SOME CHARACTERS WHICH DIFFERENTIATE THE LACTIC-ACID STREPTOCOCCUS FROM STREPTOCOCCI OF THE PYOGENES TYPE OCCURRING IN MILK

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INTRODUCTION

Much confusion exists in bacteriological literature concerning the identity of the lactic-acid streptococcus and its relation to the other chain-forming cocci which occur in milk. It is generally considered that the true lactic-acid bacteria (variously known as *Bact. lactis-acidi, Streptococcus lacticus, B. lactici-acidi, Bacterium* or *Streptococcus guntheri*, etc.) comprise a definite and rather well defined group of organisms. But no sharp points of distinction have been established between these organisms and other streptococci, notably the pyogenic streptococci, which are usually, if not always, to be found in market milk.

The descriptions given for the lactic-acid streptococcus in the older publications on systematic bacteriology, such as the books by Migula (1900) and Chester (1901), could be applied equally well to the pathogenic streptococci. The same confusion is found in the more recent literature. Weigmann (1911) includes the streptococcus of bovine mammitis in the same group as the true lactic-acid organism. Ernst (1914) says that there is no known method by which the lactic-acid streptococcus and the mammitis streptococcus can be differentiated. Jordan (1915) states that "the milk streptococcus in all its properties is extraordinarily like *Streptococcus pyogenes*." Heinemann (1906) who has extensively studied the lactic-acid bacteria, concludes that

"*Streptococcus lacticus* agrees in morphological, cultural and coagulative properties with pathogenic, fecal and sewage streptococci."

Certain morphological characters are emphasized by some workers as differentiating the true lactic-acid organisms from other streptococci. It is pointed out that the cells of the milk streptococcus are usually elongated and also that the characteristic grouping is in pairs and short chains rather than in long chains such as are usually found in the true streptococci. These points cannot be used as sufficient ground for differentiation since it is well known that many cultures of the lactic-acid bacteria appear as perfect cocci, and the formation of the long chains is not uncommon. Further, the streptococci from pathological sources are frequently of the short chain type. Chain formation, cell size and cell shape are so greatly influenced by the nature of the nutrient medium that distinctions based upon them are bound to be of doubtful value.

Hastings (1911) has called attention to the fact that the lactic-acid organism when grown in litmus milk causes a complete reduction of the litmus previous to the curdling of the milk. This is not true of the streptococci in general with which, as a rule, the action on litmus is more gradual and not so complete. This character has been used by Hastings and his associates to differentiate the true lactic-acid bacteria from other streptococci which occur in milk and cheese. Unless, however, this test can be correlated with some other points of difference, we would not be justified in accepting it alone as giving a firm basis upon which to separate the different groups of milk streptococci.

From the study of a large collection of streptococci isolated from milk, infected udders, saliva and feces of cows, Rogers and Dahlberg (1914) were able to show that the streptococci of milk resemble those which are associated with mammitis in cattle, and that the types occurring in the cows' mouths and feces are present only in small numbers and are probably relatively unimportant in milk. They further pointed out that the streptococci from infected udders had the same physiological characteristics as the well known *Streptococcus pyogenes*. It has

recently been demonstrated (Sherman and Hastings, 1914; Evans, 1916) that a large proportion of healthy milch cows harbor streptococci within their udders which are morphologically and culturally identical with the typical *Strept. pyogenes* of mammitis and other pathological conditions. Hence such organisms are probably always present in milk produced from any considerable number of animals.

From all of these facts it would appear that there are two main groups of streptococci in milk, the true lactic-acid organisms and streptococci of the pyogenes type whose chief source is the udder. The lines of cleavage between these groups should certainly be established, and it was with the object in view of finding points of differential value that the present study was undertaken.

SELECTION OF CULTURES

The ordinary way in which to attack a problem of this kind would be to isolate a large number of miscellaneous chain-forming cocci from various grades of market milk and milk products and to subject them to a detailed study with the intention of separating them in that way into their natural divisions. Our manner of approach has been different in that the cultures were obtained in such a way as would tend to limit the collection to organisms typical of the groups under consideration, and not to include a great number of closely related types which would complicate the study and make more difficult their separation into natural groups. Since the true lactic-acid organism has not been defined so as to enable it to be distinguished from other closely related bacteria, it is obviously impossible to tell just which are and which are not typical lactic streptococci. It would seem, however, that the organisms which take an active part in the natural souring of dinorary market milk could be properly designated as true lactic-acid bacteria. Proceeding on this assumption, cultures were isolated by the following method:

