakened strains therefore exhibit far steeper temperature curves than those whose vitality is unimpaired.

30. Some few strains of *Sc. cremoris* and of the betacocci can grow already at 3°, whereas *Sc. bovis* and the thermobacteria do not as a rule develop until past 20°. *Sc. fæcium* and the thermobacteria thrive well at 47½—50°; some thermobacteria, indeed, can even grow at over 50°. *Sc. glycerinaceus*, *Sc. liquefaciens*, and *Sc. thermophilus*, as well as the coli and aerogenes bacteria, grow well at 45°. The majority of the true lactic acid bacteria on the other hand exhibit poor growth already at 37½—40°, and their optimal temperature lies at 30° or below this. The pathogenic streptococci and *Bacterium bifidum* thrive best at 35—37°, and *Sc. thermophilus*, the thermobacteria and *Betabacterium longum* at 40° or over.

31. In determining the maximal temperature for life (the death temperature), the number of cells which can stand the temperatures used should be noted, as it is of far greater practical importance to know how the majority of cells behave than what the few specially resistant individuals can stand. It is only an extremely insignificant number of cells in a bacteria species which can stand the so-called death temperature.

32. The death temperature lies, for the pathogenic forms, below 60°. The betacocci are killed, when not protected by slime, at 65°, and the most common lactic acid bacteria of milk, *Sc. lactis* and *Sc. cremoris* at 70°, while most of the other lactic acid bacteria can often stand 70—75°. The greatest power of resistance to heat is shown by *Microbacterium lacticum*, which does not always perish even at 85°. The duration of the heating in these experiments was a quarter of an hour.

33. The true lactic acid bacteria — in contrast to most other bacteria — lack catalase entirely. An exception is formed by the more aerobic forms; the tetracocci and most of the microbacteria, but these two groups of bacteria cannot be reckoned entirely to the true lactic acid bacteria.

34. The true lactic acid bacteria are, in contrast to the pseudo lactic acid bacteria, unable to reduce nitrate to nitrite. The tetracocci and microbacteria are here again exceptions. It is, however, by no means all the hydrogen-peroxide-splitting lactic acid bacteria which reduce nitrate to nitrite, in closely related strains, one may reduce, and the other not.

35. The lactic acid bacteria have as a rule a great aversion to air. They form small colonies on plates, and only an extremely thin veil in streak cultures, and they grow evenly throughout the whole of the stab without any considerable surface growth. Some few thermobacteria even grow better deeper down, and *Bacterium bifidum* is obligatorily anaerobic. Exceptions are the microbacteria, which with good nitrogenous nourishment will as a rule exhibit some surface growth, and the tetracocci, which most frequently have a strong surface growth.

36. The true lactic acid bacteria are as a rule not chromogenic. Some few pathogenic streptococci can on casein peptone agar form a red colouring matter in the stab, and *Streptococcus mastitidis* forms an orange colour in casein peptone broth with soluble starch. *Mi-*

crobacterium flavum develops an orange colour on the surface, and many tetracocci form yellow, brown, orange or even red colours on the surface.

37. The magnitude and appearance of the surface growth, as well as the formation of colouring matter, are among the most variable qualities in the bacteria.

38. When lactic acid bacteria are cultivated in milk, they are at their first stage of development surrounded by a more or less distinct capsule. This can, under certain conditions (in the case of the cocci only at lower temperatures) swell up and turn into slime. The power of forming slime in milk is, however, very variable, and even though it may occur more frequently in strains of one species than in those of another, it cannot be used as species character. We have often encountered this power in *Streptococcus cremoris* and in the thermobacteria which form inactive lactic acid, and, in these cases, it was always in strains of particularly strong vitality. When, on the other hand, a strain of one of the other species has temporarily proved slimy, it has generally been defective in one respect or another.

39. The variations we have encountered in the lactic acid bacteria can as a rule be explained as a further development of already existing tendencies, or more frequently, as the result of weakness or degeneration.

40. The manner in which a bacterium is inclined to vary is often one of the most characteristic of all its qualities.

41. In a bacteria culture, most of the individuals are but slightly resistant, and as a rule weakened in one or another respect, and it is therefore necessary to inoculate abundantly in order to make sure of transferring some of the individuals of unimpaired vitality, which mark the culture as a whole.

42. Consequently, next to unsuitable composition of the nutritive substrate, and too high preservation temperature, the chief cause of the frequent degeneration met with in laboratory cultures is the slight amount of the inoculating material generally used.

43. As shown in my paper "The Main Lines in the Natural Bacteria System", out of the three morphological qualities of bacteria: shape of the cells, formation of spores, and arrangement of the flagella, the last is the one which should primarily be used. For we find that all bacteria which are able to live exclusively upon inorganic nourishment, and derive their energy chiefly through simple oxygen processes, have the flagella terminally set, whereas those requiring more complex organic food, and producing the more typical fermentation processes, have the flagella distributed throughout the whole of the cell. On this basis, therefore, I have divided the bacteria into two orders: *Cephalotrichinæ* and *Peritrichinæ*. As the true lactic acid bacteria have no flagella, we cannot determine their position in the system from their morphological qualities, but as they require as complicated food as do the animals, there is no doubt that their place is in the order of *Peritrichinæ*.

44. By taking the arrangement of the flagella together with the biological characters as the first principle of division, we obtain within the same family of bacteria both spherical and rod forms, as well as screw forms, and the old generic names, which only express the shape of the cells, are therefore no longer sufficient, but require to be supplemented by various prefixes. If, in any exceptional instance, we use the old terms, then it will at

any rate be necessary to restrict their meaning. By streptococci, for instance, we understand cocci which divide in one direction and form dextro-lactic acid. Cocci which divide chiefly in one direction, and form lævo-lactic acid, we have called betacocci, from their being most frequently found on beets. In the older bacteriology the generic name *Diplococcus* for streptococci, which fall away at once after division has already been relinquished; similarly also, the generic name *Micrococcus* should be discarded for sarcinæ without coherence. The cocci which divide in several directions, and form lactic acid, we have given the generic name *Tetracoccus*. Micrococci and sarcinæ which do not form lactic acid lie, of course, outside the scope of the present work.

45. On the basis of the present investigations, we have succeeded in dividing the true lactic acid bacteria into 5 genera and 22 species. To these should further be added *Bacterium bifidum*, which from the true ramification of the cells occupies an exceptional position. Our system of division will be seen from the table on p. 106—107. Here is also put down the group B (with 2 genera and 8 species) of related bacteria, which cannot, however, be reckoned among the true lactic acid bacteria; they only mark the boundary on the one side, as the coli and aerogenes bacteria mark the boundary on the other side.

46. Simultaneously with these investigations, we have made experiments with a view to utilisation of the isolated species. It was found that by inoculating of cabbage with *Bc. arabinosaceus* and *Sbm. plantarum*, an excellent sour cabbage was obtained, and it was also found that most important bacteria in the ripening of the not strongly heated cheeses are *Sbm. casei* together with *Sc. lactis* and *Sc. cremoris*. In the case of some few varieties of cheese, *Tc. liquefaciens* is doubtless also of importance. It has been shown in previous works that *Tbm. helveticum* ranks first in importance for the cooked hard cheeses.

INDEX

THE LACTIC ACID BACTERIA

PLATES

INDEX

All the microphotographs are magnified 1000 times.
The preparations are, where not otherwise stated, made with indian ink according to the method of *Burri*.

W = *Witte* Peptone, C = Casein Peptone, Y = Yeast Extract.

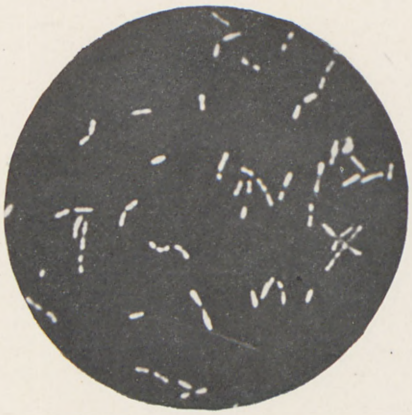
AG = Alkaline sugar-free C-Gelatin.

SG = Neutral Dextrose C-Gelatin.

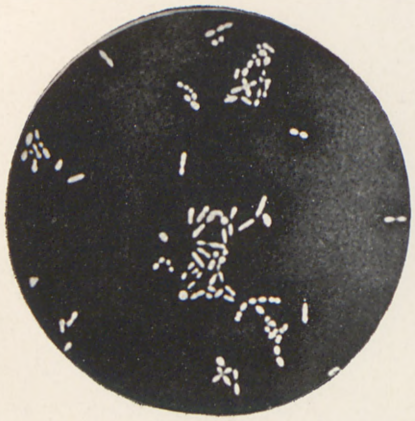
Agar = Neutral Dextrose C-Agar.

Streptococcus lactis.

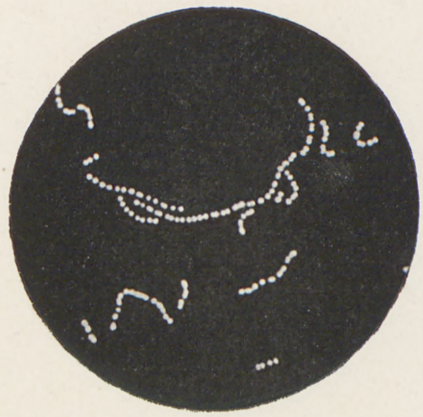
No. 4 C-Bouillon, 4 Days, 30 °.



No. 4 Agar Streak, 1 Day, 30 °.



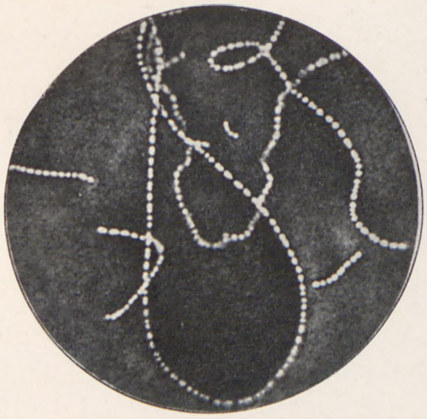
No. 4 Agar Streak, 1 Day, 37,5 °.



Nr. 4 Agar Streak, 10 Days, 10 °.

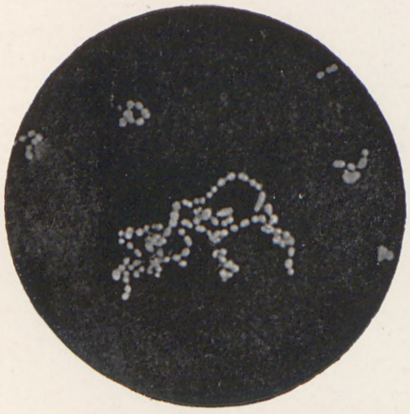
No. 4 S. G. - Plate, 4 Days, 20 °.

Streptococcus lactis.



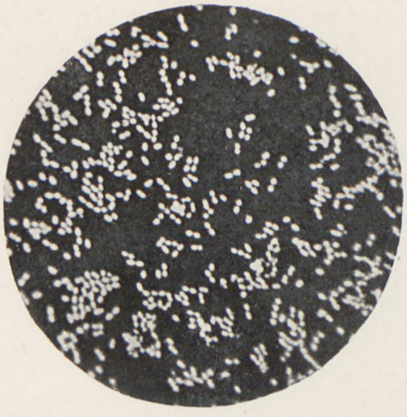
No. 7, C-Bouillon, 3 Days, 10 °.

Nr. 7, Agar Streak, 10 Days, 10 °.



No. 7, Agar Streak, 3 Days, 37,5 °.

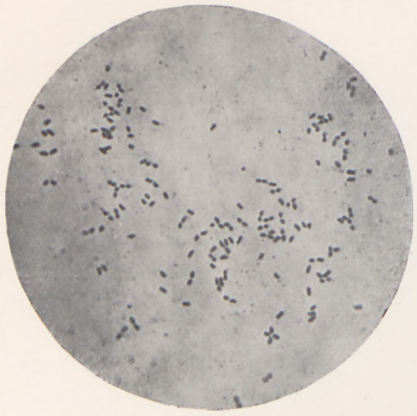
No. 7, A. G. - Plate, 5 Days, 20 °.



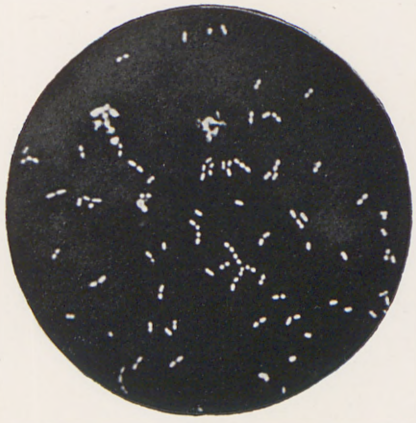
No. 17, C-Bouillon, 1 Day, 30 °.

Autotypi. Pacht & Crone's Eftflg.

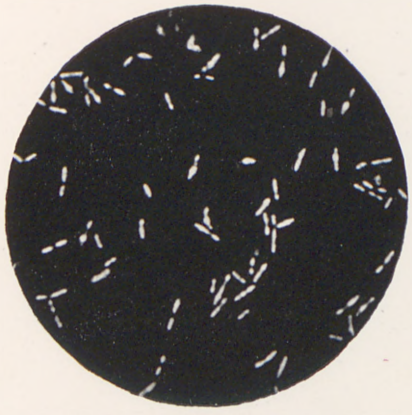
Streptococcus lactis.



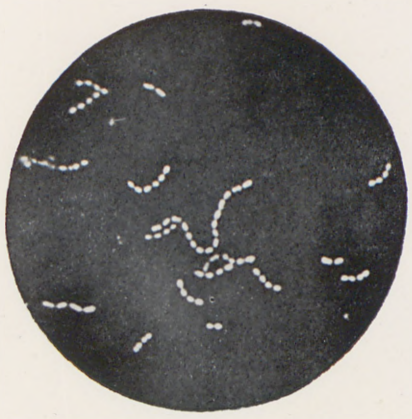
No. 13, Milk, 1 Day, 30°, *Gram* stained.



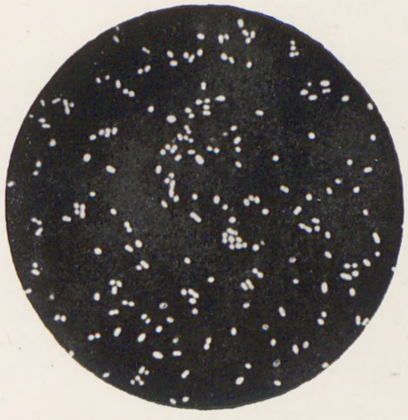
No. 13, C-Bouillon, 2 Days, 30°.



No. 13, Agar Streak, 6 Days, 30°.

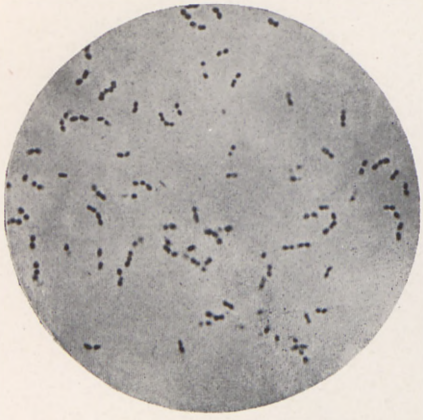


No. 13, Agar Stab, 6 Days, 45°.



No. 13, AG-Stab, 6 Days, 20°.

Streptococcus lactis.



No. 14, Milk, 2 Days, 30°, *Gram* stained.

No. 14, Agar Streak, 1 Day, 30°.

No. 14, Agar Streak, 10 Days, 10°.

Streptococcus cremoris.

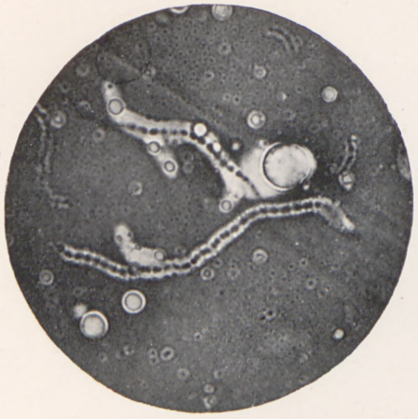
No. 1, C-Bouillon, 3 Days, 30°.

