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on the Physiology and Pathogenicity of Selected Bacteria

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Principal Investigator: Dr. Benedict T. DeCicco  
Associate Professor  
Department of Biology  
Washington, D.C. 20017

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EFFECTS OF MUTATION AND SOME ENVIRONMENTAL FACTORS ON THE PHYSIOLOGY AND PATHOGENICITY OF SELECTED BACTERIA  
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This report covers the one-year period from 1 October, 1972 through 30 September, 1973 that this grant was in effect. Much of the work described here originated and was initiated under NASA grant NGR-09-005-022, a final report of which was submitted in late 1972. Reprints of reports published in 1973 and 1974 are attached, except for one article in press, reprints of which will be forwarded when available

Our recent efforts can be divided into three sections.

1. Studies on staphylococcal virulence factors.
2. Mycobacterial pathogenicity and its relationship to autotrophic ability.
3. Studies with thermophilic bacteria and their mutants.

The first part, describing our studies with mutants of Staphylococcus aureus lacking some "Virulence Factors", has been completed. The results, summarized in Tables 1, 2, 3, and the attached abstract, suggest that the presence of deoxyribonuclease correlates with mouse pathogenicity of S. aureus, while the ability to ferment mannitol or the possession of coagulases are not required for virulence.

The studies on autotrophy in mycobacteria are also proceeding well. A complete correlation has been demonstrated between the ability to grow with hydrogen and the species of scotochromogenic mycobacterium tested. All tested strains of M. gordonae, a saprophyte, could grow autotrophically while none of the tested strains of M. scrofulaceum, a clinically important species, possessed this ability. One strain isolated by us, which grew autotrophically, was classified as intermediate between the two species. Both hydrogenase and ribulose diphosphate carboxylase

(RDPC) in M. gordonae show inducible characteristics, but the factors affecting enzyme levels differ from those seen in some other hydrogen autotrophs. A dramatic increase in cell growth was observed when M. gordonae was incubated mixotrophically, as compared to either autotrophic or heterotrophic growth conditions. Levels of hydrogenase and RDPC were low in cells grown heterotrophically with a number of different energy sources. Levels of both enzymes were high with autotrophically grown cells and were intermediate during mixotrophic growth. When cells were grown in the presence of glycerol plus hydrogen and oxygen (no carbon dioxide) hydrogenase was present and RDPC was absent, suggesting that CO<sub>2</sub> is the specific inducer for RDPC. One interesting observation is that the increased growth rates and yields observed during mixotrophic cultivation are not obtained if only hydrogen and oxygen are present. Thus these organisms seem unable to derive useful energy from hydrogen oxidation in the absence of carbon dioxide. We are looking into possible coupling effects to explain this phenomenon and are continuing a general study of autotrophy in the mycobacteria. Abstracts describing part of these studies are attached. Reprints of a more complete report, to be published in the July issue of International Journal of Systematic Bacteriology will be sent later.

Studies with thermophilic bacteria continued during this period. Reprints of an abstract and a paper describing studies with thermophilic mutants of Pseudomonas fluorescens are attached. Most of our recent studies on thermophily have been centered on selection of antibiotic resistant mutants of thermophilic members of the genus Bacillus. Three strains received from Dr. Robert Mac Elroy, NASA Ames

Research Center were labeled as follows: TDA (#1), YTP (#7), TPA (#8).

These strains grew to a maximum temperature of 71°C and had a minimum of about 40°C. All 3 strains were sensitive to low concentrations of the following antibiotics: chloromycetin, neomycin, erythromycin, kanamycin, novobiocin, penicillin, streptomycin, tetracycline, cephalothin, naladixic acid, ampicillin, furodantin, mandelamine and gentamicin. No natural resistance was observed with any tested antibiotic or chemotherapeutic agent. Three of the antibiotics, penicillin, neomycin, and erythromycin, were used for further studies

The three strains grew in tryptic-soy broth at 55°C containing up to .001 to .005 units/ml penicillin. No growth occurred in the presence of .01 unit/ml penicillin with any strain. By sequential transfer, growth in penicillin concentrations of 0.2 to 0.4 was obtained (depending upon the strain) but the resistant strains lost their resistance rapidly upon freezing or subculture in the absence of penicillin.