Ten samples of raw milk and cream were obtained from the receiving vat of the Pennsylvania State College Creamery on as many different days. These samples were of the mixed prod-

uct from a large number of different dairy farms and so might well be considered as representative of market milk or cream of ordinary quality. After collection the milks were allowed to stand at laboratory temperature until the acidity began to rise. When the acidity reached about 0.2 per cent lactic-acid the samples were plated on lactose agar in dilutions of 1/10,000,000 cc. The use of such a high dilution should result in the isolation of the organisms which are taking the most active part in the acid fermentation. The plates were incubated at 37°C. for two days, after which small, dense colonies surrounded by a hazy precipitate[1] were fished off into sterile milk tubes. Five colonies were selected from each sample making a total of fifty cultures of this type. Without exception the bacteria selected in this way produced an acid fermentation with the formation of a smooth homogeneous curd. No consideration was given to morphology in the selection of these cultures. The cultures of this group were numbered from 1 to 50 inclusive, and after being tested for purity, were added to the collection.

Cultures representing the pyogenic type of streptococci were obtained as follows: Samples were taken from the individual cows of the Pennsylvania State College dairy herd by drawing the milk directly from the udder into sterile flasks. Samples of the mixed milk from the College herd were also obtained. This milk is of a very high grade, being produced under the best of sanitary conditions, and its flora is consequently made up chiefly of the types of bacteria which come from the udder.

These milks were plated in dilutions[2] of 1/100 on lactose agar and incubated at 37°C. for two days. Colonies similar to those of the lactic-acid organisms mentioned above were transferred to glucose broth. Cultures which formed chains in broth were

[1] Bacteriologists engaged in milk studies are familiar with the precipitate formed around the colonies of the lactic-acid bacteria when grown on agar made with Witte's peptone and containing a fermentable sugar. When grown on media made with some of the American brands of peptone this characteristic is not seen. Witte's peptone was used in the preparation of the agar from which these cultures were isolated.

[2] In the case of cow 459, which had garget, the milk was plated in a dilution of 1/1,000,000 cc.

saved for study. The fifty cultures selected came from the milks of seven individual cows and from the mixed herd milk as follows:

Culture Numbers	*From Cow*
51 to 63	663
54 to 60	337
61 to 66	459
67 to 72	631
73 to 77	734
78 to 83	620
84 to 90	624
91 to 100	mixed herd milk

All of the animals from which these samples were obtained were in a healthy condition, except cow 459, which was suffering from a case of mammitis.

MORPHOLOGY

Microscopic examinations were made of all of the 100 cultures on agar, broth and bile. Although no hard and fast rules can be given, it may be said from this study that on agar and broth the group of organisms numbered from 1 to 50 showed a greater tendency to form elongated cells than did the others. Also the typical grouping, that is the grouping of the majority of cells, was usually in pairs rather than in definite chains. But chain formation was common in these cultures, and in some of them it was the predominating grouping. The grouping in pairs was frequent among the cultures numbered from 51 to 100, but in all of these the typical arrangement was in chains.

The results obtained from growth on lactose-peptone-bile were surprising in that the lactic-acid cultures made a much more luxuriant growth than did the udder streptococci. On this medium the lactic organisms grew in long chains and appeared as typical streptococci in every way. In only one of these cultures on bile were as many cells arranged in pairs as were present in chains. This is of special interest in view of the fact that many health laboratories use bile as a presumptive test for the presence of undesirable streptococci in milk. Kinyoun and Dieter (1912) consider that the formation of chains in bile

inoculated with milk is evidence of fecal contamination, while Rogers, Clark and Evans (1916) believe from their results that it is indicative of infected udders in some of the milk producing animals. In our tests the streptococci from the udder grew more feebly in bile than did the lactic-acid streptococci, and, in most cases, could be found only with difficulty in microscopic preparations. When examined after two days incubation at 37°C. bacteria were observed in only 17 of the 50 cultures. Those seen were all in typical chain formation.

In table 1 is presented a summary of the results obtained in this study. The examinations of broth and agar cultures were made after incubation at 37°C. for one day. The growth ex-

TABLE 1

Chain formation on agar, broth and bile

	CULTURES 1 TO 50	CULTURES 51 TO 100
	per cent positive	*per cent positive*
Chains on lactose agar	58	100
Chains on glucose broth	50	100
Chains on lactose-peptone-bile	100	100
Chains predominating on broth	24	100
Chains predominating on bile	98	100

amined from agar was taken from the sloped surface, not from the water of condensation. Chain formation was not recorded as positive unless definite chains of ten or more cells in length were seen.

