Temperature Adaptability of Psychrotrophic

**Pseudomonas**

PRINCE ZACHARIAH AND JOHN LISTON

*Institute for Food Science and Technology, College of Fisheries, University of Washington, Seattle, Washington 98195*

Received for publication 30 April 1973

The rate of oxidation of glucose and alanine at low temperatures by washed cells of psychrotrophic strains of *Pseudomonas* is related to growth temperature.

Psychrotrophic strains of *Pseudomonas* are common in the ocean and in other low-temperature environments and are the major cause of spoilage of chilled meats, fish, and poultry (3). These organisms derive their energy from the aerobic oxidation of organic substrates, and in fish spoilage evidence suggests that nitrogenous materials are mainly oxidized, giving rise to typical end products such as ammonia and fatty acids. The effect of temperature on the oxidative activities of spoilage *Pseudomonas* strains (26, 38, 45, 47, 55, 70) isolated from fish and a typical mesophilic strain of *P. aeruginosa* was studied. All organisms except *P. aeruginosa* were found to grow over a temperature range of 0 to 35°C, with alanine as sole source of C, N, and energy. They showed optimal growth at 25°C. *P. aeruginosa* grew over a typical mesophilic range.

The psychrotrophic strains were grown at 8 and 22°C on a medium containing KH₂PO₄ 1.3 g; Na₂HPO₄ 3.72 g; NaCl 5.0 g; CaCl₂ 0.10 g; MgSO₄·7H₂O 0.20 g; MnSO₄·H₂O 0.0061 g; FeSO₄·7H₂O 0.0150 g; yeast extract, 0.5 g; ethylenediaminetetraacetic acid, 0.6 g; DL-alanine, 2.5 g; and distilled water, 1 liter (adjusted to pH 7.0 with either 1 N NaOH or 1 N HCl), harvested in log phase, and washed. The ability of the washed cells to oxidize alanine at 8°C was tested in a Gilson Differential Respirometer by using a 0.1 M solution of DL-alanine.

Rates of oxidation of DL-alanine at 8°C by washed cells from cultures grown at 8 and 22°C, when measured respirometrically, are shown in Table 1. The oxidation activity of strains 45, 47, and 70 was the same for cells grown at 8 and 22°C, but the activity of strains 26, 38, and 55, grown at 8°C, is at least four times greater than for the same strains grown at 22°C. All strains tested at 22°C, whether pregrown at 8 or 22°C, showed equally high oxidation. This suggests that for 45, 47, and 70 the psychrotrophic system operates equally well at low and high temperatures within the growth range, but that for 26, 38, and 55 an induction (on growth) period is necessary at the lower temperature before the appropriate system for oxidation of the alanine at that temperature will operate.

A more extended study of the ability of bacteria grown at low and high temperatures to oxidize alanine and glucose over the range of −1°C to 33°C was made with the psychrotrophic *Pseudomonas* 70 and a strain of *P. aeruginosa* by using a thermal gradient incubator (6). *Pseudomonas* 70 and *P. aeruginosa* were grown in the alanine medium listed above or in nutrient broth to which 0.5% NaCl and 0.5% glucose had been added at 2 and 22°C and 13 and 37°C, respectively. They were harvested at log phase, and washed cells were added directly to tubes containing indicator, 0.5% NaCl, and substrates, 0.5% glucose and 0.25% alanine, which had been preincubated in the gradient incubator. Glucose oxidation was measured by testing for acid (4) and alanine oxidation by formazan production from 2-(p-iodophenyl)-3-(p-nitropheryl)-5-phenyl tetrazolium chloride.

The results of the thermal gradient incubator studies are shown in Fig. 1 and 2 for *Pseudomonas* 70 and *P. aeruginosa*, respectively. Oxidation of glucose by 22°C-grown cells was increasingly slower than by 2°C-grown cells at temperatures below 10°C. Alanine oxidation only exhibited a difference below 0.4°C. In accordance with the data quoted earlier for strains 45, 47, and 70, there was no difference in the rate of oxidation between cells grown at 2°C or 22°C down to 0.4°C, but below this temperature only 2°C-grown cells showed activity. Oxidation of glucose by *P. aeruginosa* did not show this type of temperature effect, but cells pregrown at 37°C oxidized glucose more rapidly.

