Viable Micro-organisms in a Fifty-Year-Old Yeast Preparation in Antarctica

During the course of our recent investigations of the microbiota of air and soil in Antarctica, a food cache which was partially covered by a snow embankment was found at Cape Evans, Ross Island. The cache was left at this base camp by Captain Robert Falcon Scott of the British Royal Navy prior to his ill-fated expedition to the South Pole in 1911, and consisted of various types of tinned foods together with a glass container of granular, dehydrated bakers' yeast (1 lb. capacity).

The bottle of yeast retained the original label, which read in part: 'Rising-up Yeast', 'Dauerhefe', and 'Levure Inalterable'. The remaining portion of the label was illegible. A wax seal covering the cork stopper was intact. The bottle of yeast was shipped under refrigeration along with other biological collections to the Department of Microbiology, University of Texas, where it was held for two months at 4° C until the laboratory investigation was initiated.

The wax seal was removed, and the outer surface of the cork stopper and mouth of the bottle were sterilized by several successive treatments with ethanol followed by merthiolate solution. The cork stopper together with the piece of 'glassine-like' paper, immediately beneath the cork, were placed in a sterile covered dish for replacement later. This exposed a 1-in.-thick layer of rancid tallow covering the surface of the yeast completely, which was used evidently for water-proofing and for preserving the yeast.

The centre portion of the tallow was removed with a sterile scalpel, so that a plug of yeast material could be withdrawn with a sterile cork borer. The bulk of the yeast material consisted of yeast cells in a plant starch medium. The tallow had permeated to within 1 in. of the bottom of the bottle, causing the granules of the yeast material to adhere together. A 5-in. plug was removed to a sterile covered dish and divided into five equal parts. Pieces of material from the centre of each of the five parts were inoculated into malt extract (Difco), plain nutrient (Difco), and prune broth tubes; and inoculated directly to malt (Difco), plain nutrient (Difco), peptone glucose-acid, prune (Difco), and Sabouraud's dextrose agar plates. All broth and agar cultures were incubated at 25° C. The broth tubes showing growth were afterwards streaked to plates of the 5 different agar media, and also incubated at 25° C. Standard bacteriological methods were used in the handling of the yeast material to ensure that the isolated organisms originated from the yeast preparation.

The uppermost part of the plug material, immediately beneath the tallow cover, produced no growth in any of the media used. The second part produced several colonies of a single species of *Rhodotorula*; no other organisms were recovered from this particular portion. From the third, fourth, and fifth parts, the following were isolated:

Yeasts[1]:

Saccharomyces cerevisiae Hansen. Many cells containing four ascospores can be seen in culture. The isolates agree well with the standard description.

Rhodotorula pallida Lodder.

Moulds:

Absidia corymbifera Lichtheim.

Rhizopus arrhizus Fischer.

Bacteria[2]:

Bacillus sp. Rods, Gram-positive, very long chains, motile, spores.

Pseudomonas sp. Rods, Gram-negative, motile, slime produced.

Micrococcaceae family. Cocci, Gram-positive, tetrads.

The fact that *Saccharomyces cerevisiae* remained viable in a yeast preparation which had been abandoned in the Antarctic for more than 50 years is unique. It is no less unique that another species of yeast, two moulds, and three species of bacteria also survived. Not any of these organisms were single isolates. The growth of *Saccharomyces cerevisiae* was noted in most of the cultures from all the lower three pieces of yeast material. The three bacterial species were also seen in most of the cultures from all the lower pieces. *Rhizopus* and *Absidia* were encountered only infrequently.

Records of longevity of micro-organisms are scanty, and opportunities for examining materials as reported here are rare.

It might have been predicted that a few viable yeast cells would be recovered from the preparation, and perhaps that contaminating bacterial endospores would survive fifty years of cold storage. It is surprising, however, that the contaminants which must have been present as a minor component in this yeast preparation have retained sufficient viability to be readily detected.

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[1] Lodder, J., and Kreger-Van Rij, N. J. W., *The Yeasts* (North-Holland Publishing Company, Amsterdam, 1952).

[2] Breed, R. S., Murray, E. G. D., and Smith, N. R., *Bergey's Manual of Determinative Bacteriology*, seventh ed. (Williams and Wilkins Co., Baltimore, 1957).

Lung Retention in Mice exposed to Airborne Micro-organisms

The respiratory minute volume of mice can be obtained from Guyton's[1] paper; but this does not tell us how much particulate material is retained in the lungs, a measurement of importance in calculating doses in respiratory infectivity experiments with micro-organisms. Harper and Morton[2] found a mean lung retention of 2·7 ml./min when mice weighing 20–22 g were exposed to clouds of single-cell particles of *Bacillus globigii* spores labelled with phosphorus-32. The exposure method used at that time was unsatisfactory in that it involved considerable restriction of the mice by neck yokes. Later (1956, unpublished work) a few tests using less restrictive methods indicated a lung retention value of approximately 7·5 ml./min.

Lung retention (the amount found in the lungs separated from the trachea at the bifurcation of the bronchi) has since been measured using improved exposure methods in which restriction is minimal.