Influence of Temperature on the Iron Metabolism of a Fluorescent Pseudomonad

J. A. GARIBALDI
Poultry Laboratory, Western Utilization R & D Division, Albany, California 94710

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The iron requirement for maximal cell yields of a fluorescent pseudomonad increases as the temperature of incubation is increased. On a succinate salts medium, maximal cell yields are attained at iron concentrations of 0.10 \( \mu g/ml \) of added iron at 20 C and at 3.0 \( \mu g/ml \) of added iron at 28 C. This bacterium does not grow in the basal medium at 31 C even in the presence of 0.01 to 10 \( \mu g/ml \) of added iron. The inability to grow at the higher temperature is due to the lack, by this organism, of its ability to biosynthesize hydroxamate iron transport compounds at temperatures of 28 C and above, since supplementation with such compounds produced by this organism at lower temperatures promoted growth at 31 C. The biosynthesis of these compounds at lower temperatures contributes to the efficient utilization of iron by the bacterium.

The concentration of the trace element, iron, is of the utmost importance in controlling longevity of the genus *Pseudomonas* (8). A fourfold difference in iron concentration, from 0.5 \( \times 10^{-4} \text{M} \) to 2.0 \( \times 10^{-4} \text{M} \), is sufficient to increase the survival of pseudomonas cells from less than 0.00001 to 100% at 4 weeks after the end of exponential growth at 37 C (8). These small changes in iron concentration were without effect at lower temperatures of incubation.

Data presented below suggest that iron transport hydroxamates produced by the fluorescent pseudomonads are probably involved in this phenomenon. The biosynthesis of these compounds, which occurs only at lower temperatures in this strain of the fluorescent pseudomonads, contributes to the efficient utilization of iron by the organism.

**MATERIALS AND METHODS**

**Organism.** An unidentified fluorescent pseudomonad (72-10) was isolated from chicken spoiled at 2 C.

**Growth conditions.** The cells were grown on a medium of the following composition: succinic acid, 10.0 g; \((NH_4)_2HPO_4, 8.0 \text{g; } K_2SO_4, 1.0 \text{g; } NaOH, 4.1 \text{g; } Mg(SO_4), 20.0 \text{mg; } Zn(ZnSO_4\cdot7H_2O), 5.0 \text{mg; } Mn(MnCl_2\cdot4H_2O), 1.0 \text{mg; } Cu(CuSO_4\cdot5H_2O), 0.5 \text{mg; } \) water to 1.0 liter. The pH of this basal medium is 5.9. The medium was dispensed at double strength in 250-ml De Long culture flasks, supplemented as desired, adjusted to a volume of 40 ml with demineralized water, capped with plastic "Kap-uts," and sterilized at 121 C for 15 min. After inoculation, the cultures were incubated on rotary shakers at either 20, 28, or 31 C. Iron was added as Fe(SO_4\cdot7H_2O).

**Inoculum.** The inoculum was grown on the unsupplemented medium at 28 C for the first experiment and at 20 C for the second experiment. The former was used without treatment. The latter was centrifuged, the cell pellet was washed twice with sterile demineralized water to remove any iron transport compound synthesized by the bacterium, and resuspended in sterile demineralized water before use.

**Growth yields.** Responses to various supplements were determined by weighing after drying at 105 C for 24 hr the twice-washed, centrifuged cell pellet from 40 ml of culture medium.

**Bound hydroxamines.** The biosynthesis and excretion of bound hydroxylamine by the bacterium into the cell-free supernatants was described by Csaky (2).

**Iron transport compounds.** Iron transport compounds were prepared from cell-free supernatant fluid from cultures grown on the basal medium at 20 C by adsorption onto Dowex 50 (Na form), followed by elution with dilute NH_4OH. These preparations form highly colored stable chelate compounds with ferric ion.

**RESULTS**

The data in Table 1 show the influence of temperature on the iron requirement and on the excretion of bound hydroxylamine by pseudomonas isolate 72-10. The iron requirement for maximal cell yield is much less at 20 C than at 28 C. Maximal cell yield at 20 C occurs at an iron concentration of 0.10 \( \mu g/ml \), whereas at 28 C, an iron requirement of at least 3.0 \( \mu g/ml \) is necessary to attain maximal cell yields. Thirty times as much iron is therefore necessary to achieve maximum cell yields at 28 C as at 20 C.

The biosynthesis of fluorescent iron transport hydroxamates is also influenced by temperature. There is no detectable excretion of these com-
pounds when the organism is grown at 28 C. However, when the organism is cultured at 20 C, copious quantities of these important compounds are excreted. Even at levels of iron of 3.0 \( \mu g/ml \), which is a 30-fold excess of that necessary to give maximal cell yields, the bacterium still synthesizes and excretes detectable amounts of bound hydroxylamine. As with the biosynthesis of iron transport compounds by other microorganisms, iron in excess represses the excretion of these compounds (5). Iron concentrations which are nutritionally limiting favor the excretion of greater amounts of these compounds.

Since the fluorescent hydroxamate iron transport compounds are not formed by this bacterium at 28 C, the influence of these important growth factors on the growth and iron requirement of this organism at 20, 28, and 31 C was determined (Table 2). Maximal cell yield at 20 C occurred at an iron concentration of 0.03 \( \mu g/ml \). A somewhat different pattern occurred at 28 and 31 C. At 28 C, there was little or no growth below an iron concentration of 0.30 \( \mu g/ml \), but growth reached a maximum at 3.0 \( \mu g/ml \). At 31 C the organism did not grow, even at iron concentrations of 10.0 \( \mu g/ml \).

