CLASSIFICATION OF THE *STREPTOCOCCUS LACTIS* GROUP

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SUMMARY

The lactic acid producing streptococci show certain differences, some of which must be of importance from the standpoint of the growth of these organisms in dairy products. A classification of this group of organisms has been proposed; it is not presumed to be complete and other types will necessarily be added. The classification is as follows:

Typical *S. lactis*

Malty *S. lactis*—*S. lactis var. maltigenes*

Ropy *S. lactis*—*S. lactis var. hollandicus*

Slow reducing *S. lactis*—*S. lactis var. anoxyphilus*

Slow coagulating *S. lactis*—*S. lactis var. tardus*

Heat resistant lactic acid streptococci—*S. thermophilus*
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The development of acid is the most common change which milk undergoes. There are many kinds of organisms that can bring this about and among these certain groups are readily recognizable. The group which is undoubtedly the most frequently encountered in the investigation of milk and other dairy products is the one ordinarily referred to as the Streptococcus lactis group. The work herein reported* represents an attempt to classify these organisms from the standpoint of the bacteriologist dealing with dairy products.

GENERAL CONSIDERATIONS

A study of any considerable number of cultures that would ordinarily be classed as S. lactis quickly shows that important differences occur among them and thus indicates that the name should be looked upon as referring to a group of closely related species or varieties. The differences involve the flavor and aroma production, the development of ropiness, reducing power, rate of coagulation and temperature requirements, and while these might be looked upon as of only minor importance by the systematic bacteriologist, the role that the organisms play in the souring of milk and cream and in the flavor and aroma development in fermented milks, butter, and cheese, makes such differences of great significance from the standpoint of the dairy industry. It seems probable that for the interpretation of bacteriological results on dairy products a division of the S. lactis group on the basis of some of these differences is of much greater importance than a division on the basis of sugar fermentations and growth on certain media. Many of the reported results of investigations of S. lactis are of less value to the bacteriologist familiar with this group than if more specific information had been given with reference to the characters of the organism studied.

LIMITATIONS OF THE S. LACTIS GROUP

Because the S. lactis group is so poorly defined it seems advisable in dealing with it to assume limits within which an organism must fall before it is to be considered as belonging to the group. The following characterization of the S. lactis group is the one adhered to in the work herein reported:

Any organism coagulating litmus milk rapidly or fairly rap-

*The authors are indebted to W. A. Cordes, who did much of the preliminary work on the study of the Streptococcus lactis group when he was assistant in dairying at the Iowa State College.
idly with reduction of the litmus but without digestion or the formation of gas and which appears in stains from milk as a gram positive coccus arranged in chains or pairs, the pairs rarely being grouped into clumps, is considered as belonging to the *S. lactis* group.

The rapid or fairly rapid coagulation of milk is a character whose inclusion in the above statement must be properly interpreted since it is not the result of a direct action of the organisms but is due to the action of one of the products formed by them. Coagulation occurs at a more or less definite acidity, depending on the composition of the milk and various other factors. It is probable that one organism might coagulate milk and that another quite like it except that it produced a few hundredths percent less acid might not; it would of course be unfortunate if these two organisms were not grouped together. Most of the *S. lactis* organisms produce acid in sufficient amounts so that the coagulation is quickly very definite, but certain organisms that should undoubtedly be classed as *S. lactis* coagulate more slowly. These latter forms can often be hastened in the time required for coagulation by a series of transfers thru milk at a favorable temperature.

The coagulation of the milk apparently influences the reduction of the litmus to a certain extent. While many cultures reduce the litmus before coagulation, others seem to reduce only as the coagulation proceeds from the bottom upward. It seems probable that the oxygen of the air which changes the colorless derivative back to litmus penetrates the milk more readily when it is liquid than after coagulation, and that some of the slower growing cultures cannot reduce the litmus except where the penetration of the oxygen is interfered with by coagulation. Variations that seem very constant occur in the reducing power of organisms, however, and certain rapidly growing cultures do not reduce until after coagulation has occurred. With all the *S. lactis* cultures the reduction is very definite if the observations are made at the proper time, even the slow reducers giving complete reduction shortly after coagulation has occurred.

In milk cultures digestion and the formation of gas are easily detected under ordinary conditions, especially when the cultures have been allowed to stand without agitation. The expression of whey, which may be confused with digestion if it is pronounced, is very unusual except after the cultures have been shaken or stirred and even then frequently does not occur. Gas production in coagulated milk results in bubbles in the curd or breaks caused by the bubbles on their way to the surface and these are ordinarily very conspicuous. Rarely *S. lactis* cultures with one or two tiny gas bubbles or breaks in the curd have been encountered, but this condition is not likely to be confused with the gas production of the typical gas formers; with the latter,
agitation or plunging a hot needle into the culture usually releases a great deal of gas, while with the former it does not. The gas so rarely evident with cultures of *S. lactis* is presumably carbon dioxide produced in the metabolism of the organism which does not readily escape from the coagulated milk.

The morphology of *S. lactis* in milk is best studied with the gram stain because of the differentiation this gives between the organisms and the casein. Frequently, where the smears are rather thick, some cells appear gram negative or poorly stained, due presumably to the protection given them by the casein, and this may give an incorrect idea of their size. It is more satisfactory to study the morphology in relatively thin areas in the smear.

