Similarity in Several Properties of Psychrophilic Bacteria Grown at Low and Moderate Temperatures

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Several properties of psychrophilic pseudomonads were studied with cells grown in batch culture in nutrient broth at 2 and 30 C. No differences were observed in the size, catalase activity, deoxyribonucleic acid, ribonucleic acid, or protein content of cells grown at either temperature. The importance of comparing physiologically similar cells is discussed.

The enzymatic properties of psychrophilic bacteria have been reported to change when the cells are grown at low temperatures. Lipase activity of Pseudomonas fragi (13) and the lipase (1) and protease (14) activities of Pseudomonas fluorescens increased when cells were grown at low temperatures. Also we reported previously that the catalase activity of two psychrophilic pseudomonads was higher in cells grown at 2 C than in cells grown at 30 C (7).

Because the chemical composition, enzymatic activity, and cell size vary at different phases of the growth curve (5), comparisons of cells grown in batch culture at different temperatures should be made between equal numbers of cells that are alike physiologically (i.e., at similar stages in the growth curve). Arpaele al. (2) have already reported that there were no significant differences in the protein or nucleic acid content of P. fluorescens cells grown at low or moderate temperatures when compared at equivalent growth stages.

In the present investigation we studied several properties of physiologically similar psychrophilic pseudomonads grown at 2 and 30 C and found that there are no differences in cell size, catalase activity, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), or protein content. Growth measurements. The organisms were grown in 50 ml of nutrient broth in 500-ml nephelometric flasks (Bellco Glass, Inc., Vineland, N.J.) at the desired temperature in a water bath shaker. Growth was measured as optical density at 600 nm, total cell counts were made with a Coulter counter, and viable counts were made with nutrient agar. Growth curves were plotted from optical density measurements and were used to determine the incubation times at 2 and 30 C, giving cells of similar physiological stages (Table 1). Comparable growth curves also were obtained from total and viable counts. The generation times at 2 and 30 C were calculated from the exponential phase of the viable count growth curves (Table 2).

Preparation of cells. Flasks containing 50 ml of nutrient broth were given small inocula and incubated at 2 or 30 C for the appropriate periods to obtain cells of several physiological ages (see Table 1). After the desired incubation time, duplicate flasks were removed for various analyses. The cells from one flask were harvested by centrifugation and assayed immediately for catalase activity. A sample was removed from the second flask and used for viable counts, total counts, and mean cell volume determinations. The remainder of this suspension was centrifuged, resuspended in 0.9 m NaCl, and kept frozen until needed for analysis of the chemical composition.

Mean cell volumes. Cell sizes were measured with a Coulter model B counter and model J particle size-distribution plotter. Because cultures of these organisms contain many undivided pairs, the size distribution plots consisted of two peaks, representing individual ("singlets") and paired ("doubllets") cells. The mean cell volume at each stage of growth was calculated from the size distribution pattern of the doubllet peak, employing procedures described previously (15). Thus, the mean volume of individual cells is one-half that calculated for the doubllet cells.

Materials and methods

Organisms. Pseudomonas strains 92 and 95, the psychrophilic cultures used in this study, were maintained as described previously (7).

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The nucleic acid and protein contents were similar in cells grown at 2 and 30°C. Strain 92 cells, when compared during the lag and exponential growth stages, have similar amounts of DNA, RNA, and protein, whereas cells compared during the stationary phase had a greater range of variation (Table 3). Comparable results (not shown) were obtained for cells of strain 95.

The catalase activity of both strains was similar in cells grown at the two incubation temperatures, when compared at equivalent physiological stages (Table 4).

### Table 1. Relationship between growth stages of the psychrophilic Pseudomonas strains and corresponding incubation time at 2 and 30°C

| Stage of growth | Incubation time (hr) | Hr of incubation |
|----------------|----------------------|-----------------|
| Lag phase      |                       | 2 C             |
|                |                       | 30 C            |
| Exponential phase |                 |                |
| Early          | 48                   | 3               |
| Middle         | 72                   | 5               |
| Late           | 120                  | 10              |
| Stationary phase |                  |                |
| Early          | 192                  | 12              |
| Late           | 264                  | 24              |

### Table 2. Growth rate of the psychrophilic Pseudomonas strains at 2 and 30°C

| Incubation temperature (C) | Generation time (hr) | Pseudomonas strain 92 | Pseudomonas strain 95 |
|----------------------------|----------------------|-----------------------|-----------------------|
|                            |                      |                       |                       |
| 2                          | 11.1                 | 12.9                  |                       |
| 30                         | 1.1                  | 1.2                   |                       |

### Chemical composition of cells

The frozen cells were thawed and fractionated according to the procedures described by Wachman (18). The DNA content was determined by a modified diphenylamine method (8), with deoxyribose as the standard; RNA by the orcinol method (3), with ribose as the standard; and protein by the method of Lowry et al. (10), with crystalline bovine albumin as the standard. Data on cell composition (Table 3) represent mean values for triplicate estimations on duplicate samples.

