BACTERIOLOGICAL STUDIES ON TWO YELLOW MILK ORGANISMS

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DAIRY SECTION

Ames, Iowa
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INTRODUCTION

The different milk constituents vary greatly in their susceptibility to the action of the bacteria that make up the flora commonly found in milk. At ordinary temperatures in raw milk, the lactose is soon attacked and acid formed, but if the acid producing organisms are destroyed by some such procedure as heating, pronounced changes soon take place in the casein. The fat is one of the milk constituents that is more resistant to bacterial action, yet even in it striking changes are observed under certain conditions. Some of the common milk organisms, even some of the acid producing types, are able to change the fat, altho frequently considerable periods of time are required for such action.

Two organisms have recently been isolated in the dairy bacteriology laboratory of the Iowa State college that are peculiar in their action on the cream layer of milk, inasmuch as they produce a decidedly yellow color in it, apparently without breaking down the fat. The common practice of producing a yellow color in butter and butter substitutes by the addition of coloring material made it seem advisable to investigate these organisms from the standpoint of their action on the other milk constituents as well as on fats foreign to butter, but used in the manufacture of butter substitutes.

HISTORICAL

Altho yellow milk is commonly mentioned in the dairy texts, the literature on the subject is not at all extensive, while the original articles for the most part date back a considerable number of years.

Micro-organisms may be the cause of yellow milk, either indirectly or directly. Among the cases resulting indirectly, come those in which yellow milk is secreted as a result of some diseased condition of the udder, the condition most frequently responsible being garget. Freeman,\(^1\) quoting Hanauer, mentions that as

early as 1732, in Braunschweig-Luneburgische, an edict requiring that the milk of the cows be poured on the grounds and buried because of an epidemic also stated that the milk was of a yellowish-red color or had yellowish-red streaks. Hess, Schaffer and Bondzynski in their studies of the physical and chemical changes in milk from inflamed udders frequently found the milk yellow.

Löhnis has pointed out that yellow milk may be secreted whether the udder infection is due to Streptococci or Staphylococci; he apparently based his ideas on the literature of the subject and refers to yellow milk accompanying udder infections due to Streptococci and to the work of Krüger who studied a sample of dirty yellow milk from an infected udder and concluded that a Staphylococcus was responsible.

According to Mohler, if there is an involvement of the udder in foot and mouth disease, the milk is yellowish in addition to being viscous.

A yellow color is sometimes produced in milk as a direct result of the growth of certain microorganisms. Ehrenberg (1840) first described a specific organism as the cause of yellow milk and named it *Vibrio synxanthus*. Fuchs (1841) studied yellow milk and found what was probably Ehrenberg’s organism; he named it *Vibrio xanthogenus*. This organism was later more carefully investigated by Schröter and named *Bacterium xanthinum* by him. Lister’s (1873) *Bacterium No. II* was also probably identical with this form; Lister states that his organism produced a peculiar golden tint on the surface of boiled milk and that there was some similar yellow material deposited at the bottom of the glass. *Bact. synxanthum* is the name commonly used at the present time but *Bacillus synxanthus* should have the preference.

According to Löhnis, Haberlandt (1875) found in milk, that had been broken down by *Oidium lactis*, yellow red globules that probably were derived from the fat and which gave a yellow to saffron red color in the upper layers. Löhnis also states that Hüppe (1884) observed intense yellow-orange spots on cream as a result of the growth of *Micro. aurantiacus*. Adametz (1890) mentioned an organism isolated by List that is very similar to *Bacillus synxanthus* and stated that he had found it on the rind of

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5 Krüger. Centbl. f. Bakt. 7: 590. 1890.
9 Schröter. Cohn’s Beiträge. 1870: 120.
12 Loc. cit.
13 Adametz. Oesterreichische Monatsschrift für Thierheilkunde. 15: 66. 1890.
Emmenthal cheese where it sometimes formed small yellow point like colonies. Barthel\textsuperscript{14} (1897) found golden yellow spots on the surface of Port-du-Salut cheese and less frequently spots of a smaller size in the depth of the cheese. These spots proved to be colonies of *Microc. flavus desidens* (Flügge) and the organisms when inoculated into sterile milk at 22° C. produced golden yellow surface colonies, without coagulation of the casein, after two days. Hohl\textsuperscript{15} (1906) isolated an organism from Galium Mollugo L. which produces a ropiness in sterile milk in 24 hours at 20-30° C. and after 8 to 10 days the milk coagulates with an amphotheric or weakly acid reaction and throws out a clear serum that is citron yellow and ropy. Unsterile milk may, on inoculation become slightly slimy, but if so the sliminess disappears when the milk coagulates as a result of the acid development. The organism was considered by Hohl to be closely related to *Bacillus synxanthus*.