A number of groups of organisms show some of the characters of the *S. lactis* forms, but most of them are easily differentiated. Certain of the organisms belonging to the genus *Lactobacillus* show essentially the same changes in litmus milk that *S. lactis* does; this is particularly true of *L. casei* while *L. bulgaricus* commonly shows less complete reduction than the typical *S. lactis*. The lactobacilli can ordinarily be readily distinguished by the morphology and the amount of acid produced in milk, but *L. casei* and *S. lactis* seem to grade into each other, which makes it sometimes difficult to tell with which of these species a culture should be placed. The organisms belonging to the rennet-acid group give in litmus milk at certain stages an appearance essentially like that given by *S. lactis* and the morphology is also quite the same; however, as the cultures age the very evident digestion that occurs makes a separation easy. Certain milk cultures of *S. paracitrovorus* show rather rapid coagulation and where this occurs reduction is usually sufficient so that the coagulated material suggests a culture of *S. lactis*. The reduction is usually less complete, however, than with the *S. lactis* organisms, and plunging a hot needle into the milk commonly releases gas in considerable quantities; in cases of doubt as to the classification, the volatile acid production serves as a final test. The morphology is of no aid in the differentiation of *S. paracitrovorus* and *S. lactis*, but the odor is helpful to persons experienced with the two groups. Certain of the micrococci produce acid in litmus milk and show a pronounced reduction, but the acid development is slow and the morphology and heavy growth on agar make it easy to distinguish these organisms from *S. lactis*.

**GENERAL BASIS FOR THE SEPARATION OF THE S. LACTIS GROUP**

Several thousand cultures of *S. lactis* have been studied in more or less detail, especially with reference to the variations that have been mentioned as of importance from the standpoint of dairying, and cultures showing certain unusual characters
have been encountered frequently enough to justify their separation into types or varieties. Altho there are correlated characters in one or two instances, with most of the types there is only one definite character on which the separation is based, so that the varietal designation is merely a method of recording some character of the organism which is important and also more or less unusual among the *S. lactis* cultures. It would be expected that an occasional culture would be found with characters that would throw it into two types and such a culture should receive two varietal designations; this need not be a cause of confusion if the type is considered to be a designation for a definite character.

**CLASSIFICATION OF THE S. LACTIS GROUP**

The cultures investigated suggest the following division of the *S. lactis* group; further study will undoubtedly show the necessity of additional types being included.

Typical *Streptococcus lactis*, showing only the common characters of this organism.

Type A, *S. lactis var. maltigenes*, (malty *S. lactis*) showing a malt-like (or caramel or burnt) flavor and odor in milk, cream, etc.

Type B, *S. lactis var. hollandicus*, (ropy *S. lactis*) showing a ropy condition in milk cultures, especially when the cultures are young.

Type C, *S. lactis var. anoxyphilus*, (slow reducing *S. lactis*) showing a comparatively slow reduction of litmus milk, etc.

Type D, *S. lactis var. tardus*, (slow coagulating *S. lactis*) showing a comparatively slow coagulation of milk.

Type E, *Streptococcus thermophilus*, (heat resistant lactic acid streptococci) showing considerable resistance to heat and growing poorly at 21°C.

A discussion of each of the types follows.

**TYPE A. STREPTOCoccus LACTIS (LISTER) LÖHNEIS VAR. MALTIGENES HAMMER AND CORDES**

Cultures varying from typical *S. lactis* by producing a malt-like flavor and aroma in milk.

Malty *S. lactis* is proposed as a common name.

McDonnell (6) named one of the lactic acid bacteria studied by him *Bacterium lactis acidi maltigenum* because it produced a malt-like flavor and odor in milk. Sadler (9) concluded that a cooked or burnt taste and an aroma resembling caramel in a sample of sour milk were due to an organism of the general type of Leichmann's '"Bacterium lactici acidi."' Hammer and Cordes (5) studied the organism producing a burnt or caramel flavor in dairy products and tentatively named it *Streptococcus lacticus var. maltigenus*; using the original species designation given to this organism by Lister, the name becomes *Streptococcus lactis var. maltigenes*.

The character which distinguishes type A is the development in milk or cream of a distinct flavor and odor that may be de-
scribed as malt-like, burnt or caramel, but which is definitely different than the overheated flavor resulting from high pasteurization exposures. In pure cultures in milk the organisms of this type can most conveniently be detected by the sense of smell; the sterilization of the milk gives such a pronounced heated taste that it tends to mask other flavors present, and moreover it is a common practice to add an indicator so that the formation of acid can readily be noted. Test tube cultures ordinarily give sufficient odor for the detection of type A, but the odor is, of course, more pronounced with larger amounts. The odor can probably be most readily detected soon after coagulation and it is frequently more pronounced when the cultures are allowed to develop at 37° C. than at lower temperatures; with repeated opening of a culture the odor rapidly becomes less pronounced.

In any series of cultures of type A grown under the same conditions there is considerable variation in the extent of the characteristic flavor and odor. This may be due in part to variations in the stage of development, but it seems that there are definite differences in the intensity of the flavor and odor produced by various cultures. The malt-like flavor and odor are not exactly the same at all stages in the development of a culture. In older cultures they both become less pronounced and less characteristic, but still definite enough to be recognized by a person experienced with them.

