# CONCERNING THE HABITAT OF STREPTOCOCCUS LACTIS

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The first streptococcus studied was *Streptococcus lactis*, which was discovered and isolated by Lister (1873, 1878) and named by him *Bacterium lactis*. Because of its technical importance this organism has been extensively investigated, but, notwithstanding the large body of scientific knowledge which has been accumulated concerning its biological properties and practical uses, the natural habitat of *Streptococcus lactis* has long been a moot question.

While utensils have been accepted as probably the most common immediate source of this organism in milk, such fortuitous objects on which growth may occur do not represent a true habitat in an ecological sense. In the older literature are to be found many claims of the occurrence of *Streptococcus lactis* in the udders, mouths and feces of cows, but such investigations were conducted before proper differential tests were at hand for the identification of *Streptococcus lactis* and its separation from other closely related types, especially *Streptococcus fecalis*.

It has long been amply established that *Streptococcus lactis* is not a normal inhabitant of the bovine udder. (Rogers and Dahlberg, 1914; Sherman and Hastings, 1915; Evans, 1916; Sherman and Albus, 1918; Orla-Jensen, 1919; Ayers and Mudge, 1922.) Although Esten (1909) reported the finding of *Streptococcus lactis* (*Bacterium lactis-acidi*) in the mouths and feces of cows, and McGuire (1915) claimed *Streptococcus lactis* to be a normal inhabitant of cow feces, Ayers and Mudge (1923), with more modern methods of study, did not find this organism among the characteristic streptococci of the bovine mouth,

throat or feces. While Esten used the characteristic reducing action in litmus milk as the criterion for *Streptococcus lactis*, his methods would not differentiate this organism from that group of fecal streptococci typified by *Streptococcus fecalis*. (Orla-Jensen, 1919; Sherman and Stark, 1931, 1934.)

## METHODS

In the examination of such substances as ears of corn (in the husks), pods of peas, heads of cabbage and lettuce, etc. aseptic methods were employed to obtain samples from their interiors.

Samples of fresh corn, young corn silks, frozen fresh corn, corn meal, wheat flour middlings, dried navy beans, and young cabbage heads were placed in sterile distilled water and held at room temperature for twenty-four hours or longer. When a stained preparation made from such an infusion indicated that Gram-positive cocci in pairs or chains predominated, the sample was plated on milk agar. If Gram-positive cocci in pairs or chains failed to predominate but were observed in small numbers, the material was inoculated in serial dilutions into tubes of litmus milk. After incubation, milk agar plates were made from the highest dilution in litmus milk which showed reduction before curdling. From the milk agar plates pure culture isolations were made.

When examining materials such as lettuce, which contained small numbers of streptococci in the presence of large numbers of Gram-negative rods, it was found desirable to add the material to sterile water containing 10 per cent of sterile litmus milk. This medium was held until sufficient acid was produced to retard the growth of Gram-negative rods and accelerate overgrowth by the streptococci. When this condition prevailed serial dilutions were made into litmus milk tubes, isolations being made by plating after the streptococci gained the ascendency in the milk cultures.

Samples from the mouths and throats of cows, taken by means of sterile swabs, were placed in sterile water containing litmus milk and held at 10°C. for a few days to prevent the growth of *Streptococcus bovis* (and its relatives), normally present in large

numbers in the cow's mouth, and to permit its overgrowth by other streptococci present in smaller numbers. Later, these samples were incubated at 30°C. until enough acid was produced to inhibit or partially destroy the Gram-negative rods present. From milk agar plates made from this material, streptococci (but not *Streptococcus lactis*) were readily isolated. Additional isolations were made after further culturing in litmus milk.

The samples of feces, soil, and other non-vegetable materials were placed in sterile water containing litmus milk, and after incubation at room temperature, transfers were successively made through litmus milk cultures as outlined above, in attempts to isolate *Streptococcus lactis*.

The streptococci isolated were tested for their ability to grow at 10°C. and 45°C., action in litmus milk, action on blood agar, liquefaction of gelatin, production of ammonia from peptone, production of carbon dioxide from glucose, limiting pH in glucose broth, and action on eleven carbohydrates and related test substances. As these methods are all now more or less standard procedures in the study of streptococci, no special description of the techniques is called for. Because of its misapplication by a few workers, however, it is desirable to say a word about the testing of bacteria for their temperature limits of growth: not only is it necessary that the incubator temperature be accurate, but the test cultures should be adjusted immediately after inoculation to the incubation temperature in a water bath, and incubation should be carried out in sealed tubes or otherwise so as to preclude a cool layer at the surface of the culture due to excessive evaporation.