437
TABLE 1. Alanine oxidation by psychrotrophic Pseudomonas

| Organism no. | Growth temp | Test temp | μl of O₂ uptake/min*
|-------------|-------------|-----------|------------------------
| 45          | 8           | 8         | 31                     |
| 45          | 22          | 8         | 33                     |
| 47          | 8           | 8         | 36                     |
| 47          | 22          | 8         | 33                     |
| 70          | 8           | 8         | 33                     |
| 70          | 22          | 8         | 33                     |
| 26          | 8           | 8         | 29                     |
| 26          | 22          | 8         | 7                      |
| 38          | 8           | 8         | 17                     |
| 38          | 22          | 8         | 4                      |
| 55          | 8           | 8         | 26                     |
| 55          | 22          | 8         | 3                      |

*a* Corrected to 10⁶ organisms.

![Fig. 1. Time to show measurable oxidation of glucose and alanine at various temperatures by washed cell suspensions of psychrotrophic Pseudomonas pregrown at 2 C (•) and 22 C (O).](image1)

![Fig. 2. Time to show measurable oxidation of glucose and alanine at various temperatures by washed cell suspensions of P. aeruginosa pregrown at 13 C (•) and 37 C (O).](image2)

between 6.5 C and 18.6 C. Surprisingly, the alanine oxidation response to P. aeruginosa was very similar to that for Pseudomonas 70.

These results suggest that temperature adaptation is a phenomenon shown by both psychrotrophic and mesophilic bacteria in their appropriate growth range. However, it appears that the effect is more marked in the case of psychrotrophic bacteria. This suggests that it may be necessary for biochemical changes to occur in cells transferring to a significantly lower growth temperature to enable them to metabolize substrate normally.

Previous studies have shown low temperature adaptability, especially with lipid and carbohydrate metabolizing systems, by psychrophiles and mesophiles (1, 5, 7). A temperature-mediated response affecting protease enzyme synthesis by psychrotrophs has also been reported (D. Bannerjee, A. M. Dollar, and J. Liston, Bacteriol. Proc. 66:10, 1966; S. B. Newton, Diss. Abstr. 26:304, 1965; and reference 8). The results reported in this study confirm that amino acid can be rapidly oxidized by psychrotrophic (spoilage) Pseudomonas strains at temperatures at which protein foods are commonly held, and that the rate of oxidation is related to previous growth temperature experience, a phenomenon appearing at higher temperatures of activity in some strains than in others. The observation of a similar temperature response in the case of P. aeruginosa suggests that this is a more general phenomenon involving some component of the system leading to alanine oxidation. Frank et al. (2) reported that growth at 2 and 30 C did not affect the amounts of macromolecules such as ribonucleic and deoxyribonucleic acid and protein synthesized by psychrophilic Pseudomonas. However, this does not rule out the induction of low-temperature enzyme synthesis or permeability changes at lower temperatures, and these seem to present the most likely explanations for the observed phenomena.

**LITERATURE CITED**

1. Evison, L. M., and A. M. Rose. 1965. A comparative study on the biochemical bases of the maximum temperatures for growth of three psychrophilic microorganisms. J. Gen. Microbiol. 48:349-364.
2. Frank, A. H., A. Reid, L. M. Santo, N. A. Lum, and S. T. Sandler. 1972. Similarity in several properties of psychrophilic bacteria grown at low and moderate temperatures. Appl. Microbiol. 24:571–574.
3. Frazier, W. C. 1967. Food microbiology, p. 252-296. McGraw-Hill Book Co., Inc., New York.
4. Harrigan, W. F., and M. E. McCance. 1966. Laboratory methods in microbiology, p. 137. Academic Press Inc., New York.
5. Jezeski, J. J., and R. H. Olsen. 1961. The activity of enzymes at low temperatures, p. 139-155. Proceedings: low temperature microbiology symposium, Campbell Soup Co., Camden, N.J.
6. Matches, J. R., and J. Liston. 1968. Low temperature growth of Salmonella. J. Food Sci. 33:641–645.
7. Nasif, S. A., and F. E. Nelson. 1953. The lipase of Pseudomonas fragi. 2. Factors affecting lipase production. J. Dairy Sci. 36:471-478.
8. Peterson, A. C., and M. F. Gunderson. 1960. Role of psychrophilic bacteria in frozen food spoilage. Food Technol. 14:413-417.