Butter sometimes shows a malt-like flavor and aroma because these are readily carried over to it from the cream and type A probably more often causes serious economic losses in this product than in any other. Pasteurization of cream may destroy the organisms causing the abnormality so that they will not be present in a living condition in the butter, but it will not entirely remove the products of their growth, and the butter may show a very definite malt-like flavor and aroma.

The malt-like flavor and odor have been frequently noted in the raw milk and cream coming into dairy plants at such low acidities (under 0.2 percent) that they would ordinarily be classed as sweet. This shows that the development of the flavor and odor may precede any considerable acid production. Hammer and Cordes determined the acidity at which the flavor was first noted in 100 c.c. quantities of sterile milk each inoculated with 0.5 c.c. of a milk culture of various strains of S. lactis var. maltigenes and held at 37° C. and found it to vary from 0.25 to 0.38 percent calc. as lactic acid. A greater development of the organisms was probably required to make the flavor evident in the case of the sterilized milk because of the masking effect of the flavor resulting from the heating.

The flavor and aroma due to type A are much more commonly encountered in the warm months than in the cool. The cream delivered to the Iowa State College Creamery frequently shows
this flavor and aroma in the spring, summer and early fall, while in the winter it is only rarely encountered. Cans of milk showing the defect are also occasionally received, and this most frequently happens when, because of a high temperature, the milk comes in containing a large number of bacteria. The malt-like flavor and aroma have been noted as especially common in milk and cream from certain farms due probably to carelessness with the utensils and to the lack of proper cooling.

When milk or cream in a raw condition shows the malt-like flavor, *S. lactis var. maltigenes* can readily be isolated. A satisfactory method for isolating is to plate on whey or beef infusion agar and then pick into litmus milk a number of colonies appearing like *S. lactis*; the cultures that show the *S. lactis* reaction are examined for such characteristics as odor and morphology, and the desired type selected. A sample of burnt cream with a malt-like flavor and aroma and having a bacterial count of 114,000,000 per c.c. as determined by the plate method, using whey agar and incubating for four days at room temperature, was examined with this procedure and 7 or 37 percent of the 19 *S. lactis* cultures picked were *S. lactis var. maltigenes*.

Two lots of butter made from raw cream with starter and having a very pronounced malt-like flavor were examined for *S. lactis var. maltigenes* by plating on whey agar and picking colonies into litmus milk; table I which gives the total *S. lactis* and *S. lactis var. maltigenes* cultures secured shows that type A made up 41 and 38 percent, respectively, of the total *S. lactis* cultures picked from the two samples.

In Iowa, cultures of *S. lactis var. maltigenes* can frequently be isolated from milk or cream which does not show the malt-like flavor and aroma, especially during the warmer weather, and this indicates that these organisms are rather common. A satisfactory method for isolating is to allow the sample to sour at a fairly high temperature, and then proceed with the technique used for isolating from material showing a malt-like flavor and aroma. Milk from each of nine producers was examined in this way and the total *S. lactis* cultures and the number of *S. lactis var. maltigenes* secured are given in table II along with the percents of the total *S. lactis* cultures that were made up of *S. lactis var. maltigenes*; these varied from 0 to 29. None of the samples of milk had a malt-like flavor or aroma, either originally or after the holding which involved three days at 21°C.

*S. lactis var. maltigenes* is readily killed by the usual pasteurization exposures so that samples of milk, cream and butter may show the malt-like flavor and aroma without containing the causative organism in a living condition.

The production of the malt-like flavor and aroma by certain *S. lactis* cultures seems to be quite constant and many cultures studied over long periods have repeatedly shown this character;
TABLE I. TOTAL S. lactis AND S. lactis var. maltigenes IN BUTTER MADE FROM RAW CREAM (WITH STARTER) AND HAVING A MALT-LIKE FLAVOR.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Total S. lactis cultures secured</th>
<th>Cultures of S. lactis var. maltigenes secured</th>
<th>Percent of total S. lactis made up of S. lactis var. maltigenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>9</td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>10</td>
<td>38</td>
</tr>
</tbody>
</table>

in a very few instances, however, the ability to produce the flavor and aroma seems to have been lost for no evident reason. The flavor and aroma producing property of the organisms is certainly constant enough and of sufficient importance so that it must be given some consideration in any classification that is to be useful to the dairy bacteriologist. Repeated attempts have been made to find some character correlated with this property but so far these have been unsuccessful.

GENERAL CHARACTERS OF TYPE A—S. lactis var. maltigenes

MORPHOLOGY:
The morphology shows nothing unusual. The organisms are frequently large, but not strikingly so; they easily stain very definitely positive with the gram stain and are ordinarily arranged in pairs with no tendency to a special grouping of the pairs.

CULTURAL CHARACTERS:
The cultural characters show nothing of special importance. The organisms are, in general, comparatively vigorous growers in the various media that are suitable for growth.

BIO-CHEMICAL FEATURES:

Acid production. In general acid is produced rather rapidly and S. lactis cultures coagulating slowly at room temperature have never been observed to give a malt-like flavor and aroma. Compared to other S. lactis cultures, type A produces rather large amounts of acid in milk. One series of 36 cultures gave an average acidity in milk of 0.82 percent after an incubation of ten days at room temperature while the maximum value was 0.98 and only a very few values fell below 0.70 percent. In another series of 11 cultures a number of acidities up to 1.10 percent were noted after an incubation of ten days at room temperature.