### Catalase activity

The catalase activity of the cell suspensions was determined by the iodometric method, as described previously (7), and specific catalase activity was expressed as the reaction rate constant at 10°C per minute per 10⁶ cells. Data on catalase activity (Table 4) represent mean values for a minimum of five separate replications.

### Results

When we compared cells grown at 2 and 30°C at equivalent physiological stages (Table 1), we found no differences in the cell size or composition. Lag and exponential cells of Pseudomonas strain 92 grown at either temperature were similar in mean cell volume and in the range of volumes (Fig. 1). Stationary phase cells at both temperatures showed a greater range of variation in size, as would be expected from a heterogeneous population containing a mixture of young and old cells. Similar results (not shown) were obtained for cells of strain 95.

### Table 3. DNA, RNA, and protein content of Pseudomonas strain 92 cells grown at 2 and 30°C

| Growth phase* | Incubation temperature (C) | DNA* | RNA* | Protein* |
|---------------|-----------------------------|------|------|---------|
| Lag phase     | 2                           | 14.5 | 291  | 840     |
| Lag phase     | 30                          | 10.8 | 227  | 848     |
| Exponential phase |                 |      |      |         |
| Early         | 2                           | 25.5 | 338  | 1,196   |
| Early         | 30                          | 24.9 | 308  | 1,228   |
| Middle        | 2                           | 13.8 | 150  | 776     |
| Middle        | 30                          | 13.5 | 162  | 780     |
| Late          | 2                           | 9.6  | 81   | 516     |
| Late          | 30                          | 11.1 | 66   | 560     |
| Stationary phase |                 |      |      |         |
| Early         | 2                           | 2.6  | 12   | 84      |
| Early         | 30                          | 9.2  | 50   | 500     |
| Late          | 2                           | 2.1  | 18   | 168     |
| Late          | 30                          | 8.2  | 33   | 408     |

* See Table 1.

### Table 4. Catalase activity in exponential and stationary phase cells of psychrophilic Pseudomonas strains grown at 2 and 30°C

| Stage of growth* | Incubation temperature (C) | Specific catalase activity* (× 10⁶) |
|------------------|-----------------------------|-------------------------------------|
|                  |                             | Pseudomonas strain 92 | Pseudomonas strain 95 |
| Middle exponential phase |                  | 2                     | 30                     | 132                  | 160                  | 144                  | 162                  |
| Late stationary phase |                  | 2                     | 30                     | 104                  | 79                   | 123                  | 110                  |

* See Table 1.

* Catalase reaction rate constant at 10°C per minute per 10⁶ cells.
DISCUSSION

These results show that several properties of psychrophilic *Pseudomonas* strains 92 and 95 are not changed by growth at low temperatures. Although their growth rate is 10-fold less (Table 2), cells grown at 2°C are similar to those grown at 30°C in size, nucleic acid and protein composition, and catalase activity.

In studying the effect of growth temperature on cell properties, one must recognize that several factors can contribute to variation in cell properties. Growth in different media, for example, can affect the size and chemical composition (4, 16, 19) of cells. In addition, the size, enzymatic activity, and RNA content differ at various stages of the bacterial growth cycle (5).

Earlier, we reported that the catalase activity of psychrophilic pseudomonads was greater in cells grown at 2°C than in those grown at 30°C (7). However, we had not considered that the differences in catalase activity could have resulted from fluctuations at various stages in the growth cycle (11). The present study shows that, when comparisons are made at the same physiological stage, cells grown at 2 or 30°C have similar catalase activities (Table 4).

The results reported in this paper appear to conflict with those reported by several other investigators who observed that chemostat-grown microbial cells were, at lower incubation temperatures, larger (4), and had more RNA (4, 9, 17), protein (4), carbohydrate (17), and respiratory enzymes (9). However, this discrepancy can also be attributed to the fact that the chemostat studies were conducted with media whose rate-limiting nutrients had been altered to obtain the same growth rate at different incubation temperatures. Mennett and Nakayama (12) reported that there were no differences in the caloric value (a measure of the combustible biomass synthesized) of *P. fluorescens* cells grown in a chemostat at maximal rates at low and moderate temperatures, indicating that the overall synthesis of cellular materials was not affected by growth rate or growth temperature.

Cellular differences occur when the growth medium is changed or if the cells are not compared at the same physiological age, and these factors could interfere with the evaluation of the effects of growth temperature on cells. Because of this, we believe that studies of temperature-induced effects on cells from batch cultures should be done with cells grown in the same medium and that comparisons should be made with cells from equivalent growth stages. In studies where comparisons have been made with physiologically similar cells, or where the same medium was used at all incubation temperatures, psychrophilic organisms grown at low temperatures were not different from cells grown at moderate temperatures in nucleic acid content, protein content (2), or in taxonomically significant properties (6). We conclude, therefore, that low growth temperature, and its concomitant decrease in growth rate, does not grossly affect the properties of facultatively psychrophilic bacteria. Perhaps facultatively psychrophilic bacteria, such as *Pseudomonas* strains 92 and 95, are able to grow at low temperatures because their crucial structural and enzymatic properties are very similar to those found at moderate temperatures. Organisms lacking such versatility would be confined to a more restricted growth temperature range.

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