These data indicate that some distinction may be drawn between these two groups of streptococci based upon cell grouping. For example, all of the cultures numbered from 51 to 100 showed chains on agar and broth, while this was true of only about one-half of the organisms from the other group. If consideration is taken of the grouping of the majority of cells a greater difference is seen. On broth in all of the streptococci of the udder type the predominating arrangement was in chains whereas this was the case in only about one-fourth of the other cultures.

Although it might appear that chain formation is of some value in distinguishing the lactic-acid bacteria from other streptococci, we do not wish to make that claim. Such a differentiation would at best be a doubtful one. The method used in collecting cultures for this work was such as would tend to introduce an error in this phase of the study; only cultures which showed typical chain arrangement were selected as representing the udder-type of streptococcus, while no attention was paid to morphology in selecting the lactic-acid type. As was noted before, chain formation is not a very constant character. In this study there was apparently no correllation between the lactic cultures which grew in chains on broth and those which

TABLE 2

Action on milk

	CULTURES 1 TO 50	CULTURES 51 TO 100
	per cent positive	*per cent positive*
Milk curdled	100	100
Milk curdled in twenty-four hours	94	20
0.75 per cent lactic acid in milk	80	8

did so on agar, and it is doubtful if they would show much constancy in this respect on the same medium.

ACTION ON MILK

The amount of acid produced in milk, the presence of coagulation and the time required for curdling were noted. All of the cultures produced sufficient acid to cause the coagulation of milk when tested at 37°C. As may be seen from table 2, the organisms of the first group (cultures 1 to 50) showed a more prompt clotting of milk than did those of the second group. At 37°C. all but 3 in the first group curdled milk within twenty-four hours, while only 10 of the other group acted so promptly.

The maximum acidity produced in milk was tested by incubating the cultures for ten days at 35°C. The acidity developed

at this temperature would not be so high as if a lower incubation temperature were used, but for comparing the two classes of organisms it should serve the purpose. By this test it was found that the lactic-acid type of streptococci (cultures 1 to 50) as a group produced considerably larger amounts of acid in milk than the udder cultures. If an arbitrary standard of 0.75 per cent lactic acid is taken (table 2) it will be seen that the two groups are divided quite well by the acid producing powers of

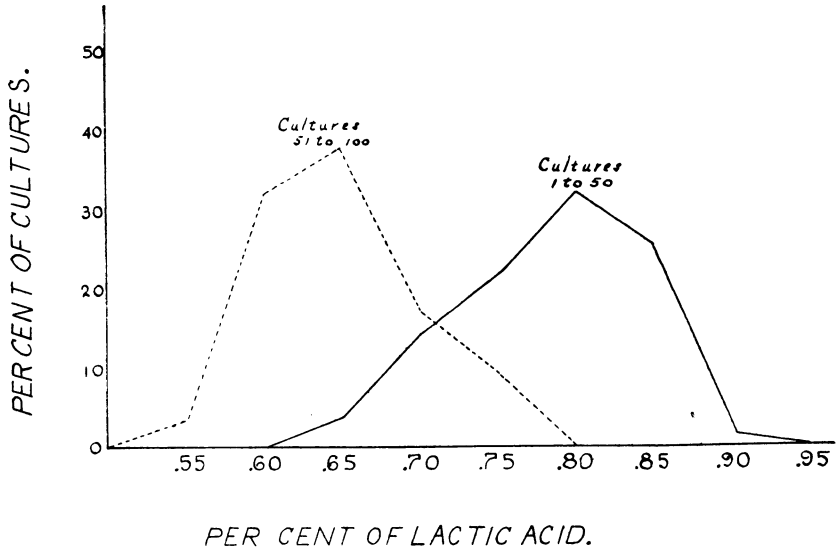

FIG. 1. FREQUENCY CURVE SHOWING ACID PRODUCTION IN MILK

their members. The difference in acid production is better shown by the frequency curves presented in figure 1.

These curves show a marked difference in the modes of the two groups as to acid production in milk. The same thing is true with respect to acid formation in lactose-peptone-bile. As was noted before, the lactic cultures appeared to grow more vigorously in bile than did the other streptococci, and the difference in the amounts of acid formed by the two types in this medium gave further evidence of that fact.

