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Acquisition of Iron by *Aeromonas salmonicida*

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The ability of six typical and three atypical strains of *Aeromonas salmonicida* to sequester Fe^{3+} from the high-affinity iron chelators ethylenediaminedihydroxyphenylacetic acid, lactoferrin, and transferrin was determined. Typical strains were readily able to sequester Fe^{3+} and used two different mechanisms. One mechanism was inducible and appeared to involve production of a low-molecular-weight soluble siderophore(s). Iron uptake by this mechanism was strongly inhibited by ferricyanide. One virulent strain displayed a second mechanism which was constitutive and required cell contact with Fe^{3+}-lactoferrin or -transferrin. This strain did not produce a soluble siderophore(s) but could utilize the siderophore(s) produced by the other strain. Fe^{3+} uptake by this stripping mechanism was strongly inhibited by dinitrophenol. Atypical strains displayed a markedly reduced ability to sequester iron from high-affinity chelators, although one of them was able to utilize the siderophores produced by the typical strain. In all strains examined, Fe^{3+} limitation resulted in the increased synthesis of several high-molecular-weight outer membrane proteins.

Aeromonas salmonicida is an important pathogen, causing the systemic disease furunculosis in fish (11). In acute forms of the disease, the organism grows rapidly in the major body organs, producing a terminal septicemia which is often accompanied by severe tissue necrosis. Although best recognized as producing disease in the salmonidae, the organism is also able to infect a wide range of other fish species (11). In recent years, a variety of atypical strains of *A. salmonicida* have been isolated, especially from carp species. These strains differ from typical *A. salmonicida* strains in several biochemical characteristics (30) and by their poor growth on conventional laboratory media.

The primary virulence factor of this fish pathogen is a surface protein array with tetragonal symmetry (A-layer) (14, 17). This protein array appears to have an important role in protecting the organism from the host defenses (17). However, in addition to withstanding host defense mechanisms, successful systemic pathogens such as *A. salmonicida* must obtain essential growth factors. Bacteria have an absolute requirement for iron (18, 19, 29). In host animals, iron is tightly bound by glycoproteins such as transferrin and lactoferrin which have a very high affinity for ferric iron (5, 32). Consequently, the free iron levels are well below the limits required for bacterial growth (3, 5). So, to survive and grow in the host, pathogens such as *Escherichia coli, Neisseria meningitidis*, and *Vibrio anguillarum* have evolved highly specific mechanisms which allow them to obtain Fe^{3+} from host iron-binding proteins (2, 5, 6).

Because of its ability to grow rapidly in fish and produce disease, *A. salmonicida* clearly should have highly efficient mechanisms for iron acquisition. We therefore investigated the ability of *A. salmonicida* to obtain iron from various chelating agents with high association constants for Fe^{3+}.

MATERIALS AND METHODS

Bacteria. Strains of *A. salmonicida* used in this study are listed in Table 1. *V. anguillarum* strains 775 and 775-E39 were provided by M. H. Schiewe (Northwest Fisheries Center, National Marine Fisheries Service, Seattle, Wash.). Strain 775 was virulent in salmonid fishes and carried the 47-megadalton plasmid pJM1, coding for a high-affinity iron-sequestering mechanism (6). Strain 775-E39 was an ethidium bromide-cured plasmidless derivative of 775(pJM11), pJM11 being a transposition derivative of plasmid pJM1 (7). This strain was avirulent and unable to grow under conditions of iron limitation (6). *Salmonella typhimurium* strain LT2 was obtained from K. D. Sanderson (Department of Biology, University of Calgary, Alberta, Canada).