Type of lactic acid produced. Hammer and Cordes reported results on a number of cultures of type A which showed that the lactic acid

TABLE II. TOTAL S. lactis AND S. lactis var. maltigenes IN MILK FROM NINE PRODUCERS AFTER IT HAD SOURED WITHOUT SHOWING A MALT-LIKE FLAVOR OR AROMA.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Total S. lactis cultures secured</th>
<th>Cultures of S. lactis var. maltigenes secured</th>
<th>Percent of total S. lactis made up of S. lactis var. maltigenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>31</td>
<td>5</td>
<td>16</td>
</tr>
</tbody>
</table>
produced was of the d type. These findings have been confirmed by data obtained on a number of additional cultures.

**Carbon dioxide production.** The CO₂ produced* from 10 c.c. of milk in ten days at room temperature was equivalent to 1.6 c.c. of N/10 Ba (OH)₂ as an average for 35 cultures, while the individual values varied from 0.8 to 4.5 with most of them a little over 1 c.c.

**Temperature relationship.** *S. lactis* var. maltigenes grew well at 21°, 30° and 37° C. Acid production was slower at 21° than at 30° or 37° C, but there did not seem to be any regular difference between the two latter temperatures. The more rapid growth at a comparatively high temperature undoubtedly explains why the malt-like flavor and aroma are more pronounced at 37° C than at room temperature and also why the causative organism is more common during the warm weather than during the cold.

**Source of malt-like flavor and odor.** The malt-like flavor and aroma are readily produced in skim milk, whole milk and cream. In sterilized whey, whether secured with rennet or with acetic acid, the odor could often be noted, but in lactose bouillon, even after making additions of various kinds, it could not alto the growth was sometimes very good. It is probable that the products responsible for the malt-like flavor and odor are elaborated most readily in milk or in some milk derivative such as whey.

**Cause of malt-like flavor and aroma.** The nature of the material responsible for the malt-like condition has not been determined. It is apparently highly volatile because when milk fermented by type A is distilled by the method** used for determining the volatile acidity of fermented milk a malt-like odor can readily be detected at the end of the condenser tube early in the distillation and can also be noted in the distillate.

**TYPE B. STREPTOCOCCIUS LACTIS (LISTER) LÖHNIS VAR. HOLLANDICUS BUCHANAN AND HAMMER**

Cultures varying from typical *S. lactis* by producing a ropiness in milk.

Ropy *S. lactis* is used as a common name.

A number of species of organisms include occasional cultures that produce ropiness in milk and accordingly it would be expected that ropy *S. lactis* cultures would be encountered. Such cultures have been secured by different investigators from a wide variety of sources; a number of these isolations have been summarized by Buchanan and Hammer (3) and these authors established the name *Streptococcus lacticus* var. *hollandericus*.

The character which distinguishes type B is the production of a ropy or viscous condition in milk or cream which is quite distinct from the thickening of the milk due to the coagulation of the casein. Several different cell arrangements have been found accompanying the ropiness and there does not seem to be any morphological character regularly correlated with this condition. The ropiness can often be detected by the tendency of the material to hang together when a milk culture is shaken, but more

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*The CO₂ production was measured by means of the tubes used by Eldredge and Rogers. Centh. f. Bakt., etc. 49. 1914, p. 5. See also la. Agr. Exp. Sta. Res. Bul. 81, p. 29.

certain procedures are to plunge a sterilized wire loop into the fermented milk and slowly withdraw it or to pour the material from a tube or other container. The time of examination of the cultures is important; some cultures show the ropiness before coagulation of the milk while others do not and in old cultures the ropiness decreases and often disappears entirely.

The degree of the ropiness is extremely variable and ranges from scarcely detectable to a very tough and doughy condition. While some of this variation is due to differences in age, very definite differences between cultures also exist.

The temperature of growth of cultures is important as regards the development of ropiness and certain cultures which give a definiteropy condition at 21° C. fail to do this at 37° C.

The ropy type of *S. lactis* is of practical importance in connection with the development of ropiness in starters carried in dairy plants. For no apparent reason starters that have been entirely satisfactory suddenly show ropiness, which may be very pronounced, and, from these, ropy *S. lactis* cultures can commonly be secured by plating and picking colonies into litmus milk. In trials involving the carrying of such ropy starters for considerable periods under good conditions it was found that usually the ropiness was lost, then regained, and a variation with respect to this condition continued. These results indicate that getting rid of the ropiness in starters is a rather uncertain proposition and that ropy starters should be discarded instead of attempting to get them into a satisfactory condition. Occasionally starters are encountered which suggest ropiness but which are not definitely viscous and the distinction between very slight ropiness and no ropiness is sometimes difficult to make.

Buchanan and Hammer reported that starters from a certain manufacturing concern quite regularly became ropy under creamery conditions, and that this tended to increase thru the transfers, altho the commercial cultures failed to show any evidence of ropiness. Starters that were carried for a considerable period eventually lost their ropiness, but the ropiness could not be at once overcome by holding transfers at 37° C. as can often be done with ropy *S. lactis* cultures.