No. 1, Agar Streak, 1 Day, 30°.

No. 1, Agar Streak, 10 Days, 10°.

Autotypi. Pacht & Crone's Eftflg.

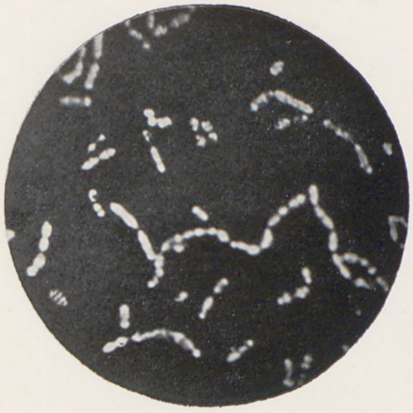
Streptococcus cremoris.



No. 5, Milk, 1 Day, 30°, Methylene Blue.

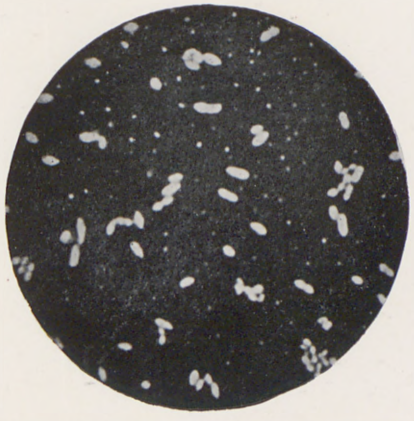
No. 5, Milk, 1 Day, 30°, *Gram* stained.

No. 5, C-Bouillon, 1 Day, 30°.



No. 5, Agar Streak, 1 Day, 30°.

No. 5, Agar Stab, 2 Days, 30°.

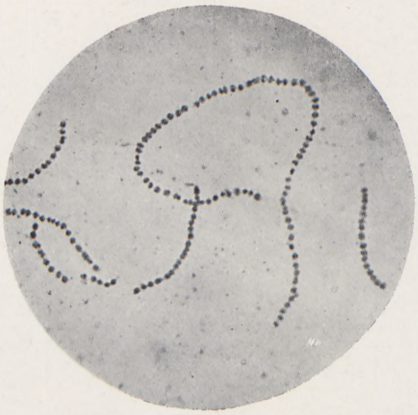


No. 5, S G-Plate, 8 Days, 20°,

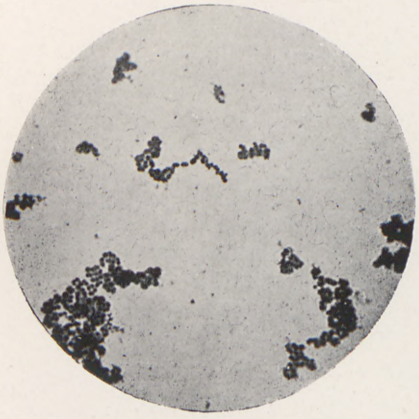
Autotypi. Pacht & Crone's Eftflg.

Streptococcus cremoris.

Nr. 7, Agar Streak, 2 Days, 30°.



No. 18, Milk, 1 Day, 30°, *Gram* stained.



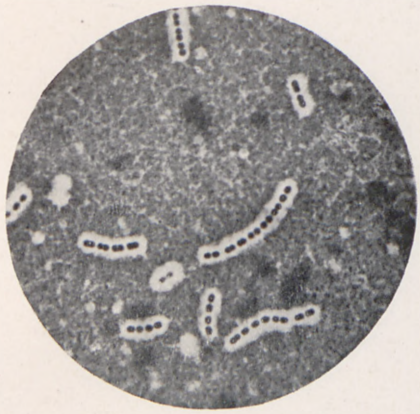
No. 18, C-Bouillon, 1 Day, 30°, Fuchsin.

No. 18, Agar Streak, 19 Days, 10°.

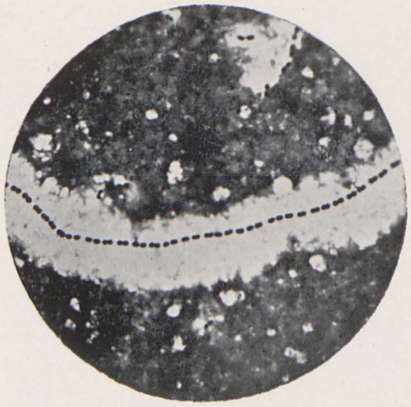
No. 18, S G - Plate, 4 Days, 20°.

No. 18, A G - Plate, 7 Days, 20°.

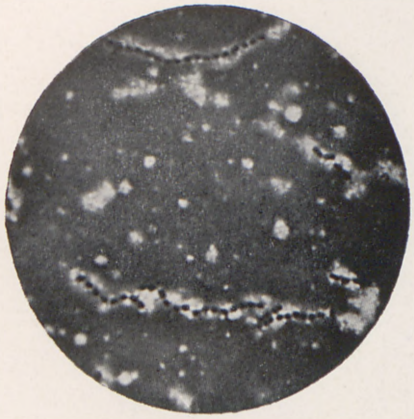
Streptococcus cremoris.



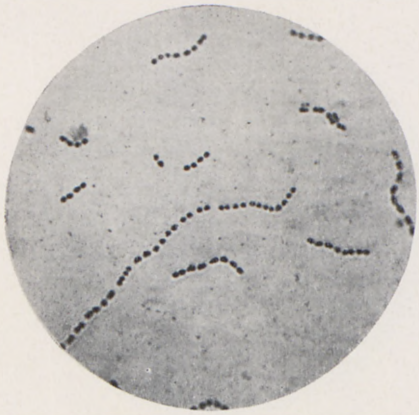
No. 19, Milk, 1/2 Day, 20°, Fuchsin.



No. 19, Milk, 1 1/2 Days, 20°, Fuchsin.



No. 19, Milk, 3 Days, 20°, Fuchsin.



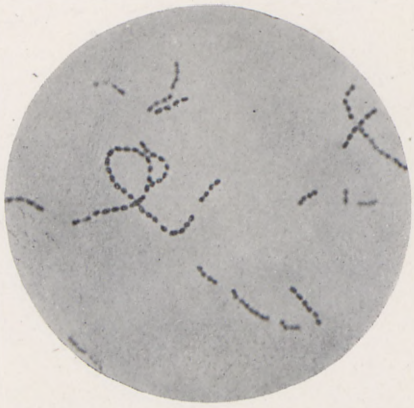
No. 19, Milk, 5 Days, 20°, *Gram* stained.

No. 19, C-Bouillon, 1 Day, 30°.

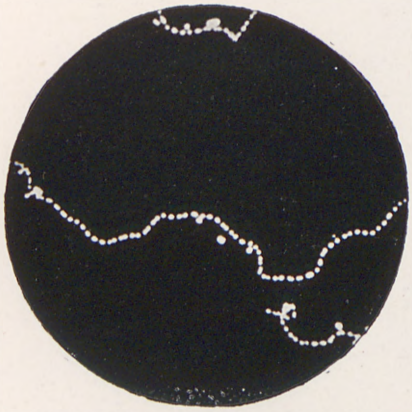
No. 19, Agar Streak, 30 Days, 5°.

Streptococcus cremoris.

No. 19, S G - Plate, 6 Days, 20 °.



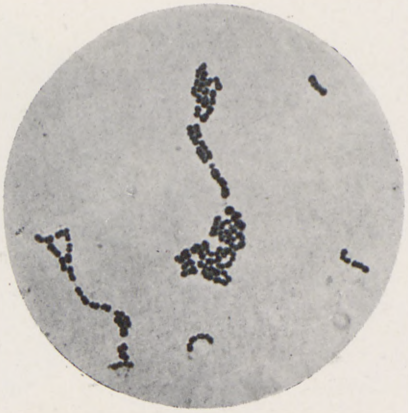
No. 20, Milk, 1 Day, 30 °, *Gram* stained.



No. 20, C-Bouillon, 2 Days, 30 °.

No. 20, Agar Streak, 27 Days, 5 °.

No. 20, Agar Streak, 1 Day, 30 °.



No. 20, Agar Streak, 1 Day, 30 °, Fuchsin.

Autotypi. Pacht & Crone's Eftfg.

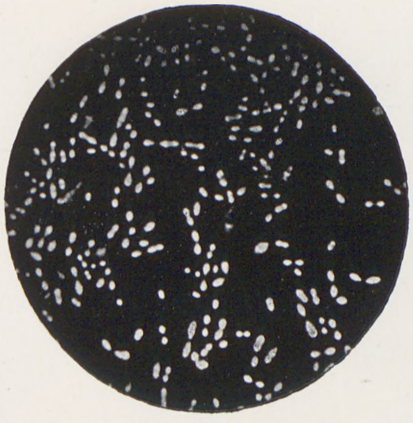
Streptococcus cremoris.

No. 20, S G - Stab, 6 Days, 20°.

No. 20, A G - Stab, 6 Days, 20°.

No. 2, C-Bouillon, 2 Days, 30°.

No. 2, Agar Streak, 1 Day, 30°.

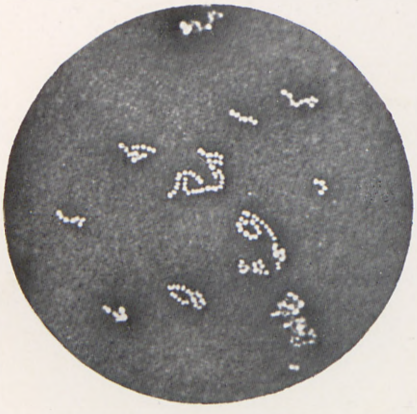


No. 2, Agar Streak, 2 Days, 37,5°.

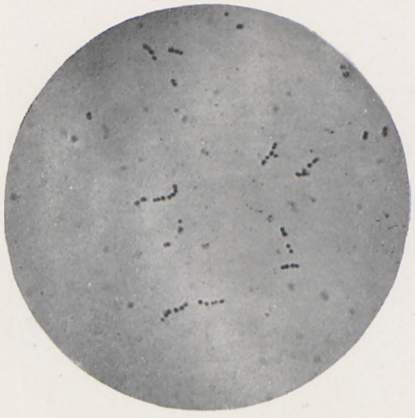
Streptococcus mastitidis.

No. 2, C-Bouillon, 2 Days, 30°.

No. 2, S G - Stab, 8 Days, 20°.

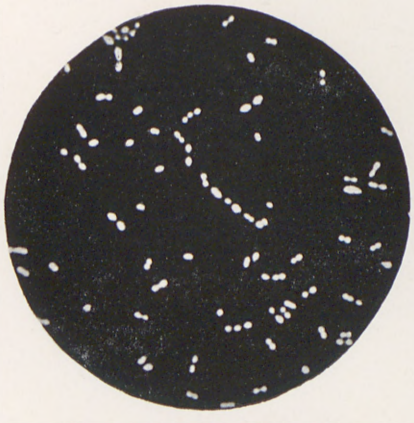


No. 2, A G - Stab, 8 Days, 20°.



No. 3, Milk, 2 Days, 30°, *Gram* stained.

Streptococcus thermophilus.



No. 5, Agar Streak, 2 Days, 30°.

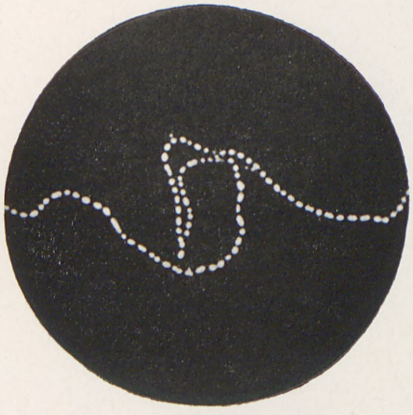
No. 5, Agar Streak, 2 Days, 45°.

Autotypi. Pacht & Crone's Eftflg.

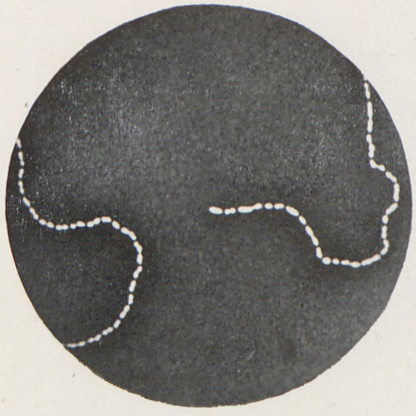
Streptococcus thermophilus.

No. 3, Agar Streak, 10 Days, 30°.

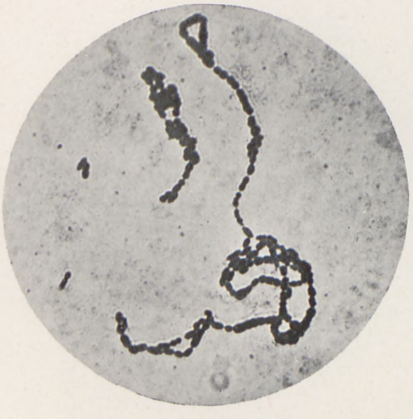
No. 3, Agar Streak, 10 Days, 45°.



No. 3, C-Bouillon, 4 Days, 45°.



No. 4, C-Bouillon, 4 Days, 45°.

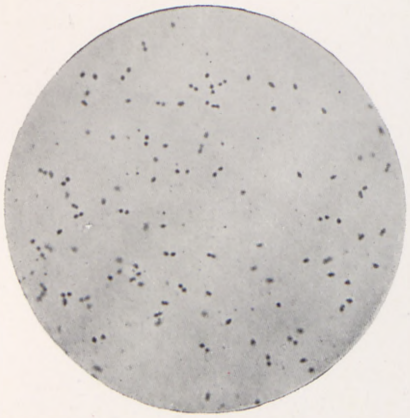


No. 4, Agar Streak, 4 Days, 45°, Fuchsin.

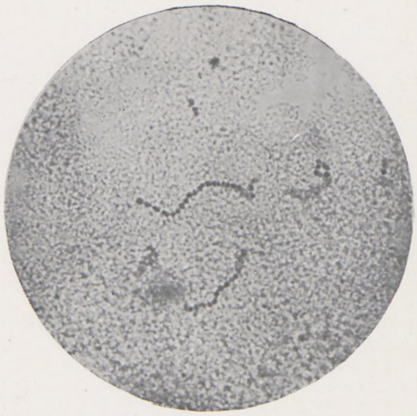
No. 4, Agar Streak, 4 Days, 45°.

Autotypi. Pacht & Crone's Eftflg.

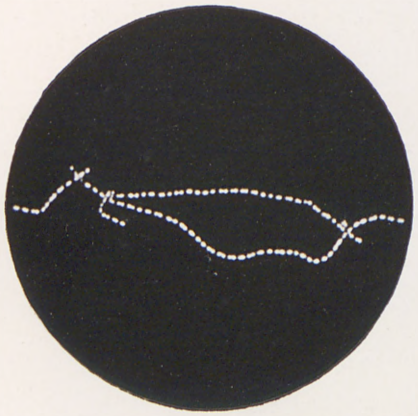
Streptococcus thermophilus.



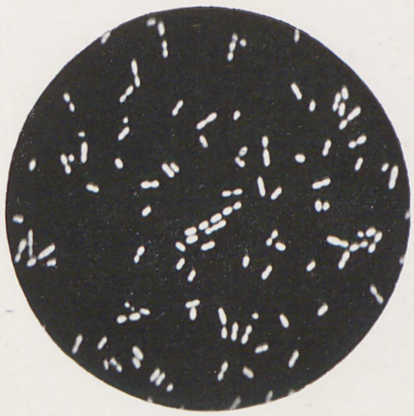
No. 2, Milk, 1 Day, 30°, *Gram* stained.



No. 2, Milk, 1 Day, 45°, *Gram* stained.

