Conversion of Mesophilic to Psychrophilic Bacteria

Abstract. *The minimum temperature of growth of the mesophilic bacterium* Pseudomonas aeruginosa *has been significantly lowered from approximately 11° to 0°C. This shift in the minimum temperature of growth was accompanied by a corresponding decrease in the maximum temperature of growth. Transfer of this genetic characteristic by a transducing phage grown on a psychrophile or by ultraviolet mutagenesis was used to accomplish these shifts in range of growth temperature.*

As a consequence of temperatures of optimum growth, bacteria have been grouped as psychrophiles, mesophiles, or thermophiles. Although various species within a group may differ somewhat with regard to minimum and maximum growth temperature, they tend to form a group with regard to optimum growth temperature. When the physiological characteristics of these groups have been studied, the emphasis usually has varied. With thermophiles, maximum temperature of growth and resistance to heat have received the most attention. With psychrophiles, the primary emphasis has been on their growth and activities near 0°C or at their maximum temperature of growth, which is characteristically lower than for mesophilic bacteria. Mutations in mesophilic bacteria resulting in the synthesis of temperature-sensitive enzymes and a lowering of the maximum growth temperature are commonly used in the elucidation of reaction sequences mediated by cellular enzymes or in studies related to the synthesis of noncatalytic macromolecules. Changes in the minimum temperature of growth after mutagenesis of bacteria have been reported (*1*).

However, in both these and other studies where changes in the minimum or maximum temperatures of growth have been observed, shifts in the entire range concomitant with changes in the determinant of minimum or maximum growth temperatures, to our knowledge, have not been reported. The alteration of the determinant of either the minimum or maximum temperature of growth has resulted in a diminution of the range of growth temperature of the organism in question. We now report shifts in the entire range, which occur when mesophilic bacteria are subjected to either mutagenesis by ultraviolet irradiation or to transduction and become psychrophilic.

Mutation of mesophilic bacteria to psychrophily has been reported by Azuma *et al.* (*2*). However, it is unclear from their report whether a lowering of the maximum temperature of growth accompanied the acquisition of the ability to grow near 0°C. We have attempted to repeat their results, and our psychrophilic mutants do show shifts in their entire growth temperature range. During our studies, we used appropriate controls to eliminate the possibility of contamination and spontaneous mutations. At least twice as many control platings as mutant selective plates were made, and the controls were uniformly negative. The minimum and maximum growth temperatures for mesophiles were changed from approximately 11° and 44°C to 0° and 32°C, respectively. Thus, the total growth temperature range was not significantly affected. For this work, we used several strains of *Pseudomonas aeruginosa*. With some strains we were unable to recover mutants at any time, whereas with other strains, such as ATCC 12633 [used by Azuma *et al.* (*2*)], and strain R 629 and strain 1 received from B. W. Holloway, we regularly produced psychrophiles. The mutation rate to psychrophily after ultraviolet irradiation in an amount sufficient to kill more than 99 percent of the cells was approximately 1 per 10^8 unirradiated bacteria. Two hundred psychrophilic mutants were selected by plating at 3°C on tryptone, glucose, yeast-extract agar (*3*); these generally did not form visible colonies before 7 days of incubation at 3°C. Our results essentially confirm those of Azuma *et al.* (*2*) except that we found a proportional lowering of the maximum growth temperature along with the acquisition of the ability to grow near 0°C. The doubling time for the growth of these mutants at 3.5°C was generally 4 to 6 hours, which is comparable to growth rates for wild-type psychrophiles (*4*). Concomitant with their mutation many of the psychrophilic isolates became auxotrophic or they no longer showed their characteristic phage type, perhaps reflecting the effects of the heavy dosage of ultraviolet irradiation required to produce them. At any rate, the fact that we observed such a radical change in the properties of the organism—mutation to the psychrophilic state—prompted us to consider that growth temperature range may be defined by a limited number of genetic loci. To support this concept, we next attempted, using *Pseudomonas* bacteriophage PX4, to transduce psychrophily.