Media and culture conditions. Strains were maintained at −80°C in tryptic soy broth (GIBCO Diagnostics, Madison, Wis.) containing 15% (vol/vol) glycerol as the cryoprotectant. Bacteria were cultured on tryptone soy agar (GIBCO) or in tryptone soy broth (100 ml in 500-ml conical flasks) with agitation (orbital shaker at 150 rpm; New Brunswick Scientific Co., New Brunswick, N.J.). *A. salmonicida* and *V. anguillarum* were cultured at 20 and 25°C, respectively,

TABLE 1. Strains of *Aeromonas salmonicida*

Strain	Source[a]
Typical	
A438	T. P. T. Evelyn (strain 76-30)
A449	C. Michel (strain TG36/75)
A449-3	Attenuated derivative of A449 (17)
A450	C. Michel (strain TG72/78)
A450-3R	Attenuated derivative of A450 (17)
A470	D. Rockey (strain AsSC14)
Atypical	
A400	This laboratory (30)
A406	This laboratory (30)
A419	E. B. Shotts, Jr. (30)

[a] T. P. T. Evelyn, Pacific Biological Station, Nanaimo, B.C., Canada; C. Michel, Laboratoire d'Ichtyopathologie, Thiverval-Grignon, France; D. Rockey, University of Wyoming, Laramie, Wyo.; E. B. Shotts, Jr., University of Georgia, Athens, Ga.

whereas *S. typhimurium* was cultured at 37°C. For anaerobic culture, strains were incubated in a carbon dioxide-hydrogen atomosphere (GasPak; BBL Microbiology Systems, Cockeysville, Md.).

Iron chelation. Iron (Fe^{3+})-limited culture conditions were provided by the addition of 0.28 mM ethylenediaminedihydroxyphenylacetic acid (EDDA), lactoferrin (1 mg/ml), or transferrin (5 mg/ml). Fe^{3+} limitation was determined by culturing *V. anguillarum* strain 775-E39 in concentration gradients of chelator in tryptone soy broth; minimum inhibitory concentrations were used for further study. In addition, a similar protocol was employed to determine the amount of $FeCl_3$ required to saturate chelator Fe^{3+}-binding capacity (0.54 mM). Before experimentation, strains were subcultured three times across media containing chelator.

Iron chelators used in this study were obtained from Sigma Chemical Co. (St. Louis, Mo.). Contaminating Fe^{3+} was removed from EDDA by the method of Rogers (25) and from glycoproteins by the method of Aisen et al. (1).

Uptake of $^{59}Fe^{3+}$. $^{59}FeCl_3$ (833 μCi/mol) was obtained from Amersham Corp., Oakville, Ontario, Canada. Kinetics of $^{59}Fe^{3+}$ uptake were monitored by providing mid-exponential-phase cultures with $^{59}Fe^{3+}$ bound to EDDA or to lactoferrin. Cellular $^{59}Fe^{3+}$ was determined by applying 20-μl volumes of culture medium to nitrocellulose filters (0.45-μm pore size; Millipore Corp., Bedford, Mass.) and washing with 5 ml of sterile tryptone soy broth-EDDA medium. The cell-associated $^{59}Fe^{3+}$ on these filters was quantitated in a gamma counter (model 8000; Beckman Instruments, Inc., Fullerton, Calif.). Values obtained by applying $^{59}Fe^{3+}$ solutions alone to Millipore filters were subtracted from test data.

In some experiments, the effects of metabolic inhibitors were tested on cells grown aerobically or anaerobically in tryptone soy broth-EDDA to the exponential phase. After 30 min, $^{59}Fe^{3+}$ bound to EDDA was added, and after a further 30 min, cells were collected and cellular radioactivity was determined. The inhibitors tested were iodoacetamide (Sigma Chemical Co., St. Louis, Mo.), *p*-chloromercuribenzoic acid (*p*CMB, Sigma), 2,4-dinitrophenol (DNP, Sigma), $K_3Fe(CN)_6$, NaN_3, NaCN, NaF, $NaAsO_2$, and *N,N'*-dichlorohexylcarbodiimide (DDCD) (Aldrich Chemical Co., Milwaukee, Wis.).

Binding of $^{59}Fe^{3+}$ to lactoferrin. Fe^{3+} was removed from lactoferrin by the method of Aisen et al. (1) before reacting it with $^{59}FeCl_3$ (2 μCi/mg of lactoferrin) at 4°C (2 h). $^{59}Fe^{3+}$-lactoferrin complexes were dialyzed against distilled water (4°C) until extraneous activity approached background radioactivity. The inherent activity of $^{59}Fe^{3+}$-lactoferrin was found to be 8×10^4 cpm/mg.