Although these results would appear to indicate that the true lactic-acid organisms as a class are capable of enduring a higher degree of acidity and of producing a more rapid coagulation of milk than the pyogenic streptococci, it is desirable to qualify this conclusion. The manner of securing the lactic streptococci was such as would tend to result in the collection of strains which were especially vigorous in their growth and action on milk. As is well known, the amount of acid produced

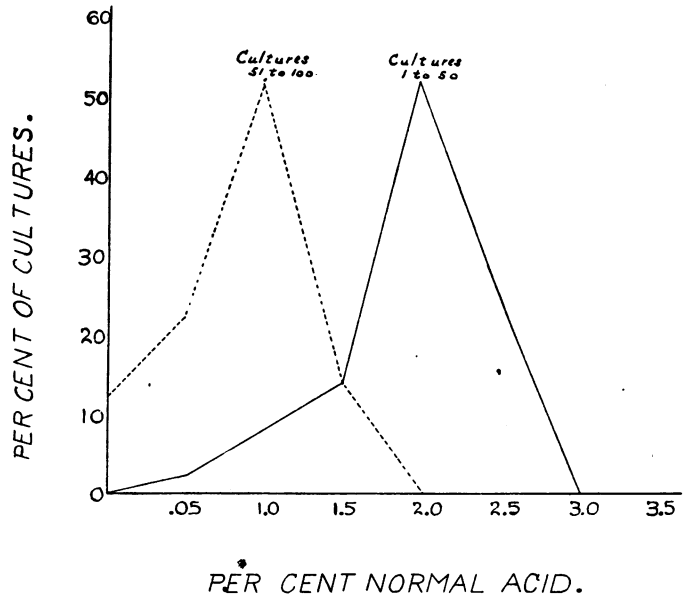

FIG. 2. FREQUENCY CURVE SHOWING ACID PRODUCTION IN LACTOSE-PEPTONE-BILE

in milk is not a very stable character and may be greatly altered by various factors such as, for example, growth on laboratory media. However, the degree of titratable acidity developed is probably of some differential value and it is not unlikely that determinations of actual hydrogen-ion concentrations, such as were made by Clark and Lubs (1915) with the colon-aerogenes bacteria and by Ayers (1915) with pathogenic and non-pathogenic streptococci, would reveal a fundamental difference between these two groups.

FERMENTATIVE CHARACTERISTICS

The value of fermentation tests with carbohydrates and related compounds in systematic bacteriology has become well established. The fermentative characteristics have been made use of especially in studies on streptococci, and by their means this very evasive group has been subdivided into a number of fairly well defined types. These tests have not been used, successfully at least, to distinguish between streptococci of the pyogenic and lactic-acid types.

In this study glucose, galactose, levulose, maltose, lactose, sucrose, raffinose, dextrin, inulin, starch, glycerine, mannit and salicin were used. The medium used for the fermentation tests had the following composition:

	per cent
Beef extract	0.3
Peptone	1.0
Dibasic potassium phosphate	0.5
Test substance	1.0

In the tests made with glucose, galactose, levulose and maltose the dibasic potassium phosphate was omitted.

In making the tests the cultures were incubated at 33°C. and then titrated against $\frac{N}{20}$ NaOH, with phenolphthalein as indicator, and the results expressed as per cent of normal acid. An increase above the control tube of 1 per cent normal acid was regarded as a positive test for fermentation. The sugars were incubated seven days and the other compounds two weeks before titration. In cases in which less than 25 per cent of the organisms were either positive or negative the minority cultures were retested. The results of this study are summarized in table 3.

A review of these data shows that the fermentative properties of the two groups are, generally speaking, quite similar. Although resembling each other on the whole, a very noticeable break in the similarity is found in the case of sucrose. It is seen that 76 per cent of the udder organisms attacked this substance as against only 6 per cent of the other group. The fermentative powers of the cultures are presented graphically in figure 3.

In this graph only the test substances are given which showed a difference of at least 5 per cent between the two groups.

From this it is evident that aside from sucrose none of the substances show sufficient difference to be of differential value. Exception might be taken to this statement in the case of maltose, in which all of the udder streptococci reacted positively while about one-fourth of the cultures of the other group were negative, but this could hardly be considered of great value for purposes of identification. Though none of the udder cultures of this collection fermented mannit, that fact is probably of no

TABLE 3

Fermentation of test substances

	CULTURES 1 TO 50	CULTURES 51 TO 100
	per cent positive	*per cent positive*
Glucose	100	100
Galactose	84	100
Levulose	94	100
Maltose	76	100
Lactose	100	96
Sucrose	6	76
Raffinose	0	0
Dextrin	8	18
Inulin	0	0
Starch	0	2
Glycerine	0	8
Mannit	28	0
Salicin	28	16

significance from the point of view of classification because it is well known that the ability to attack this substance is not uncommon among streptococci of the pyogenes type. In the work of Rogers and Dahlberg (1914) about one-fourth of the udder streptococci (from infected udders) fermented mannit, and this character was also common among cultures from other sources.

