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Influence of Temperature on the Biosynthesis of Iron Transport Compounds by *Salmonella typhimurium*

J. A. GARIBALDI

Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710

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The biosynthesis of phenolate iron transport compounds by *Salmonella typhimurium* Tm-1 is temperature-sensitive. As the temperature of incubation is raised from 31.0 to 36.9 C, the organism excretes less iron transport compound into the medium. The organism in unable to grow at 40.3 C on a 1% succinate-salts medium unless supplemented with such iron transport compounds. The iron requirement for maximum cell yields on this medium is 0.10 μg/ml. The biosynthesis of phenolate iron transport compounds is suppressed at iron concentrations greater than 3.0 μg/ml.

The importance of iron transport compounds in the metabolism of salmonellae has been emphasized by the report describing the crystallization and function of enterobactin (8), by the discovery of 2,3-dihydroxybenzoyl serine and its role in the growth of *Salmonella typhimurium* in blood sera (11), and by the role played by these phenolate compounds in the spoilage of shell eggs by this pathogenic bacterium (3). If the production or presence of this type of compound is a requisite for the organism to establish itself in the host, it is of utmost importance to determine the environmental conditions which either promote or suppress its biosynthesis. The reports above (8, 11) establish, as is true with other iron transport compounds (5, 9), that accumulation of the chelator occurs only under nutritional conditions which are limiting with respect to iron. It is evident, however, that this is the case in most, if not all biological systems, where iron exists principally as neither free ferrous ion nor free ferric ion. In the human it is usually bound in some organic molecule such as heme or transferrin or is present as an insoluble complex of ferritin and as such is probably unavailable to the bacterium. Microorganisms which require iron for growth must therefore be able to compete with these forms for iron before they can establish themselves in the host.

We have recently shown that the biosynthesis of iron transport compounds by a fluorescent pseudomonad is temperature-sensitive (4). Although the organism grew at both 20 and 28 C under the nutritional conditions of our experiments, it synthesized the iron transport compounds only at the lower temperature and did not grow at 31 C unless the medium was supplemented with siderochromes synthesized by this same organism at lower temperatures (20 C).

In the present study, we report that the biosynthesis of enterobactin by *S. typhimurium* Tm-1 is similarly temperature-sensitive, with little or no excretion occurring at a temperature of 40 C or above.

MATERIALS AND METHODS

Bacteria. *S. typhimurium* Tm-1 is from our own culture collection.

Medium. The bacteria were cultured on a medium of the following composition: succinic acid, 10.0 g; $(NH_4)_2HPO_4$, 8.0 g; K_2SO_4, 1.0 g; NaOH, 4.1 g; Mg ($MgSO_4 \cdot 7H_2O$), 100 mg; Zn ($ZnSO_4 \cdot 7H_2O$), 1.0 mg; Mn ($MnCl_2 \cdot 4H_2O$), 5.0 mg; Cu ($CuSO_4 \cdot 5H_2O$), 0.5 mg; water to 1.0 liter. The *p*H of this basal medium was 5.9. The medium was dispensed at 2× strength in 250 ml. DeLong culture flasks, supplemented as desired, were adjusted to volume 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 31.0, 34.7, 36.9, or 40.3 C.

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 a noted volume of culture medium.

Supplements. (i) Iron was added as a solution of $FeSO_4 \cdot 7H_2O$. (ii) Salmonella iron transport compounds, phenolates (parent compound 2,3-dihydroxybenzoyl^N-serine), were prepared by ether extraction of acidified cell-free supernatant fluids (8) of *S. typhimurium* Tm-1 grown on the above medium which is limiting with respect to iron.

Phenolates excreted. The biosynthesis and excretion of phenolate-type compounds by the bacteria into the cell-free supernatant fluid was determined by the procedure of Arnow (1). 2,3-Dihydroxybenzoic acid was used as a standard.

RESULTS

The effect of iron concentration on the cell yields of *S. typhimurium* Tm-1 at various temperatures in the presence and absence of phenolate iron transport compounds is given in Table 1. At the lower temperatures, namely 31.0, 34.7, and 36.9 C, the organism reached maximum cell yields at an iron concentration of approximately 0.10 μg/ml irrespective of whether the medium had been supplemented with phenolate iron transport compounds. At the highest temperature, 40.3 C, significant growth occurred only at iron concentrations of 3.0 μg/ml or greater in the absence of added phenolate compounds. With phenolate supplementation, however, growth at 40.3 C was the same as that at the lower temperatures at all iron concentration. A similar response occurred when the basal medium was supplemented with ferrichrome, the iron transport compound produced by *Ustilago sphaerogena*.

The effect of iron concentration and temperature on the biosynthesis and excretion of phenolate iron transport compound by *S. typhimurium* Tm-1 is shown in Table 2. As has been shown previously, excretion of phenolate iron transport compounds is greatest at intermediate iron concentrations. The biosynthesis of these compounds under the nutritional conditions of our experiments is repressed by concentrations of iron in excess of 3.0 μg/ml.

Although growth yields at 31.0, 34.7, and 36.9 C were comparable (Table 1), the excretion of the chelator was reduced significantly as the temperature of incubation was increased. At 36.9 C the amount of phenolate excreted was only 40% of that excreted by 31.0 C. At 40.3 C there was no detectable excretion of the phenolate, and therefore growth of the organism was not significant at iron concentrations below 3.0 μg/ml.