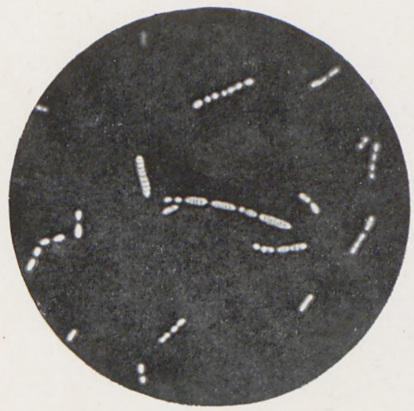


No. 2, C-Bouillon, 1 Day, 30°.



No. 2, Agar Streak, 1 Day, 30°.

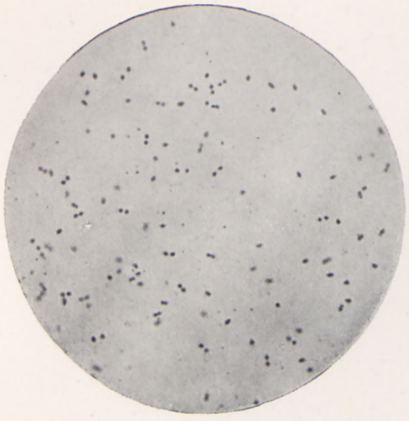
No. 2, S G-Plate, 31 Days, 20°.



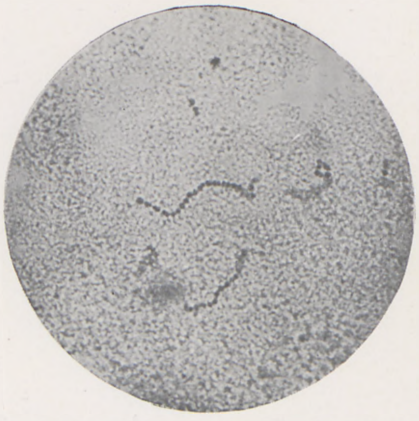
No. 2, Agar Streak, 1 Day, 45°.

Autotypi. Pacht & Crone's Eftflg.

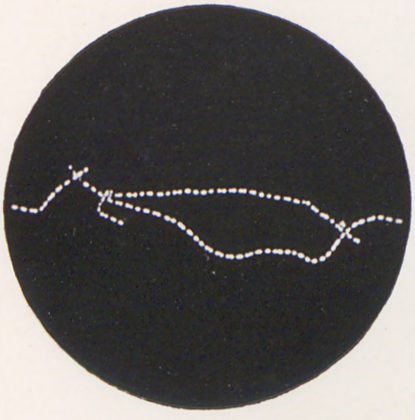
Streptococcus thermophilus.



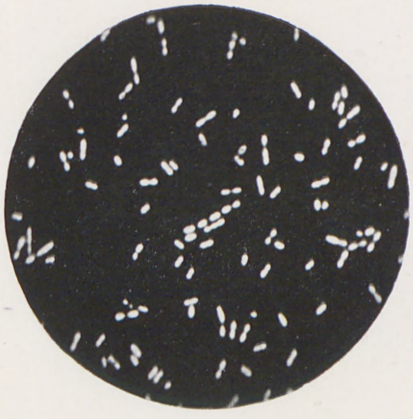
No. 2, Milk, 1 Day, 30°, *Gram* stained.



No. 2, Milk, 1 Day, 45°, *Gram* stained.

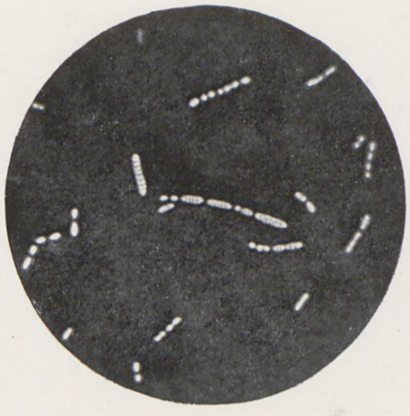


No. 2, C-Bouillon, 1 Day, 30°.



No. 2, Agar Streak, 1 Day, 30°.

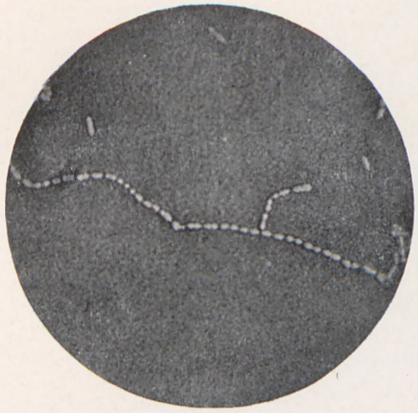
No. 2, S G-Plate, 31 Days, 20°.



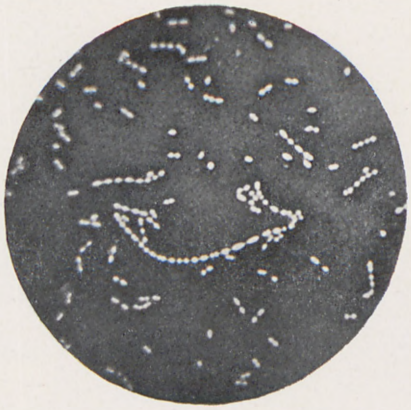
No. 2, Agar Streak, 1 Day, 45°.

Autotypi. Pacht & Crone's Eftflg.

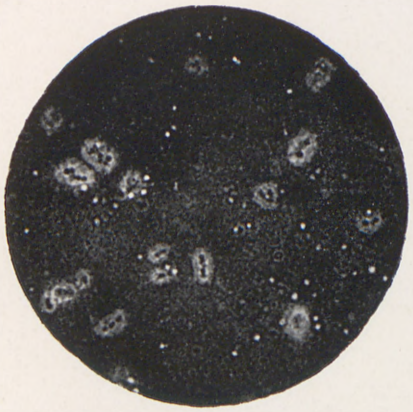
Streptococcus bovis.



No. 1, C-Bouillon, 1 Day, 30°.

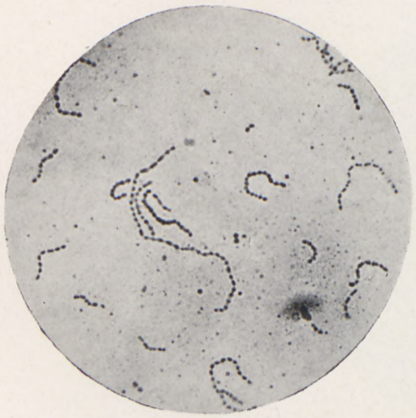


No. 1, Agar Streak, 2 Days, 30°.



No. 3, Milk, 2 Days, 30°, Methylene Blue.

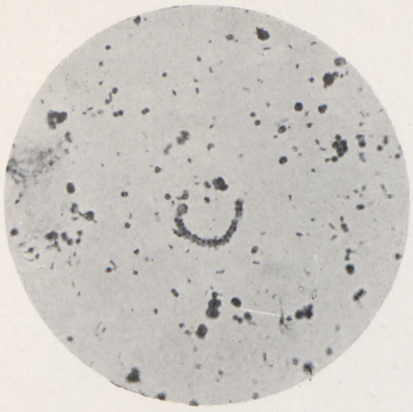
No. 3, Agar Stab, 2 Days, 30°.



No. 5, Milk, 2 Days, 30°, Methylene Blue.

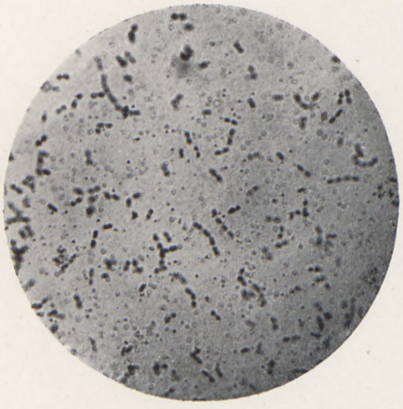
No. 5, Agar Streak, 2 Days, 30°.

Autotypi. Pacht & Crone's Eftflg.



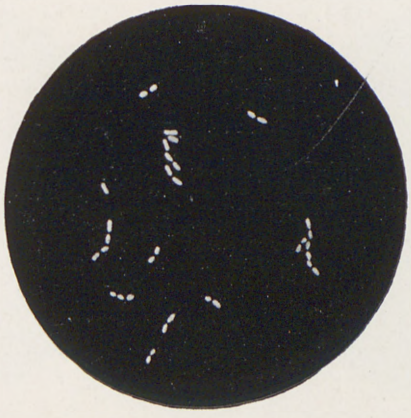
Streptococcus bovis and Micrococci in Cowdung, *Gram* stained.

Streptococcus inulinaceus.



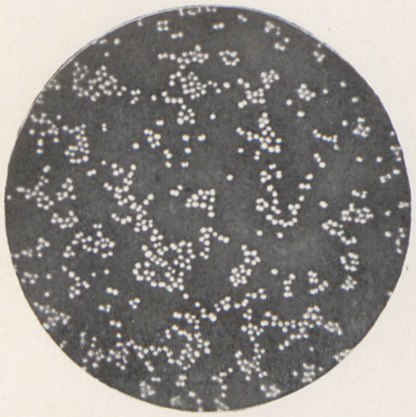
No. 5, Milk, 1 Day, 30°, *Gram* stained.

No. 5, C-Bouillon, 2 Days, 30°.

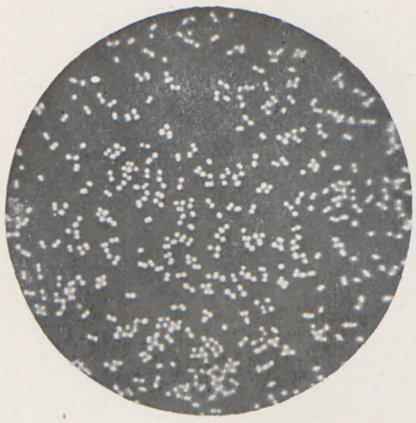


No. 5, Agar Streak, 1 Day, 30°.

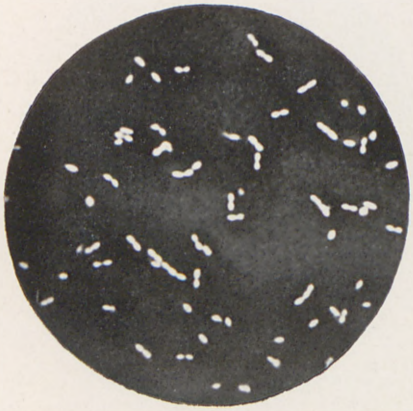
Streptococcus faecium.



No. 2, Agar Streak, 1 Day, 30°.

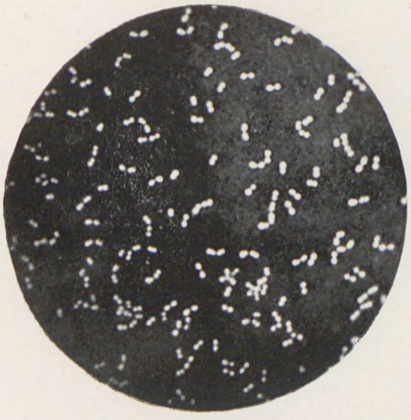


No. 3, Agar Streak, 1 Day, 30°.



No. 6, A G-Plate, 7 Days, 20°.

No. 8, Milk, 2 Days, 30°, *Gram* stained.

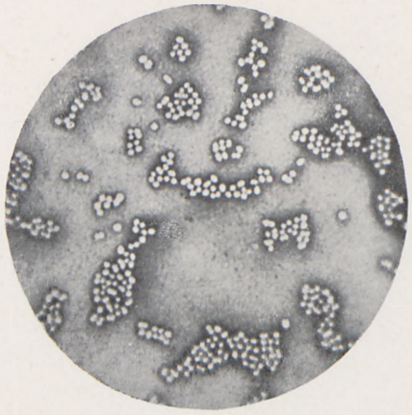


No. 8, Agar Streak, 1 Day, 45°.

No. 8, Agar Stab, 2 Days, 30°.

Streptococcus faecium.

No. 11, Agar Streak, 1 Day, 30°.



No. 16, Agar Streak, 1 Day, 30°.

No. 12, C-Bouillon, 1 Day, 30°.

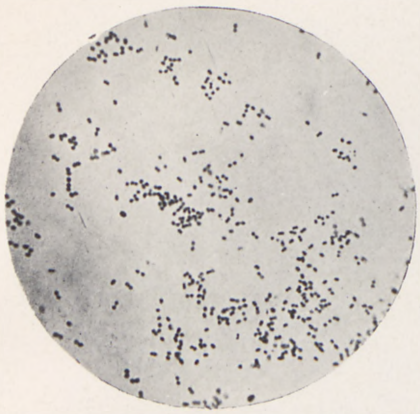
No. 12, C-Bouillon, 7 Days, 45°.

Streptococcus glycerinaceus.

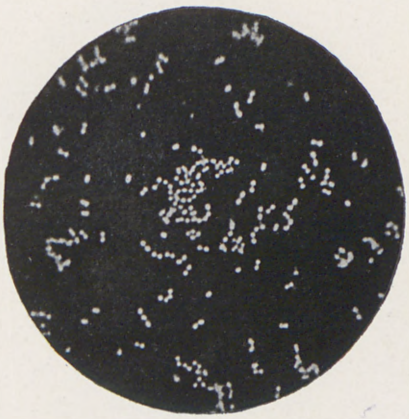
No. 1, C-Bouillon, 1 Day, 30°.

No. 1, Agar Streak, 1 Day, 30°.

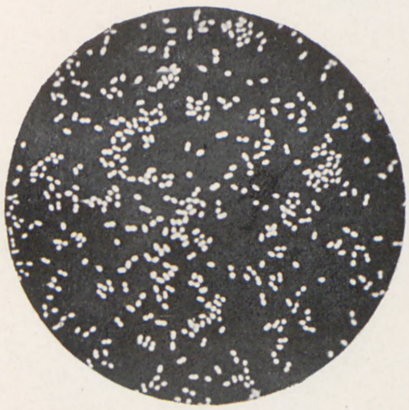
Streptococcus glycerinaceus.



No. 4, Milk, 2 Days, 30°, *Gram* stained.

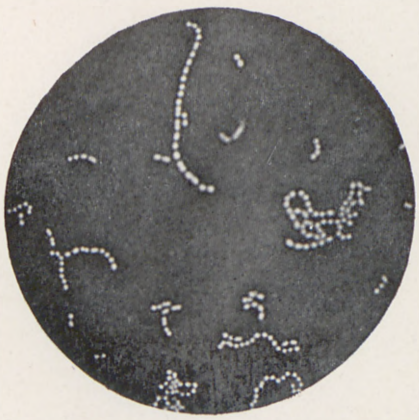


No. 4, C-Bouillon, 1 Day, 30°.

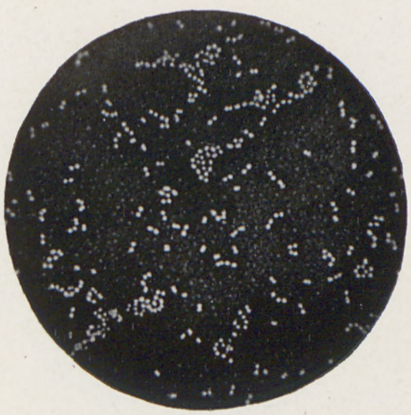


No. 4, Agar Streak, 1 Day, 30°.

No. 4, Agar Streak, 4 Days, 45°.



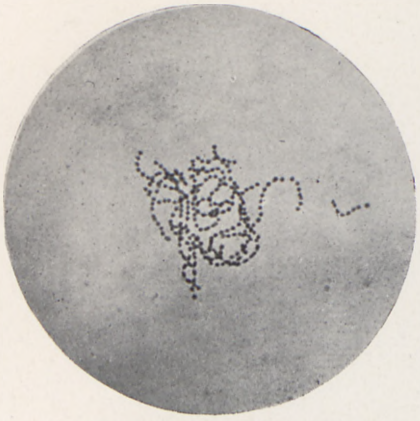
No. 5, C-Bouillon, 1 Day, 30°.



No. 5, Agar Streak, 1 Day, 30°.

Autotypi. Pacht & Crone's Eftflg.

Streptococcus glycerinaceus.