With neomycin the three strains originally grew in concentrations of .005 to .05 ug/ml and strains capable of growth in 0.1 to 2.5 ug/ml could be obtained, but, like the penicillin resistant cells, these strains lost some resistance after subculture.

By far the most favorable results were obtained with erythromycin. All three strains were originally inhibited by 0.05 to 0.2 ug/ml of erythromycin. By repeated transfer to higher concentrations all three strains became tolerant to a concentration of 100 ug/ml. The three strains were taken from tubes containing 30 ug/ml of the antibiotic (TSB medium) and grown through five subcultures at 68°C without erythromycin. The subcultured organisms were then inoculated into TSB plus 30 ug/ml of erythromycin at 55°C and all three strains grew. We therefore can conclude that

resistance to high levels of this antibiotic can be maintained even after many transfers in the absence of the antibiotic; this characteristic therefore can be used as a stable genetic marker for these thermophilic bacilli.

A culture labelled TD-80, also obtained from Dr. MacElroy, was run through a similar procedure with erythromycin and gave differing results. The growth that occurred in higher concentrations of erythromycin only became evident after incubation for a number of days ( at 60<sup>0</sup>C) and was not transferable to new antibiotic containing tubes. It appeared that this growth was due to the gradual decomposition of erythromycin at 60<sup>0</sup>C. Since this antibiotic is bacteristatic rather than bactericidal, the inhibited cells were able to grow after the thermal inactivation of the erythromycin.

We were not able to use the DNA extracted from the marked strains for successful genetic homology studies among the thermophilic bacilli because preliminary experiments showed the spectrophotometric technique we intended to use was not precise enough for accurate homology determinations, and there was insufficient time before this grant terminated to prepare isotopically labeled DNA for homology studies using the membrane filter method .

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## Thermophilic Mutants of *Pseudomonas fluorescens*

B. T. DeCicco and K. F. Noon

Department of Biology, The Catholic University of America, Washington, D.C. 20017

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*Summary.* A series of heat tolerant mutants of *Pseudomonas fluorescens* were obtained which can grow at temperatures up to 54°C, in contrast to a maximum growth temperature of 37°C for the wild type. The minimum temperatures allowing growth of the mutant strains increased to the same extent as their maximum temperatures. Antibiotic sensitivity patterns suggested the mutants had altered ribosomes, but the purified mutant ribosomes showed no significant increase in thermostability. The virulence of the wild and mutant strains for mice correlated with their relative abilities to grow at the mouse body temperature of approximately 37°C.

The capacity for normal laboratory microorganisms to adapt to extreme environments is still largely unknown. This almost certainly depends to some extent on the particular environmental factor in question. The fact that drastic physiological changes are thought to be uncommon is evidenced by the use of environmental factors such as salt tolerance, maximum and minimum growth temperatures, and utilization of various substrates as indices of taxonomic relatedness. While many individual changes can be brought about in microorganisms through minor changes in cell structure (e.g. phage or antibiotic resistance) or the loss of certain enzymes (nutritional mutants), some other types of mutational changes such as in salt tolerance (Ingram, 1957) and base composition (Garrity *et al.*, 1969) have been intentionally sought without success. The physiological limitations imposed by genetic adaptations to one environmental extreme were demonstrated when Olsen and Metcalf (1968) reported that bacterial mutants capable of growing near 0°C had decreased abilities to grow at higher temperatures. The minimum temperature for growth of the mutants was lowered by the same amount as was the maximum temperature; thus the range of temperature over which growth occurred was not changed. There is evidence demonstrating that a limited range of tolerance may apply to other environmental factors as well; for example salt tolerant halophiles cannot grow in the absence of salt (Larsen, 1962) and thermophilic bacteria cannot grow at normal temperatures (Imšenecki and Solnzeva, 1945; Brock and Freeze, 1969).