Uptake of $^{59}Fe^{3+}$ across a dialysis membrane. Ability of strains to obtain across a dialysis membrane iron bound to lactoferrin was examined by placing cultures (10^9 bacteria in 1 ml) opposite a dialysis membrane (14,000-molecular-weight exclusion limit) separating them from lactoferrin (1 mg/ml, in tryptone soy broth) saturated with $^{59}Fe^{3+}$. Samples (200 μl) were taken after 3.0 h of incubation.

Siderophore detection. Hydroxamate synthesis was examined by the method of Csáky (8) with and without the modification of Gibson and Magrath (10) and Holzberg and Artis (12); culture supernatants, concentrated (×10) by freeze-drying, were analyzed with hydroxylamine hydrochloride (Sigma) as a standard (10).

Possible catechol production was assessed in ethyl acetate extracts of culture supernatants (21, 22) by the colorimetric method of Arnow (4) with dihydroxybenzene (Sigma) as a standard.

Cross-feeding. Cells of *A. salmonicida* or *S. typhimurium* were grown in tryptone soy broth-EDDA for 30 h and removed by centrifugation (4,000 × *g*, 30 min). A 1-ml amount each of DNase (Sigma) and RNase (Sigma) was added per ml, and the supernatant fluid was sterilized by filtration through a 0.25-μm filter (Millipore). A 1-ml volume was added to 5 ml of tryptone soy broth-EDDA, which was then inoculated with atypical strain A406. Growth was assessed by determination of culture absorbance at 590 nm.

Isolation of outer membranes. Cells were harvested from agar plates or broth cultures, washed in 20 mM Tris-hydrochloride (Sigma) (pH 7.2), and resuspended in this buffer containing 10 mM EDTA (Sigma) and 0.1 μg each of DNase and RNase per ml. After disruption of cells by three passages through a precooled French pressure cell (16,000 lb/in^2), unbroken cells were removed by centrifugation at 4,000 × *g* for 30 min. The total cell membrane (envelope) fraction was then collected by centrifugation at 40,000 × *g* at 4°C for 30 min. Cytoplasmic membranes were selectively solubilized with sodium lauryl sarcosinate (W. R. Grace and Co., Organic Chemicals Division, Nashua, N.H.) by the method of Filip et al. (9), and the remaining outer membrane fraction was sedimented by centrifugation at 40,000 × *g* for 1 h. The outer membrane was washed in 20 mM Tris-hydrochloride (pH 7.2) and stored at −20°C until required.

Outer membrane samples were prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and proteins were separated according to the method of Laemmli (16). Proteins solubilized in sample buffer (62.5 mM Tris-hydrochloride [pH 6.8/5%], [vol/vol] 2-mercaptoethanol, 3% [wt/vol] sodium dodecyl sulfate) were stacked in 4.5% acrylamide (10 mA) and separat-

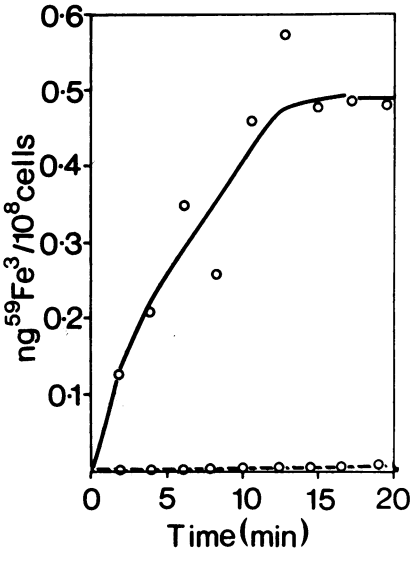

FIG. 1. $^{59}Fe^{3+}$ uptake from $^{59}Fe^{3+}$-EDDA by cells of *A. salmonicida* A450. Cells were grown to exponential phase (10^8 cells per ml) in 10 ml of broth plus 0.28 mM EDDA (- - - -) or in broth (———), 1 μM $^{59}Fe^{3+}$ was added to the growth medium, and cell-associated radioactivity was determined.

ed with 12.5% acrylamide (20 mA). Proteins were visualized by staining with Coomassie blue (31).