Organisms which digest milk to a yellowish material are not infrequently encountered among the organisms found in ordinary milk samples and Cohn and his associates have described a number of these in their classification of dairy bacteria. In the plating of market milk, yellow colonies, some of which are intensely colored are also commonly observed. On transferring such colonies to tubes of sterile milk, the usual result is to get a small amount of yellow sediment in the medium, as the first observable change; these organisms on account of their slow development in milk are of no importance in the market milk industry.

Yellow spots have been observed on butter, although they are of little significance under ordinary conditions. According to Wiegmann\textsuperscript{16} they occur most frequently when the butter is exposed, the causative organisms apparently coming from the air. Krueger\textsuperscript{17} (1890) observed a case of yellow butter from which he isolated a spore forming yeast, *Saccharomyces flava lactis*. The yellow color occurred only on the outside of the butter and the organism when inoculated into milk, formed a yellow membrane there. The production of yellow spots on and in cheese has also been reported and the work of Barthel\textsuperscript{18} (1897) along this line has already been mentioned. A yellow color in either butter or cheese is much more uncommon than a red color; a number of organisms causing the latter change in both materials have been described.

The yellow fermentation of milk and milk derivatives has evidently been of only very little practical importance; the organisms responsible for this change grow comparatively slowly

\textsuperscript{14} Barthel. Jahresber. u. d. Fort. d. Lehre v. d. Gährungs-Org. 8: 204. 1897. (Abs. by Leichman.)
\textsuperscript{16} Weigmann. Mykologie der Milch. 79.
\textsuperscript{17} Krueger. Centbl. f. Bakt. 7: 468. 1890.
\textsuperscript{18} Loc. cit.
and the production of acid in milk apparently prevents their development there, under ordinary conditions.

**ORGANISM 1**

Organism 1, which was isolated during some work on bitter milk, produces an intense yellow to orange color in cream or in the cream layer of milk. The sample of milk from which the organism was obtained came from a cow that was kept in one of the smaller towns of Iowa. The milk was drawn with considerable care, although no attempt was made to exclude all outside contamination, and was plated on the agar recommended by the American Public Health association for milk work. After incubating the plates at 20°C, a number of colonies were picked off and inoculated into milk, an attempt being made to secure all of the types. None of the agar colonies showed a pronounced color and it was not until the inoculations into sterile whole milk were made that the color producing ability of the organism was known. Only one culture of this organism was secured although it is possible that there may have been others on the plates.

The action of the organism on milk and milk derivations received the most attention. Skim milk was quite rapidly digested to a greenish yellow material, the digestion being well started after 48 hours at 20°C, and being nearly complete after 5 or 6 days at this temperature. The coagulation of the casein also occurred, the time of its appearance depending on the temperature at which the culture was held. At room temperature it was commonly present in 48 hours while at 37°C, it did not occur until later. The curd was never firm and the coagulation would not have been noticed unless the cultures had been examined carefully. A tenacious dirty white pellicle formed on the digested milk within 2 or 3 days and eventually became quite heavy; the pellicle floated even after it was detached from the glass. In cultures a number of weeks old there still remained a very small amount of undigested casein or some casein derivative. In old cultures the digested material also showed a moderate degree of ropiness.