## EXPERIMENTAL FINDINGS

In all, 200 cultures which were identified as *Streptococcus lactis* have been isolated from various plant sources. Both the grains and silks from fresh corn at the proper stage for human consumption were found in every instance tested to contain *Streptococcus lactis*. Only one sample each of frozen corn and corn meal was tested, but *Streptococcus lactis* was isolated from each of these. Several samples of dried navy beans were tested and found in each

case to contain *Streptococcus lactis*, which could be isolated with ease. It was also obtained from cabbage, head lettuce, garden peas, and wheat middlings, but because of the presence of larger numbers of other types of bacteria the isolation of *Streptococcus lactis* from these sources was more difficult.

The characteristics of the 200 cultures isolated from plants, together with their sources, are given in table 1. Included in the table for comparative purposes are the characteristics of 35 cultures of *Streptococcus lactis* which were recently isolated from milk and milk products. As may be noted from the table, the characteristics of the organisms from plants agree with those

TABLE 1

*Characteristics of cultures of Streptococcus lactis from plants and from milk*

| SOURCE | NUMBER OF CULTURES | GROWTH AT 10°C. | GROWTH AT 45°C. | LITMUS MILK REDUCED BEFORE CURDLING | BETA TYPE OF HEMOLYSIS ON BLOOD AGAR | FINAL pH OF 4.2 TO 4.0 IN GLUCOSE BROTH | AMMONIA PRODUCED FROM PEPTONE | CARBON DIOXIDE PRODUCED FROM GLUCOSE | LIQUEFACTION OF GELATIN | ARABINOSE | XYLOSE | GLUCOSE | LACTOSE | MALTOSE | SUCROSE | RAFFINOSE | INULIN | GLYCEROL | MANNITOL | SALICIN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Corn | 82 | + | − | + | − | + | + | − | − | + | + | + | + | + | ± | − | − | − | + | + |
| Navy beans | 48 | + | − | + | − | + | + | − | − | ± | ± | + | + | + | ± | − | − | − | ± | + |
| Cabbage | 20 | + | − | + | − | + | + | − | − | − | + | + | + | + | − | − | − | − | + | + |
| Wheat | 19 | + | − | + | − | + | + | − | − | − | + | + | + | + | ± | − | − | − | ± | + |
| Garden peas | 18 | + | − | + | − | + | + | − | − | − | + | + | + | + | − | − | − | − | − | + |
| Head lettuce | 13 | + | − | + | − | + | + | − | − | − | + | + | + | + | + | − | − | − | + | + |
| Milk | 35 | + | − | + | − | + | + | − | − | − | ± | + | + | + | ± | − | − | − | ± | + |

obtained from milk and with the properties of *Streptococcus lactis*, as this species or group has been described and limited by recent authorities.

While negative findings in a study of this type should be accepted with some reserve, it is interesting to note the sources from which we failed to isolate the organism. *Streptococcus lactis* was not obtained from: mature corn, 12 samples; aged heads of cabbage, 2 samples; lima beans, 2 samples; alfalfa, 2 samples; soil, 12 samples; intestines of earth worms, 6 samples; mouths and throats of cows, 4 samples; bovine feces, 12 samples; human feces, 7 samples.

It should be noted that *Streptococcus fecalis*, which has been very generally confused with *Streptococcus lactis*, was obtained from the mouths and throats of cows, bovine feces and human feces.

From the data given in table 1 it is seen that all of the 235 cultures from plants and milk agree perfectly with the standard descriptions of *Streptococcus lactis*. Without exception, they were found to have the following characteristics: Growth took place at 10°C.; there was no growth at 45°C.; litmus in milk cultures was reduced before curdling took place; true hemolysis of the beta type was not produced in blood; a low final pH was produced in glucose broth; ammonia was produced from peptone; carbon dioxide was not produced from glucose; gelatin was not liquefied; glucose, maltose, lactose and salicin were fermented; but raffinose, inulin and glycerol were not fermented.

Fermenting and non-fermenting strains were found with arabinose, xylose, sucrose and mannitol, which finding is in keeping with the results of all workers who have used these test substances with the *Streptococus lactis* group. It should be noted also that strains which do not ferment maltose or salicin are well known, but were not encountered in the present study. Likewise, Ayers, Johnson and Mudge (1924) have reported strains of *Streptococcus lactis* which do not produce ammonia from peptone, while Hammer and Baker (1926) and others have recognized varieties based on characters not used in this study. In connection with the variable characters found in the group, it should be mentioned that while *Streptococcus lactis* has never been found to produce true hemolysis of the beta type on blood agar, strains which give either the alpha (usually weak) or gamma reaction are common, and both types were represented in the collection used in this study. In addition, some strains of *Streptococcus lactis* cause a slight hydrolysis of starch.