The biochemical basis for thermophily has not been resolved. Evidence based on comparisons between thermophiles and mesophiles has implicated thermostable enzymes and proteins (Campbell, 1955; Koffler and Gale, 1957), ribosomes (Friedman *et al.*, 1967; Pace and Campbell, 1967; Zeikus and Wolfe, 1972), and cell membranes (Brock, 1967; Shen *et al.*, 1970) as distinctive components. Until recently the commonly known heat tolerant organisms were strains of gram positive spore-forming bacteria, blue green algae, and fungi. Then Brock and Freeze (1969) reported the isolation of a gram negative extremely thermophilic bacterium capable of growing at temperatures up to 79°C, which they named *Thermus aquaticus*. However, because of its highly specialized physiology the taxonomic relationship between this organism and known mesophiles was not obvious. The taxonomic (and evolutionary) relationship between recently isolated extremely thermophilic strains of *Bacillus* (Weerkamp and Heinen, 1972) and *Methanobacterium* (Zeikus and Wolfe, 1972) and mesophilic strains of the same genera can be more easily estimated.

We decided to seek thermophilic bacterial variants because this environmental factor can be closely controlled, its latitude is wide enough to allow for more than one sequential mutation, and a comparison of the mutant and wild strains would afford a means for studying the biochemical basis for thermophily.

### Materials and Methods

*P. fluorescens* (ATCC 13525) was obtained from the American Type Culture Collection. For mutant isolation, growth rate and heat tolerance studies, and mouse pathogenicity experiments, cultures were grown aerobically in 1.5% tryptic soy broth (TSB; Difco, Detroit, Mich.) on an incubator shaker at the desired temperature, which remained constant within 0.2°C. Doubling times were determined from the growth curves plotted from optical density measurements at 540 nm. For determination of the wild and mutant strains' capacities to utilize single substrates as sole carbon and energy sources, 0.1% organic substrate was added to a mineral salts solution composed of 0.1%  $(\text{NH}_4)_2\text{SO}_4$  and 0.02%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  in 0.04 M potassium phosphate buffer, pH 6.8.

Cytochrome oxidase, lipase, and lecithinase activities were detected as described by Stanier *et al.* (1966), as was the determination of the mechanism of aromatic ring cleavage.

Ribosomes were isolated and purified according to the method of Zeikus *et al.* (1970). Thermal denaturation of purified ribosomes suspended in 0.01 M tris(hydroxymethyl) aminomethane (Tris) buffer, pH 7.8, containing 0.01 M  $\text{MgCl}_2$  and 0.06 M KCl, was determined using a Beckman DU Spectrophotometer, attached thermospacers, and Gilford model 220 accessories. Absorbance and temperature (measured by a linear thermosensor) were recorded using a Varian model G10 recorder.

Pathogenicity of wild type *P. fluorescens* and three mutant strains was determined by intraperitoneal injection of standardized cell suspensions into ICR strain male mice. Cell suspensions were standardized by correlating cell numbers with optical density and then adjusting the cell densities to the desired values. Different

**M 311** Pathogenicity of Staphylococcus aureus Mutants Lacking Some "Virulence Factors." NORMA SNELLINGS\* and B. T. DE CICCIO. Catholic University of America, Washington, D. C.

Three mutant types, each lacking a different "virulence factor," were obtained from Staphylococcus aureus ATCC 12600 following mutagenesis with nitrosoguanidine. Separate mutants lacking the capacity to (A) ferment mannitol, (B) produce free and bound coagulase, or (C) produce extracellular deoxyribonuclease (DNase) were isolated using appropriate differential media. Each type of mutant retained the normal characteristics of the parent strain with the exception of the selected character. The mouse virulence of each mutant type was compared to S. aureus ATCC 12600 and S. epidermidis ATCC 14990 using the intraperitoneal route. With  $7 \times 10^9$  colony forming units of each strain, the proportion of killed mice to total mice injected was as follows: S. aureus ATCC 12600, 14/15; S. epidermidis ATCC 14990, 0/9; mannitol nonfermenting strain, 12/13; coagulase negative strain, 12/13; DNase negative strain, 1/15. The difference in virulence between the wild strain of S. aureus and the DNase negative mutant is highly significant statistically ( $P < .001$ ). Our results suggest that the production of an extracellular DNase is important for normal virulence of S. aureus in mice while the presence of free and bound coagulase may play no significant role.



**G 116** Chemolithotrophic Growth of Mycobacterium gordonae with Hydrogen. S.S. PARK\* and B.T. DECICCO.

Catholic University of America, Washington, D.C.