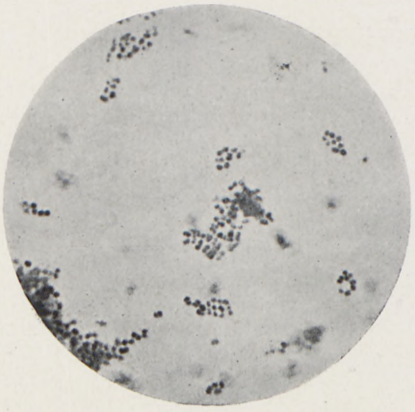


No. 6, Milk, 2 Days, 30°, *Gram* stained.

No. 6, W-Bouillon, 2 Days, 30°.

Streptococcus liquefacieus.

No. 1, C-Bouillon, 1 Day, 30°.

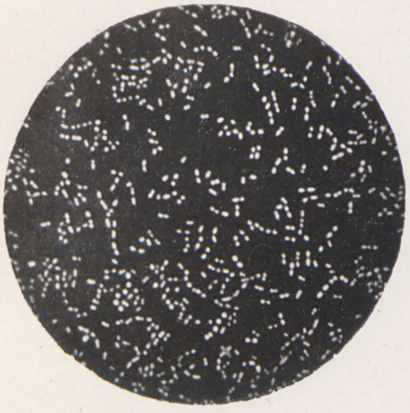


No. 1, Agar Streak, 4 Days, 30°, Fuchsin.

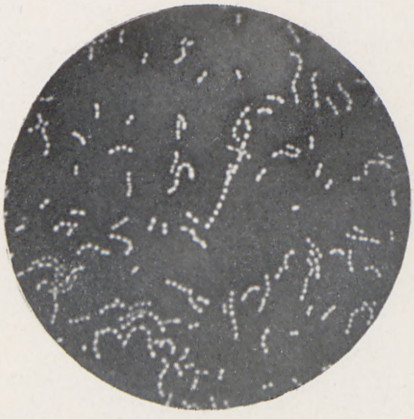
No. 3, Agar Streak, 4 Days, 45°.

No. 5, C-Bouillon, 1 Day, 30°.

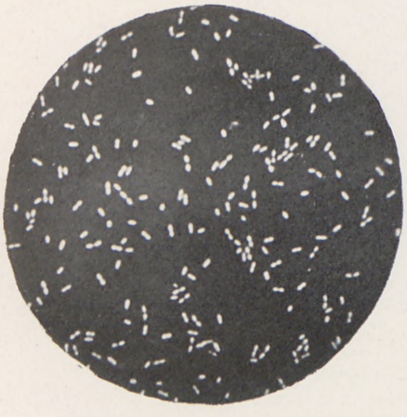
Remaining Saprophytic Streptococci.



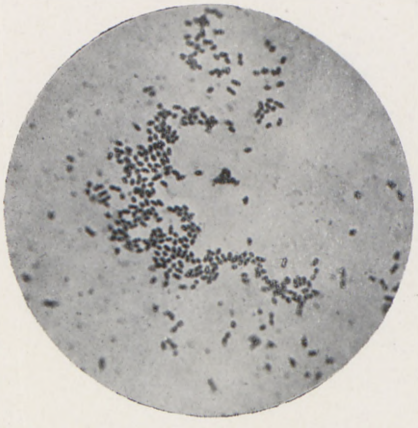
No. 1. Agar Streak, 1 Day, 30°.



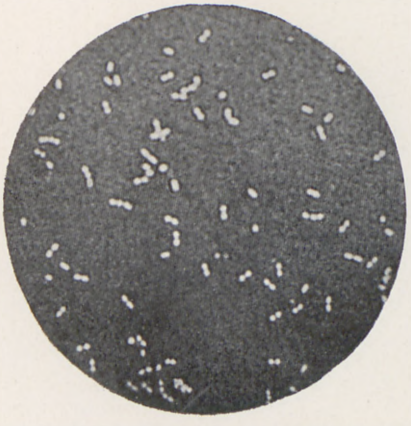
Nr. 3, Agar Streak, 1 Day, 30°.



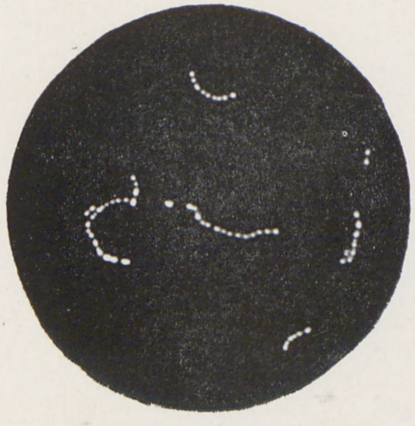
Nr. 5, Agar Streak, 1 Day, 30°.



No. 7, Milk, 1 Day, 30°, *Gram* stained.



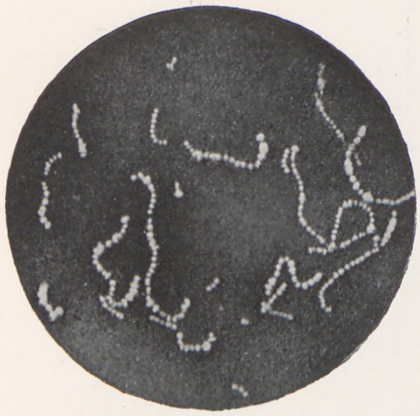
No. 7, C-Bouillon, 1 Day, 30°.



No. 7, Agar Streak, 7 Days, 10°.

Autotypi. Pacht & Crone's Eftflg.

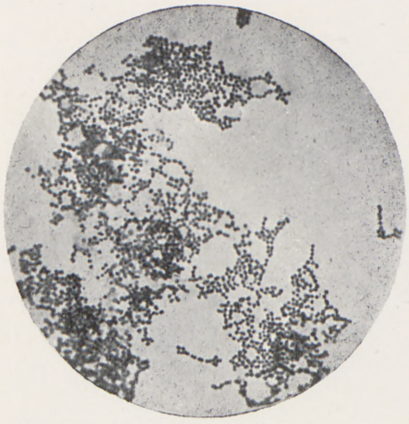
Remaining Pathogenic Streptococci.



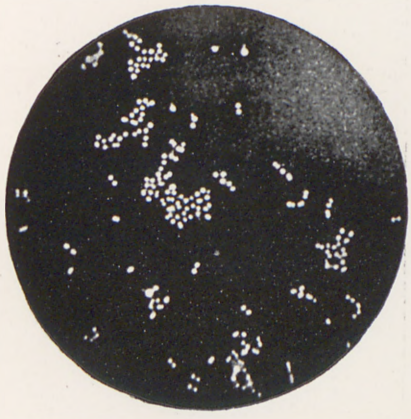
No. 2, C-Bouillon, 2 Days, 30°.

No. 6, C-Bouillon, 2 Days, 30°.

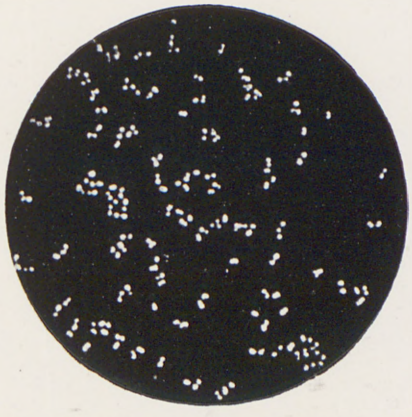
No. 6, Agar Streak, 1 Day, 30°.



No. 8, C-Bouillon, 2 Days, 30°, *Gram* stained.



No. 10, C-Bouillon, 2 Days, 30°.



No. 10, Agar Streak, 1 Day, 30°.

Autotypi. Pacht & Crone's Eftflg.

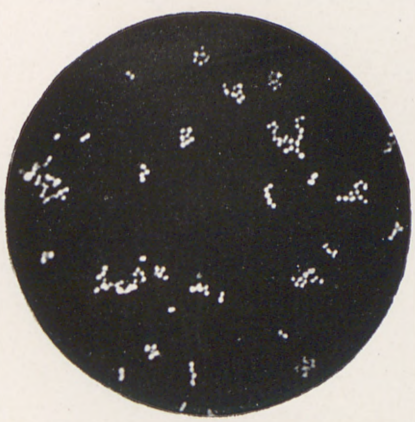
Betacoccus arabinosaceus.

No. 4, Agar Streak, 4 Days, 10°.

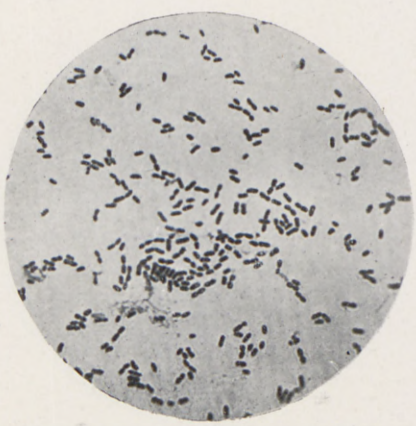
No. 4, Agar Streak, 3 Days, 30°.

No. 9, C-Bouillon, 2 Days, 30°.

No. 9, Agar Streak, 1 Day, 30°.



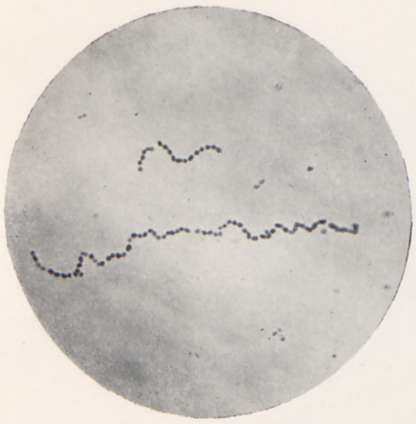
No. 12, C-Bouillon, 3 Days, 30°.



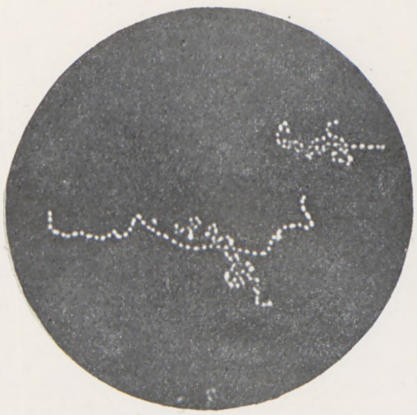
No. 12, Beet Gelatin Stab, 1 Day, 20°, Fuchsin.

Autotypi. Pacht & Crone's Eftflg.

Belacoccus arabinosaceus.

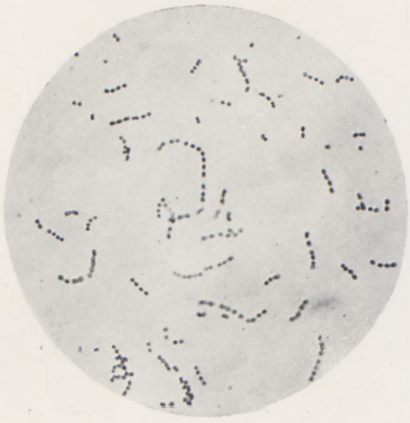


No. 11, Milk, 3 Days, 30°, *Gram* stained.

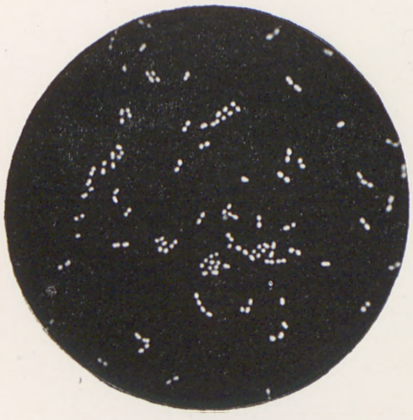


No. 11, C-Bouillon, 3 Days, 30°.

No. 11, Beet Galatine Stab, 1 Day, 20°, Fuchsin.



No. 7, Milk, 2 Days, 30°, *Gram* stained.

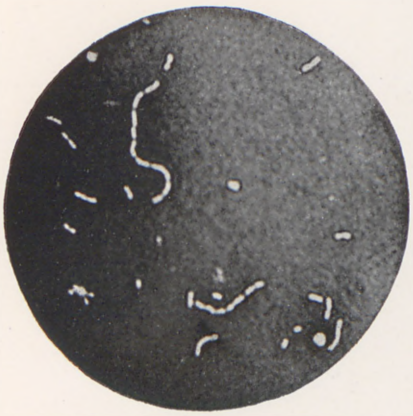


No. 7, Agar Streak, 1 Day, 30°.

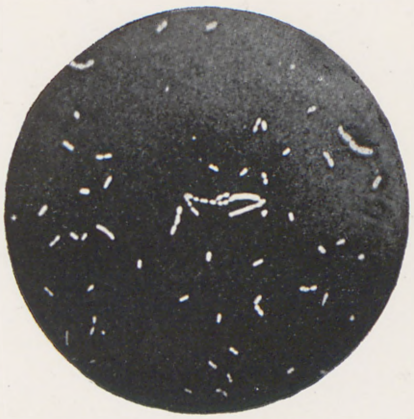
No. 7, Agar Stab, 2 Days, 30°.

Autotypi. Pacht & Crone's Eftflg.

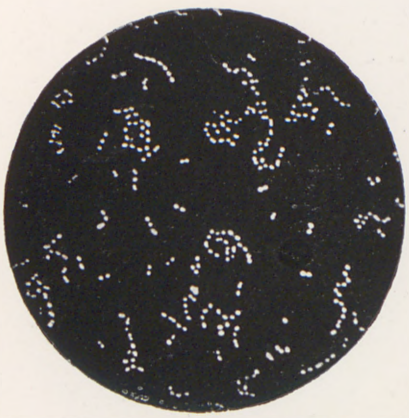
Betacoccus bovis.



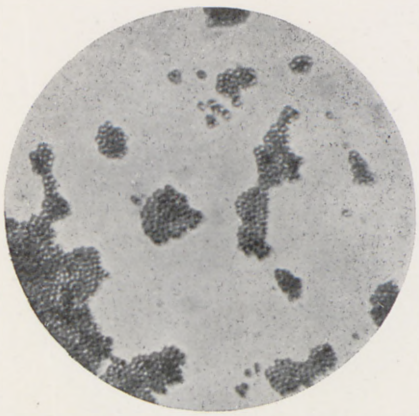
No. 33, C-Bouillon, 3 Days, 30°.



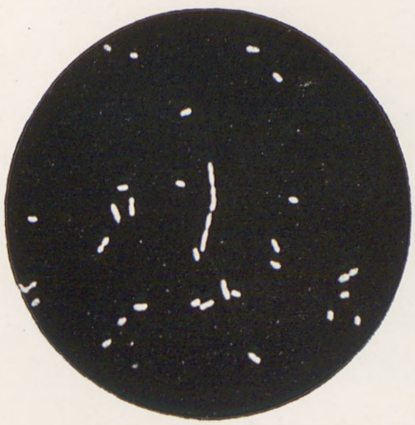
No. 33, Agar Streak, 1 Day, 30°.



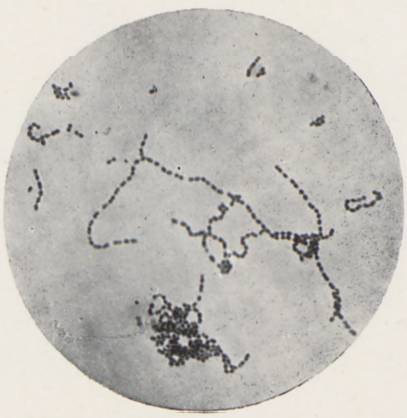
No. 35, Agar Streak, 1 Day, 30°.



No. 35, Agar Streak, 10 Days, 35°, Fuchsin.



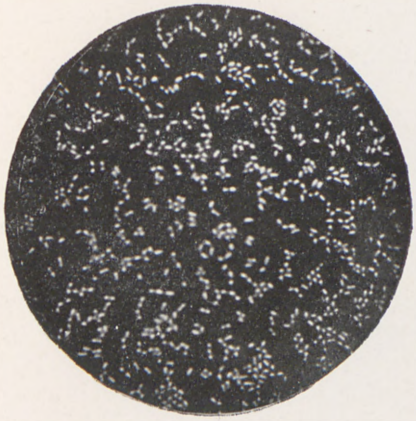
No. 36, Agar Stab, 2 Days, 30°.



