Catalase Activity of Psychrophilic Bacteria Grown at 2 and at 30 C

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ABSTRACT

FRANK, HILMER A. (University of Hawaii, Honolulu), SANDRA T. ISHIBASHI, ANN REID, and JUNE S. ITO. Catalase activity of psychrophilic bacteria grown at 2 and at 30 C. Appl. Microbiol. 11:151–153, 1963.—Catalase activity was measured in resting-cell suspensions of psychrophilic bacteria grown at 2 and at 30 C. Enzyme activity decreased in both cell-suspension types as harvest age increased. At comparable physiological age, cells grown at 2 C had more catalase than cells grown at 30 C.

Although psychrophilic bacteria have been studied for many years, growth ability of this group at near-freezing temperatures that inhibit most microorganisms has not been satisfactorily explained. Recently, several investigators have studied the influence of low growth temperatures on some physiological characteristics of psychrophilic bacteria. Brown (1957), Ingraham and Bailey (1959), and Sultzer (1961) reported that, when test temperatures were reduced, carbohydrate oxidation rates of several psychrophilic bacteria decreased less than oxidation rates of mesophilic bacteria. Only a few studies have compared the effect of low and moderate temperatures on enzyme production in the same bacterial strain. Nashif and Nelson (1953) found that lipase production in Pseudomonas fragi was high between 8 to 15 C and practically absent at 30 C and above. Alford and Elliott (1960) reported lipase production per cell in P. fluorescens to be the same at 5 and at 20 C but only slight at 30 C, despite good growth. Lipase activity, on the other hand, was optimal at 40 C, regardless of growth temperature. Also, Peterson and Gunderson (1960) observed that production of proteolytic enzymes in a psychrophilic P. fluorescens strain was higher at lower growth temperatures. Because most psychrophilic bacteria are highly aerobic

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(Brown and Weidemann, 1958; Ingraham and Stokes, 1959; Ayres, 1960; Witter, 1961; Sultzer, 1961), and because catalase is universally present in aerobic microorganisms (Callow, 1923; McCarthy and Hinshelwood, 1959), this study was begun to investigate the relationship of this important enzyme to growth at low temperatures. This report contains the results of a comparative study of catalase activity in psychrophilic bacteria grown at 2 and at 30 C.

MATERIALS AND METHODS

Organisms. The following psychrophilic cultures were obtained from John A. Alford: P. fluorescens strain 6 (ATCC 11251); P. fragi strain 43 (NRRL B-25); Pseudomonas sp. strain 92 (originally from M. A. Bernard); and Pseudomonas sp. strain 95 (originally from J. C. Ayres). Stock cultures were maintained on nutrient agar (Difco) slants.

Preparation of cell suspensions. Two types of suspensions were prepared from each strain and tested for catalase activity: (i) cells harvested after growth at 2 C and (ii) cells grown at 30 C. Lengths of incubation periods varied and are given below. Intact, resting cells were prepared by inoculating nutrient agar surfaces in petri dishes, removing resultant growth with sterile 0.01 M potassium phosphate buffer (pH 7.0), washing twice by centrifugation, and diluting with sterile buffer to the desired concentrations.

Estimation of catalase activity. During preliminary parts of this investigation, enzyme activity was measured by a method based on oxygen liberation from catalatic breakdown of hydrogen peroxide (Gagnon, Hunter, and Esselen, 1959). Small paper discs saturated with catalase-containing solution (or suspension of catalase-positive bacteria) become buoyant after submersion in test tubes containing hydrogen peroxide solution. The interval elapsing before the disc rises to the surface is called the "flotation time" and is inversely related to catalase activity. Instead of saturating the discs by dipping them into test suspensions (Gagnon et al., 1959), more uniform results were obtained by pipetting 0.1 ml of the suspension directly on each disc. In these early studies, viable cell concentrations were estimated by the plate-count method, using nutrient agar and incubation for 3 days at 30 C. Test systems contained 10 ml of aqueous 3% hydrogen peroxide solution per tube. Determinations were carried out in
water baths maintained at the temperatures specified. Flotation times represent mean values taken from seven to ten replications.