The presence of fat in milk, however, modified the appearance of the fermented milk materially. The digestion of the casein with the formation of a greenish yellow material went on as in the case of the skim milk, although the change seemed to be delayed and less complete, while the fat, or the upper layer only if there was considerable, became an intense yellow to orange in color. After 48 hours at 20°C, the color was quite pronounced but was by no means as abundant as it was in older cultures. The most intense color did not occur at the surface where there was a free exposure to the air but just beneath this surface layer.
As a result, the color observed when looking down into the mouth of a flask of fermented milk was more of a yellow and less intense than the pronounced orange observed when the flask was viewed from the side. In very old cultures there was a decrease in the intensity of the color of the fat layer with practically a total disappearance of the orange; the fat also tended to fall slightly while a small amount of serum collected above it.

The reaction change observed in milk is shown in table I, the acidities being calculated as lactic acid. From the results presented it will be seen that apparently room temperature was more favorable for acid production than a temperature of 37°C, skim milk at 37°C, evidently developing no detectable acid. More acid seemed to be produced in whole milk than in skim milk, but in all cases there was eventually a decrease in the acidity, the skim milk at 37°C becoming alkaline after a number of days.

**TABLE I. REACTION CHANGE IN MILK INOCULATED WITH ORGANISM 1. ACIDITIES CALCULATED AS LACTIC ACID**

<table>
<thead>
<tr>
<th>Period of Growth in Days</th>
<th>At 37°C</th>
<th>At Room Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skim Milk</td>
<td>Whole Milk</td>
</tr>
<tr>
<td>1</td>
<td>.15% acid</td>
<td>.15% acid</td>
</tr>
<tr>
<td>2</td>
<td>.12% acid</td>
<td>.18% acid</td>
</tr>
<tr>
<td>20</td>
<td>Alkaline</td>
<td>.11% acid</td>
</tr>
<tr>
<td>24</td>
<td>Alkaline</td>
<td>.13% acid</td>
</tr>
</tbody>
</table>

In some of the flasks of whole milk in which there was a good color production, small black granules were observed at the surface and next to the glass. At first these were thought to be a contaminating mold but a microscopic examination disproved this as they were found to be structureless. It is believed that they represent one of the products that is sometimes, but not constantly, produced by the organism.

As would be expected with an organism that produced such a rapid digestion of the casein, the odor of the fermented milk, either skim or whole, was very disagreeable and suggestive of putrefaction; the same is true of the fermented creams. The odor was so strong that a few small flasks of fermented milk were responsible for an offensive odor throughout most of the laboratory. In skim milk the odor was equally as strong as in whole milk or cream so it seems that the production of the odor is associated with the breaking down of the casein and not with any action on the fat.

The action of the organism on materials other than butter fat was studied.* Inoculations directly onto oleo oil and neutral lard failed to give a perceptible growth, undoubtedly because of the lack of moisture and perhaps nutrient material, as well.

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*G. L. Noble, a student in advanced dairy bacteriology, assisted in this work and also in the work on the manufacture of butter from cream in which Organism 1 had grown.
Other inoculation made onto oleo oil, neutral lard, and cotton seed oil, to which in each case peptone had been added, also gave no perceptible growth, and here it seems likely that the lack of moisture was responsible. Oleo oil, neutral lard and cotton seed oil were separately used with skim milk, the milk being sterilized after the addition of the foreign materials. Altho color production did not occur as quickly, proceed as rapidly nor reach such an intensity as when whole milk was used, a definite yellowish color was obtained in each case. With the oleo oil and neutral lard, the distribution of the color was much the same as in whole milk, but with cotton seed oil the color production seemed to occur between the milk and the oil floating above. In some of the flasks with the oleo oil or neutral lard a yellowish green color developed while in others the color was more of a yellowish orange. In the skim milk to which the different materials had been added there was a digestion of the casein with the production of a disagreeable odor much the same as there was in whole milk or cream.

Oleo oil, neutral lard, cotton seed oil and butter were also used with plain bouillon and with lactose bouillon. The organism in these materials failed to produce a color, except in some cases a faint green, and here the materials added were not believed to be in any way connected with the color production. The bouillon developed a heavy turbidity and sediment, with probably considerable growth near the surface and in contact with the added materials.

The production of color, as is so frequently the case, seemed to be dependent to a large extent on the temperature. At 37° C. in whole milk there was only a very slight color production, the intensity being much less than the intensity in the same milk at room temperature or at 20° C.

