APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1980, p. 584-587
0099-2240/80/03-0584/04$02.00/0

Vol. 39, No. 3

Effect of Temperature on Mineralization by Heterotrophic Bacteria

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When pure cultures of the bacteria *Pseudomonas fluorescens* (a psychrotroph), *Escherichia coli* (a mesophile), and SRL 261 (a thermophile) were shifted away from temperatures to which they were adapted, the percentage of substrate mineralized increased (percent mineralized = [substrate respired to CO_2]/[substrate respired to CO_2 + substrate incorporated into biomass] × 100). The increase in the percent mineralized was larger for larger temperature shifts. Similar responses were observed when natural heterotrophic bacterial populations from sediments of Lake George, N.Y., and a thermophilic algal-bacterial mat community at the Savannah River Plant, Aiken, S.C., were subjected to temperature shifts. These results suggest that an increase in the percent mineralized may be an indication of thermal stress in bacterial populations.

The importance of determining the amount of substrate mineralized in heterotrophic potential determinations was recognized by Hobbie and Crawford (6). The wide range of the percentage of substrate mineralized (% Min = [substrate respired to CO_2]/[substrate respired to CO_2 + substrate incorporated into biomass] × 100) observed by various investigators in various ecosystems has been summarized by Hoppe (8).

Berman et al. (1) suggested that temperature may have affected the % Min observed in Lake Kinneret. Nutrient availability was suggested by Griffiths et al. (4) as a possible factor affecting the % Min by planktonic and sediment heterotrophic communities of the Beaufort Sea.

Höfle (7) reported that sudden temperature shifts resulted in decreased rates of formation of oxidation products of glucose; however, the effect of the temperature shifts of respiration rates was not determined.

We report here the results of temperature shifts on % Min by pure cultures of psychrotrophic, mesophilic, and thermophilic bacteria and by naturally occurring microbial communities. The possibility that changes in the % Min may be an indication of stress in microbial communities is discussed.

MATERIALS AND METHODS

Escherichia coli K-12 was obtained from C. Hurwitz, Veterans Hospital, Albany, N.Y. *Pseudomonas fluorescens* (ATCC 13525) was obtained from the American Type Culture Collection. SRL 261 was isolated from a natural algal-bacterial mat community in a thermal effluent at the Savannah River Plant, Aiken, S.C.

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These organisms were grown in 200 ml of nutrient broth (Difco Laboratories) in 500-ml flasks shaking at 125 rpm. Cultures were grown to an absorbancy at 750 nm of approximately 0.2. A 5-ml amount of culture was placed in 20-ml serum vials, equilibrated to the temperature of the culture, which were then sealed with serum stoppers. Care was taken to avoid temperature changes during these manipulations. Triplicate samples for incorporation of substrate into biomass and for mineralization of added substrate along with Formalin-killed controls were transferred to water baths at the various test temperatures and allowed to equilibrate for 15 min. The stoppers were then pierced with a hypodermic needle to release any excess pressure inside the vials resulting from the temperature shifts. A 5-μCi amount of [*U*-^{14}C]glucose (New England Nuclear Corp., Boston, Mass.) was injected into each vial. Samples were incubated for 15 min, after which 0.2 ml of neutralized Formalin was added to each previously unfixed sample.

Samples and controls for determining mineralized substrate were acidified with 0.2 ml of 2 N HCl. The $^{14}CO_2$ was trapped on phenethylamine-methanol (1:1)-saturated filter paper wicks by the method of Harrison et al. (5). Samples were placed on a shaker at 125 rpm for at least 1 h at ambient temperature to ensure the complete removal of $^{14}CO_2$ from solution. The wicks were then removed and placed into scintillation vials containing 20 ml of Omnifluor scintillation cocktail (New England Nuclear). Internal $NaH^{14}CO_3$ standards were included to determine the efficiency of $^{14}CO_2$ recovery. Recovery efficiencies were 80 ± 5% for both pure cultures and environmental samples.