A strongly acid fast, slow growing, scotochromogenic Mycobacterium was isolated from a medium specific for hydrogen utilizing chemolithotrophs. The organism grew well in pure culture in simple mineral salts media under an atmosphere of hydrogen, oxygen and carbon dioxide. No growth occurred in the absence of the gas mixture unless organic substrates were added. The hydrogenase enzyme was induced by hydrogen; the enzyme level during heterotrophic growth being only 5-10% of that present during autotrophic incubation. Two major colony types were observed. During autotrophic cultivation a flat, rough form was dominant while heterotrophic cultivation caused a population shift to a smooth, domed variety. A number of strains of M. tuberculosis, M. kansasii, M. marinum, M. flavescens, M. gordonae, M. scrofulaceum, M. avium, M. intracellulare, M. xenopi, M. chelonae, and M. fortuitum were tested for their capacity to grow autotrophically with hydrogen. All 4 strains of M. gordonae grew autotrophically while no tested strains of the other species possessed the capacity. The ability to grow autotrophically could be a useful characteristic for distinguishing the saprophytic scotochromogens (M. gordonae) from the more pathogenic strains (M. scrofulaceum).

dilutions were injected into groups of 5 to 14 mice and the number of dead and surviving mice was used to estimate  $LD_{50}$  values, using the method of Reed and Muench (1938). For statistical analyses the ratio of mice killed by  $1 \times 10^9$  cells of each mutant strain was compared to the wild strain using the "exact" test (Bailey, 1959).

### Results

*P. fluorescens* is unable to grow at even moderately high temperatures; its inability to grow at  $41^\circ\text{C}$  is used to distinguish it from *P. aeruginosa*. The neotype strain of *P. fluorescens* (ATCC 13525) normally cannot grow at temperatures above  $37^\circ\text{C}$ . When  $3 \times 10^9$  cells were inoculated into flasks containing 100 ml of TSB and incubated at  $45^\circ\text{C}$  with shaking, an increase in turbidity was noted in 24–36 h. Smaller inocula did not yield organisms capable of growth at this temperature even after lengthy incubation, and may reflect the low frequency of heat tolerant mutants in the normal population, as was noted with some species of *Bacillus* (Allen, 1953). Appropriate sterile controls and cloning of the wild strain were employed to eliminate the possibility of selecting for air contaminants or members of mixed populations. Isolates from the heavily inoculated flasks were capable of rapid growth at  $45^\circ\text{C}$  and subsequently were inoculated into TSB at  $48^\circ\text{C}$  and  $50^\circ\text{C}$ . Growth was evident in the  $48^\circ\text{C}$  flask after 24 h whereas no increase in turbidity occurred at  $50^\circ\text{C}$  even after 7 days. An isolate from the  $48^\circ\text{C}$  flask was transferred to fresh media at  $50^\circ\text{C}$ . Growth was evident after 48 h and an isolate was transferred to media at  $52^\circ\text{C}$ . This produced a fourth isolate. Subsequent transfers to media incubated at  $55^\circ\text{C}$  or above yielded no additional mutants. The four isolates were designated strains *M 45*, *M 48*, *M 50* and *M 52* to correspond to the temperature of incubation at which each was obtained, and were then studied as separate strains. Strain *M 50* showed some instability and, since its physiological characteristics were quite similar to *M 48*, it was omitted from some of the studies.

Although *P. fluorescens* consistently produced heat tolerant mutants, two other bacterial species, *Escherichia coli* and *P. aeruginosa*, seemed unable to yield mutants capable of growth at temperatures above the wild strains' maxima. A fourth species, *Bacillus megaterium* yielded a single step mutant with a maximum growth temperature about  $5^\circ\text{C}$  above that of the wild strain. Similar mutants of some *Bacillus* species have been reported (Allen, 1953).

The growth rates of *P. fluorescens* 13525 and strains *M 45*, *M 48*, and *M 52* at different temperatures are shown in Fig. 1. It is evident that the four strains differ significantly in their response to temperature of incubation. The mutant strains have lost the ability to grow at low temperatures and, like the psychrophilic mutants of *P. aeruginosa* (Olsen and Metcalf, 1968), maintained an overall temperature span of growth of approximately  $35^\circ\text{C}$ .