While all agree that individual strains of the *Streptococcus lactis* group vary in their respective actions on arabinose, xylose, sucrose and mannitol, for our present purpose it is desirable to show whether or not the cultures obtained from plants correspond with those from milk and milk products with respect to these characters. These data are assembled in table 2.

It is seen that the cultures fall into nine groups based upon their actions on these four test substances. The plant strains are found in eight groups, while the small collection of only 35 cultures from milk fall in six groups, five of which coincide with groups from plants. Three groups (1, 3 and 4) are represented by plant strains but not by any of the milk cultures included in this collection. However, reference to the published data of other workers reveals cultures of *Streptococcus lactis* from milk or milk products which fit into these groups: Group 1, Anna Orla-Jensen and Hansen (1932); group 3, S. Orla-Jensen (1919); and group 3, S. Orla-Jensen (1919), and Anna Orla-Jensen and Hansen (1932).

TABLE 2

*Cultures of Streptococcus lactis from plants and milk grouped according to their abilities to ferment arabinose, xylose, sucrose and mannitol*

| GROUP | ISOLATED FROM | ARABINOSE | XYLOSE | SUCROSE | MANNITOL |
|---|---|---|---|---|---|
| 1 | Corn, navy beans, milk* | + | + | + | + |
| 2 | Lettuce, wheat, milk | − | + | + | + |
| 3 | Corn, milk* | + | + | − | + |
| 4 | Cabbage, wheat, milk* | − | + | − | + |
| 5 | Milk | − | − | + | + |
| 6 | Wheat, milk | − | + | + | − |
| 7 | Navy beans, milk | − | − | − | + |
| 8 | Garden peas, wheat, milk | − | + | − | − |
| 9 | Navy beans, milk | − | − | − | − |

* Milk cultures in groups 1, 3 and 4 obtained from data of other workers.

Although somewhat aside from the present problem, it is interesting to note that of the sixteen possible groupings with the use of the above four test substances, nine groups are represented in the collection of 235 cultures used in this study. As a matter of fact we have been able to find representatives of the remaining seven groups in the data of other workers. In other words, those who are inclined to divide the *Streptococcus lactis* group into additional species on the basis of slight variations would obtain sixteen "species" by the use of these four test substances. If the other known variable characters were included, the resulting number of possible "species" would become absurd.

## DISCUSSION

The data presented in this paper are sufficient evidence, we believe, that *Streptococcus lactis* occurs on plants and that, therefore, this source is the most probable one from which it finds its way into milk. This finding is not surprising, as doubtless many others have also suspected that this organism is associated with plants, rather than animals, in nature. There have been many reports of the occurrence of *Streptococcus lactis* among the lactic acid bacteria present in fermenting vegetable materials, but such a finding is no more remarkable, nor ecologically significant, than its occurrence in sour milk.

Hüttig (1931) claimed the conversion of *Streptococcus lactis* into *Bacterium herbicola*, and *vice versa*, by heating the cultures at 58°C. for twenty minutes, combined with aging and other manipulations. He considered *Streptococcus lactis* to be a dissociative form of *Bacterium herbicola*. While not to be interpreted as a reflection upon the interesting results of Hüttig, our observations while isolating *Streptococcus lactis* from plant materials may have some pertinence in this connection. These two organisms were frequently observed growing together in mixed cultures on agar plates made from plant infusions, and not infrequently in the same well-isolated colony. Sometimes, discrete colonies of *Streptococcus lactis* were observed growing within and up through the colonies of *Bacterium herbicola*. However, we experienced no difficulty in separating the two organisms by reisolation, and it should be emphasized that we observed no such radical dissociation among the pure cultures of *Streptococcus lactis* which were used in the present investigations.

Although it appears to be established that *Streptococcus lactis* occurs on plants, conclusions concerning the natural habitat of an organism should not be lightly drawn. *A priori*, one might expect upon the discovery of a true habitat of a bacterium to find there the organism in large or fairly abundant numbers. We have no evidence that this is true in the case of *Streptococcus lactis* on plants. On the other hand, many bacteria are found in only small numbers in what we believe to be their natural habitats, as, for examples, certain soil and water forms and those

which occur on the skin, within the udder, etc. In the course of evolution, many organisms have found habitats under environmental conditions which serve admirably for the preservation of the species but place rigid restrictions upon reproduction. In the present case, we feel that the data presented are suggestive but do not warrant dogmatic conclusions concerning the true habitat of *Streptococcus lactis*.

## SUMMARY

*Streptococcus lactis* has been repeatedly isolated from certain plants but not from all plants examined.

It was not found in the mouths nor throats of cows, bovine feces, human feces, nor in soil.

It is suggested that plants may represent the natural habitat of *Streptococcus lactis*.

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