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An aromatic bacillus of cheese

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During the fall of 1892 my attention was called to a rot of cabbage which was doing serious damage. An isolation of the germs responsible for the disease was undertaken, since none of the higher fungi seemed to be present. Among the germs present was one that had a very striking and peculiar odor when grown in bouillon. It was not unlike that peculiar to Limburger cheese. It resembled old and well-cured cheese. The same germ grown in agar had a pleasant, aromatic and nut-like odor, wholly different than the odor in bouillon. I suggested to Mr. Wallace, who was then working in the laboratory, to try an experiment in making cheese using this germ. During the winter and spring several more were made by Mr. McKay and Mr. Fairfield. In some of these experiments the milk was not previously heated, so that of course the lactic acid germs were allowed to develop along with the Bacillus aromaticus. In another case the milk was heated sufficiently to destroy most of the lactic acid germs, shown by the fact that lactic acid developed very slowly.

In one cheese made in this experiment the milk was not heated; the cheese, however, was very different from those made at the same time in the ordinary way; it was of a very superior quality. It was, as Mr. McKay stated, something like a white clover cheese. This fact is mentioned here simply to call attention to the fact that possibly the excellence of June cheese may be due quite as much to the various saprophytic germs that find lodgment on the leaves of clover and other green plants, and thus get into the milk. This germ was isolated, as noted above, from green and partially rotting cabbage.

In a second experiment two more were made, and in this lot the milk was pasteurized. They were cured for several
months and then tested. It had a flavor of its own, some-
what sharper and stronger than ordinary cheese, and to
some perhaps not desirable, but it suited my tastes. Others
who tested the cheese also praised it in the highest terms.
It will be seen later on that the germ used in this experiment
produces an abundance of gas. It is a well-known fact that
when lactic acid is not developed sufficiently before the cheese
is pressed, holes will appear. In some cases the quantity
may be so large that the cheese swells. Our cheese began to
swell, owing to the presence of gas; a section through the
cheese showed numerous holes from the imprisoned gas.

The germ was isolated from cabbage in the ordinary way,
using Esmarch roll agar tubes. A small portion of the cab-
bage was placed in melted agar* and then solidified on the
sides of the tube. Among the cultures coming up was a
whitish colony, which later was transferred to agar.

AGAR.—In agar it grows best at the temperature of the room.
It does not respond readily to the temperature of blood when
placed in an incubator. It follows along the track of the
needle, forming a whitish growth which spreads over the sur-
face as well as down in the medium (Fig. 1); the germ is, in
fact, a facultative anaerobe, that is growing in the absence of
oxygen as well as in the presence of the same. On agar, if
the growth is rapid, large lens-shaped gas bubbles are formed
(Fig. 3). The growth radiates out in wide streams. As the
culture becomes older, usually in from one to three weeks,
it assumes a yellowish white color, with a slight tinge of
brown. It maintains its vitality for some time. An old agar
culture, started on March 7th, was transferred on May 20th;
though the agar had dried up somewhat, the new culture
grew readily.

GELATIN.—It grows rapidly in gelatin, forming at the ordi-

* Agar is a substance derived from certain marine algae (sea weeds) from India.
It is an excellent medium for growing bacteria.
in twenty-four hours. It settles to the bottom, forming a yellowish white sediment, but slightly tenacious. The odor is strong and reminds one of Limburger cheese. The product undoubtedly belongs to the aromatic series, and probably forms some butyl compounds. It gives an acid reaction with litmus paper, but this acid develops rather slowly.

In bouillon containing potassium nitrate it grows only on the surface, the upper surface becoming very cloudy in twenty-four hours. This substance falls to the bottom, forming a more or less flocculent white sediment. The odor is the same as in ordinary bouillon.

Gas.—Gas is formed very rapidly in sugar bouillon when placed in fermentation tube. Considerable of the liquid is displaced in twenty-four hours. After it has produced this gas from four or five days it ceases to develop. The gas bubbles may be seen to rise as very fine bubbles. In bouillon containing potassium nitrate gas is not formed, and the organism becomes an aerobe.