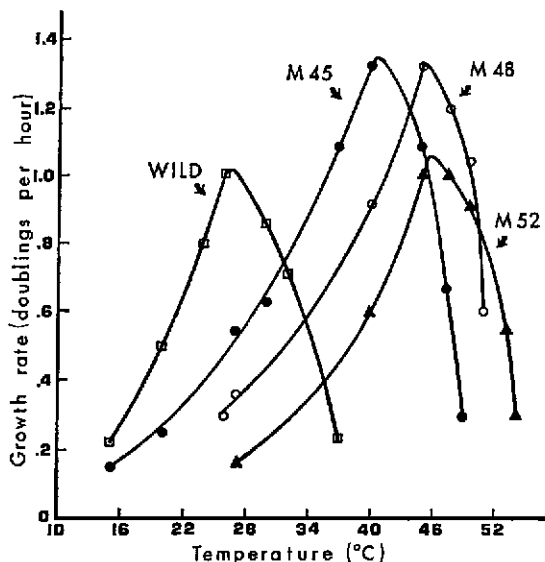


Fig. 1. Effect of incubation temperature on growth rates of wild and mutant strains of *Pseudomonas fluorescens*

The heat tolerance of the mutant strains showed dramatic increases with each sequential isolation. At 60°C the wild strain yielded a four log kill (0.01% survivors) in 25 sec. *M 45* underwent the same kill in 4.5 min while the values for *M 48* and *M 52* were 34 and 43 min, respectively.

The mutant strains retained most of the physiological, morphologic and biochemical characteristics of the wild strain. All were small, motile, gram negative rods. Although the characteristic green pigment of the wild strain was usually absent in the mutants, strains *M 45* and *M 48* produced the pigment if grown in glycerol medium or at temperatures near their minima. Some characteristics of the wild and mutant strains of *P. fluorescens* are shown in Table 1. All strains catabolized p-hydroxybenzoate using ortho cleavage, as is typical of *P. fluorescens* (Stanier *et al.*, 1966). The mutants lost detectable lecithinase and the capacity to utilize protocatechuate, quininate and possibly valine as sole carbon and energy sources.

The strains were assayed for their sensitivity to a number of antibiotics by the disc method. The wild strain was sensitive to kanamycin, tetracycline and streptomycin and resistant to cephalothin, erythromycin, ampicillin, lincomycin and penicillin G. The four mutant strains showed virtually identical sensitivities as compared to the wild strain

Table 1. Biochemical properties of wild and heat tolerant mutant strains of *Pseudomonas fluorescens*

Character <sup>a</sup>	Strain			
	<i>P. fluorescens</i>	<i>M 45</i>	<i>M 48</i>	<i>M 52</i>
Enzyme production				
Oxidase	+	+	+	+
Catalase	+	+	+	+
Lipase (tween 80)	+	+	+	+
Lecithinase	±	—	—	—
Utilization of				
Arginine <sup>b</sup>	+	+	+	+
Phenylalanine	+	+	+	+
Tryptophan	+	+	+	+
Leucine	+	+	+	
Proline	+	+	+	+
Serine	+	+	+	
Histidine	+	+	+	+
Isoleucine	+	+	+	
Alanine	+	+	+	
Valine	+	±	±	
Dextrose	+	+	+	
Lactate	+	+	+	
Benzoate	+	+	+	+
Protocatechuate	+	—	—	
Quinate	+	—	—	—
p-Hydroxybenzoate	+	+	+	
Glycerol	+	+	+	+

<sup>a</sup> Enzyme production was recorded as + (present), — (absent), or ± (weak). Each strain's ability to utilize the listed organic compounds as sources of carbon and energy for growth was determined by turbidometric measurements. + indicates significant growth; —, no growth; ±, slight or very slow growth.

<sup>b</sup> All amino acids tested were of the L configuration.

except for erythromycin and streptomycin. All four mutant strains were resistant to streptomycin and sensitive to erythromycin.

The thermal stability of purified ribosomes from the wild strain and strains *M 45*, *M 48* and *M 52* was determined by following the increase in absorbance at 260 nm upon raising the temperature. Ribosomes from all four organisms began to denature at 59°C to 60°C and displayed  $T_m$ 's (temperature at which 50% of the hyperchromicity occurs) of between 67.6°C (wild strain) and 69.2°C (*M 52*). Ribosomes from strains *M 45* and *M 48* had an intermediate  $T_m$  of 68.0°C. With a mean variation for duplicate determinations of ± 0.5°C the differences among the four strains were probably not significant.