Incorporation of substrate into biomass was determined by filtering 2.5 ml of the samples onto 0.2-μm pore size membrane filters (Nuclepore Corp., Pleasanton, Calif.) and washing with 0.02 M glucose. The filters were then placed into scintillation vials, and the samples were solubilized with 1 ml of Soluene 350 (Packard Instrument Co., Downers Grove, Ill.). A 10-ml amount of Omnifluor was then added to each vial. The amount of radioactivity was determined by using

an SL 350 liquid scintillation spectrometer (Intertechnic, Dover, N.J.).

Sediment from a depth of 1 m in the littoral zone of Lake George, N.Y. was collected with an Ekman dredge. Slurries were made by mixing equal volumes of sediment and lake water. Samples were prepared and analyzed as described above, except that the samples were incubated with [*U*-^{14}C]glucose for 30 min in the dark.

Slurries from an algal-bacterial mat growing on a 15-m-long plywood trough microcosm receiving flow directly from a thermal effluent at the Savannah River Plant, were made by suspending two 6-cm^2 cores of mat in 400 ml of water from the sample site. Samples were prepared as described above and incubated with [*U*-^{14}C]glucose for 10 min in the dark. After fixation and processing as described above, the samples for incorporation of substrate into biomass were combusted by using a Packard Tri-Carb sample oxidizer (Packard Instrument Co., Downers Grove, Ill.). The amount of radioactivity was determined by using an LS 250 liquid scintillation spectrometer (Packard Instrument Co.).

RESULTS

The results of laboratory temperature shift experiments with pure cultures of *P. fluorescens*, *E. coli*, and SRL 261 adapted to different temperatures are shown in Fig. 1, 2, and 3, respectively. These results show that as these bacteria were shifted away from the temperature to which they were adapted they responded by increasing the % Min of glucose. The further these organisms were shifted from their adapted temperature the greater the % Min. At nonpermissive growth temperatures, 37 and 45°C for *P. fluorescens*, 5°C for *E. coli*, and 15 and 60°C for SRL 261, and the % Min increased dramatically. The data in panels B and C of Fig. 1 through 3 show that at permissive growth temperatures the amount of glucose mineralized was relatively constant, regardless of the temperature. However, the amount of glucose incorporated into biomass appears to be much more sensitive to thermal perturbations. Thus, the observed effect of temperature on the % Min appears to be due to the effect of temperature on biosynthetic processes rather than degradative processes responsible for CO_2 production.

The results of temperature shift experiments using natural microbial communities from Lake George sediments (Fig. 4A) and a thermophilic algal-bacterial mat community from the Savannah River Plant (Fig. 4B) show the same trends as those observed with pure bacterial cultures. That is, the % Min increased as these natural communities were shifted away from the in situ temperature.

DISCUSSION

These data show that increased % Min occurred during periods of adaptation to thermal

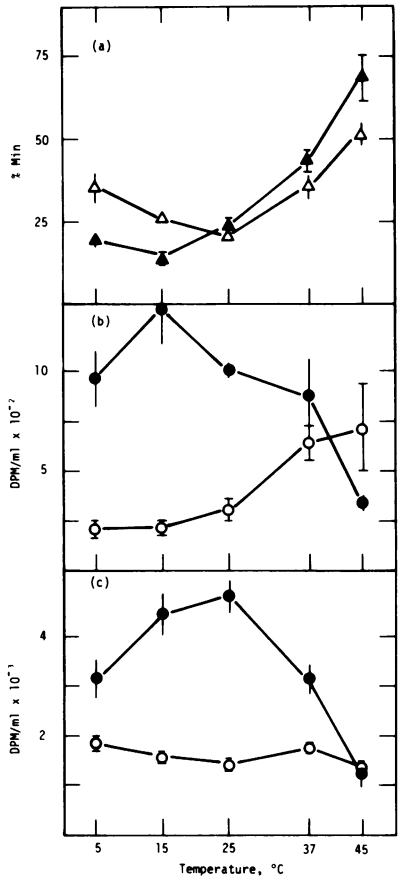

FIG. 1. *% Min of glucose by P. fluorescens adapted to 5°C (▲) and 25°C (△) after shifts to various temperatures (A). Amount of glucose mineralized (○) and incorporated into cellular material (●) by P. fluorescens adapted to 5°C (B) and 25°C (C) after temperature shifts. Bars represent ±1 standard deviation from the mean of triplicate samples.*