RESULTS

Growth under Fe^{3+} limitation. The ability of typical and atypical strains of *A. salmonicida* to grow under conditions of Fe^{3+} limitation was tested on media containing the high-affinity Fe^{3+} chelators EDDA, lactoferrin, and transferrin. The chelators produced Fe^{3+} limitation in the medium as demonstrated by the inability of *V. anguillarum* 775 E-39 to grow in the absence of added Fe^{3+}. All typical strains of *A. salmonicida* tested grew well in the presence of each of the Fe^{3+} chelators. In contrast, the growth of the normally slower growing atypical strains tested was perceptibly reduced under conditions of Fe^{3+} limitation, as imposed by EDDA. In the case of atypical strain 406, growth was totally inhibited by EDDA. The addition of $FeCl_3$ to the medium alleviated growth inhibition in all cases.

Uptake of $^{59}Fe^{3+}$. The ability of *A. salmonicida* cells to take up $^{59}Fe^{3+}$ was demonstrated by using mid-exponential-phase cells of strain A450 growing in tryptone soy broth-EDDA (Fig. 1). When these cells were provided with excess $^{59}Fe^{3+}$ (1 μM) bound to EDDA, the cells immediately began to take up the radiolabeled Fe^{3+}, and this uptake continued linearly for 10 min, at which time cells became saturated with iron. In a parallel experiment, total cell membranes (envelopes) and outer membrane fractions were isolated from cells collected at the various time intervals, and the membrane-associated $^{59}Fe^{3+}$ was determined. A linear increase in membrane-associated $^{59}Fe^{3+}$ was seen during the first 10 min of cell exposure to $^{59}Fe^{3+}$ (data not shown). By 10 min, 32 ng of $^{59}Fe^{3+}$ per mg of protein was envelope associated, of which only 14% was associated with the outer membrane. Exponential-phase cells growing in broth without EDDA and membrane fractions isolated from these cells failed to accumulate $^{59}Fe^{3+}$ over the same time period.

Inducibility of Fe^{3+} uptake systems. Cells were grown to exponential phase under Fe^{3+} limitation in tryptone soy broth-EDDA or Fe^{3+}-sufficient broth, harvested by centrifugation, and suspended in broth containing $^{59}Fe^{3+}$ bound to EDDA (Fig. 2). Strain A449 showed immediate uptake of $^{59}Fe^{3+}$, regardless of the previous culture conditions. In contrast, cells of strain A450 which had been grown in the Fe^{3+}-sufficient medium were unable to immediately take up $^{59}Fe^{3+}$. These noninduced cells experienced a 90-min lag before taking up substantial $^{59}Fe^{3+}$.

Siderophore production. The ability to produce a low-molecular-weight soluble siderophore(s) was determined by measuring $^{59}Fe^{3+}$ uptake from $^{59}Fe^{3+}$-lactoferrin across a dialysis membrane. When a broth-EDDA culture of strain A450 was separated from the lactoferrin-bound $^{59}Fe^{3+}$, a preformed soluble siderophore(s) in the culture medium allowed cells of A450 to acquire $^{59}Fe^{3+}$ (Table 2). Predictably, a broth culture of strain A450 was unable to acquire $^{59}Fe^{3+}$ because of the absence of a preformed siderophore(s) in the culture medium. Cells of A449 were unable to obtain $^{59}Fe^{3+}$ from lacto-

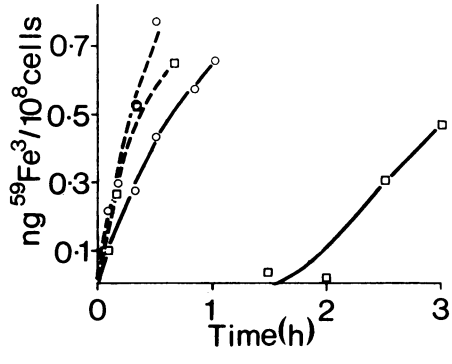

FIG. 2. Inducibility of iron uptake mechanisms of *A. salmonicida*. Cells were grown to mid-exponential phase (10^8 cells per ml) in 10 ml of broth (□) or broth plus 0.28 mM EDDA (○), harvested by centrifugation, and resuspended in 10 ml of broth plus 0.28 mM EDDA with 1 μM bound Fe^{3+}. Cell-associated radioactivities of strain A449 (- - - -) and strain A450 (———) were determined.

