GROWTH AND SURVIVAL OF MICROORGANISMS 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

THE EFFECT OF THE SERUMS OF VARIOUS ANIMALS UPON DIFFERENT SPECIES OF CERCARIAE

Species of cercariae	Group of cercariae	Vertebrate serums						
		Man	Rat	Cat	Herring gull	Water snake	Green frog	Bullhead fish
Cercaria of *Schistosomatium douthitti*[3]	Schistosome	o	o	o	o	+	+	
Undescribed cercaria	Schistosome	⊕	+	o	⊕	+	+	
Cercaria elephantis[4]	Schistosome	+	+	o	+	+		
Cercaria physae[5]	Strigeid	+	+	o	+			+
Cercaria of *Diplostomum flexicaudum*[6]	Strigeid	o	⊕	o	⊕	⊕	+	⊕
Cercaria of *Diplodiscus temperatus*[7]	Amphistome	+	+	o	+	+	+	

+ : all cercariae killed ; ⊕ : some cercariae killed ; o : no cercariae killed.

that the serums of many animals have a definite cercaricidal action, the parasites readily losing their characteristic activity and finally becoming so distorted in shape and appearance as the result of contact with the serum that they could not be recognized as cercariae. The substance in the serum responsible for the cercaricidal action is inactivated by heating or desiccating the serum and is quickly lost by the serum in storage.

The relative potency of the cercaricidal action of a serum can be estimated by determining the greatest dilution of serum in physiological salt solution which, added to an equal volume of a suspension of cercariae, kills all the organisms after the mixture is incubated for 1 hour at 37° C. A total volume of fluid of 1 cubic centimeter containing from 25 to 75 cercariae generally proves satisfactory in the test.

Serums of representatives of all classes of vertebrates manifest the cercaricidal action, the following, among others, having been tested for the property: mammals—man, cat and white rat; birds—domesticated duck and herring gull (*Larus argentatus smithsoniaus*); reptile—water snake (*Natrix sipedon sipedon*); amphibian—green frog (*Rana clamitans*); and fish—bullhead (*Ameiurus nebulosus*). All these serums, save that of the cat, were found active against at least some species of cercariae, although the cercaricidal titers varied between broad limits. For example, against a strigeid, *Cercaria physae*,[1] the titers of human, rat, cat and duck serums were, respectively, 1:48, 1:192, zero and 1:24. Representatives of each of the different groups of cercariae tested proved susceptible to the action of the serum, although they differed much in their susceptibility to specific serums. If, finally, conclusive evidence can be adduced for the existence of a relationship between the cercaricidal action of the serum and the resistance of a given vertebrate to infection with a specific cercaria, the cercaricidal test with serum would assume importance in the selection of vertebrate hosts in experiments to complete trematode life histories.[2]

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3 Cort, *Jour. Parasitol.*, 1: 65, 1914.

4 Cort, *Jour. Parasitol.*, 4: 49, 1917.

5 Cort and Brooks, *loc. cit.*

6 *Idem.*

7 Krull and Price, Occasional Papers of the Museum of Zoology, University of Michigan, Ann Arbor, No. 237; 1, 1932.

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