changes. After these organisms had adapted to a new temperature the % Min at that temperature decreased significantly. This can be seen with *P. fluorescens* at 5°C, *E. coli* at 15°C and SRL 261 at 25°C before and after adaptation to these temperatures. It is interesting to note that at the optimum growth temperature for each of these organisms, 25°C in the case of *P. fluorescens*, 37°C for *E. coli*, and 45°C for SRL 261, the % Min was relatively unchanged regardless of the temperature to which the organisms were adapted.

The increased % Min observed when *P. fluorescens*, *E. coli*, and SRL 261 were shifted away from temperatures to which they were adapted resulted, for the most part, from changes in the amount of glucose incorporated into cellular material. Rates of macromolecular synthesis, e.g., deoxyribonucleic acid, ribonucleic acid, and pro-

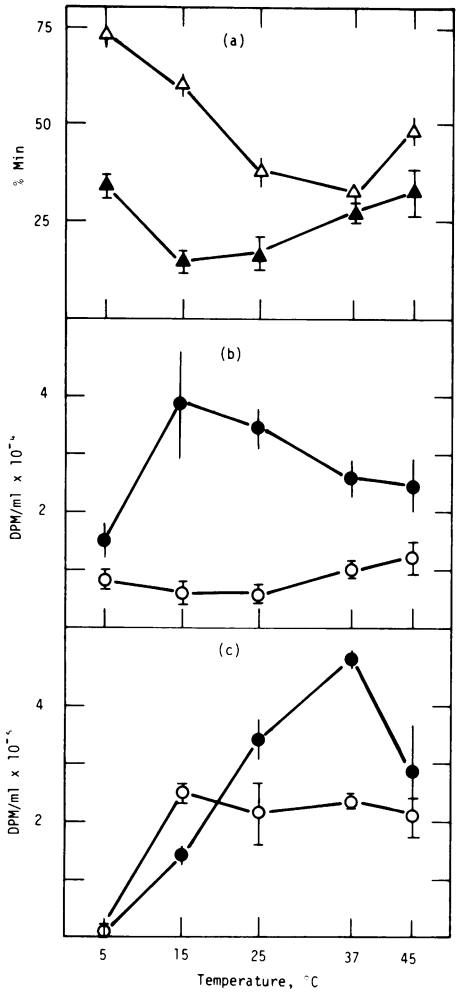

FIG. 2. *% Min of glucose by E. coli adapted to 15°C (▲) and 37°C (△) after shifts to various temperatures (A). Amount of glucose mineralized (○) and incorporated into cellular material (●) by E. coli adapted to 15°C (B) and 37°C (C) after temperature shifts. Bars represent ±1 standard deviation from the mean of triplicate samples.*

tein, by *P. fluorescens* (R. J. Soracco and D. H. Pope, unpublished data) and *E. coli* (R. J. Broeze and D. H. Pope, unpublished data) were decreased when these organisms were shifted away from their adapted temperatures. The effect on macromolecular synthesis was most marked during periods of adaptation after temperature shifts. As a result of decreased biosynthetic activity during periods of adaptation and the apparent insensitivity of degradative CO_2-generating processes, an increase in the % Min is observed.

Although Hobbie and Crawford (6) recognize the importance of bacterial respiration in heterotrophic potential measurements, they indicate that incubation temperature was not an important factor in their studies. Our data indicate that incubation temperature does affect the % Min and is an important factor to consider, especially during short-term incubations before adaptation occurs.