The relative pathogenicity for mice of the wild and mutant strains was estimated by determining the  $LD_{50}$  dosage for each bacterial strain and by measuring the proportion of mice killed by  $1 \times 10^9$  cells of each

Table 2. Mouse pathogenicity of wild and mutant strains of *Pseudomonas fluorescens*

Strain	LD <sub>50</sub>	Proportion of mice killed by 1 × 10 <sup>9</sup> cells	P <sup>a</sup>
13525	1 × 10 <sup>9</sup>	7/14	—
M45	3 × 10 <sup>8</sup>	7/7	0.03
M48	6 × 10 <sup>8</sup>	6/6	0.045
M52	1 × 10 <sup>10</sup>	0/8	0.02

<sup>a</sup> Significance level of data shown in column 3. Each mutant strain was compared statistically to wild strain 13525.

strain (Table 2). Strains *M 45* and *M 48* were more virulent than the wild strain, while *M 52* was much lower. The differences between each mutant strain and the wild strain were statistically significant at the 0.05 level.

### Discussion

Of the four bacterial species tested, only *B. megaterium* and *P. fluorescens* demonstrated a readiness to spontaneously mutate to heat tolerance and only *P. fluorescens* showed a substantial increase in the maximum growth temperature. While *B. megaterium* increased its maximum temperature by about 5°C, the *P. fluorescens* mutants extended the upper limit of growth by 17 degrees, from 37°C to 54°C. Allen (1953) reported that a substantial number of the *Bacillus* strains which she tested were capable of growing at 55°C after mutation. The ability to undergo this type of mutation was apparently highly strain specific, and no thermophilic mutants were obtained from genera other than *Bacillus*, although a small number of other bacteria were tested. Our results also indicate a high selectivity for this characteristic, although only a small number of cultures were examined and we did not employ mutagens or extremely large inocula. Thus our results may only indicate that *P. fluorescens* 13525 undergoes mutation to thermophily at a greater rate than do some other organisms. The stable growth profiles shown in Fig. 1 plus the changes in antibiotic sensitivity and heat resistance indicate that the heat tolerance of strains *M 45*, *M 48* and *M 52* is due to genetic changes and is not simply physiological adaptation to heat, as was reported by Dowben and Weidenmüller (1968) in *B. subtilis*.

Since the wild and mutant strains had very different optimum growth temperatures, the wild strain was routinely grown at room temperature while the mutant strains were grown at 37°C. Thus the mutants' loss of detectable lecithinase and their inability to utilize protocatechuate, quinate, and possibly valine may be due to the inactivation of thermolabile enzymes or proteins associated with the transport or catabolism of these substrates during incubation at 37°C. We are investigating the possibility that biochemical differences between thermophilic mutants

and the parent strains may be useful for identifying thermolabile enzymes and pathways. Thus the pathway involved in the catabolism of protocatechuate and quinate may possess a thermolabile enzyme.

The four mutant strains possessed increased resistance to streptomycin and increased sensitivity to erythromycin as compared to the wild strain. Both of these antibiotics interfere with protein synthesis by binding to ribosomal subunits (Davies, 1964; Mao and Putterman, 1969). These observations suggested that the original mutation(s) enabling *P. fluorescens* to grow at 45°C may have produced some alteration of the ribosome. This is especially significant when related to the findings of McDonald and Matney (1963) which demonstrated close linkage between the genetic locus for the ability of *B. subtilis* to grow at 55°C and the streptomycin-resistance locus. Also, it has recently been reported that psychrophilic mutants of *P. aeruginosa* had altered ribosomal proteins (Kulpa and Olsen, 1971). Altenburg and Saunders (1971) demonstrated that the 50s ribosomal subunit plays the greater role in the thermostability of the ribosome, and this is also the subunit affected by erythromycin. Our results with purified ribosomes from the wild strain and three of the mutants revealed no major difference in thermostability (as evidenced by ultraviolet hyperchromic effects). In fact, the *P. fluorescens* ribosomes are very similar in thermostability to *E. coli* ribosomes, which begin to denature at 60°C and display a T<sub>m</sub> of 71°C (Zeikus *et al.*, 1970). We can speculate that the 50s subunit of the mutant ribosomes may have been altered so as to increase the mutant strains' protein synthesizing capacities in the critical temperature range (37–54°C) without significantly affecting the ultraviolet thermal denaturation profiles. However, a number of other mechanisms might also explain the basis for the thermal tolerance of the mutants.