The cream coming into creameries occasionally shows ropiness and in the case of sour cream this is sometimes due to ropy *S. lactis*. In sweet cream and sweet milk, other species, such as *Achromobacter viscosum* and *Aerobacter aerogenes*, are ordinarily the cause of the condition since it appears that *S. lactis* cannot produce ropiness without sufficient growth to cause a definite acidity increase.

Hammer (4) secured some non-ropy *S. lactis* cultures when plating out ropy cultures and picking colonies and in one instance when plating out one of these non-ropy cultures ropy cultures were secured. This indicates that sudden variations may
occur in the ropy character and may explain the occasional development of ropiness in starters.

The ropy cultures of *S. lactis* studied have always been secured from ropy material and among the large number of *S. lactis* colonies picked from sources other than ropy starter and cream ropy cultures have never been encountered.

Different cultures of ropy *S. lactis* may show differences in morphology and at least three microscopic pictures have been obtained; these are pairs with chains, pairs with certain of the cells grouped in clumps, and pairs with no significant larger grouping. These three conditions grade into each other, however, and there is no sharp distinction between them. In certain starters which go ropy, chains of cells, some of which may be rather long, are observed in the smears, altho usually the portion of the total number of cells that are in chains is not large; non-ropy *S. lactis* cultures that show distinct chain formation are also encountered. Cultures showing ropiness and a tendency to chain formation can often be isolated from starters showing these conditions.

Hammer reported that ropy *S. lactis* cultures studied by him showed large numbers of cells arranged in pairs and an occasional clump made up of pairs of cells irregularly packed together; some of the clumps were small while others seemed to contain hundreds of pairs. However, non-ropy cultures secured from plates poured with ropy ones also showed the same general morphology and the ropy and non-ropy cultures could not be distinguished on the basis of morphology. Clumps made up of what were evidently pairs of organisms have occasionally been observed in non-ropy cultures of *S. lactis* and, in the instances tried, a series of transfers with such cultures failed to result in the development of ropiness.

In some ropy cultures of *S. lactis* secured from starters there was no evidence of either chains or clumps, but there seemed to be unusually large numbers of organisms in pairs. Such cultures, in the trials made, persisted in their ropiness and did not develop any significant grouping of the pairs of cells.

**GENERAL CHARACTERS OF TYPE B—*S. lactis var. hollandicus***

**MORPHOLOGY:**

Ropy *S. lactis* cultures show definite variations in the cell groupings, the pairs sometimes being in chains, sometimes in clumps and sometimes without special arrangement. The cells in different cultures may also show considerable variation in size; they stain readily with the gram stain.

**CULTURAL CHARACTERS:**

Aside from the production of ropiness in milk *S. lactis var. hollanicus* shows nothing unusual in its cultural characters. Ropiness is not produced in bouillons, etc.
BIO-CHEMICAL FEATURES:

Acid production. The ropy *S. lactis* cultures studied have all produced acid rather rapidly.

Type of lactic acid produced. Hammer reported that the ropy cultures showing clumps of pairs which he studied produced *d* lactic acid. Ropy *S. lactis* cultures, none of which showed a definite grouping of the pairs of cells, were isolated from several ropy starters and also found to produce *d* lactic acid.

**TYPE C. STREPTOCOCCUS LACTIS (LISTER) LÖHNIIS VAR. ANOXYPHILUS**

Cultures varying from typical *S. lactis* by a comparatively slow reduction of litmus.

Slow reducing *S. lactis* is proposed as a common name.

While pronounced powers of reducing litmus are commonly looked upon as an important character of the *S. lactis* organisms the litmus reducing powers vary and cultures that are comparatively slow reducers have been occasionally encountered.

The character that distinguishes type C is the comparatively slow reduction of litmus so that commonly coagulation precedes complete reduction. Observations must be made at the proper time if cultures of this type are to be detected. With most cultures of *S. lactis* reduction precedes coagulation so that litmus milk may be white, except for a small band at the top, before coagulation takes place, but with type C coagulation occurs before the litmus becomes white. When Janus Green is employed as an indicator in milk type C commonly causes coagulation when only the first stage of reduction has occurred so that the milk appears pink; with this medium cultures of *S. lactis* showing the usual reducing power have the reduction complete before coagulation occurs. With both litmus milk and Janus Green milk type C completes the reduction soon after coagulation and the cultures then have quite the usual white area in the lower part of the tubes. No other character has been found correlated with the slow reduction.

The only way so far found for securing type C is to isolate *S. lactis* cultures and then make careful and very frequent observations as to the relationship between coagulation and reduction; Janus Green milk is more satisfactory for this than litmus milk.

The work that has been carried out indicates that type C is only occasionally found in milk and cream in Iowa. In some instances several cultures have been picked from the same plate which suggests that this type multiplies in milk or cream when it happens to gain entrance. Coagulation of milk with type C is quite rapid so that it seems it would have a good opportunity for growth in mixtures of various *S. lactis* types.