Though having no relation to the purpose of this paper, it is interesting to note that among the udder organisms all of the six cultures from one cow (no. 631) fermented salicin while only

two cultures of the remaining forty-four had that property. Of greater interest perhaps is the fact that five of the six cultures taken from cow 459, the only animal which had an infected udder, fermented dextrin. Whether this was merely a coin-

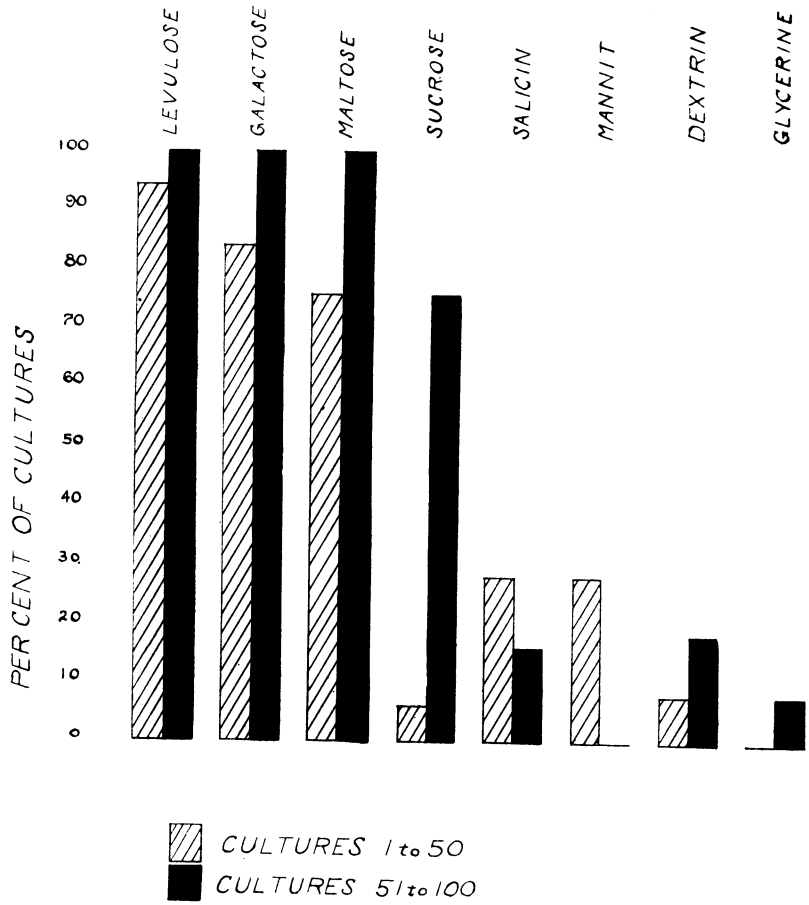

FIG. 3. GRAPH SHOWING DIFFERENCES IN FERMENTATIVE PROPERTIES OF CULTURES STUDIED

cidence or whether there exists a relation between the ability to attack dextrin and pathogenicity would require further study to answer. Two of the udder cultures failed to ferment lactose. Lactose negative streptococci have been shown by Andrewes

and Horder (1906) and by Winslow and Palmer (1910) to be numerous in the intestinal contents of the horse. In the present case, the two cultures in question not only caused an acid fermentation in milk, but produced such a vigorous one as to coagulate the casein. It seems, therefore, that these organisms were able to ferment lactose under favorable conditions but could not attack it for some reason when growing in the nutrient broth employed for the tests. A similar idiosyncrasy is noted in a few of the cultures reported by Rogers and Dahlberg (1914).

If it is conceded that the organisms of this collection numbered from 1 to 50 are representative of the group of true lactic-acid streptococci some significant points are brought out. It is generally stated in bacteriological texts[3] that the lactic organism ferments sucrose, while the statement is also found that mannit is one of the substances attacked, but according to our results the group is typically negative with both of these substances though some strains can ferment them. The typical *Strept. lacticus*, it would appear, ferments glucose, galactose, levulose, maltose and lactose. Of the cultures which failed to ferment either levulose or galactose none attacked maltose. Among the fifty cultures used there were fourteen which acted upon mannit and a similar number which fermented salicin. Nine of these attacked both compounds, thus showing quite a marked correlation between the fermentation of mannit and salicin. In most cases the cultures which fermented sucrose or dextrin also attacked both mannit and salicin.