Other environmental perturbations resulting in stress to microbial communities which might require adaptation may also result in increased % Min. Increased % Min may be an indicator not only of thermal stress but also of stress due to other physical factors, nutrient limitation, or environmental pollutants such as pesticides and heavy metals. Wood and Chua (9) found that the % Min of glucose by microbial communities in sediments of Lake Ontario was related to pollution stress. Goulder et al. (3) found no

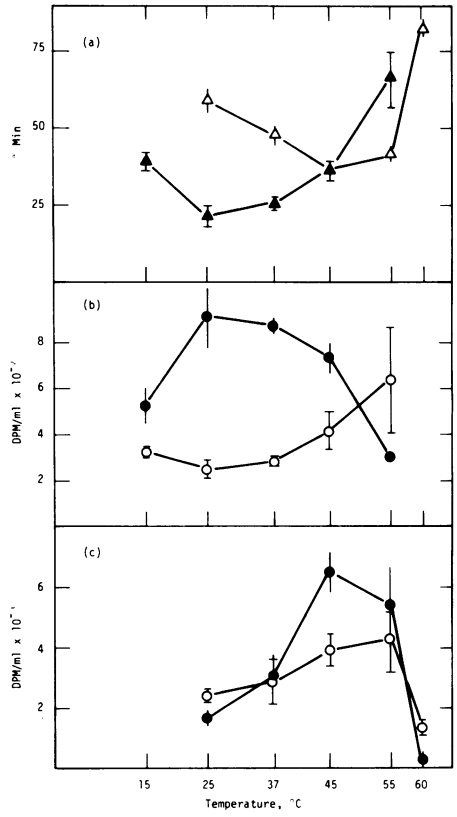

FIG. 3. *% Min of glucose by SRL 261 adapted to 25°C (▲) and 55°C (△) after shifts to various temperatures (A). Amount of glucose mineralized (○) and incorporated into cellular material (●) by SRL 261 adapted to 25°C (B) and 55°C (C) after temperature shifts. Bars represent ±1 standard deviation from the mean of triplicate samples.*

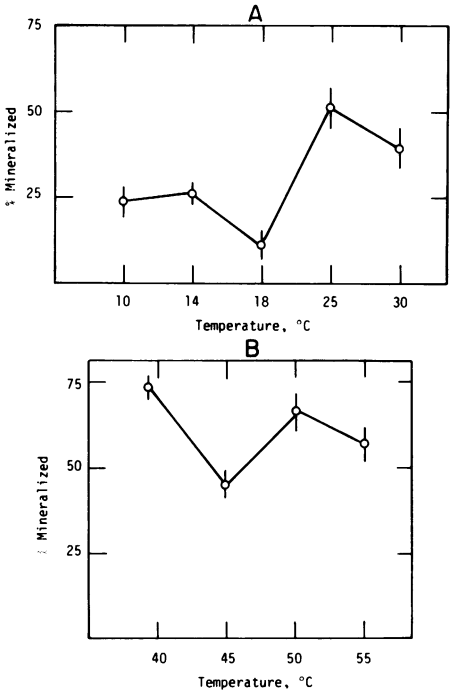

FIG. 4. *(A) % Min of glucose by a natural microbial community from the sediment of Lake George, N.Y. The in situ temperature was 18°C. (B) % Min of glucose by a thermophilic algal-bacterial mat community from 45°C in a thermal effluent at the Savannah River Plant, Aiken, S.C. Bars represent ±1 standard deviation from the mean of triplicate samples.*

correlation between copper concentration and the % Min of glucose by planktonic estuarine microorganisms, however.

It should be kept in mind, however, that the % Min by an unstressed microbial community is dependent on the metabolic capability of that community to a given substrate. The % Min will vary according to the species composition of a given community and the substrate being tested. Crawford et al. (2) and Hobbie and Crawford (6) have shown that the % Min by estuarine microorganisms was different for different substrates. Therefore, it will be necessary to establish a base-line % Min of a substrate by a given community before any conclusions regarding % Min and stress on that community can be made.

ACKNOWLEDGMENTS

We thank C. B. Fliermans of the Savannah River Laboratory, E. I. du Pont de Nemours & Co., Inc., Aiken, S.C., for many helpful discussions and for the use of his laboratory facilities at the Savannah River Plant. We also thank R. D. Fallon for critically reviewing the manuscript.

This work was supported, in part, by National Environmental Research Park funds under contract no. AT(07-2)-1 with the U.S. Department of Energy.

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