The slower reduction of litmus or Janus Green with essentially
TABLE III. RESULTS SECURED ON ZINC SALTS OBTAINED FROM THE NON-VOLATILE ACID PRODUCED IN MILK AT ROOM TEMPERATURE BY \textit{S. lactis} var. \textit{anoxyophilus}.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Percent H\textsubscript{2}O Det. A</th>
<th>Percent H\textsubscript{2}O Det. B</th>
<th>Average</th>
<th>Rotation</th>
<th>Percent ZnO$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.04</td>
<td>13.02</td>
<td>13.03</td>
<td>1</td>
<td>33.73</td>
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<tr>
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<td>33.68</td>
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<tr>
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<td>12.84</td>
<td>13.14</td>
<td>13.09</td>
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<td>33.47</td>
</tr>
<tr>
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<td>13.14</td>
<td>13.09</td>
<td>1</td>
<td>33.66</td>
</tr>
</tbody>
</table>

$^*$Theoretical ZnO in zinc lactate is 33.46.

the same rate of acid formation shows that type C has a somewhat different oxygen relationship than the other types. In either litmus milk or Janus Green milk type C resembles the other types in showing reduction and coagulation in the extreme bottom before it does in the higher layers.

Slow reduction is a character which is quite constant and it has been found to persist thru a rather extended series of transfers in litmus milk and Janus Green milk. Observation at the proper time is very necessary if type C is to be recognized and delayed observation may suggest that the slow reduction character has been lost when this is not the case.

No evidence has so far been secured indicating that type C is of significance in dairy practice. It seems, however, that under certain conditions of oxygen supply this type might be of particular importance.

GENERAL CHARACTERS OF TYPE C—\textit{S. lactis} var. \textit{anoxyophilus}

MORPHOLOGY:

The morphology shows nothing unusual; the cells stain readily with the gram stain, and there is no tendency to a special grouping.

CULTURAL CHARACTERS:

The tendency for the color to persist thruout tubes of milk containing such indicators as litmus or Janus Green at the time of coagulation is the only cultural character out of the ordinary.

BIO-CHEMICAL FEATURES:

Acid production. All the cultures of type C studied have been rather rapid acid producers. The average acidity produced in milk after ten days at room temperature for 36 cultures was 0.79 percent.

Type of lactic acid produced. The results secured on the zinc salts obtained from the non-volatile acid produced in milk at room temperature by five cultures of \textit{S. lactis} var. \textit{anoxyophilus} are given in table III; the water of crystallization, the optical activity, and the percent ZnO indicate that the acid formed was lactic while the rotation of the Zn salt shows that the acid was of the $d$ type.

Carbon dioxide production. The CO\textsubscript{2} produced from 10 c.c. of milk in ten days at room temperature was equivalent to 1.8 c.c. of N/10 Ba (OH)$_2$ as an average for 35 cultures; the individual values ranged from 0.85 to 3.4 with most of them between 1 and 2 c.c.

Temperature relationship. All of the cultures of type C studied grew well at 21º, 30º and 37º C.; growth was regularly somewhat slower at 21º C. than at the higher temperatures.
TYPE D. **STREPTOCOCCUS LACTIS** (LISTER) LÖHNIS
VAR. TARDUS

Cultures varying from typical *S. lactis* by a comparatively slow coagulation of milk.

Slow coagulating *S. lactis* is proposed as a common name.

Variations regularly occur in the time required for coagulation when an *S. lactis* culture is inoculated into milk. These are in part due to such factors as the amount of inoculation, the temperature of holding and the stage of development of the inoculating material, but when these are all controlled striking differences still occur. Certain cultures, even when transferred under the most favorable conditions, show a comparatively slow coagulation. In general the slow coagulating cultures are also slower in their reduction than the more rapid coagulating cultures.

The rate of coagulation among these slow coagulating cultures varies considerably and with some coagulation is greatly delayed; frequently also with a given culture variations among the transfers occur and one transfer may not coagulate at all while the ones preceding and following it do.

When starters or even supposedly pure cultures of *S. lactis* are plated out and colonies picked variations in the rate of coagulation occur among the *S. lactis* cultures secured and often some coagulate only very slowly. Rogers and Davis (8) have pointed out that Schierbeek (1900) found that by replating and making subcultures new cultures could be obtained, some of which followed the active fermentation of the original, while others were slow in acid production. It accordingly seems necessary to recognize that slow coagulating cultures of *S. lactis* do exist instead of assuming that all the streptococci producing acid slowly in milk belong to other species.

Rogers and Davis reported that in their studies the cultures curdling milk tardily or not at all multiplied slowly and never attained the numbers reached by the cultures curdling milk in a short time. These authors believe that differences in the acid production in milk are due to a variation in the vitality rather than to a variation in the particular function of forming acid from sugar.

The rate of coagulation of milk is a character that apparently undergoes definite fluctuations. Cultures that coagulate rapidly may change to slow coagulating types even when transferred under conditions which would be expected to maintain the vitality of the organisms. Slow coagulating cultures can sometimes be secured from rapid coagulating ones by holding for a considerable time between transfers but in other cases such a change does not occur and on holding the rapid coagulating culture continues to give a rapid coagulating growth until it dies as a result of the exposure to acid. Slow coagulating cultures can sometimes be changed to rapid coagulating ones by a series of fre-
quent transfers while in other cases this is apparently impossible and even after long periods of transferring the cultures continue to show only a comparatively slow coagulation.