TEMPERATURE RELATIONS

It is generally thought that the true lactic-acid streptococcus grows better at a lower temperature than the optimum for most pathogenic bacteria. Although it has not been demonstrated with any considerable number of cultures, the statement is usually made that the lactic organism has an optimum temperature of from 30°C. to 35°C. Stowell, Hilliard and Schlesinger

[3] See Weigmann's Mikologie der Milch; Marshall's Microbiology and Buchanan's Household Bacteriology.

(1913) have shown that streptococci from milk are, as a rule, more active at 20°C. than those isolated from the human throat.

Experiments at low temperatures of incubation were conducted, by inoculating litmus milk with the cultures and incubating at the desired temperature. Growth was determined by the presence or absence of visible changes in litmus milk. At 10°C. the two groups were differentiated perfectly; all of the cultures numbered from 1 to 50, which were supposed to represent the *Strept. lacticus* type, grew, while none of the group representing the *Strept. pyogenes* type did. The latter showed no change in the litmus milk after six weeks, but when put in a 37°C. incubator at the end of this period the tubes all turned acid, thus indicating that the cultures were alive but their

TABLE 4

Temperature relations

	CULTURES 1 TO 50	CULTURES 51 TO 100
	per cent positive	*per cent positive*
Growth at 10°C.	100	0
Growth at 43°C.	6	82

growth had been inhibited. The lactic streptococci all showed visible signs of growth within one week at 10°C.

Tests similar to the above were also made with high temperatures of incubation. At 43°C. was found a temperature which separated the two groups quite well, but the separation was not so perfect as at 10°C. In this case it was the pyogenic type which grew and the lactic type which failed to grow.

REDUCTION OF DYES

The use of stains as an aid in the identification of bacterial groups is not uncommon. Some familiar examples are the employment of neutral red, fuchsin and brilliant green for differentiating members of the colon-typhoid group of organisms. The reduction of neutral red was one of the characters advocated by Gordon (1905) as being of value in the separation of strep-

tococci. Rogers and Davis (1912) considered the reduction of this compound of differential value in their study of the lactic-acid bacteria of milk. As was noted earlier, Hastings (1911) has used the action on litmus in milk to distinguish the lactic-acid organism from other streptococci.

A preliminary survey of the various stains indicated that methylene blue, neutral red, litmus and indigo carmine might be of service in the present work. According to Fred (1912), bacteria, at least those types common in milk, reduce stains more actively in a milk medium than in broth, and a few tests made at the beginning of this study verified this conclusion. Tests were made by adding the dye to sterilized whole milk, the advantage of unskimmed milk being that the fat forms a layer over the surface which excludes the air quite effectively and thus reduction is not hindered. The litmus milk was prepared in the ordinary way by adding sufficient litmus solution to the milk to give a rather dark lavender color and then sterilizing. The other dyes were made as follows:

Methylene blue

Medicinal methylene blue	0.5 gram
Distilled water	1000 cc.

Indigo carmine

Indigo carmine (Kahlbaum's)	1.0 gram
Distilled water	1000 cc.

Neutral red

Neutral red (Grübler's)	0.1 gram
Distilled water	1000 cc.

The stain solutions and milk were sterilized separately and then mixed in the proportion of 1 cc. of stain to 10 cc. of milk.

In making the tests twenty-four hours old cultures of the organisms in milk were used to inoculate from, and the stain culture so prepared was incubated at 37°C. Observations were then made on three points, (1) reduction of stain, (2) time required to reduce, and (3) whether reduction was before or after curdling of milk. When no reduction was evident the cultures were allowed to remain six days before final examination was made.

As may be seen from table 5 the reduction of dyes proved to be an efficient test to differentiate between these two groups of bacteria. Methylene blue differentiated the groups perfectly, all of the true lactics causing a complete reduction of the stain within twenty-four hours and previous to curdling, while the udder streptococci failed entirely to cause reduction. After six days the tubes containing the latter class were not curdled and there was no evidence that growth had taken place. Some of these cultures were transferred to plain milk tubes but no growth occurred, thus indicating that not only were they unable

TABLE 5

Reduction of dyes

	CULTURES 1 TO 50	CULTURES 51 TO 100
	per cent positive	*per cent positive*
Methylene blue	100	0
Methylene blue (within twenty-four hours)	100	0
Methylene blue (before curdling)	100	0
Litmus	100	100*
Litmus (within twenty-four hours)	100	0
Litmus (before curdling)	100	0
Indigo carmine	100	100*
Indigo carmine (within twenty-four hours)	100	0
Indigo carmine (before curdling)	100	0
Neutral red	92	0
Neutral red (within twenty-four hours)	84	0
Neutral red (before curdling)	4	0

* Reduction not complete.

to reduce methylene blue, but that, in the concentration used, it had entirely inhibited growth and led to their destruction. The longest time required for any of the lactic-acid streptococci to reduce was ten hours, while a large majority caused a complete decolorization within eight hours after inoculation.