On account of the presence of the color in the fat, some of the fermented cream was churned in order to see the effect of the color on the resulting butter. Because of the low acidity developed by the organism in question, Bact. lactis acidi was added after there had been a good development of yellow color in the cream. Small amounts of the cream were churned in a bottle and the butter washed as much as 4 times, but the disagreeable odor present in the cream could not be overcome. The taste also was very objectionable, being decidedly biting, but the butter had a decidedly golden yellow color which gave it a good appearance.

The effect of various conditions on this yellow butter was then studied. At room temperature in a glass jar and in the presence of light the intensity of the color increased, and the increase in color was greatest on the side towards the light, the jars having been placed against a wall. In a sealed jar the change in color was much the same throughout the depth of the jar, but in an un-
sealed jar, only the lower two-thirds of the butter layer showed the golden yellow color and the upper one-third became a dirty gray, shading to a dirty pink at the surface. When held in a cool room in the dark, both the sealed and unsealed jars failed to undergo any change except a decrease in the intensity of the color; when held in the dark at room temperature there was apparently an additional decrease in the intensity of the color.

In order to determine whether or not there was a decrease in the amount of fat in milk inoculated with this organism, fat determinations were made after cultures had stood for various lengths of time. The results obtained by the Babcock method are given in table II and indicate that the fat is not broken down by the organism even during considerable periods of time.

**TABLE II. EFFECT OF ORGANISM I ON THE PERCENTAGE OF FAT IN MILK**

<table>
<thead>
<tr>
<th>Period of Growth in Days</th>
<th>% Fat in Control Flask</th>
<th>% Fat in Inoculated Milk Trial 1</th>
<th>% Fat in Inoculated Milk Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>5.0</td>
<td>4.9</td>
<td>5.0</td>
</tr>
<tr>
<td>31</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>
DESCRIPTION OF ORGANISM 1

The organism was studied morphologically, culturally, and biochemically with the following results:

MORPHOLOGY.

Form — The organism was found to be rod-shaped.
Size — In a 24 hour agar culture the organisms were from .5 to .6 microns wide and from 1.3 to 2.2 microns long on the average, although these values by no means represent the extremes encountered. Milk cultures 24 hours old showed organisms of approximately the same size, while in old milk cultures the organisms were not so large.
Arrangement — The organisms were frequently observed isolated but a paired arrangement was common even in old cultures, while in young cultures it was a very common arrangement.
Motility — Young bouillon cultures showed numerous actively motile organisms.
Staining Reactions — The organism stained well with the stains commonly used, although quite irregularly with Loeffler’s methylene blue, two organisms side by side frequently taking the stain very differently. The Gram stain was negative, *Bact. lactic acidii* being used as a positive control.
Spore Formation — Although there was frequently an appearance suggesting a non-uniform staining of a single cell, no preparations suggestive of spores were observed. The heat test, using exposures of 80° C. for 5 minute and 10 minute periods on both young and old cultures, showed the absence of spores.

CULTURAL CHARACTERISTICS.

Agar Streak — Considerable growth was present after 24 hours at either 37° or 20° C. The growth was white, increased on standing and after a few days at either temperature was very heavy and slightly brown, the color being more pronounced at 20° than at 37° C. The growth showed a smooth edge, was raised, shiny and neither viscid nor tenacious. The water of condensation showed a turbidity.
Agar Stab — After 24 hours, growth was evident along the line of puncture but was heaviest nearest the surface. In cultures several days old there was a very heavy light brown surface growth that showed a pronounced tendency to spread; the amount of growth decreased rapidly from the surface downward.
Agar Plate Colony — Colonies were evident after 24 hours at 20° C. but were small. The mature (72 hours) surface colonies were large, spreading, transparent and had a bluish cast by reflected light. Under the low power the colonies appeared brown and granular with a smooth edge. The mature subsurface colonies were white and varied from oval to round, while under the low power they were brown and granular with a smooth edge.
Gelatine Stab — After 24 hours at 20° C. growth was evident along the entire line of puncture, but was most abundant at the surface where liquefaction had apparently begun. After 72 hours liquefaction was complete at the surface but was not complete beneath the surface. In the liquefied material, there was a heavy turbidity and a sediment that was flocculent in nature. In cultures two weeks old liquefaction was almost complete, the liquified material showing a greenish tinge, in addition to a heavy flocculent sediment and a partial membrane and ring.
Bouillons — In plain bouillon, as well as bouillons to which various materials had been added, there was, after 24 hours, considerable turbidity, which seemed to be heaviest near the surface. After 48 hours the development of a pellicle had begun and in older cultures a heavy white, brittle membrane that fell in part on agitation, was present. In these older cultures there was also a heavy sediment and turbidity. In the bouillons to which various materials were added no intense colors were observed although a green was quite frequently encountered. The green was probably most pronounced in dextrose, galactose, maltose, mannit and sucrose, but also occurred to a lesser degree with other materials. In the case of both dextrose and galactose bouillon, the membrane in old cultures was sometimes covered with black granules that gave the appearance of a mold growth. These granules have already been mentioned in connection with milk cultures. The greatest bouillon acidities (see reaction change under bio-chemistry) were observed in galactose and dextrose bouillons and it seems probable that there is some relationship between the acidity and the formation of these granules.