In the presence of microgram quantities (3.0 \( \mu g/ml \)) of a preparation of iron transport compounds synthesized by this same organism at 20 C, a different response occurs. The pattern at 20 C is quite similar to that in unsupplemented medium, although the maximal cell yield may be somewhat lower. At 28 C an immediate response is seen, even when no extra iron is added to the basal medium. The maximal cell yield under these nutritional and environmental conditions is reached at 0.10 \( \mu g/ml \) of added iron. At 31 C, where no significant growth occurred in the basal medium even when supplemented with 10.0 \( \mu g/ml \) of iron per ml, the iron transport compounds stimulated the bacterium to grow and reach maximum cell yields at an iron level of 3.0 \( \mu g/ml \). At 31 C under these nutritional conditions, this organism has a strict requirement for an iron transport compound.

### DISCUSSION

The biosynthetic capability of the unidentified fluorescent pseudomonads 72-10 at temperatures of 20 C and below enable it to meet its iron requirement at levels of iron 30-fold less than that at temperatures 8 degrees higher. The ability to produce iron transport compounds must certainly be a factor in the efficient utilization of this important trace nutrient. The functioning of the various iron-containing enzymes in highly aerobic organisms is well recognized. The longevity of pseudomonas may well depend on the full complement of such iron enzymes.

This organism excretes compounds containing bound hydroxylamine, even in the presence of iron, in excess of that necessary to reach maximal cell yield. The organism, therefore, probably has a need for iron in excess of that necessary for cell production. This need may result in an iron storage compound and contribute to the bacterium’s survival in times of iron restriction.

Iron transport compounds produced at lower temperatures are essential growth factors for this organism at higher temperatures. As with other microorganisms which biosynthesize and excrete these iron-binding compounds (6), the pseudomonads also produce them in multiple forms. Since some forms may be growth factors, others may be inactive, and still others may be bacteriostatic, it is not surprising that the response in the presence of added iron transport compound(s) at 28 C and 31 C does not reflect exactly the growth

### TABLE 1. Influence of temperature on the iron requirement and on the production of bound hydroxylamine by isolate 72-10

<table>
<thead>
<tr>
<th>Supplement to basal medium(^a)</th>
<th>20 C</th>
<th>28 C</th>
<th>31 C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell yield(^b)</td>
<td>Bound NH(_2)OH(^c)</td>
<td>Cell yield(^b)</td>
</tr>
<tr>
<td>None</td>
<td>37</td>
<td>7.6</td>
<td>7</td>
</tr>
<tr>
<td>0.03</td>
<td>84</td>
<td>12.3</td>
<td>30</td>
</tr>
<tr>
<td>0.30</td>
<td>92</td>
<td>5.2</td>
<td>73</td>
</tr>
</tbody>
</table>

\(^a\) Milligrams of iron per milliliter.

\(^b\) Milligrams of dry cells per 40 ml of culture.

\(^c\) Micrograms of NH\(_2\)OH per milliliter of cell-free supernatant.

### TABLE 2. Influence of temperature and hydroxamate iron transport compound(s) on the growth and iron requirement of pseudomonads isolate 72-10

<table>
<thead>
<tr>
<th>Supplement to basal medium(^a)</th>
<th>20 C</th>
<th>28 C</th>
<th>31 C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell yield (mg of dry cells/40 ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No ITC(^d)</td>
<td>+ITC</td>
<td>No ITC(^d)</td>
</tr>
<tr>
<td>None</td>
<td>54</td>
<td>69</td>
<td>2</td>
</tr>
<tr>
<td>0.01</td>
<td>60</td>
<td>74</td>
<td>2</td>
</tr>
<tr>
<td>0.03</td>
<td>90</td>
<td>76</td>
<td>2</td>
</tr>
<tr>
<td>0.10</td>
<td>90</td>
<td>80</td>
<td>2</td>
</tr>
<tr>
<td>0.30</td>
<td>87</td>
<td>78</td>
<td>12</td>
</tr>
<tr>
<td>1.00</td>
<td>85</td>
<td>77</td>
<td>69</td>
</tr>
<tr>
<td>3.00</td>
<td>84</td>
<td>77</td>
<td>74</td>
</tr>
<tr>
<td>10.00</td>
<td>85</td>
<td>87</td>
<td>69</td>
</tr>
</tbody>
</table>

\(^d\) Micrograms of iron per milliliter of medium.

\(^e\) Hydroxamate iron transport compounds (ITC) at 3.0 \( \mu g/ml \).
at 20 C in unsupplemented medium. The forms which are either innocuous or bacteriostatic may bind the available iron more firmly than the form which has growth factor activity. It follows, therefore, that a growth response will occur only with the iron that is bound by the active form.

The results of Chan and co-workers on the growth of Arthrobacter citreus at elevated temperatures (1, 7, 9) should be considered in light of the data presented above. The promotion of growth of A. citreus at elevated temperatures when grown associated with either a pseudomonad, A. niger, S. cerevisiae, or Streptomyces scabies (1) probably reflects the ability of these associated organisms to biosynthesize and excrete iron transport compounds into the surrounding environment. Work at this laboratory has shown that fluorescent pseudomonads, as a group, have this biosynthetic capability. A. niger is known to excrete a compound identical to ferrichrome (J. A. Garibaldi, Ph.D. Thesis, University of California, Berkeley, 1958), and the biosynthetic ability of the genus Streptomyces to synthesize iron transport compounds is well recognized (4).

In addition, our strain of Pseudomonas, like A. citreus, responds to a chelating agent at higher temperatures. It would be of interest to determine whether A. citreus, like other species of this genus (3), biosynthesizes iron transport compounds and, if so, whether our strain of pseudomonas it is also temperature dependent.

LITERATURE CITED