S. typhimurium Tm-1, like our fluorescent isolate 72-10, loses its biosynthetic ability to produce iron transport compounds and is therefore unable to grow under these nutritional conditions at higher temperatures.

DISCUSSION

As has been previously shown for the hydroxamate iron transport compound of a fluorescent pseudomonad (4), the biosynthesis of the phenolate iron transport compound, enterobactin, is temperature-sensitive. The critical temperature for the salmonella is, as would be expected, above that of the psychrophilic pseudomonad (40 C versus 28 C). Because this critical temperature is within the range of most physiological systems, it is of interest to speculate on the importance of this observation as it may relate to the control of pathogenic microorganisms in the various hosts they may invade.

In a discussion on the value of fever, Best and Taylor (2) state that although fever is usually a herald of serious disease it should not be looked upon as a reaction detrimental in itself. On the contrary, they indicate that its occurrence is an important aid to the body in

TABLE 1. *Influence of temperature and phenolate iron transport compounds (ITC) on the growth and iron requirement of Salmonella typhimurium Tm-1*

Supplement to basal medium (μg of Fe/ml)	Cell yield (mg of dry cells/40 ml)							
	31.0 C		34.7 C		36.9 C		40.3 C	
	No ITC	+ITC[a]	No ITC	+ITC	No ITC	+ITC	No ITC	+ITC
None	6	33	6	24	3	27	0	14
0.01	38	80	49	91	33	63	0	43
0.03	88	120	116	122	87	112	0	90
0.10	117	127	132	130	101	113	14	103
0.30	116	124	105	126	97	114	6	110
1.00	121	126	126	123	112	96	9	113
3.00	129	135	127	128	125	115	95	124
10.00	128	134	129	124	122	134	66	116

[a] Phenolate ITC added equivalent to 4.0 μg of 2,3-dihydroxybenzoic acid/ml.

TABLE 2. *Influence of temperature and iron concentration on the production of phenolate iron transport compounds (ITC) by Salmonella typhimurium Tm-1*

Supplement to basal medium (μg of Fe/ml)	Phenolate iron transport compounds[a]							
	31.0 C		34.7 C		36.9 C		40.3 C	
	No ITC	ITC[b]	No ITC	ITC	No ITC	ITC	No ITC	ITC
None	<0.005	0.07	<0.005	0.06	<0.005	0.02	<0.005	<0.005
0.01	0.10	0.15	0.09	0.06	0.06	0.04	<0.005	0.025
0.03	0.20	0.17	0.30	0.17	0.18	0.16	<0.005	0.025
0.10	0.34	0.22	0.20	0.32	0.20	0.10	<0.005	0.06
0.30	0.52	0.17	0.34	0.17	0.20	0.05	<0.005	0.03
1.00	0.50	0.26	0.21	0.13	0.12	<0.005	<0.005	<0.005
3.00	0.31	0.10	0.06	0.03	0.03	<0.005	0.09	<0.005
10.00	0.04	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005

[a] Expressed as microequivalents of 2,3-dihydroxybenzoic acid per milliliter of cell-free supernatant fluid.
[b] ITC added equivalent to 4.0 μg of 2,3-dihydroxybenzoic acid/ml.

its combat with the disease. They also state that the role fever plays in the defensive process is unknown. Because the concentration of readily available iron in the human is low, any invading microorganisms requiring iron must have a mechanism to compete for the various forms of chelated iron present in the host. Salmonella has such a mechanism but, as we have indicated above, the mechanism is sensitive at temperatures only slightly above normal body temperature. For this reason we suggest that one role that fever may play in the defensive process which aids the body in its combat of disease is to raise the temperature to the critical point which prevents the biosynthesis of iron transport compounds which may be a mandatory requirement if the invading microorganism is to establish itself in the host. Since this blockage would restrict the growth of the pathogen severely, the other defensive mechanisms of the body could more readily eliminate the disease.

This sensitivity to temperature may also contribute to the inability of many strains of salmonella to establish themselves in chickens and turkeys. It is well documented that the average body temperatures of birds range from approximately 105 to 111 F (10) depending on the species. This range is above the critical temperature for the biosynthesis of enterobactin by *S. typhimurium* Tm-1. Preliminary results in this laboratory suggest that such may be the case for other strains of salmonella tested. It follows that strains whose critical temperature is below the normal body temperature of the bird would be unable to establish themselves in the host.

An additional observation on the greater susceptibility of young poults to infection by microorganisms again (7) may be related to the fact that the average body temperature of birds less than 10 days old is 2 to 3 F less than that of the adult bird (6).

Although the sensitivity of the biosynthesis of iron transport compounds to temperature has been shown for only *Salmonella* and our *Pseudomonas* isolate 72-10, it may be common to all other iron transport-synthesizing systems. It would be interesting to determine whether such is the case for the production of the mycobactins which recent data have suggested to have survival value in the iron-deficient environment which the organism encounters in serum and tissues of the host animal (I. Kochan and D. L. Cahall, Bacteriol. Proc., p. 87, 1971).

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