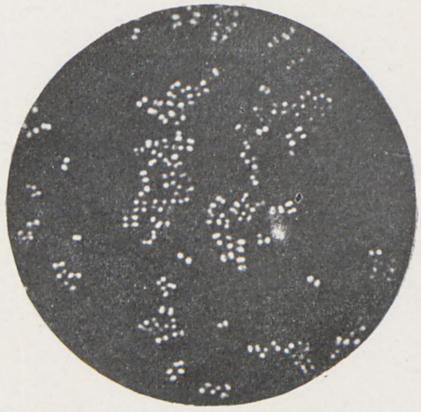
No. 42, C-Bouillon, 1 Day, 30°, *Gram* stained.

Autotypi. Pacht & Crone's Eftflg.

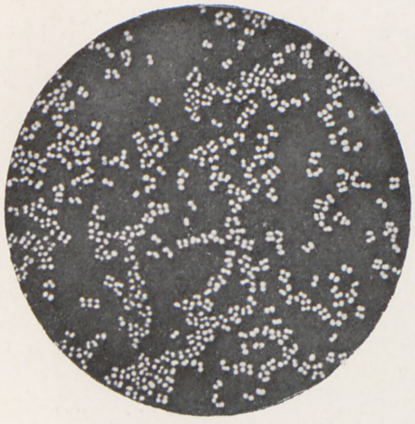
Betacoccus bovis.



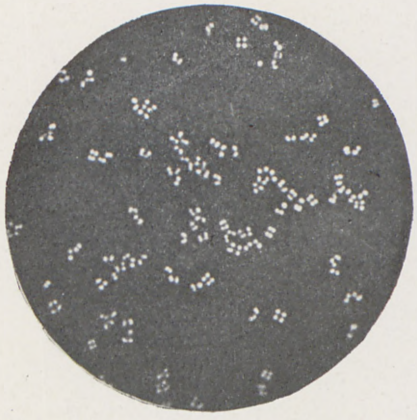
No. 44, A G-Plate, 8 Days, 20°.



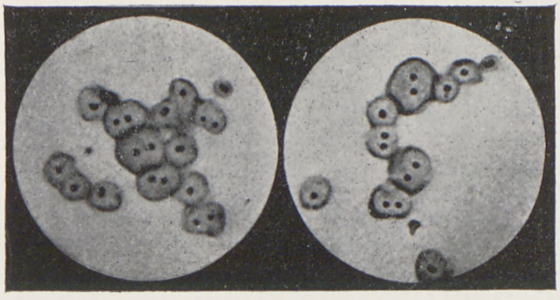
No. 46, C-Bouillon, 2 Days, 45°.



No. 46, Agar Streak, 2 Days, 30°.



No. 46, C-Bouillon, 2 Days, 30°.

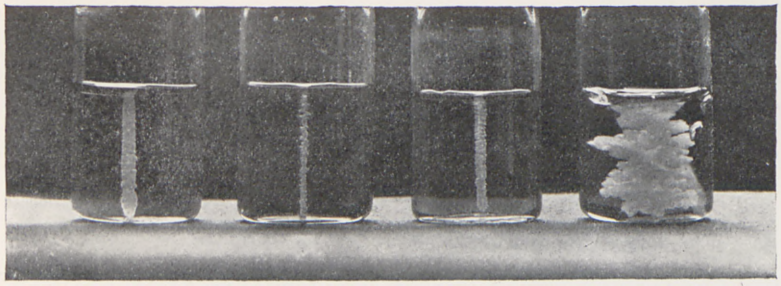


„Aller broat", Saccharose Bouillon, 1 Day, Antimony Mordant and Fuchsin. From *Zettnow*.

Saccharose Gelatin Stab Culturs of Betacocci.

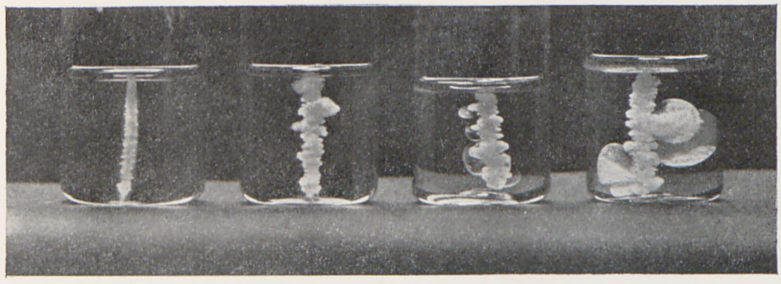
25 Days, 20°, Natural Size.

Beet Gelatin.

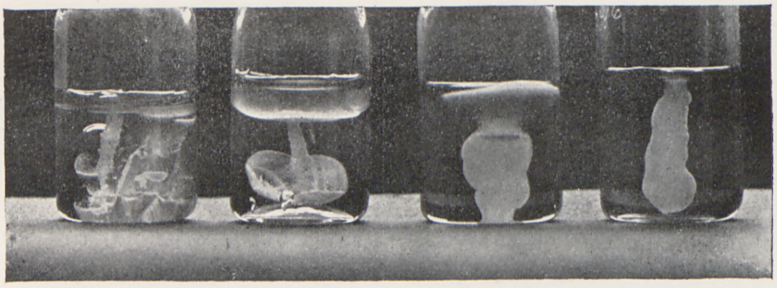


No. 35. No. 4. No. 5. No. 12.

C-Gelatin.

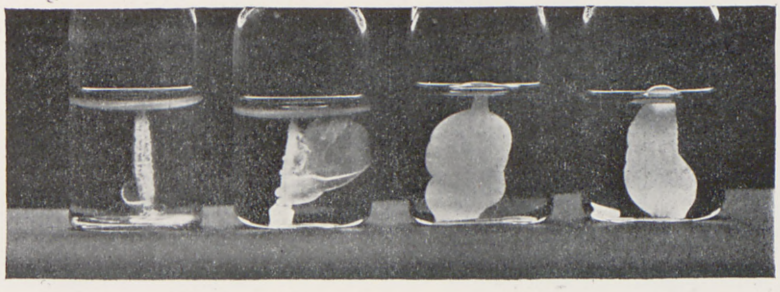


Beet Gelatin.



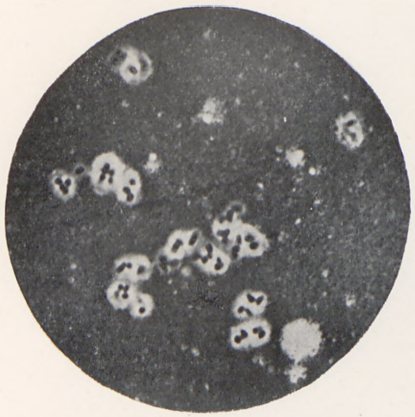
No. 11. No. 6. No. 42. No. 41.

C-Gelatin.

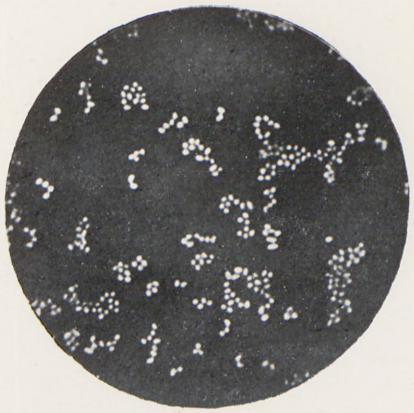


Nos. 11 and 12 prefer the Beet Gelatin.
Nos. 6 and 11 liquefy the Beet- and C-Gelatin with Saccharose.

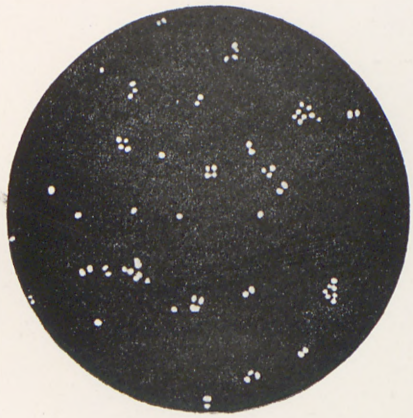
Tetracocci.



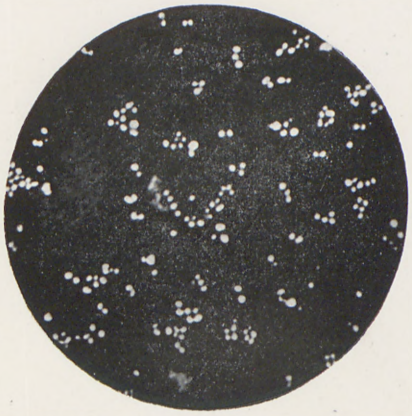
No. 5, Milk, 4 Days, 30°, Fuchsin.



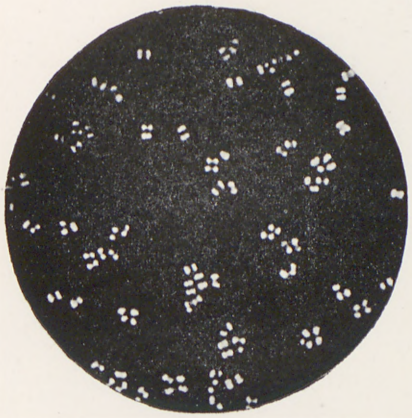
No. 5, Agar Streak, 3 Days, 30°.



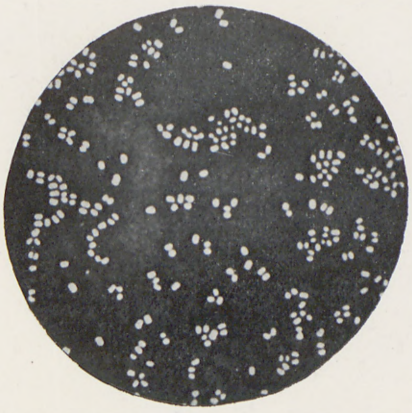
No. 9, Agar Plate, 5 Days, 30°.



No. 10, Agar Streak, 3 Days, 30°.



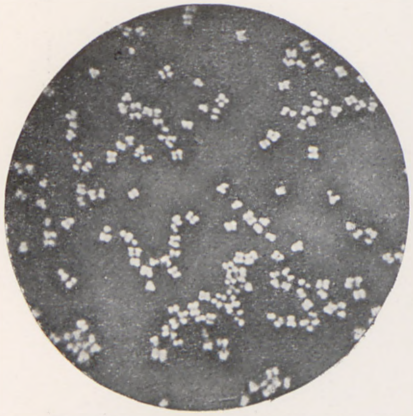
No. 11, C-Bouillon, 2 Days, 30°.



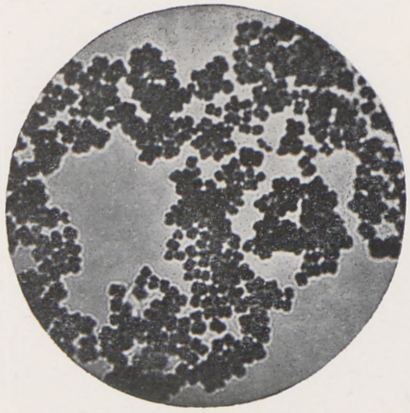
No. 11. Agar Plate, 5 Days, 30°.

Autotypi. Pacht & Crone's Eftflg.

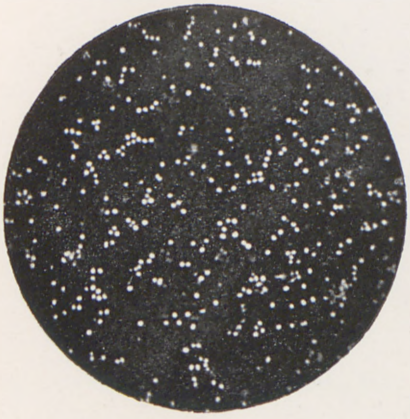
Tetracocci.



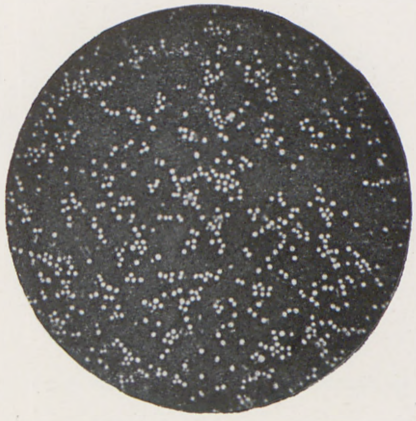
No. 3, Agar Streak, 1 Day, 30°.



No. 8, C-Bouillon, 3 Days, 30°, Methylene Blue.

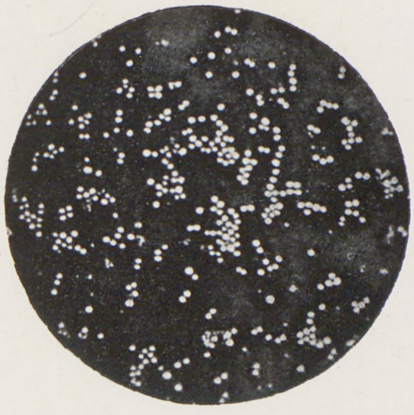


No. 13, Agar Stab, 28 Days, 20°.



No. 14, Agar Stab, 30 Days, 20°.

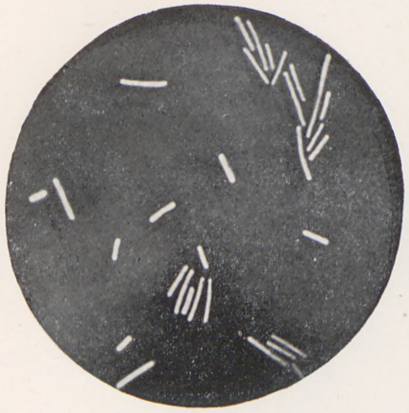
No. 29, Agar Streak, 1 Day, 30°.



No. 31, Agar Stab, 28 Days, 20°.

Autotypi. Pacht & Crone's Eftflg.

Thermobacterium cereale.



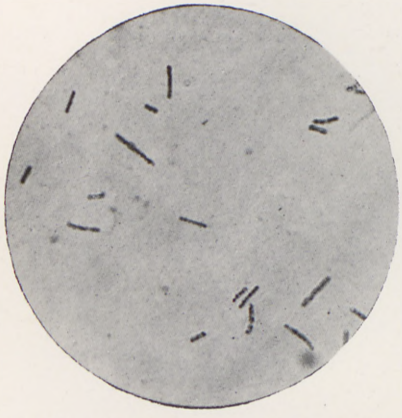
No. 3, Y-Malt Bouillon, 2 Days, 40°.

No. 4, Y-Malt Bouillon, 2 Days, 40°.

No. 5, Agar Stab, 2 Days, 40°, Methylene Blue.

No. 5, Y-Malt Agar Stab, 2 Days, 40°, Methylene Blue.

Thermobacterium lactis.



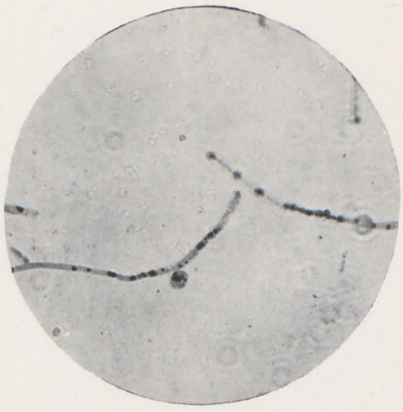
No. 7, Milk, 4 Days, 40°, *Gram* stained.

No. 7, Agar Streak, 1 Day, 40°, Methylene Blue.

Autotypi. Pacht & Crone's Eftflg.

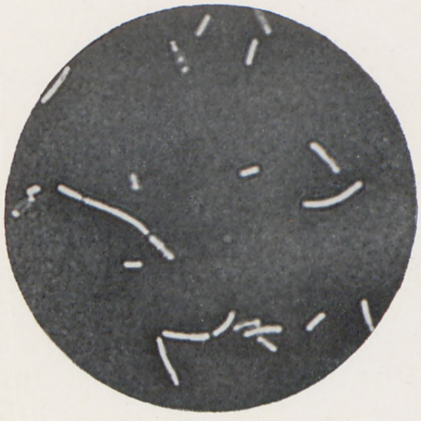
Thermobacterium lactis.