Pseudomonas bacteriophage PX4 is a generalized transducing phage. This was shown by the following procedures: PX4-transducing lysates were prepared for the growth of other *Pseudomonas* phages (*3*). However, the following modification was imposed to eliminate the possibility of transformation by bacterial deoxyribonucleic acid from the phage-propagating host. Lysates of phage were treated with yeast ribonuclease (4 μg/ml) and beef pancreas deoxyribonuclease (4 μg/ml) in the presence of $MgSO_4$ (0.002 mole/liter), after initial clarification of the lysate by centrifugation at 8000*g*. For transductions, approximately 10^9 cells and 5×10^8 phage were mixed in broth and allowed to adsorb for 10 minutes at 25°C. The cells were then centrifuged and suspended in saline to volume. Portions (0.2 ml) were spread on the surface of 20 plates. Phage-sterility and bacterial controls were treated similarly. Transductions were scored after a 48-hour incubation period at 25° and 37°C or after 6 days at 3.5°C. Phage PX4 can transduce a variety of auxotrophic markers (*5*), and the behavior of this system is similar to that reported for two other phages used to transduce saphrophytic *Pseudomonas* species (*6*). For this work we used *P. fluorescens* 14 as the donor of psychrophily and *P. aeruginosa* 2, a tryptophan-requiring auxotroph, as the recipient. These bacterial strains and phages have been described (*3*).

Over 200 interspecies recombinants were selected for either tryptophan independence at 25°C on glucose minimal medium [Vogel and Bonner (*4*)] or for psychrophily at 3.5°C on tryptone, glucose, yeast-extract complete medium. When this was done, frequencies of transduction for psychrophily (selected at 3.5°C) or for tryptophan independence (selected on minimal medium at 25°C) were usually from 2.5×10^{-7} to 6×10^{-8} per viable phage. Approximately 50 percent of the psychrophiles were cotransduced for tryptophan independence and similarly 50 percent of the isolates selected for on minimal media at 25°C were psychrophiles. When selection for tryptophan independence was done at 37°C on minimal medium, none of the prototrophic isolates were psychrophiles. Accordingly, testing psychrophiles for

growth temperature range showed an inability to grow above 33°C. Thus, the psychrophilic transductants resembled psychrophilic mutants in the shift of their growth temperature maxima and minima to that characteristic of wild-type psychrophilic pseudomonads of the soil. Cotransduction of tryptophan independence shown here may be trivial to the causal basis for psychrophily. However, this observation indicates that the involvement of many scattered loci as contributing to psychrophily is unlikely. From these and the mutagenesis experiments, it would seem that the growth temperature range may be prescribed by a limited number of genetic loci or a closely linked constellation of characters.

Hence, of fundamental importance is the relationship of these findings to causal factors responsible for the growth temperature range of these organisms. Although it has been repeatedly shown that psychrophiles have temperature-sensitive enzymes limiting growth much above 32° to 33°C (*8*), it seems unlikely that either mutagenesis by ultraviolet irradiation or transduction would change the total enzymic makeup of the cell sufficiently to promote psychrophily, if psychrophily were dependent on a whole array of temperature-sensitive enzymes. Consequently, it may be that, throughout the evolution of these species, mutation toward thermal sensitivity may have occurred and been maintained in the absence of counterselection at warm temperatures not prevalent in the usual environment of psychrophilic bacteria. Thus, psychrophily and perhaps the delineation of the growth temperature range of mesophilic bacteria may reflect the temperature response of the products of a limited number of genetic loci whose primary function is the regulation of cell division in response to temperature fluctuations.

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References and Notes

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5. We have used bacteriophage PX4 to transduce isoleucine plus valine (ilv) or leucine (leu) independence in addition to tryptophan (try) independence and psychrophily (psy), referred to in this paper. The transduction frequency for ilv or leu independence was approximately the same as for try independence or psy. However, cotransduction of ilv and psy or leu and psy was less than 1 percent when tested as described for try and psy cotransduction. Some (two- to fivefold) stimulation in the transduction of ilv after irradiation of phage suspension sufficient to cause a 100-fold decrease in phage titer occurred. However, leu, try, or psy transduction frequencies decreased in parallel fashion with the number of surviving lytic phage particles as the ultraviolet exposure time was lengthened.
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9. Supported by NIH grant AI-07533 and NSF grant GB-693.