The actions on litmus and indigo carmine were apparently identical. In both cases the lactic-acid streptococci caused a prompt reduction; with litmus the longest time taken was eleven hours, while with indigo carmine thirteen and one-half hours was the longest time required. The udder cultures, on

the other hand, caused a reduction but it did not take place until after two or more days and then it was never absolutely complete. The important distinction, however, is that the reduction in the lactic cultures took place previous to curdling, whereas the pyogenic streptococci caused no reduction until after coagulation. This we believe represents a fundamental physiological difference between the two groups and it is to be considered, therefore, of value as a differential test. Since the litmus, indigo carmine, methylene blue and low temperature tests are all perfectly correlated, it would appear that the method of distinguishing between lactic-acid organisms and other milk streptococci based upon whether they cause reduction in litmus milk before curdling is sound.

Neutral red, which has been used considerably in studying streptococci, proved the least valuable of the four stains employed. Though the two groups were separated quite well, the distinction based upon this dye was not a perfect one. The lactic-acid organism did not cause as prompt reduction with neutral red as with the other stains, it requiring from twelve to fifty hours to decolorize this compound; and the reduction took place in most cases after curdling. Only four of the lactic cultures failed to reduce while all of the other streptococci gave negative reactions.

DISCUSSION

From the data which have been presented, it seems justifiable to conclude that the organisms studied represent two classes of streptococci; cultures numbered 1 to 50 the true lactic-acid streptococcus, and the other group (cultures 51 to 100) the pyogenic type. A review of the tests made will show a number of characters which differentiate the cultures into those two main types. The diagram given in figure 4 shows very clearly some of the fundamental differences between these groups. In this graph are presented only those tests which appeared to be of considerable differential value, those showing only slight differences between the two types being omitted.

