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Food accessory substances and the nitrite bacteria.

By T. J. MURRAY.

[*From the Department of Bacteriology, Rutgers College, New Brunswick, N. J.*]

In the isolation of nitrifying bacteria, soil is inoculated into a synthetic solution containing $(NH_4)_2 SO_4$ as a source of nitrogen for the "nitrite" bacteria and into one containing $NaNO_2$ for the "nitrate" bacteria. The solutions are incubated and when a test for nitrites is found in one case or a test for nitrates in the other, a small amount of the liquid is transferred to a new synthetic solution. This transferring is continued for some time. Then the material is plated out on silica jelly containing suitable inorganic salts. From the colonies developing, inoculations are made into synthetic solution and if nitrites are formed in the one containing $(NH_4)_2 SO_4$ a "nitrite" bacteria has been isolated or if nitrates are formed where $NaNO_2$ is the source of nitrogen a "nitrate" bacteria has been isolated.

Great difficulty was experienced in isolating cultures by the above procedure. At the beginning, in no case where inoculations from the colonies on silica jelly were made into the synthetic solution, were positive tests subsequently obtained. If, however, a solution giving a positive test were centrifuged and the sedi-

ment inoculated on silica jelly by means of a loop and from the colonies that developed, inoculations were made into synthetic solutions, positive tests would be obtained. This applied to both the "nitrite" and the "nitrate" organisms. Now if these positive cultures were inoculated on nutrient agar, growth would appear. This growth would not cause nitrification, showing the cultures in question were not pure. In one experiment the synthetic solution for nitrate bacteria which gave a positive test was centrifuged and inoculated on a gypsum block partly immersed in the synthetic solution. From the growth later obtained, dilutions were made and plated on silica jelly. Ten colonies were picked. Five grew on nutrient agar and five did not. The ten cultures were inoculated into the synthetic solution for nitrate formation. The five that did not grow on nutrient agar never developed any nitrates, the other five that were shown contaminated by the growth on nutrient agar all gave positive tests for nitrate after incubation. The procedure has been varied in many ways, but whenever and by whatever method used, when a positive test was obtained after incubation in the synthetic solution, organisms were present that grew on nutrient agar.

Two possibilities occurred to the writer. Are the nitrifying organisms filterable or do they get some substance from the bacteria that accompany them, that grow on nutrient agar.

No nitrification was ever obtained from a Berkefeld filtrate of a solution giving a positive test for nitrites or nitrates no matter what was added to the synthetic solution in addition to the inorganic constituents.

The work in connection with the second hypothesis was done only with the nitrite organisms, those oxidizing ammonia to nitrite compounds. In transferring cultures about two c.c. are added to 100 c.c. of the synthetic solution. In a week a strong positive test is obtained, when transfers are again made. If less inoculum is used a longer time is required.

One c.c. of a very weak culture of nitrite bacteria was added to synthetic solutions that had quantities of the following added, Berkefeld filtrate of nutrient agar bacteria, nutrient agar bacteria, dead nutrient agar bacteria, fresh soil, sterilized soil, and Berkfeld filtrate of soil solution, as well as to the check (synthetic solution). It was found after incubation that more nitrite was formed in every case in a given time than in the check. The

fresh soil gave the most brilliant test. Then a series was run as follows: check (synthetic solution), solution plus fresh soil, solution plus sterile soil and solution plus fresh soil. Three-tenths c.c. of nitrite culture was added to the first three. Fresh soil gave best results, sterilized soil not quite as good. Fresh soil wihout the culture gave a slight test after long incubation, showing that the beneficial result was not due to any nitrifying bacteria already in the soil.

It is evident that there is some substance present, in the bacteria that grow on nutrient agar, and in the soil itself, that stimulates these still impure cultures of the "nitrite" bacteria. It was thought that this substance might be in the nature of a vitamin. In order to test this out, to the synthetic solution, small amounts of the following were added: dried autolised yeast, dried yeast, yeast cake, alfalfa and soil. Two sets were run. One set was sterilized and the other not. It was found that all these substances, sterile or not, stimulated the "nitrite" bacteria. Larger amounts of nitrite were formed in all cases, in a given time, than in check. Since the above substances are rich in vitamin A and B, the writer feels there may be a definite relation between food assessory substances and the "nitrite" organisms.

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Some temperature studies on B. acidophilus milk.

By NICHOLAS KOPELOFF AND PHILIP BEERMAN.

[*From the Department of Bacteriology, Psychiatric Institute, Ward's Island, New York City.*]

Since the beneficial effects of *B. acidophilus* appear to depend upon a transformation of the intestinal flora, it follows that a mass inoculation is desirable. This means a maximum number of viable organisms per c.c. of pabulum. The usual recommendation on commercial preparations of fermented milk is that such milk be kept in a cool place preferably in the ice-box. Consequently, the influence of low temperature on the number of viable *B. acidophilus* in milk seemed worth investigating.