Microscopic examinations of slow coagulating cultures quite regularly show a comparatively small number of cells and this agrees with the idea of Rogers and Davis that the slow coagulation is due to a slow growth of the organisms. The cause of the slow growth is difficult to determine since apparently cells exposed in the same way may be differently influenced; in certain instances it seems that it may be due to an exposure to conditions unfavorable for growth because slow coagulating cultures may sometimes be secured when there is a delay in transferring.

In certain instances slow coagulating cultures of *S. lactis* seem to be rather common among the cultures picked from old sour cream and this suggests that they may be the result of exposure to conditions not satisfactory for growth. It would seem that the slow acid producers would be of little importance in the souring of milk or cream when the usual flora was present but it is possible that they may be of significance under special conditions; e.g. their presence in old sour cream in certain instances in unusual numbers suggests that they may be playing a part in the acid increase under these severe conditions.

Cultures which produce acid in milk without coagulating it are commonly encountered and it would seem that certain of these might be *S. lactis* cultures with which the rate of acid formation had been still more decreased than with *S. lactis var. tardus*.

**GENERAL CHARACTERS OF TYPE D—** *S. lactis var. tardus*

**MORPHOLOGY:**
Smears made from milk cultures show comparatively small numbers of organisms; these stain readily with the gram stain and there is no tendency to a special grouping of the pairs. The size of the cells seems to vary considerably in different cultures; even with the same culture there is considerable variation with the old cultures showing smaller cells than the young ones.

**CULTURAL CHARACTERS:**
The slow development both from the standpoint of acid production and reduction is the only unusual cultural character.

**BIO-CHEMICAL FEATURES:**
Acid production. In general, slow coagulating cultures produce rather small amounts of acid in milk even when the holding period is prolonged.

Type of lactic acid produced. Only one slow coagulating culture was studied as to the type of lactic acid formed and this produced d acid.

Carbon dioxide production. The CO₂ produced from 10 c.c. of milk in ten days at room temperature was equivalent to 2.3 c.c. of N/10 Ba (OH); as an average for 28 cultures, while the individual values varied from 0.75 to 4.9, with most of them between 1 and 2 c.c.
TYPE E. STREPTOCOCCUS THERMOPHILUS ORLA-JENSEN

Cultures varying from typical S. lactis mainly in the resistance to heat and in the growth temperature.

Heat resistant lactic acid streptococci is proposed as a common name.

Altho the S. lactis organisms isolated from raw sour milk or cream are ordinarily easily destroyed by heat, various investigators have found lactic acid producing streptococci which were quite heat resistant; Ayres and Johnson (1 and 2) have reviewed a number of such instances. These authors in one of their investigations found a lactic acid organism whose thermal death point was 74.4°C. (166°F.) in broth and 75.6°C. (168°F.) in milk when heated in Sternberg bulbs for 30 minutes and in another secured an organism whose thermal death point was 79.4°C. when a broth culture was heated in a Sternberg bulb for 30 minutes.

The character which primarily distinguishes type E is the resistance of the organism to high temperatures. Along with the heat resistance the organism ordinarily shows two other characters that are not common with the S. lactis types; these are much slower growth at room temperature than at higher temperatures such as 37°C, and less complete reduction.

The heat resistant lactic acid streptococci grow rapidly at 37°C. but rather slowly at lower temperatures. At 37°C. coagulation of litmus milk ordinarily occurs rather quickly—24 to 48 hours with a fairly heavy inoculation—but at 21°C. coagulation requires considerable time if it occurs at all; at the lower temperatures, however, there is commonly a definite growth with reddening of the litmus and in some cases where coagulation is not complete there is some coagulation in the bottom of the tube of milk.

While at 37°C. there is usually definite reduction in the bottom of a tube of litmus milk in which type E has grown, this reduction does not extend to as near the surface as with the other S. lactis types and often at about the time of coagulation the reduced portion does not involve over one-third of a two to three inch depth of milk. The very narrow pink band at the surface of a litmus milk tube, which is so common with most types of S. lactis, is not observed. At room temperature reduction is frequently very slight; with only a small amount of coagulation it is common to find the entire coagulated portion reduced but where coagulation is complete the reduction never extends to near the surface.

The importance of type E lies in its relationship to pasteurized milk and cream. It is practically always found in these products and plays a part in the changes that occur in them on standing, especially at a fairly high temperature. The organism may undoubtedly be important in certain other dairy products, such as
Swiss cheese in the press, where the temperature is rather high for a considerable period.

*S. thermophilus* can readily be isolated by taking pasteurized milk or cream collected so as to exclude outside contamination, allowing it to stand until there is a coagulation and then plating on whey or infusion agar and after incubation of the plates picking colonies into litmus milk; the plates may be incubated at room temperature or 37°C, altho the latter gives the more rapid colony development. The inoculated litmus milk should be held at 37°C; the cultures which coagulate milk fairly rapidly without digestion or the formation of gas and with some reduction of the litmus and which show the usual morphology are commonly *S. thermophilus* but should be checked further as to heat resistance, comparative growth at 37° and 21°C, and possibly in other ways since contamination may have resulted in types that are not heat resistant being present.

In pasteurized milk or cream held at rather high temperatures (e.g. 37°C) type E does not control the fermentation in the way that some of the *S. lactis* cultures usually do at 21°C and pronounced changes other than acid development are often present. Gas formation, due to anaerobic spore formers, digestion and off flavors are frequently evident but commonly do not seriously interfere with the isolation of *S. thermophilus* altho the plates show a much greater variety of colonies than plates poured with milk soured at 21°C. It must be recognized that contamination after pasteurization may result in the presence of types of *S. lactis* other than that resistant to heat.

