GROWTH AND SURVIVAL OF MICRO-ORGANISMS AT SUB-FREEZING TEMPERATURES

WITH the consumption of frozen fruits and vegetables steadily increasing, the attention of a great number of food technologists is focused upon the behavior of microorganisms at low temperatures.

In July, 1933, during a routine examination of frozen strawberries, raspberries and cherries packed in Oregon in June, 1930, it was noted that while the number of viable microorganisms to be found in the fruit, after three years' storage at 15° F., was so small as to be practically negligible (average less than 70 per gram), there were an unusually large number of interesting species to be seen on the plates. These organisms, representing many species of bacteria, yeasts and molds, were studied in pure culture form and later identified as closely as possible with known species.

Largely from curiosity concerning the behavior of these forms on artificial media at low temperatures, freshly made beef infusion agar, adjusted to pH 7.0, slant cultures of each of them were placed in the 16° F. room of the cold storage building at the Arlington Experiment Farm, Virginia. The cultures were placed in this room within 30 minutes from the time transfers were made. The cold storage room is carefully controlled and maintains a fairly even, recorded temperature of 16° F. (−8.89° C.).

The cultures were examined every month, and it was not until the end of the third month that slight but definite signs of growth were observed on three of the slants, all of which were yeasts. In all three of these species of yeasts, the morphological and cultural characters would place them among the true yeasts, in the family *Saccharomycetes*.

Hitherto the lowest temperature known to the author at which growth takes place in any yeast was found in the recent data of Berry and Magoon,[1] who reported growth taking place in *Torula* sp. at −4° C.

The amount of growth in these yeast cultures at the end of a year at −8.89° C. was still slight, being about equal to the amount formed in 18 hours at room temperature.

Between the fifth and seventh months, more cultures —namely, *Bacillus aterrimus* Lehmann u. Neumann, *B. fluorescens* Ford, *B. mycoides* Flügge, *B. ruminatus* Gottheil and *Penicillium* sp.—showed slight but definite growth. In these cultures as well as in the yeast, growth at the end of a year while thin was sufficiently extensive to leave no doubt as to its presence.

At the end of the year all the cultures were brought into the laboratory, allowed to thaw out and incubate at room temperature for twenty-four hours; *Dematium* sp., *Monilia* sp., *Oidium* sp., *Penicillium* sp. (second strain ?), and an unidentified yeast failed to grow. The others, including those mentioned above as showing positive signs of growth at −8.89° C., produced an exceptionally large amount of characteristic growth in 24 hours.

The species showing this abundant growth on the same slants at room temperature after a year's storage at −8.89° C., but showing no signs of growth while held at this temperature were: *Bacillus albolactis* Löffler (Migula), *B. cereus* Frankland, *B. graveolens* Gottheil, *B. lobatus* Bergey, *B. aterrimus* Lehmann u. Neumann (second strain ?), *B. mycoides* Flügge (second strain ?), *B. polymyxa* (Prazmowski) Gruber, *B. subtilis* (Ehrenberg) Cohn, *B. vulgatus* Trevisan, two species of *Penicillium* and two species of unidentified yeast.

To summarize: twenty-six species of bacteria, yeasts and molds which were able to keep alive in frozen fruit held at 15° F. for three years were isolated, studied in pure culture form and identified with known species as closely as possible. Freshly made agar slant cultures of each species were held at 16° F. (−8.89° C.) for one year. Eight species were able to produce growth at this temperature, thirteen species, while showing no signs of growth at 16° F., did produce abundant growth when the cultures were removed to room temperature and allowed to incubate 24 hours. Only five species out of the twenty-six failed to survive the storage period of one year on artificial media at 16° F. These findings make it plain that many species of microorganisms have remarkable faculties for survival as well as for adapting themselves to changes in environment and must be taken as a warning against careless methods in the preparation of frozen foods.

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[1] J. A. Berry and C. A. Magoon, *Phytopathology*, 24: 7, 780–796, July, 1934.

A NEW ANTAGONISTIC PROPERTY OF NORMAL SERUMS: THE CERCARICIDAL ACTION

THE destructive action which normal serum manifests against many bacteria, some kinds of protozoa and certain filterable viruses is well known. To our knowledge, however, no such behavior of the normal serum against multicellular organisms has been described. During the past summer (1935), it was our privilege in the Helminthology Laboratory of the University of Michigan Biology Station at Douglas Lake, Michigan, to test *in vitro* the normal serum of divers animals upon a number of species of cercariae, one of the larval stages of trematode parasites. It was found