Milk.—In milk it grows readily, at the ordinary temperature of the room. There are no perceptible changes in the milk in twenty-four hours, except the characteristic odor found in bouillon. In forty-eight hours the odor is stronger, and the milk is curdled, consisting of fine, rounded masses. After this the test for acid was very evident. The curdled mass began to separate out into a liquid, with the precipitated casein at the bottom. This gradually becomes dissolved.

Blood Serum.—Grows readily, forming a dirty whitish growth along the track of the needle. Soon spreads over the whole surface liquefying the medium. The odor in this medium is much pleasanter than in bouillon or gelatin. It seems to be a purer odor, resembling fruit, with a combination of butyl some compound.

Potato.—On potato it forms a yellowish white growth on the surface, following the scratch of the needle somewhat invisible, unless the potato is moist. When potato has considerably moisture, it forms round yellowish white colonies; having an odor of fermenting potatoes.

Need of Oxygen.—The organism is a facultative anaerobe. It usually grows in the presence of oxygen, but will
also grow in the absence of oxygen. Buchner's method of growing anaerobic germs was used, which is as follows:

The germ is inoculated in a fresh agar tube. This tube is placed in a larger tube, which contains ten cubic centimeters of ten per cent solution of caustic potash, to which one gramme of pyrogallic acid is added. This solution absorbs the oxygen, so that only strict anaerobic germs grow in the agar tube. A culture prepared in this way made some growth, but no color was developed.

**Thermal Death Point.**—65°C. for ten minutes did not destroy its vitality completely, growth appeared after five days. It lost, however, some of its peculiar odor. 58°C. for ten minutes did not destroy its vitality. It took longer for the culture to develop than when heated to 47°C. 61°C. for ten minutes, vitality not destroyed. 60°C. for ten minutes did not destroy its vitality, odor as at 58 and 65°C. At 70°C. for ten minutes vitality was destroyed.

**Disinfectants.**—The subject of disinfectants has little value from a practical standpoint in this connection, although it is of some importance to the biologist. We therefore tried a few disinfectants. Much has been written concerning the antiseptic properties of peroxide of hydrogen, it being much used in surgery. In the laboratory I have used it on several germs, but in no case did it prevent the growth of the germs tested. Mr. Leo Thurlimann who tested a fresh bottle of the peroxide, used in the botanical laboratory, found that it contained two per cent of the peroxide of hydrogen.

The following proportions were used:

1 cc. of peroxide of hydrogen, to which was added 250 cc. of water. The germ was in contact with this solution ten minutes, after contact a quantity was removed to a tube containing bouillon. The characteristic odor appeared in forty-eight hours.

We then made some experiments with corrosive sublimate, which is well known, as a powerful disinfectant. In these experiments sterilized water was used, and the corrosive sublimate diluted to the required amount. A quantity of the germ in the loop of a platinum needle was transferred to a tube containing the corrosive sublimate, the germ in each
case was allowed to remain in contact with the solution the desired time.

1—1,000..........................ten minutes..........................vitality destroyed.
1—1,000..........................just touched..........................vitality not destroyed.
1—2,000..........................ten minutes..........................vitality destroyed.
1—2,000..........................just touched..........................vitality not destroyed.
1—5,000..........................ten minutes..........................vitality destroyed.
1—5,000..........................just touched..........................vitality not destroyed.
1—8,000..........................ten minutes..........................vitality not destroyed.
1—8,000..........................just touched..........................vitality not destroyed.
1—9,000..........................ten minutes..........................vitality not destroyed.
1—9,000..........................just touched..........................vitality not destroyed.
1—16,000..........................ten minutes..........................vitality not destroyed.
1—16,000..........................just touched..........................vitality not destroyed.

Corrosive sublimate somewhat changed the character of the odor. In 24—48 hours it had a somewhat unpleasant burnt fetid-like odor, but this gradually changed in four days to the characteristic butyl odor.

MORPHOLOGY.—It is a non-motile bacillus with rounded ends, from .9—1.20 u. long and .3—.45 u. wide.

DETECTION OF THE GERM IN THE CHEESE TO WHICH IT HAD BEEN APPLIED.

Milk pasteurized before the germ was applied, and then cured for several months, still contained the germ in quantity. It was by no means the only species present. Cultures were made by the usual methods, a germ was obtained which had the same morphological characters, liquefied gelatin, had the same growth on agar and the same peculiar and characteristic odor. There is no question, therefore, concerning the identity of the germ.