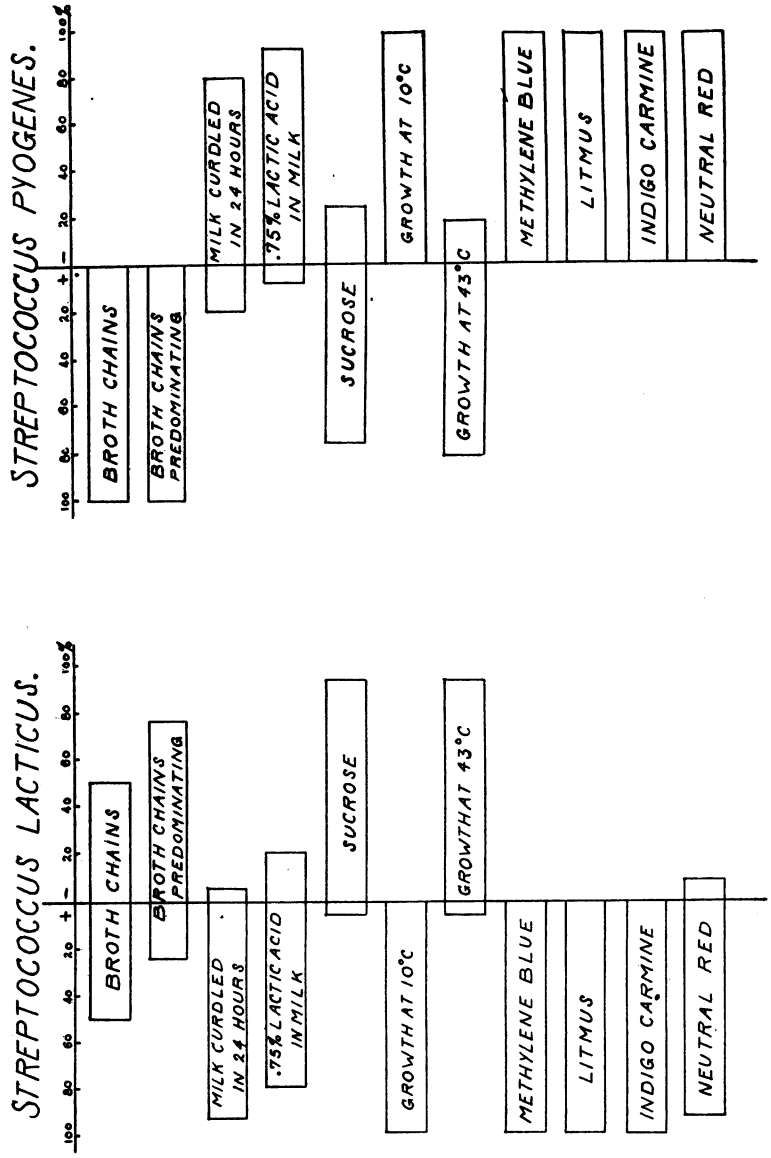

FIG. 4. DIAGRAM SHOWING DIFFERENCES BETWEEN THE LACTIC-ACID AND PYOGENIC STREPTOCOCCI

These tests, it is thought, are of sufficient value to make possible the separation of these two groups of streptococci, but it would be advantageous further to limit the number of tests and to establish the most constant ones. The differences in the groups relative to chain formation and action on milk are, in all probability, significant, but for reasons given earlier in this paper, it is not desirable to emphasize them greatly. The remaining characteristics, however, we believe to be stable ones —as stability occurs in the physiological characters of bacteria. Among the dyes neutral red did not give an absolute differentiation of the cultures studied and so might be eliminated in favor of methylene blue. As the action on indigo carmine was identical in all cases with that on litmus, it also might be discarded. The five remaining tests we wish especially to recommend as furnishing a ready and simple means of separating the milk streptococci. Growth at 10°C., reduction of methylene blue in milk, and the reduction of litmus (or indigo carmine) in milk previous to curdling are characters which have divided perfectly, in our study, the two types; *Strept. lacticus* having in all cases reacted positively while the *Strept. pyogenes* cultures, without exception, failed so to act. Absolute differentiation was not obtained with the other two tests—growth at 43°C. and the fermentation of sucrose—but the *Strept. pyogenes* type, in the great majority of cases, gave positive reactions whereas the lactic-acid streptococci in both instances were usually negative.

Although the object of this work was only to establish points of difference between the two groups of organisms, it should, aside from its main purpose, be of value in helping to define more clearly the characteristics of the *Strept. lacticus* (*Bact. lactis-acidi*) group of bacteria. As was pointed out previously, our results do not substantiate some of the generally accepted ideas concerning its fermentative properties. The facts established with reference to its temperature requirements and reducing ability, should so identify the true lactic-acid organism as to enable more reliable work than has been possible in the past concerning morphology, physiology, natural habitat and pathogenicity.

SUMMARY

A study was made of 100 cultures of organisms isolated from milk. The collection was so made that 50 of these cultures, it is believed, represented the true lactic-acid streptococcus and the other 50, streptococci of the pyogenes type.

Morphological observations were made from agar, broth and bile. The tendency to form chains on agar and on broth was not so marked among the cultures of the *Strept. lacticus* group as among the organisms of the *Strept. pyogenes* type. On lactose-peptone-bile, however, the *Strept. lacticus* cultures grew readily and formed long, typical streptococcic chains.

Among the cultures studied, the representatives of the *Strept. lacticus* type as a class had a more vigorous action on milk than did the other streptococci; coagulation was usually more prompt and larger amounts of acid were formed.

The fermentative characteristics of the two groups were quite similar with all of the substances used except sucrose. This compound was attacked by 38 of the 50 cultures of the pyogenic streptococci, while all but three of the lactic-acid streptococci failed to ferment it.

At 10°C. all cultures of the lactic-acid bacteria grew while none of the cultures of the *Strept. pyogenes* type were able to develop at this temperature. At 43°C. only 3 of the lactic organisms grew, whereas the pyogenic streptococci developed in 84 per cent of the cases.

The reduction of stains proved a valuable means of distinguishing these groups. With methylene blue all of the lactic streptococci caused reduction, while all of the pyogenic streptococci failed to reduce. Litmus and indigo carmine in milk were completely reduced before curdling by all of the *Strept. lacticus* cultures; the cultures of *Strept. pyogenes* caused no decolorization of these compounds previous to curdling, and the reduction after coagulation was slow and never absolutely complete. Neutral red, though of value, did not give as perfect a differentiation between the two types as did the other dyes.

Differences in chain formation and action on milk are doubtless of some importance, but differentiation based on these characters is probably not to be recommended. The reduction of stains, the fermentation of sucrose and the temperature tests, on the other hand, are believed to represent more constant characters and to offer means by which these two groups of streptococci may be differentiated.

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