TABLE 2. Uptake of $^{59}Fe^{3+}$ from $^{59}Fe^{3+}$-lactoferrin across a dialysis membrane[a]

Strain	Culture condition, broth with:	Cell-associated radioactivity (total cpm/10^9 cells)
A450	0.28 mM EDDA	697 ± 49[b]
	No EDDA	10 ± 14
A449	0.28 mM EDDA	18 ± 16
	No EDDA	6 ± 7
	EDDA culture supernatant from A450	751 ± 70

[a] Exclusion limit, 14,000 molecular weight.
[b] Arithmetic mean of eight determinations ± standard deviation of the mean.

ferrin-bound $^{59}Fe^{3+}$ across a dialysis membrane regardless of whether they are growing in the presence or absence of EDDA; however, this strain was able to use the siderophore(s) produced by strain A450 to obtain glycoprotein-bound Fe^{3+} across a dialysis membrane (Table 2).

Procedures used to detect phenolate siderophores in *E. coli* (20, 21) were then applied to EDDA-culture supernatants of strains A449, A450, and four other typical strains. All failed to give the positive Arnow reactions (4) obtained with control supernatants of *S. typhimurium* LT2. Furthermore, the Csáky assay (9) and various modifications (10, 12) failed to detect a siderophore of the hydroxamate class.

The ability of the siderophore(s) produced by strain A450 to cross-feed atypical *A. salmonicida* strain A406 was also tested. This strain displayed no growth in medium containing low Fe^{3+}, but when it was provided with excess Fe^{3+} or broth-EDDA culture supernatant from strain A450 (30-h culture), normal growth was displayed (Fig. 3). Other experiments showed that the extent of A406 growth was directly related to the amount of A450 supernatant added and that broth-EDDA culture supernatants from strains A438 and A470 also cross-fed atypical strain A406 (data not shown). Growth of A406 in the presence of EDDA was promoted to a lesser extent by cell-free supernatants from broth-EDDA cultures of *S. typhimurium* LT2 (Fig. 3). Culture supernatants from EDDA-grown A449 failed to facilitate growth of A406, as did culture supernatants from strains A450, A438, and A470 cultured in plain broth (data not shown). In these experiments, DNase and RNase were added to the various supernatants to minimize the possibility that genetic transfer, such as of plasmid DNA, might be involved in subsequent growth of A406.

Inhibition of Fe^{3+} uptake. Differences in the mechanisms of aerobic uptake by strains A449 and A450 were shown with the metabolic inhibitors 2,4-DNP and potassium ferricyanide (Table 3). The iron uptake mechanism of strain A449 was sensitive to 2,4-DNP, whereas that of strain A450 was sensitive to potassium ferricyanide. Both aerobic mechanisms were also inhibited by the sulfhydryl inhibitors *p*CMB and iodoacetamide, by NaN_3, and by NaCN (15). The anaerobic uptake by strain A450 was also inhibited by iodoacetamide, *p*CMB, NaCN, and DCCD but unaffected by potassium ferricyanide.

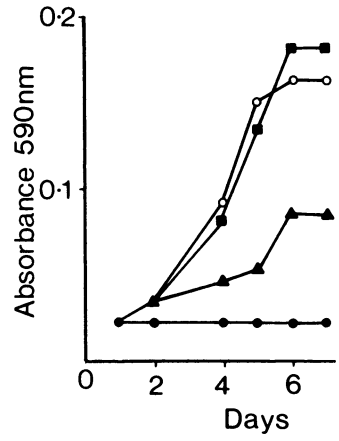

FIG. 3. Cross-feeding of atypical *A. salmonicida* strain A406. Symbols: ●, growth in 6 ml of broth-EDDA; ○, growth in broth-EDDA with 0.45 mM Fe^{3+} added; ■, Growth in 6 ml of broth-EDDA containing 1 ml of strain A450 broth-EDDA culture supernatant; and ▲, growth in 6 ml of broth-EDDA containing 1 ml of *S. typhimurium* broth-EDDA culture supernatant.