Subsequently, catalatic reaction rates were measured at 10 °C, using intact cell suspensions, 50 mmoles of initial peroxide substrate, and the iodometric method of Herbert (1955). In these latter studies, total cell counts were estimated by the direct microscopic method, using a Petroff-Hausser counting chamber. Specific catalase activities of the various suspensions were calculated as reaction, rate constant at 10 °C per min per 10^8 cells, resembling the Katalase-fähigkeit generally used for comparing activities of enzyme solutions (Maehly, 1954; Hartree, 1955).

**Results and Discussion**

**Preliminary studies using the flotation method.** Catalase activities were studied at 2, 20, 30, and 40 °C with cells grown at 30 °C and prepared after 1 to 2 days of incubation and with cells grown at 2 °C and prepared after 14 days. Based on observations made of these cultures growing in nutrient broth and on nutrient agar slants, these incubation periods were chosen to obtain ample cell yields with high catalase concentrations. Cole and Hinshelwood (1947) and McCarthy and Hinshelwood (1959) previously reported that catalase content in *Bact. lactis aerogenes* increased during the logarithmic growth phase, reached a maximum just prior to the stationary growth phase, and then declined exponentially.

Figure 1 shows the catalatic activity of *Pseudomonas* 95 tested at 2 °C; this is typical of curves obtained by the flotation method. In each of the four cultures tested, fewer cells grown at 2 °C than cells grown at 30 °C were needed for a given flotation time at any test temperature, indicating that 2-°C suspensions had greater catalatic activity per cell. Discrepancies resulting from using plate counts to estimate cell concentrations do not account for the large differences noted between catalatic activities of the two cell-suspension types. Viable counts generally comprised from 70 to 90% of total cells in suspensions tested by the flotation method.

Enzyme activity was destroyed by heating cell suspensions for 5 min at 100 °C. Although optimal temperature for enzyme activity was not determined directly, catalase activity always increased with test temperature and was highest at 40 °C. Growth of these strains, however, was optimal at 30 °C and generally poor at 40 °C. Lower catalase activity in cells grown at 30 °C was not due to lack of substrate penetration. Cells of strains 6 and 95 grown at 30 °C did not show any increased catalatic activity after toluene treatment (Clayton, 1959) to increase cellular permeability to hydrogen peroxide.

**Catalatic studies using the iodometric method.** The effects of growth temperature and of culture age on catalase activity were studied in strains 92 and 95 (Table 1). At both growth temperatures, strain 95 cells had greater catalase activity than strain 92 cells of the same age. Because it is difficult to detect separate growth phases in agar surface cultures (Henrici, 1928), sets of physiologically identical cells grown at 2 and 30 °C cannot be selected from our data. Nevertheless, reasonable comparisons can be made by considering that incubations for 1, 2, and 3 days at 30 °C give cells that are similar to those grown at 2 °C for 5, 11, and 14 days, respectively. On this assumption,

![FIG. 1. Effect of growth temperature on catalase activity of *Pseudomonas* strain 95 at 2 °C.](http://aem.asm.org/)
cells grown at 2 C had two to three times as much catalatic activity as physiologically comparable cells grown at 30 C. However, enzyme decay proceeded at similar rates in both cell-suspension types, about 50 to 60% of initial activity remaining after 3 days at 30 C and 14 days at 2 C.

Our observation that catalase production is higher at the low growth temperature agrees with the observations made on P. fragi lipase by Nashif and Nelson (1953) and on P. fluorescens proteinases by Peterson and Gunderson (1960). These results also agree with those reported by Cole and Hinshelwood (1947), McCarthy and Hinshelwood (1959), and White (1962) concerning the marked decrease in catalatic activity with increased cell age. It is also apparent from Table 1 that the 14-day incubation at 2 C used during the preliminary portion of this study was not ideal for maximal catalase content of cells grown at 2 C.

Increased catalase synthesis may be essential for growth of aerobic microorganisms at low temperatures. Possibly, the increased oxygen solubility at low temperatures contributes to increased hydrogen peroxide production by highly aerobic microorganisms. Coupled with a reduced catalatic activity rate at lowered temperatures, more catalase would be required for survival of psychrophilic aerobes. Not all bacteria would require increased catalase production for low-temperature growth. Psychrophilic bacteria growing under strict anaerobiosis, such as those reported by Schmidt, Lechovich, and Folinazzo (1961) and Upadhya and Stokes (1962), would not need catalase at any growth temperature. It will be interesting to see whether production of other enzymes in aerobic microorganisms, as well as among the anaerobes, increases at low growth temperatures.

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LITERATURE CITED