*S. thermophilus* is rarely encountered in the isolation of *S. lactis* cultures from raw milk, either sweet or sour, and this is probably due to the slow growth of this type in competition with the other types at the common holding temperatures. Its resistance to heat, however, makes it the type that is present in heated dairy products, from which it can be readily isolated after enrichment.

**GENERAL CHARACTERS OF TYPE E—*S. thermophilus***

**MORPHOLOGY:**

The morphology shows nothing unusual. The organisms stain definitely gram positive and show no special grouping of the pairs.

**TABLE IV. RESULTS ON ZINC SALTS OBTAINED FROM THE NON-VOLATILE ACID PRODUCED IN MILK AT 37°C. BY *S. thermophilus***

<table>
<thead>
<tr>
<th>Culture</th>
<th>Percent H₂O</th>
<th>Percent ZnO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Det. A</td>
<td>Det. B</td>
<td>Average</td>
</tr>
<tr>
<td>1</td>
<td>12.90</td>
<td>12.84</td>
</tr>
<tr>
<td>2</td>
<td>12.86</td>
<td>12.73</td>
</tr>
<tr>
<td>3</td>
<td>12.74</td>
<td>12.81</td>
</tr>
<tr>
<td>4</td>
<td>12.99</td>
<td>12.85</td>
</tr>
<tr>
<td>5</td>
<td>13.31</td>
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<td>12.89</td>
</tr>
<tr>
<td>7</td>
<td>13.15</td>
<td>12.96</td>
</tr>
<tr>
<td>8</td>
<td>13.98</td>
<td>13.01</td>
</tr>
<tr>
<td>9</td>
<td>12.91</td>
<td>12.87</td>
</tr>
<tr>
<td>10</td>
<td>12.84</td>
<td>12.83</td>
</tr>
</tbody>
</table>
Sometimes the cells seem to be rather small and few in number but not strikingly so.

**CULTURAL CHARACTERS:**
The less complete reduction of litmus milk is the cultural character in which type E varies definitely from the other *S. lactis* types.

**BIO-CHEMICAL FEATURES:**

Acid production. At 37°C. type E produces acid quite rapidly, but at room temperature the increase in the acidity is very slow. Eighteen cultures were tried out as to the acidity produced in ten days at 37°C.; the individual results varied from 0.59 to 0.91 percent and averaged 0.76 percent.

Type of lactic acid produced. The results secured on the zinc salts obtained from the non-volatile acid produced in milk at 37°C. are given in Table IV and indicate the formation of lactic acid of the d type.

Carbon dioxide production. The CO₂ produced from 10 c.c. of milk in ten days at room temperature was equivalent to 2.1 c.c of N/10 Ba (OH)₃ as an average for 18 cultures while the individual values varied from 1.05 to 4.95 with most of them between 1 and 2 c.c.

Temperature relationship. Temperatures such as 37°C. are much more favorable than lower temperatures.

**CULTURES WHICH SHOULD BE CLASSED UNDER TWO VARIETIES**

It has already been suggested under the heading “General basis for the separation of the *S. lactis* group,” that an occasional culture showing characters which would class it under two varieties is to be expected. Several cultures have been isolated which produced a malt-like flavor and aroma in milk and which also were slow reducing but these constitute the only real instances of cultures showing such a combination of characters that they should be classed under two varieties. It would seem that certain combinations of characters would probably be encountered, e.g. heat resistant cultures which produce a malt-like flavor and aroma would be expected because *S. thermophilus* and *S. lactis* var. *maltigenes* both are favored by a comparatively high temperature, but thus far these anticipated combinations have not been observed.

The cultures showing characters which put them into two varieties should presumably receive a combination of varietal designations. Those producing a malt-like flavor and aroma and which at the same time were slow reducing might be designated as *S. lactis* var. *maltigenes* and var. *anoxyphilus*; while this is an awkward name it is less cumbersome than a statement of the characters of the organisms and the information conveyed by it might be very important under certain conditions.
DISCUSSION

The *S. lactis* types that have been suggested are distinct and constant enough in their characters so that it would seem they are entitled to definite designations. The differences between them are important, at least from the standpoint of the dairy bacteriologist, and there should be some way of indicating which one of the types is meant. The list of types proposed is not intended to be complete and includes only some of the more common ones. *S. lactis* cultures which do not fall in with any of the types suggested have been encountered, e.g. cultures which fail to grow at 37°C, but which coagulate rapidly at 21°C. However, these have been isolated so infrequently that even an incomplete understanding of their general characters is impossible and accordingly their addition to the list of types must await a more detailed study of them.

It is often difficult to know whether two organisms should be put into separate species or into varieties within a species. In the suggested classification *S. thermophilus* is recognized as a separate species because of the very considerable differences between it and the other organisms, while the other types are put on a varietal basis. Further study may suggest a different treatment but the close relationship of the organisms listed under *S. lactis* must not be lost sight of. Because of the great differences in opinions on the classification of organisms an attempt has been made to keep the proposed classification on a conservative basis.

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