25 July 1968; revised 30 September 1968

Dictyostelium discoideum: A Gamma-Ray Resistant Organism

Abstract. *The vegetative cells of the slime mold* Dictyostelium discoideum *are extremely resistant to cobalt-60 gamma rays. The survival curve has a shoulder at low doses and a 10 percent survival dose of 300 kilorads in air. Dose fractionation experiments indicate that repair of sublethal damage is an important factor in this resistance.*

The cellular slime mold *Dictyostelium discoideum* is an excellent model system for studying the molecular and cellular mechanisms associated with development and differentiation (*1*).

Vegetative amoeboid cells of *Dictyostelium discoideum* NC-4, a stable haploid strain (*2*), were grown in an aerated liquid suspension of *Escherichia coli* B/r (10^{10} cell/ml) in tris-saline solution (TSS) (0.01*M* NaCl; 0.01*M* KCl; 0.04*M* tris, *p*H 7) at 23°C (*3*). The cell doubling time was 2.5 hours. At 10^6 cell/ml (exponential growth phase), the suspensions were cooled to 0°C and irradiated with Co^{60} gamma rays (Gammacell-200) at a dose rate of 13.1 krad/min with aeration. Irradiating in the presence or absence of the bacteria did not substantially alter the results. After irradiation, the cells were either immediately diluted in TSS for plating or held for further treatment. The nutrient plates (*4*) were spread with 0.1 ml of the diluted *D. discoideum* suspension along with 0.1 ml of an unirradiated *E. coli* B/r suspension (5×10^9 cell/ml). As each deposited viable cell multiplied, it consumed the surrounding bacteria and after many divisions gave a large clear colony or "plaque" in the bacterial lawn. The plates were incubated at 23°C and counted at 2, 3, 4, and 5 days, after which no new plaques appeared. For dose fractionation experiments, irradiated suspensions were aerated at 23°C for various times in the presence of *E. coli* (10^{10} cell/ml, generally irradiated with *D. discoideum*) and then irradiated again.

Figure 1 ("0 hr" curve) gives the percent survival of plaque-forming ability as a function of unfractionated gamma ray dose. A threshold dose of about 200 krad is needed before survival swings sharply downward, becoming exponential at higher doses (*5*) (Fig. 1). These cells are extremely gamma-ray resistant compared to most other organisms (*6–10*) and in fact rank with some of the most radiation-resistant organisms known, including *Micrococcus radiodurans* (*11*), some protozoa (*12*), and a Hartmannellid amoeba (*13*). Although a shoulder exists on the survival curve, the initial slope is not zero, possibly indicating a heterogeneous population with respect to extent of shoulder (*6*). This may be the consequence of irradiating a non-

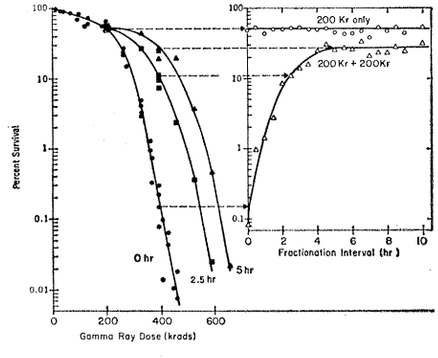

Fig. 1. Percentage survival of colony-forming ability of *Dictyostelium discoideum* vegetative cells as a function of total gamma ray dose in kilorads. Zero-hour curve (—●—): unfractionated doses; 2.5-hour (—■—) and 5-hour (—▲—) curves: obtained by initially irradiating with 200 krad, then incubating the suspension for 2.5 or 5 hours before the remainder of the dose was given. Inset: Survival as a function of time of incubation at 23°C with aeration before plating after a single dose of 200 krad (—○—); and recovery of survival as a function of incubation time between an initial dose of 200 krad and a later dose of 200 krad (—△—).