Potato — In 24 hours cultures held at 20°C there was a brown growth. After 5 or 6 days the brown growth covered all of the potato plug that was above the water line, the sides as well as the sloped surface being overgrown. The edge of the plug resting in the water had a greenish tinge.

Dunham’s Solution — There was a slight turbidity after 24 hours at 20°C. In cultures 72 hours old there was a tendency to pellicle formation, while in still older cultures there was a slight sediment, and sometimes a very faint green color. The growth in this medium, however, was always scanty as compared with the growth in bouillons.

Uschinsky’s Medium — After 24 hours, there was considerable turbidity with the growth most abundant near the surface. In older cultures there was a small amount of sediment, a slight membrane, and a heavy turbidity. Near the surface there was a greenish color.

Litmus Milk — Cultures held 24 hours at 20°C showed no change. In cultures somewhat older coagulation had occurred and digestion of the casein had begun. There was a reduction of the litmus at the upper edge of the undigested material but at the lower edge the color remained. The digested material commonly had a greenish tinge and there was a heavy membrane that fell in part on agitation.

Plain Milk — The changes produced in milk, both whole and skim, have already been discussed.

BIO-CHEMICAL FEATURES

Gas Production — No gas production was observed in bouillon in the presence of glycerine, dextrose, levulose, galactose, lactose, sucrose, maltose, mannit, salicin, raffinose or inulin.

Oxygen Relation — There was no growth in the closed arm of the fermentation tubes.

Indol Production — The addition of sulphuric acid to old cultures of the organism in Dunham’s solution increased the green color if a green was already present and if not the acid caused the appearance of a green color. The addition of nitrite solution destroyed the green without the production of a pink color.

Reaction Change — The reaction change in milk has already been mentioned. The reaction produced by the organism in plain bouillon and in bouillon containing various additions (2%) is shown in the table III. The bouillons before inoculation were practically neutral.
### TABLE III. REACTION CHANGE SECURED IN BOUILLONS WITH ORGANISM 1

(Figures represent the c. c. of n/20 NaOH required for 5 c. c. of bouillon)

<table>
<thead>
<tr>
<th>Period of Growth</th>
<th>Plain</th>
<th>Glycerine</th>
<th>Dextrose</th>
<th>Levulose</th>
<th>Galactose</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Maltose</th>
<th>Mannit</th>
<th>Dulcine</th>
<th>Salicin</th>
<th>Raffirose</th>
<th>Inulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk. at 20° C.</td>
<td>a*</td>
<td>.5</td>
<td>1.3</td>
<td>.5</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
<td>a*</td>
<td>.1</td>
<td>.5</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>18 da. at 20° C.</td>
<td>a</td>
<td>a</td>
<td>.3</td>
<td>1.1</td>
<td>2.0</td>
<td>a</td>
<td>a</td>
<td>a*</td>
<td>.4</td>
<td>...</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>1 wk. at 37° C. plus 2 wks. at room to get maximum growth</td>
<td>...</td>
<td>a</td>
<td>1.4</td>
<td>.7</td>
<td>1.8</td>
<td>...</td>
<td>a</td>
<td>...</td>
<td>...</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

*a* = alkaline.