No. 6, Milk, 1 Day, 40°, Methylene Blue

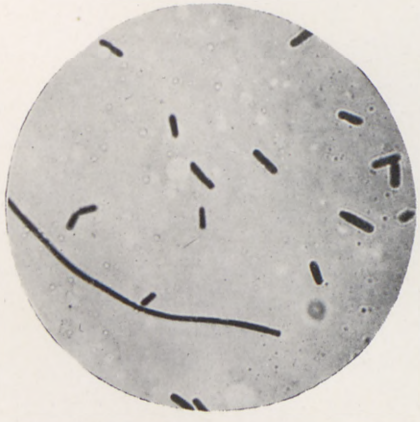


No. 6, Milk, 3 Days, 40°, Methylene Blue.

No. 6, Agar Streak, 1 Day, 40°, Methylene Blue.



No. 6, Agar Stab, 2 Days, 40°.



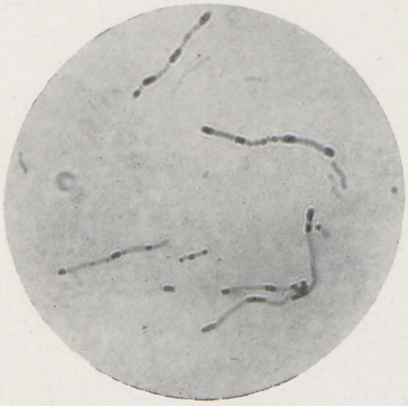
No. 10, Milk, 2 Days, 40°, *Gram* stained.

No. 10, Agar Stab, 1 Day, 40°.

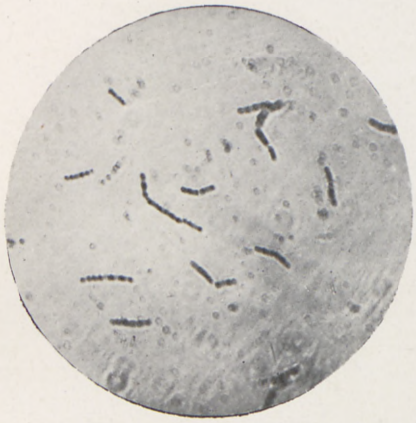
Autotypi. Pacht & Crone's Eftflg.

Thermobacterium lactis.

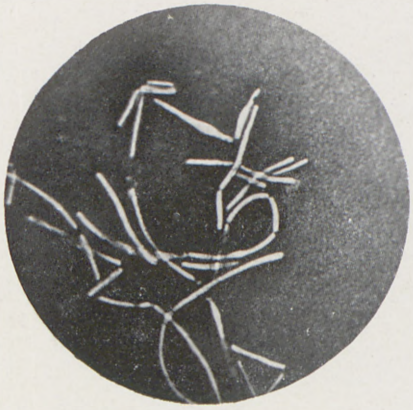
No. 9. Agar Stab, 1 Day, 40°, Methylene Blue.



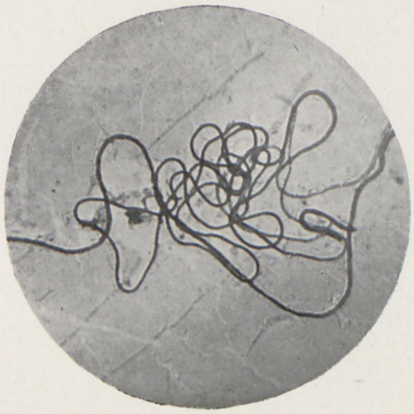
No. 11, Milk, 2 Days, 40°, Methylene Blue.



No. 8, Milk, 3 Days, 30°, Methylene Blue



No. 8, Agar Streak, 1 Day, 40°.



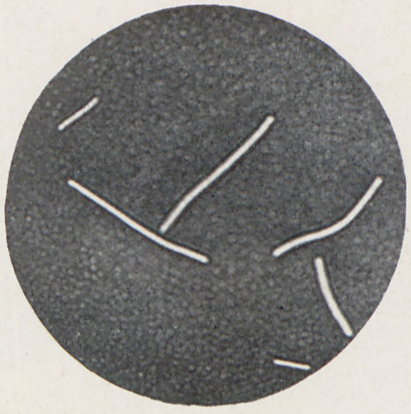
No. 8, Y-Agar Plate, 3 Days, 40°, Fuchsin.

Autotypi. Pacht & Crone's Eftflg.

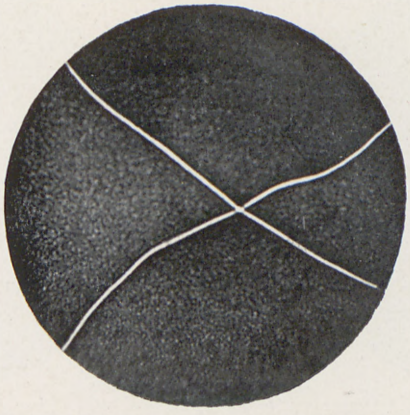
Thermobacterium helveticum.

No. 12, Milk, 1 Day, 40°, *Gram* stained.

No. 12, W-Whey, 2 Days, 40°.



No. 12, Agar Stab, 4 Days, 40°.

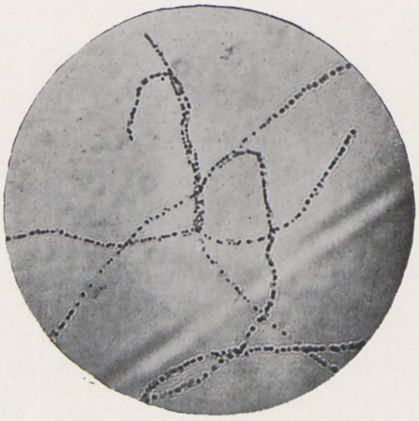


No. 12, Agar Tube, 3 Days, 40°.

Autotypi. Pacht & Crone's Eftflg.

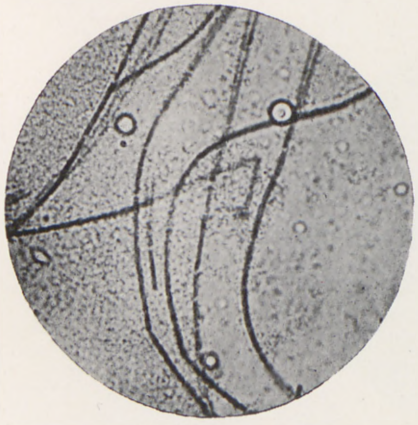
Thermobacterium Jugurt.

No. 13, Milk, 1 Day, 40°, *Gram* stained.

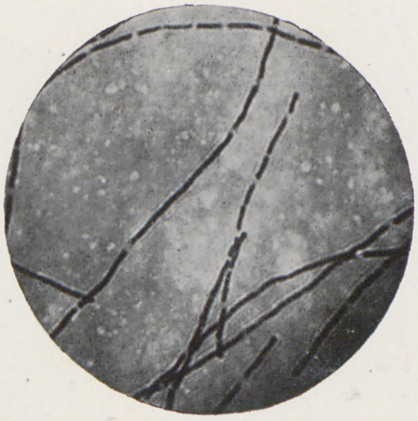


No. 13, Milk, 1 Day, 30°, *Gram* stained.

No. 13, Milk, 1 Day, 40°, Fuchsin.



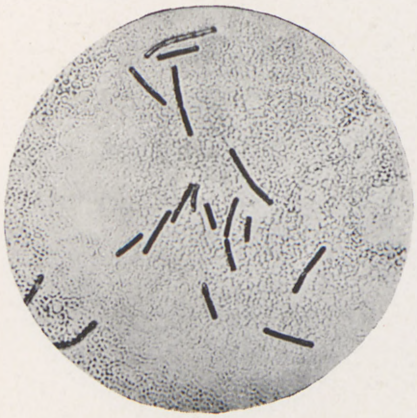
Nr. 13, Milk, 2 Days, 30°, Methylene Blue.
Preparation in Water.



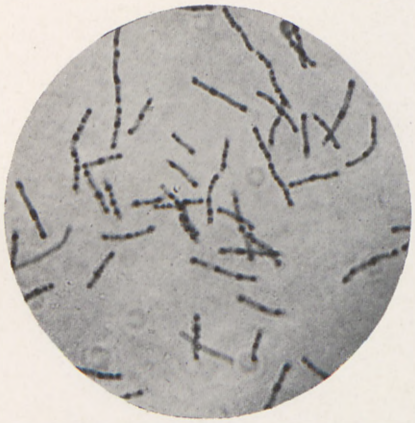
Nr. 13, Milk, 2 Days, 30°, Methylene Blue.
Preparation in Canada Balsam.

Autotypi. Pacht & Crone's Eftflg.

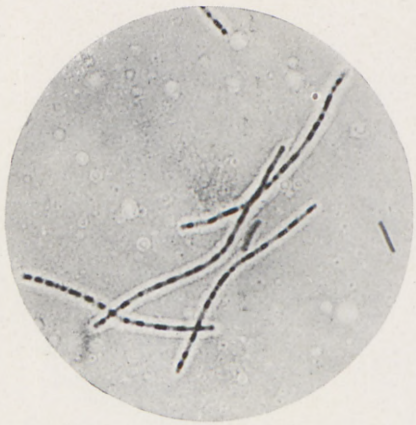
Thermobacterium bulgaricum.



No. 14, Milk, 2 Days, 40°, *Gram* stained.



No. 14, Milk sterilised, 1 Day, 40°, Methylene Blue.



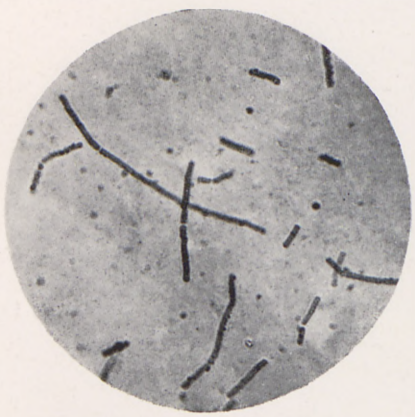
No. 14, Milk, pasteurised at 80°, 1 Day, 40°, Methylene Blue.

No. 14, Agar Tube, 4 Days, 40°.

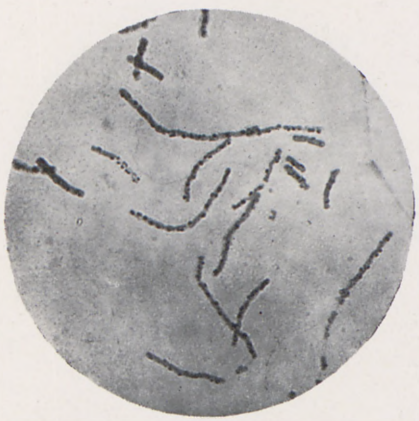
Autotypi. Pacht & Crone's Eftflg.

Thermobacterium ?.

No. 15. Milk, 1 Day, 30°, Fuchsin.



No. 15. Milk, 1 Day, 30°, *Gram* stained.

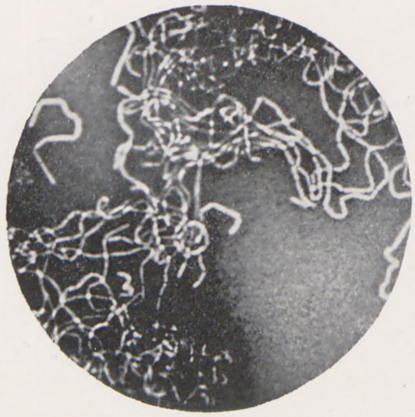


Nr. 15. Milk, 5 Days. 30°, *Gram* stained.

Nr. 15. Milk, 5 Days, 30°, Methylene Blue.

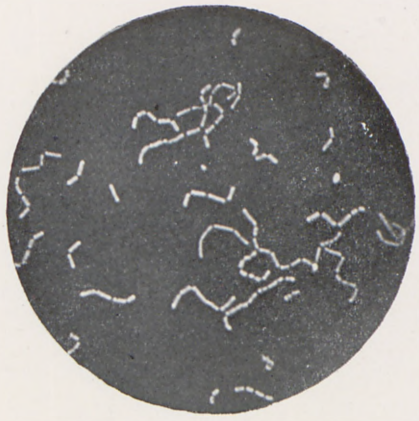
Streptobacterium casei.

No. 1, C-Bouillon, 2 Days, 30°.



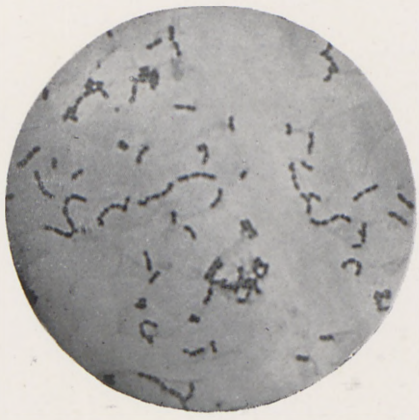
No. 5, C-Bouillon, 2 Days, 30°.

No. 2, C-Bouillon, 2 Days, 30°.



No. 2, Agar Tube, 4 Days, 30°.

No. 4, Agar Streak, 2 Days, 30°.



No. 4, A G-Plate, 8 Days, 20°, Fuchsin.

Autotypi. Pacht & Crone's Eftflg.

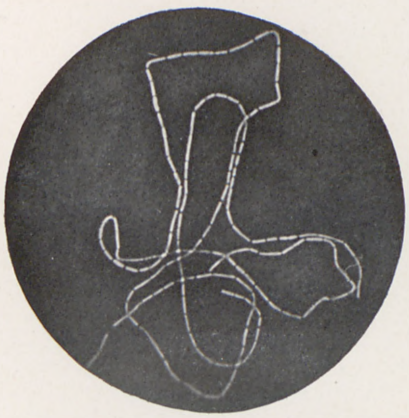
Streptobacterium casei.

No. 7, Agar Streak, 2 Days, 30°.

No. 7, S G - Plate, 8 Days, 20°.

No. 8, Milk, 4 Days, 30°, *Gram* stained.

Nr. 8, C-Bouillon, 2 Days, 30°.



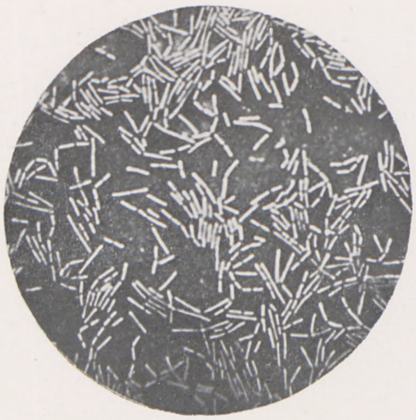
No. 10, C-Bouillon, 2 Days, 30°.

No. 10, Agar Streak, 4 Days, 30°.

Autotypi. Pacht & Crone's Eftflg.

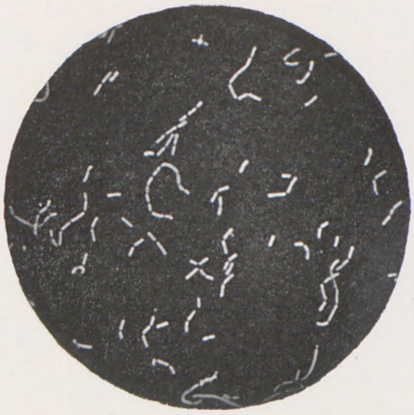
Streptobacterium casei.

No. 9, C-Bouillon, 2 Days. 30°.



No. 9, Agar Tube, 4 Days, 30°.

No. 9, A G-Plate, 10 Days, 20°.



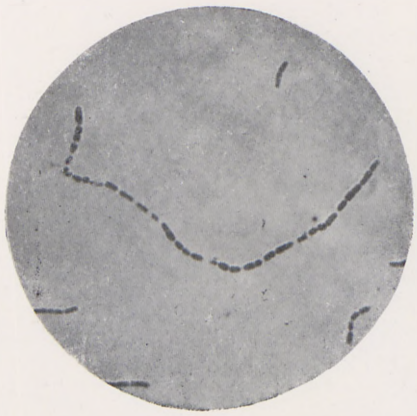
No. 15, Agar Streak, 2 Days, 30°.