Effect of Fe limitation on outer membrane proteins. When outer membranes prepared from *A. salmonicida* strains A449 and A450 cultured

TABLE 3. Inhibition of $^{59}Fe^{3+}$ uptake in *A. salmonicida* strains A449 and A450

Inhibitor	Concn (mM)	% Inhibition[a]		
		A449 Aerobic	A450 Aerobic	A450 Anaerobic
Iodoacetamide	1	74	58	16
	0.1	57	38	16
*p*CMB	1	94	100	66
	0.1	44	30	41
DNP	1	76	53	0
	0.1	50	0	0
$K_3Fe(CN)_6$	1	0	100	0
	0.1	0	71	0
NaN_3	10	93	82	0
	1	73	42	0
NaCN	10	39	48	39
	1	0	14	0
DCCD	1.0			58
	0.1			32

[a] Incubation conditions are described in the text.

in the presence and absence of EDDA were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, several proteins of high apparent molecular weight were seen in cells grown under Fe limitation. These proteins were, however, poorly resolved (Fig. 4A) because they ran in the same region of the electrophoretogram as the high-molecular-weight fraction of *A. salmonicida* lipopolysaccharide (17). This interference was avoided by using an A-layer-deficient derivative of A450 which lacked this high-molecular-weight lipopolysaccharide fraction (17). This deep rough lipopolysaccharide mutant, A450-3R, was indistinguishable from A450 in its ability to grow under conditions of iron limitation. The results in Fig. 4B show that under Fe limitation, three outer membrane polypeptides with apparent molecular weights of 83,200, 77,700 and 76,600 were induced.

DISCUSSION

A. salmonicida is a highly efficient pathogen of fish (11). Intramuscular injection of as few as 10^2 virulent cells can produce death within 96 h. Similarly, asymptomatic carrier fish subjected to environmental stress can die within 24 h with fulminant furunculosis. In both cases, postmortem examination has revealed the presence of large numbers of bacteria in the tissues, implying that the organism is readily able to grow in the low-Fe^{3+} environment found in vivo. This study suggests that one important contributor to this ability to grow rapidly in fish is the efficiency with which typical strains of *A. salmonicida* can sequester iron. In this property, it is similar to the other systemic pathogen of fish, *V. anguillarum*, in which a high-affinity iron-sequestering mechanism has been demonstrated to be an important virulence attribute (6). Unlike *V. anguillarum*, however, virulent strains of *A. salmonicida* have an additional mechanistic problem to overcome in the form of a regular surface protein array. This layer protects the cell from bacteriophage (13) and serum complement (17), as well as from several proteolytic enzymes (unpublished data). Although the exclusion limit of this layer is not currently known, studies with other surface arrays suggest that these structures act as barriers for molecules with molecular weights larger than ca. 3,000 (28). If this is the case with the A-layer, then iron-carrying glycoproteins such as lactoferrin or transferrin with a molecular weight of 90,000 would certainly be excluded from direct access to the outer membrane.