### ORGANISM 2

Organism 2 was isolated from a sample of farm butter that had developed a very undesirable flavor, described as bitter by the person who submitted it. The organism was encountered only in small numbers and was not believed to have been causally related to the deterioration of the product. No information regarding the method of manufacture of the butter or the source of the cream was available, the sample having been sent to the laboratory with but a brief history. The statement was made, however, that the butter had been fair in quality and had developed the undesirable flavor while being held at quite a low temperature.

With Organism 2 as with Organism 1, the action on milk was the principal thing studied. After considerable periods, the milk was coagulated, the coagulation apparently occurring earlier at 37° C. than at room temperature, while the curd tended to settle out, leaving a small amount of clear serum next to the fat. In sterilized whole milk a yellow color appeared in the course of 6 to 12 days, commonly before coagulation had occurred, and seemed to be above, rather than in the upper layers of the fat. On skim milk in flasks there was, in general, no color produced at the surface, but on skim milk in tubes it was common to get color produced in this location; in those few tubes in which there was no color at the surface a yellowish sediment was present. Agitation of the tubes of skim milk generally caused the falling of the membrane.

These observations seemed to clear up the situation and it is probable that in whole milk in flasks or tubes the fat remaining at the surface supports the membrane formed by the growth of the organisms, while in skim milk in flasks, with practically no fat, there is a tendency for the membrane to fall; skim milk in tubes, presumably on account of the better opportunity for the membrane to cling to the glass, generally showed a color at the surface.

Oleo oil, neutral lard, cotton seed oil and butter were added to both plain and lactose bouillon in flasks and the mixtures ster-
ilized, after which the organism under consideration was added. In all flasks, except those to which cotton seed oil had been added, yellow spots, varying in size, were produced. These spots were commonly most abundant around the edge of the floating fat and sometimes were suggestive of a band of yellow here. The bouillons in all cases showed a turbidity. It seems probable that the cotton seed oil prevented the formation of a membrane because of the lack of nutrients at the surface, the oil acting as a sort of seal. In the case of the materials that are solid at room temperature this is not true, cracks in the fat affording the organisms an opportunity to get the nutrients required.

The slow development and the small amount of the yellow color, as well as the lack of uniformity in its distribution, prevented the making of butter from cream on which this organism had grown.

The reaction change in milk is shown in table IV, the acidities being calculated as lactic acid. From the results presented it will be seen that in whole milk at both 37° C. and at room temperature there was some production of acid; in skim milk at 37° C. the normal reaction of the milk was changed to alkaline, while in skim milk at room temperature there was a slight increase in acidity following a slight decrease.

**TABLE IV. REACTION CHANGE IN MILK INOCULATED WITH ORGANISM 2. ACIDITIES CALCULATED AS LACTIC ACID**

<table>
<thead>
<tr>
<th>Period of Growth in Days</th>
<th>At 37° C.</th>
<th>At Room Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skim Milk</td>
<td>Whole Milk</td>
</tr>
<tr>
<td>1</td>
<td>.15% acid</td>
<td>.15% acid</td>
</tr>
<tr>
<td>2</td>
<td>.11% acid</td>
<td>.19% acid</td>
</tr>
<tr>
<td>6</td>
<td>.05% acid</td>
<td>.27% acid</td>
</tr>
<tr>
<td>20</td>
<td>.02% acid</td>
<td>.31% acid</td>
</tr>
<tr>
<td>34</td>
<td>Alk.</td>
<td></td>
</tr>
</tbody>
</table>

The effect of the organism on the percentage of fat in milk is shown in table V; the results were secured with the Babcock method. The data given show that the organism did not break down the fat present even after considerable periods of time.