No. 16, C-Bouillon, 3 Days, 30°.

No. 16, S G-Plate, 11 Days, 20°.

Streptobacterium casei.

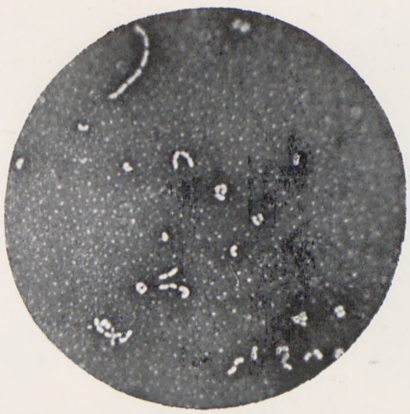
No. 18, C-Bouillon, 2 Days, 30°.



No. 18, A G - Stab, 8 Days, 20°, Methylene Blue.

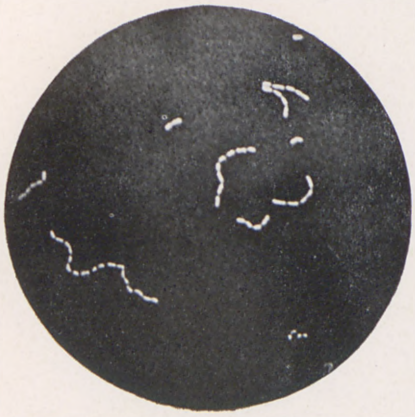
No. 28, C-Bouillon, 1 Day, 30°.

No. 29, Agar Tube, 3 Days, 30°.



No. 31, Agar Streak, 2 Days, 30°.

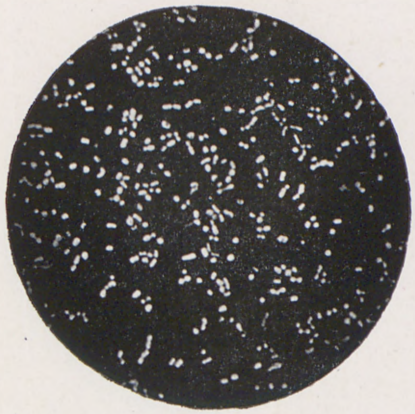
Streptobacterium casei.



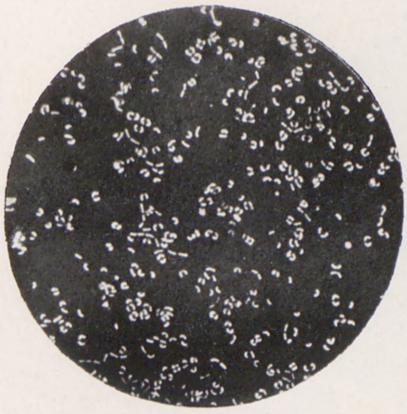
No. 32, C-Bouillon, 2 Days, 30°.

No. 32, C-Bouillon, 3 Days, 45°.

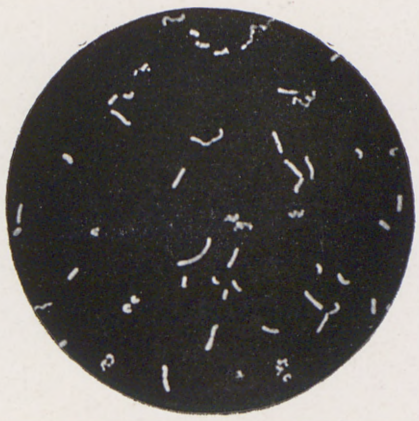
No. 32, S G - Stab, 15 Days, 20°.



No. 32, A G - Stab, 30 Days, 20°.



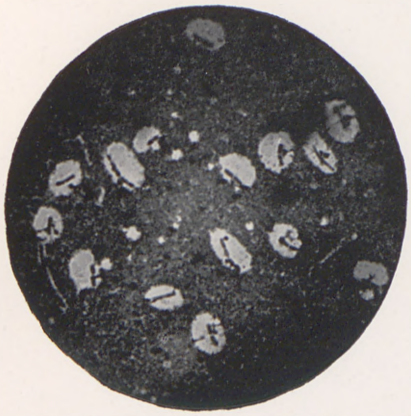
Nr. 33, C-Bouillon, 1 Day, 30°.



Nr. 33, A G - Stab, 30 Days, 20°.

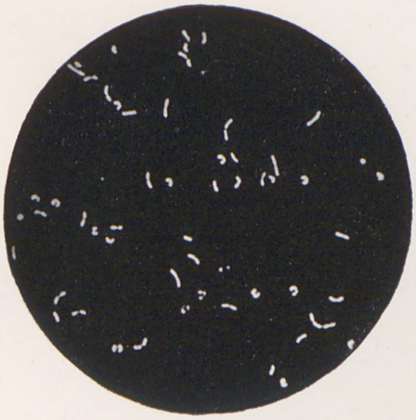
Autotypi. Pacht & Crone's Eftflg.

Streptobacterium casei.

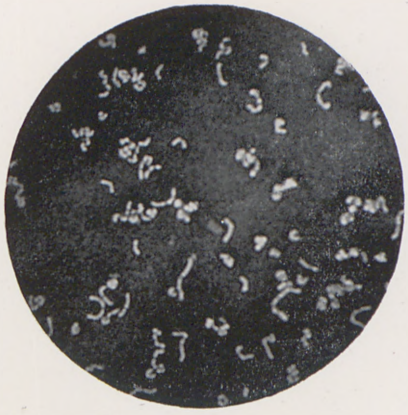


No. 31, Ropy Variety, Milk, 2 Days, 30°, Fuchsin.

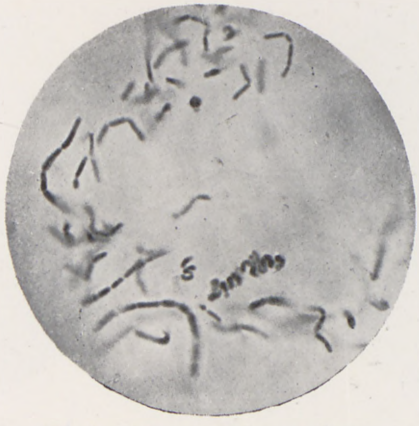
No. 34, Ropy Variety, C-Bouillon, 2 Days, 30°.



No. 34, Agar Streak, 2 Days, 30°.

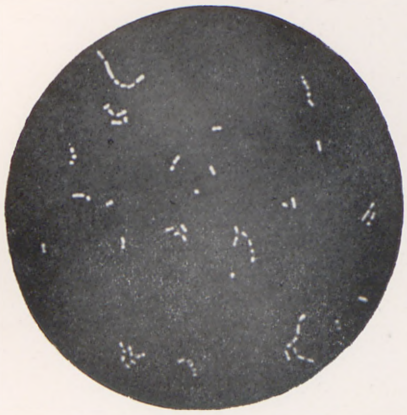


No. 34, Agar Streak, 19 Days, 30°.

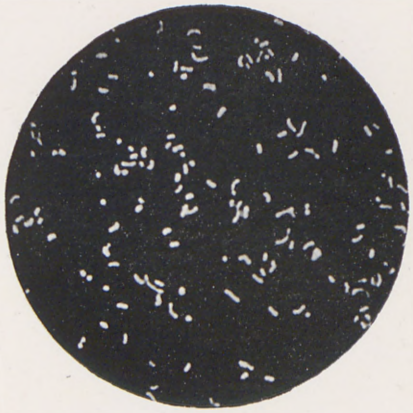


No. 34. Agar Streak, 1 Day, 45°, Fuchsin.

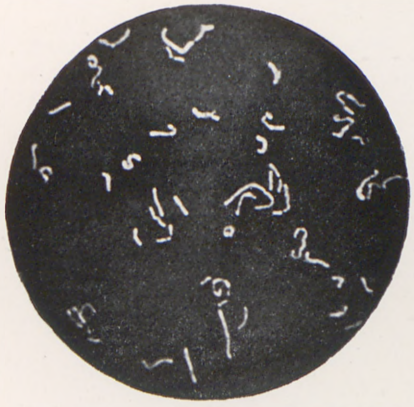
Streptobacterium plantarum.



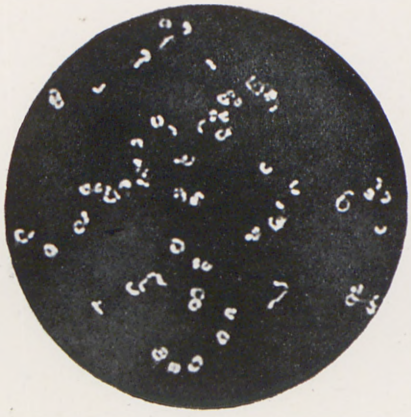
No. 1, C-Bouillon, 3 Days, 30°.



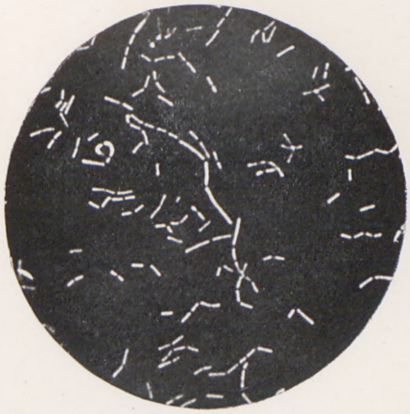
No. 1, C-Bouillon, 14 Days, 30°.



No. 1, Agar Streak, 14 Days, 30°.



No. 1, S G - Plate, 14 Days, 20°.



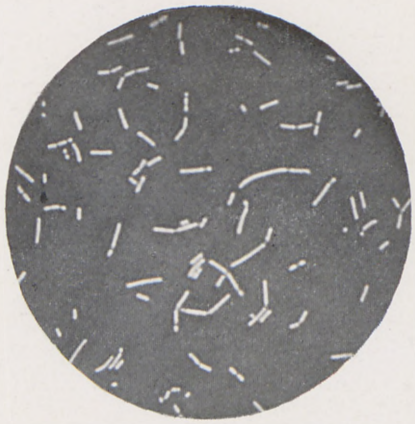
No. 20, Agar Streak, 3 Days, 30°.

No. 20, Agar Tube, 2 Days, 30°.

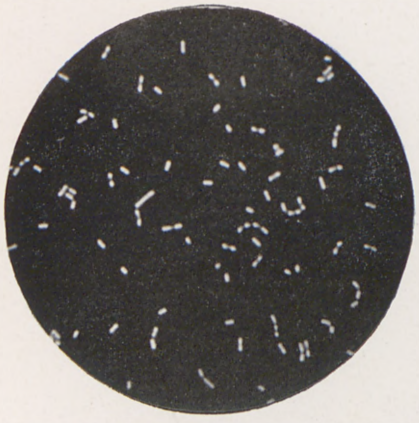
Autotypi. Pacht & Crone's Eftflg.

Streptobacterium plantarum.

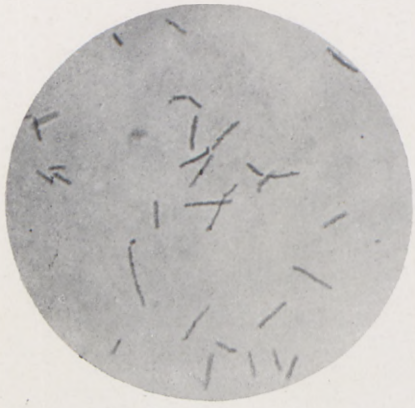
No. 2, C-Bouillon, 2 Days, 30°.



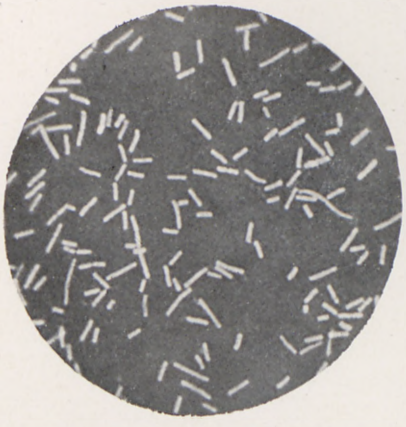
No. 2, Agar Streak, 2 Days, 30°.



No. 2 S G · Plate, 11 Days, 20°.



No. 18, Milk, 6 Days, 30° *Gram* stained.



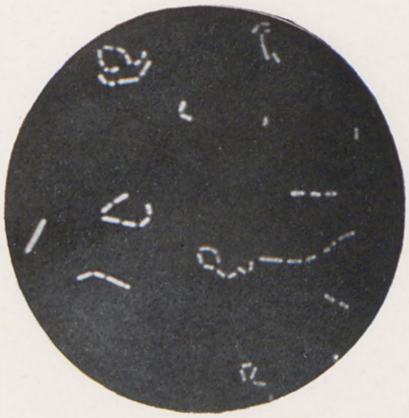
No. 18, C-Bouillon, 1 Day, 30°.

No. 18, Agar Streak, 1 Day, 30°.

Autotypi. Pacht & Crone's Eftflg.

Streptobacterium plantarum.

No. 5, Milk, 5 Days, 30°, *Gram* stained.



No. 5, C-Bouillon, 2 Days, 30°.

No. 5, Agar Streak, 3 Days, 30°.

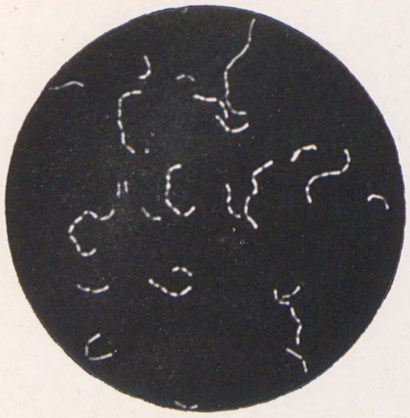
No. 5, Agar Tube, 3 Days, 30°.

No. 21, Agar Streak, 2 Days, 30°.

No. 21, Agar Streak, 2 Days, 37,5°.

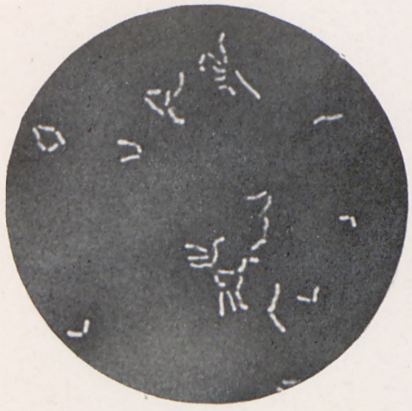
Autotypi. Pacht & Crone's Eftflg.

Streptobacterium plantarum.

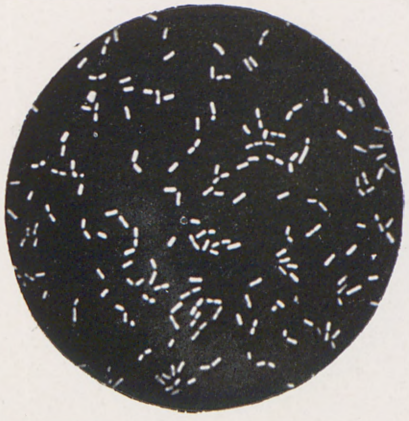


No. 30, Agar Streak, 3 Days, 30°.

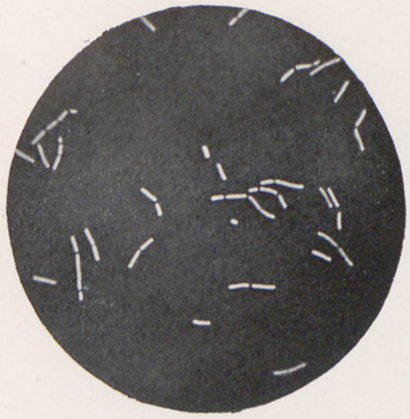
No. 30, Agar Streak, 3 Days, 37,5°.



No. 42, C-Bouillon, 2 Days, 30°.



No. 42, S G - Stab, 8 Days, 20°.