A comparison of the virulence of the wild and mutant strains with the growth profiles in Fig. 1 suggests a correlation between each strain's capacity to grow at 37°C (the mean body temperature of mice is 37.2°C) and its virulence for mice. This suggests that the low maximum growth temperature for wild type *P. fluorescens* may be one reason for its low pathogenicity as compared to *P. aeruginosa*, which is more pathogenic for mice with an LD<sub>50</sub> between 10<sup>7</sup> and 3 × 10<sup>7</sup> cells (Liauw *et al.*, 1970), and can grow to temperatures of 44°C (Olsen and Metcalf, 1968).

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Dr. B. T. DeCicco  
 Department of Biology  
 The Catholic University of America  
 Washington, D.C. 20017, U.S.A.

TABLE 1

COMPARISON OF BIOLOGICAL CHARACTERISTICS OF S. aureus MUTANTS  
WITH WILD TYPE S. aureus and S. epidermidis

Character- istic	<u>S. aureus</u> ATCC 12600	Mannitol Mutant	DNase Mutant	Coagulase (-) Mutant	<u>S. epi.</u> ATCC 14990
Gram stain & morphology	typical	typical	typical	typical	typical
Glucose fer- mentation	+	+	+	+	+
Catalase pro- duction	+	+	+	+	+
Pigment	golden	golden	golden	golden	white
Mannitol fer- mentation	+	-	+	+	-
Soluble co- agulase	+	+	+	-	-
Bound co- agulase	+	+	+	-	-
DNase acti- vity	+	+	-	+	-
Hemolysin production	+	+	+	+	-



TABLE 2  
COMPARISON OF LD<sub>50</sub> IN MICE INOCULATED IP WITH  
S. aureus AND S. epidermidis

Organism	LD <sub>50</sub> (CFU)
<u>S. aureus</u> ATCC 12600	$6 \times 10^9$
<u>S. epidermidis</u> ATCC 14990	$3 \times 10^{10}$

TABLE 3  
IP VIRULENCE OF S. aureus, S. epidermidis, AND NTG-INDUCED S. aureus MUTANTS (approx.  $7 \times 10^9$  CFU)

Strain	Deaths/Number injected	p <sup>a</sup>
<u>S. epidermidis</u> ATCC 14990	0/9	$8 \times 10^{-6}$
<u>S. aureus</u> ATCC 12600	14/15	—
Mannitol nonfermenter	12/13	.52
Coagulase negative mutant	12/13	.52
DNase negative mutant	1/15	$1.5 \times 10^{-5}$

a. P = significance level of data compared to S. aureus 12600  
Exact test (1)

Hydrogenase and Ribulose Diphosphate Carboxylase  
in Autotrophic, Scotochromogenic Mycobacteria.

S.S. PARK\* and B.T. DECICCO. Catholic University of America, Washington, D.C.

Four strains of Mycobacterium gordonae plus a new isolate of a strongly acid fast, slow growing, scotochromogenic Mycobacterium were capable of chemolithotrophic growth in a mineral salts medium under an atmosphere of hydrogen, oxygen and carbon dioxide. A number of characterized mycobacterial strains of other species were unable to grow autotrophically. Utilization of hydrogen and carbon dioxide was observed manometrically and correlated with each strain's autotrophic capabilities. In autotrophic strains both hydrogen consumption and total gas consumption increased after preincubation under the autotrophic atmosphere. Levels of ribulose diphosphate carboxylase (RDPC) present in cell extracts from autotrophic cultures were 10 to 20 times higher than in extracts from the same strains after heterotrophic growth. Although the newly isolated strain seemed intermediate between M. gordonae and M. scrofulaceum in biochemical and physical characteristics, no tested strain of M. scrofulaceum grew autotrophically or possessed significant hydrogenase or RDPC activity after either heterotrophic or mixotrophic cultivation. Possible mechanisms interacting between chemolithotrophic and heterotrophic metabolism in the autotrophic mycobacteria will be discussed.

E&A  
G  
GP  
M  
MM  
P  
V

A  
 R  
 RT