**TABLE V. EFFECT OF ORGANISM 2 ON THE PERCENTAGE OF FAT IN MILK**

<table>
<thead>
<tr>
<th>Period of Growth in Days</th>
<th>% of Fat in Control Flask</th>
<th>% of Fat in Inoculated Milk—Trial 1</th>
<th>% of Fat in Inoculated Milk—Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.2</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td>48</td>
<td>4.2</td>
<td>4.2</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Judging by the appearance of the milk, both before and after coagulation, there was no digestion of the casein and, in conformity with this, it was found that the odor produced by the organisms in milk was not suggestive of putrefaction.
DESCRIPTION OF ORGANISM 2

The organism was studied morphologically, culturally and biochemically with the following results.

MORPHOLOGY.

Form — The organism was found to be rod-shaped.

Size — In young agar cultures the majority of the organisms were about .7 or a micron wide and 1.5 microns long. In young milk cultures the organisms were slightly larger and in old cultures of both milk and agar smaller.

Arrangement — The organisms were very frequently found in pairs although single organisms were common. In hanging drop preparation made from bouillon cultures in which there was a heavy growth, clumps were often observed.

Motility — The organisms in young bouillon cultures showed an active motility. Clumps made up of many organisms frequently showed a movement also.

Staining Reaction — The organism stains readily with the common stains. With Loeffler's methylene blue the organisms frequently showed an irregular staining. The Gram stain was negative, Bact. lactis acidi serving as a positive control.

Spore Formation — The microscopic picture gave no evidence of spores although organisms staining unevenly were sometimes encountered. An exposure to 80° C. for either 5 or 10 minutes was sufficient to cause the death of the organism, whether taken from young or from old cultures.
CULTURAL CHARACTERISTICS.

Agar Streak — After 24 hours at 20° C. the growth was rather small in amount, raised, with a smooth edge, and from white to a yellowish white in color. The growth increased rapidly on standing and soon assumed a decidedly yellow color, although the edge of the growth was frequently yellowish white when the older portion was yellow. Cultures 48 hours or more old often showed a wrinkled growth that was membranous in character although the younger portions frequently had no wrinkled appearance. At 37° C. growth with color production occurred altho not as rapidly as at room temperature.

Agar Stab — Cultures showed a white beaded growth along the line of inoculation as well as a slight surface growth, yellow in color, after 24 hours at 20° C. The surface growth increased rapidly in amount and in intensity of color, although the edge of the growth was often a yellowish white. The growth later assumed a wrinkled appearance and became membranous in character.

Agar Plate Colony — After 24 hours at 20° C. the colonies were extremely small. The mature surface (72 hours) colonies were large, raised, non-viscid and yellow except at the edges which were a yellowish white. Under the low power the edge was seen to be smooth and the colony yellowish brown in color. The mature sub-surface colonies were round to oval, quite small and yellow in color and the low power showed the edge to be smooth. Surface colonies that were older than 72 hours sometimes assumed a wrinkled appearance particularly on plates containing but few colonies and which accordingly afforded a good opportunity for continued growth.

Gelatine Stab — After 24 hours at 20° C. growth was slight along the line of inoculation. On standing a heavy surface growth developed which became irregular in shape and wrinkled. The growth along the stab was beaded and yellow but never became heavy in amount. No liquefaction was observed.

Bouillons — Plain bouillon and bouillons with the addition of various materials showed a slight turbidity after being held 24 hours at 20° C. After 48 hours the growth had increased and was particularly abundant in the monoses in which there was a heavy turbidity and in some tubes a pellicle and sediment. The growth in the other tubes varied and not infrequently was present as a flocculent precipitate. The sediment observed in the bouillons sometimes showed a yellow color.

Potato — There was a slight growth, yellow in color after 24 hours at 20° C. The growth increased rapidly and eventually the greatest portion of the potato was covered with a bright yellow, glistening, non-viscid growth, while there was considerable yellow sediment in the water around the base of the potato plug.

Dunham’s Solution — Cultures held at 20° C. showed considerable flocculent precipitate after 24 hours. The growth increased on standing and the precipitate eventually became tinged with yellow.

Uschinsky’s Medium — Growth was evident after 24 hours at 20° C. and occurred mainly at the surface where membrane formation had begun. In older cultures the membrane was very definite, tenacious but not stringy, slightly tinged with yellow and firmly adherent to the wall of the tube, with practically no growth beneath. When the membrane was torn from the wall by agitation, it fell to the bottom of the tube.