No. 43, C-Bouillon, 2 Days, 30°.

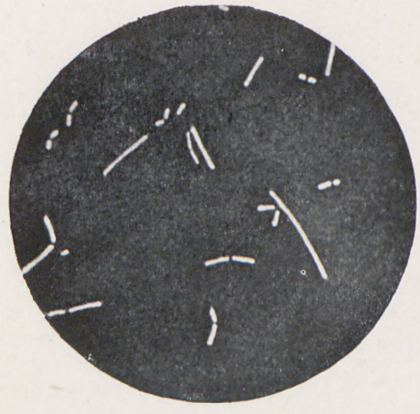
No. 43, Agar Streak, 2 Days, 30°.

Streptobacterium plantarum.

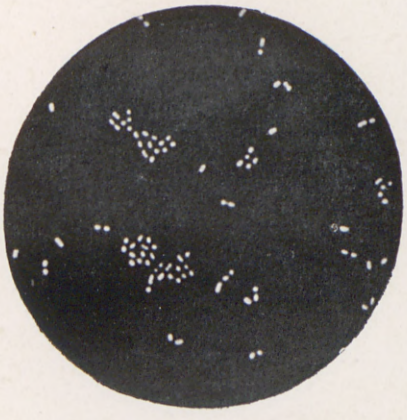
No. 44, C-Bouillon, 2 Days, 30°.

No. 44, Agar Tube, 3 Days, 30°.

No. 44, S G - Stab, 10 Days, 20°.



No. 44, S G - Plate, 10 Days, 20°.

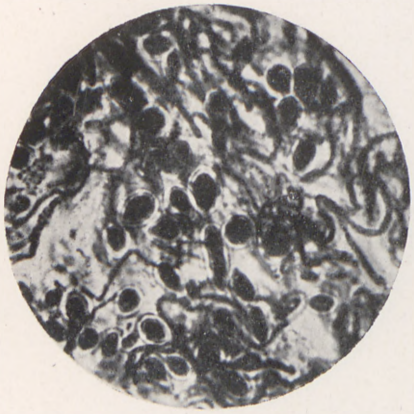


No. 44, A G - Stab, 10 Days, 20°.

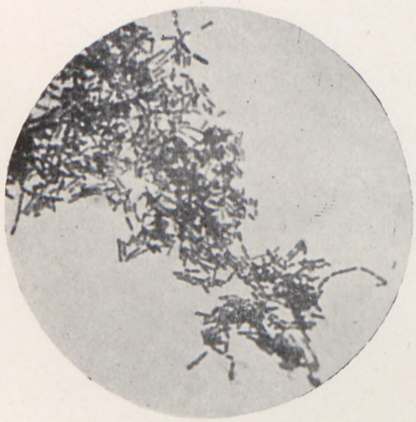
No. 8, Agar Streak, 2 Days, 30°.

Autotypi. Pacht & Crone's Eftflg.

Betabacterium caucasicum.



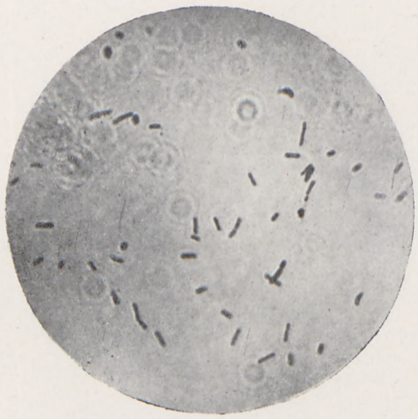
Cut through a Kefir Grain, Methylene Blue.



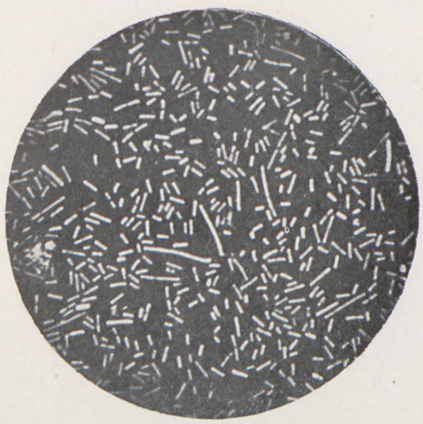
No. 2, Y-Bouillon, 4 Days, 30°, Methylene Blue.

No. 2, Y-Agar Tube, 10 Days, 30°.

Betabacterium breve.



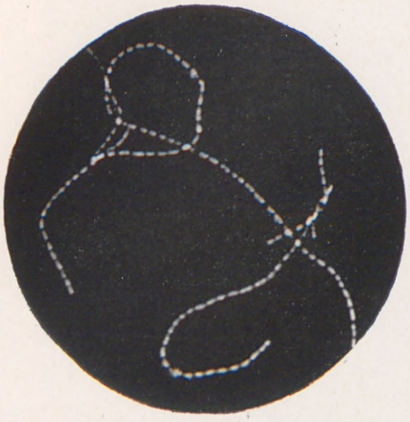
No. 5, Milk, 14 Days, 30°, *Gram* stained.



No. 5, Agar Streak, 4 Days, 30°.

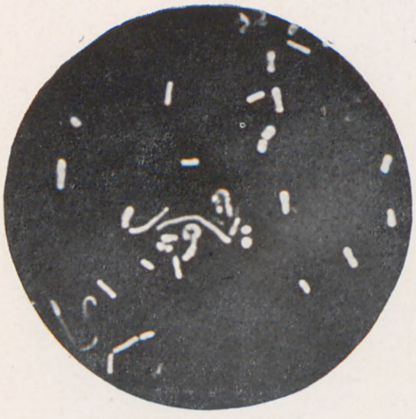
No. 5, S G-Plate, 8 Days, 20°.

Betabacterium breve.

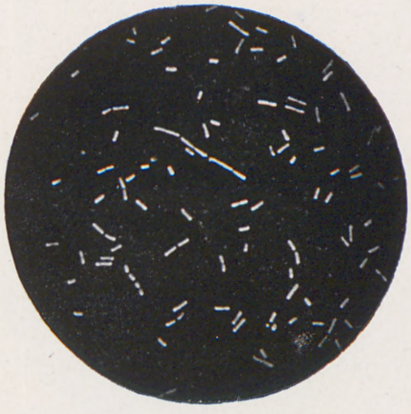


No. 3, C-Bouillon, 1 Day, 30°.

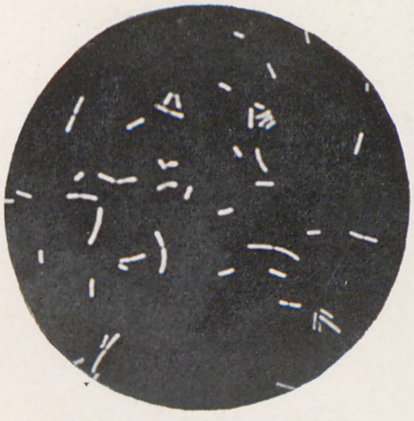
No. 8, Agar Streak, 4 Days, 30°.



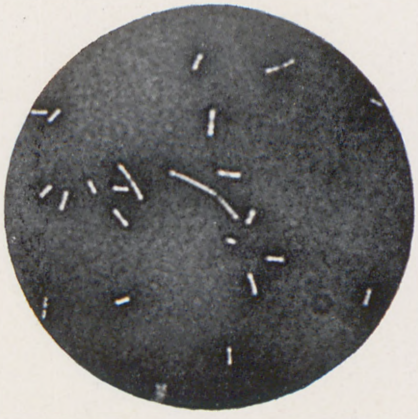
No. 9, Agar Streak, 3 Days, 30°.



No. 10, Agar Streak, 4 Days, 30°.



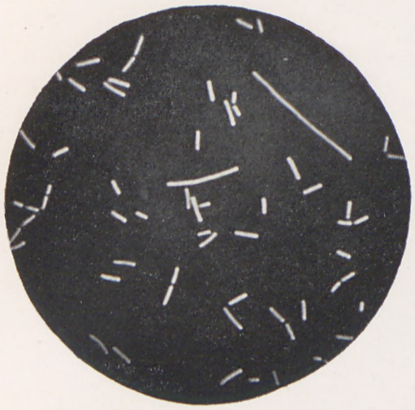
No. 10, Agar Streak, 5 Days, 30°.



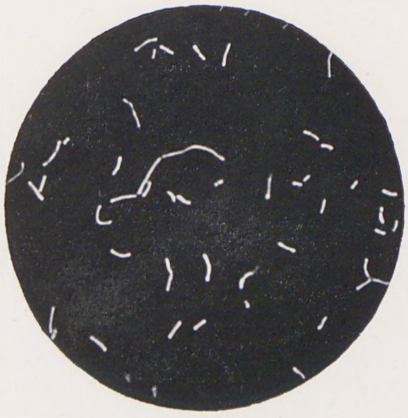
No. 10, S G-Plate, 10 Days, 20°.

Autotypi. Pacht & Crone's Eftflg.

Betabacterium breve.



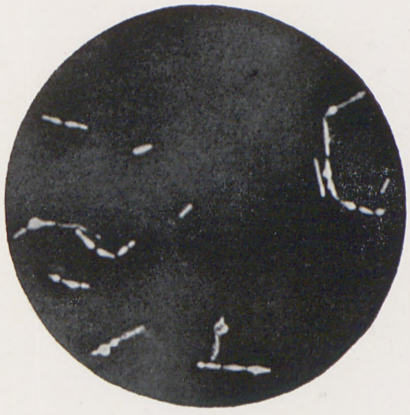
No. 11, C-Bouillon, 2 Days, 30°.



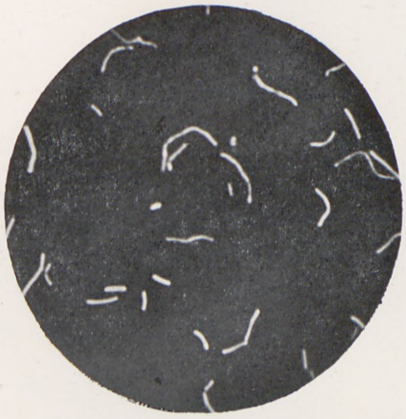
No. 11, Agar Streak, 3 Days, 30°.

Betabacterium longum.

No. 22, C-Bouillon, 1 Day, 30°.



No. 22, C-Bouillon, 2 Days, 30°.



No. 30, Agar Streak, 2 Days, 40°.

Nr. 33. Agar Streak, 1 Day, 40°.

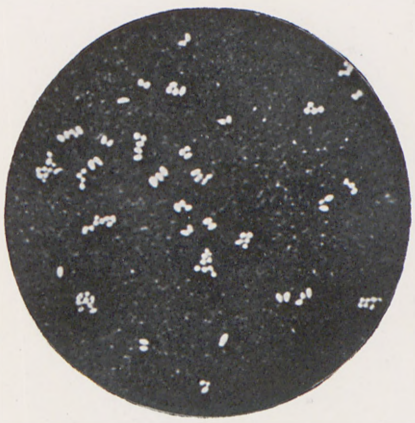
Autotypi. Pacht & Crone's Eftflg.

Microbacterium lacticum.

No. 1. Agar Streak, 4 Days, 30°.

No. 1. Agar Plate, 4 Days, 30°.

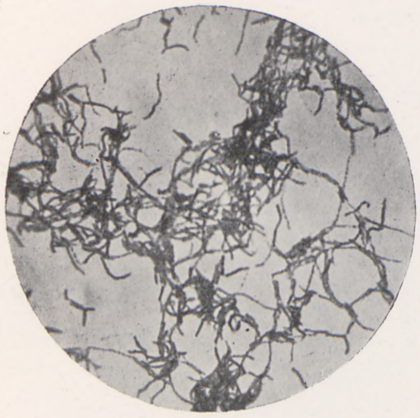
No. 1. A G - Plate, 10 Days, 20°.



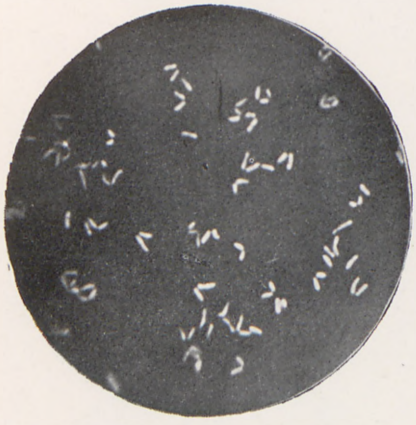
No. 4. Agar Plate, 4 Days, 30°.

No. 5. Agar Plate, 4 Days, 30°.

Microbacterium mesentericum.



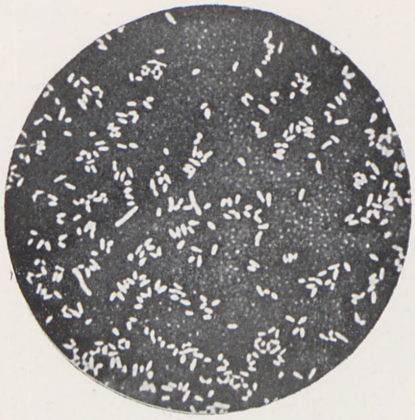
No. 7, Agar Streak, 1 Day, 30°, *Gram* stained.



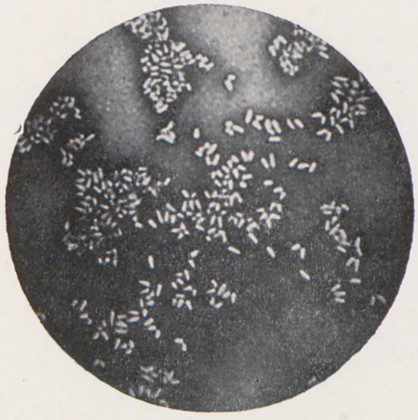
Nr. 7, Agar Plate, 3 Days, 30°.

Nr. 7, A G-Plate, 10 Days, 20°.

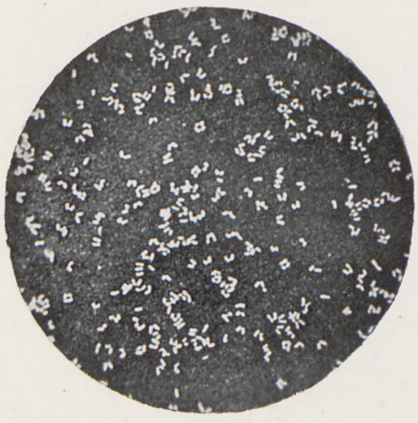
Microbacterium flavum.



No. 9, Agar Plate, 3 Days, 30°.



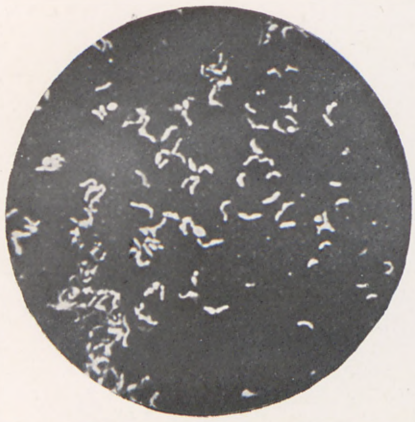
No. 10, Agar Streak, 2 Days, 30°.



Microbacterium liquefaciens.
Agar Plate, 3 Days, 30°.

Autotypi. Pacht & Crone's Eftflg.

Bacterium bifidum.

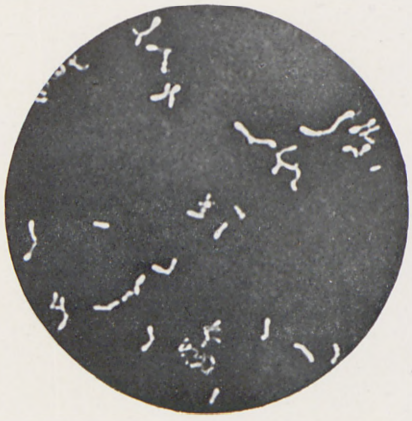


From Infant II, Agar Stab, 7 Days, 37,5°.

From Infant III, Agar Stab, 7 Days, 37,5°.

Bacterium acidi propionici.

x, Agar Stab, 2 Days, 30°.



b, Agar Stab, 2 Days, 39°.