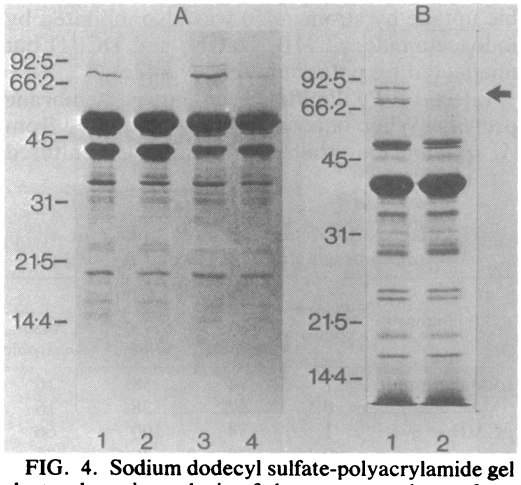

FIG. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the outer membrane fraction isolated from cells of *A. salmonicida* grown in the presence of 0.28 mM EDDA or 0.28 mM EDDA + 0.54 mM $FeCl_3$. Proteins were stained by Coomassie blue, and molecular weight standards (×1,000 daltons) are given on the left of the gels. (A) Strain A449 grown with EDDA (lane 1) and EDDA + Fe^{3+} (lane 2) and strain A450 grown with EDDA (lane 3) and EDDA + Fe^{3+} (lane 4). (B) Strain A450-3R grown with (lane 1) and without EDDA (lane 2). Induced outer membrane proteins are indicated by the arrow.

This study has shown that typical strains of *A. salmonicida* utilize two distinct strategies to sequester iron, one involving production of a soluble low-molecular-weight siderophore(s) and one which does not involve soluble siderophore production. Three of the four typical strains tested produced the siderophore(s). In the case of *A. salmonicida* A450, the siderophore(s) gave neither positive Arnow nor positive Csáky reactions, suggesting that it may not belong to either phenolate or hydroxamate classes. The siderophore(s) clearly differs from those produced by *Aeromonas hydrophila*, which are detectable by the Arnow test (unpublished data) and have been reported to be indistinguishable from enterobactin (C. R. Andrus and S. M. Payne, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, D13, p. 61). The iron-sequestering system of strain A449 was noninducible and appeared not to involve the production of a soluble siderophore(s). This strain appeared to require cell contact with iron-chelator complexes such as the glycoprotein lactoferrin and was able to directly strip the iron from this chelator. In this regard, it resembles the strains of *N. meningitidis* studied by Simonson et al. (26, 27), which also required cell contact with the iron chelator. As was the case when *N. meningitidis* acquired Fe^{3+} from transferrin, the iron-sequestering activity of *A. salmonicida* strain A449 was energy dependent, with the A449 system being strongly inhibited by the proton gradient uncoupler 2,4-DNP. Although apparently unable to produce soluble siderophores, *A. salmonicida* strain A449 was able to utilize the siderophore(s) produced by

strain A450. This ability readily distinguished strain A449 from the direct-iron-stripping strains of *N. meningitidis*, which were unable to use Fe^{3+} from four common siderophores.

As is the case with other bacterial species, *A. salmonicida* displays iron-repressible outer membrane proteins. The role of these outer membrane proteins in *A. salmonicida* is unclear. However, in the case of *E. coli*, some of the low-Fe^{3+}-induced proteins are involved in the binding of Fe^{3+}-siderophore complexes (20, 24). Owing to the size (ca. 700 daltons) and the hydrophilic nature of ferric enterobactin, *E. coli* requires an outer membrane component to transport the iron ligand into the cell. The first step in this transport of ferric enterobactin involves the binding of the ligand to an outer membrane receptor, the *fepA* gene 81,000-dalton protein product (20). Since the low-Fe^{3+}-induced outer membrane proteins of *A. salmonicida* are in the same subunit size range as this iron chelator receptor of *E. coli* and also of other bacteria, they may well have the same function.

The atypical strains of *A. salmonicida* were perceptibly poorer in their ability to sequester iron under conditions of iron limitation. However, like typical strain A449, atypical strain A406 was able to utilize the siderophore(s) in strain A450 supernatant fluid. Indeed, the generally poorer growth of these atypical strains even in the presence of soluble siderophores may indicate that they require their Fe^{3+} in a different form altogether. Perry and Brubaker (23) have reported that *Yersinia pestis* is able to obtain iron from hemin without the benefit of excreted siderophores, and preliminary evidence suggests that this may be the case with atypical strains of *A. salmonicida* (Ishiguro et al., unpublished data). This apparently restricted ability of atypical strains of *A. salmonicida* to acquire Fe^{3+} may also be reflected in their pathogenesis. The atypical strains commonly produce a slowly progressing infection with surface lesions and little or no systemic involvement until late in the disease process.

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