Milk — Already discussed.
BIO-CHEMICAL FEATURES

Gas Production — No gas formation was observed in bouillons containing glycerine, dextrose, levulose, galactose, lactose, sucrose, maltose, mannit, salicin, raffinose, or inulin.

Oxygen Relation — There was no growth in the closed arm of the fermentation tubes.

Indol Production — In old cultures indol could be detected.

Reaction Change — The reaction change produced by Organism 2 in plain bouillon and in bouillons with various additions (2%) is shown in Table VI.

### TABLE VI. Reaction Change Produced in Bouillon with Organism 2
(Figures represent the c. c. of n/20 NaOH required for 5 c. c. of bouillon)

<table>
<thead>
<tr>
<th>Period of Growth</th>
<th>Plain</th>
<th>Glycerine</th>
<th>Dextrose</th>
<th>Levulose</th>
<th>Galactose</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Maltose</th>
<th>Mannit</th>
<th>Dulcite</th>
<th>Salicin</th>
<th>Raffinose</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 Days at 20° C.—First Trial...</td>
<td>a*</td>
<td>.1</td>
<td>1.8</td>
<td>1.2</td>
<td>2.6</td>
<td>a</td>
<td>2.9</td>
<td>.9</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>19 Days at 20° C.—Second Trial...</td>
<td>a</td>
<td>1</td>
<td>2.1</td>
<td>1.4</td>
<td>2.6</td>
<td>a</td>
<td>1.4</td>
<td>.3</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

*a = Alkaline.

### DISCUSSION

It is recognized that the two organisms that have been described in the preceding pages are not to be regarded as typical milk organisms, but their more or less uncommon action on milk is believed to be of some little importance.

Butter making must be looked upon as a typical fermentation industry and there is no reason why the production of a desirable color in butter by the growth of harmless micro-organisms should be more objectionable than the production of a desirable flavor by the same means. The growth of Organism 1 in cream gave a very good color to the resulting butter, but the aroma and flavor produced by the action of the organism on the constituents of milk and cream other than fat were very objectionable and made its use for the production of a color in butter out of the question. Organism 2, on account of its slow color production as well as the location of the colored material, was likewise of no value for the production of color in butter. It is possible, however, that an organism may eventually be found that will bring about desirable color changes in cream without having any undesirable action on the various cream constituents.

*B. synxanthus* has apparently been described only incompletely. Hohl regarded the organism isolated by him as a form related to *B. synxanthus* and Weigmann mentions a variety that produces a yellow color in the cream and a red color in the serum. From a comparison of the characteristics of Organism 1 of this paper with the available reports of the characteristics of *B. synxanthus*...
which are, as already stated, rather incomplete, it is believed that Organism 1 must be regarded as the same type and should not be looked upon as a new species. There are certain characteristics in which Organism 1 differs from some of the published descriptions of *B. synxanthus*, among which are the lack of yellow color on agar and gelatine and the lack of acid production in lactose; taking into account, however, the irregularities that are commonly found when the same organism is described by different investigators, these variations cannot be considered as being of any great importance.

Organism 2, as far as it has been possible to determine, has never been described. As has been previously pointed out, this cannot be regarded as a typical milk organism and it is highly probable that its presence in the sample of butter studied was entirely accidental. It produces changes in milk only comparatively slowly, and the production of the yellow color at the surface of cultures in whole milk, and under certain conditions in skim milk also, is not peculiar to milk but occurs on agar surfaces and on bouillon surfaces as well if there is any material present that can float the growth that is produced. The organism has been named *B. aurantinus*.

**SUMMARY**

*B. synxanthus* was isolated from a sample of milk secured in one of the smaller towns of Iowa. The action of this organism on various materials has been studied and the results obtained with cream indicate that the odor and flavor produced are so objectionable that the organism cannot be used for the production of color in butter.

A microorganism that produces a yellow color on the surface of whole milk was isolated from a sample of butter. The organism is believed to be a new species and has been described and named *B. aurantinus*. Inasmuch as it acts very slowly on milk its use for the production of color in butter is out of the question.

The study of these two organisms indicates that eventually an organism may be found that can be used for the production of color in butter.
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