JOURNAL OF BACTERIOLOGY, Oct. 1997, p. 6201–6204 Vol. 179, No. 19
0021-9193/97/$04.00+0

Ferrous Iron Uptake by a Magnesium Transport System Is Toxic for *Escherichia coli* and *Salmonella typhimurium*

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Received 16 April 1997/Accepted 29 July 1997

At low magnesium concentrations, *Escherichia coli* and *Salmonella typhimurium* LT2 accumulate ferrous iron independent of the ferrous iron transport system *feo*. Mutant strains with mutations in the magnesium transport gene *corA* accumulated less ferrous iron than the parent strains. *corA*$^+$ and *corA* strains also differed in their sensitivity to ferrous iron under oxic conditions. *corA* mutants were more resistant to ferrous iron than their parent *corA*$^+$ strains. Part of the ferrous iron accumulated can be chased by the addition of magnesium. Much less iron was chased when ferric iron was taken up by the siderophore ferrichrome. These results may indicate that the intracellular metabolism of the iron taken up by these systems differs and that it depends on the uptake route of the iron.

Magnesium is the most abundant divalent cation in bacterial and eukaryotic cells. Several transport systems that function under different conditions allow the maintenance of the required amount of Mg^{2+}. The best-characterized bacterium in this regard is *Salmonella typhimurium*, which has three magnesium uptake systems. The main magnesium transporter is encoded by the *corA* gene, which is expressed constitutively. The transport system is relatively nonspecific and also allows uptake of Mn^{2+}, Co^{2+}, and Ni^{2+}, especially at low Mg^{2+} concentrations in the medium (8). Co^{2+} in large amounts is toxic for the cell and has been used to select mutants in the CorA (cobalt resistance) transport system. In addition, the CorA system allows efflux of Mg^{2+} under certain conditions (4). Two high-affinity uptake systems, encoded by *mgtA* and *mgtB*, are induced during magnesium starvation (20). From uptake studies (14, 16) and sequence similarities (2, 3), it is certain that at least two of the proteins of the magnesium uptake systems, CorA and MgtA, also exist in *Escherichia coli* K-12. Genes homologous to *corA* have also been found in many other bacteria, and CorA is assumed to be a ubiquitous transporter (19).

The gene region from *E. coli* equivalent to *mgtB* in *S. typhimurium* has been sequenced (3); however, a corresponding gene was not detected. Instead an MgtE-type transporter was postulated for *E. coli* (24), but we did not find an *mgtE*-like gene in the completed *E. coli* genome sequence by a BLAST search in the database (National Center for Biotechnology Information). We were interested in determining whether ferrous iron, in addition to Mg^{2+}, Co^{2+}, Ni^{2+}, and Mn^{2+}, is also transported by CorA. In *E. coli* and *S. typhimurium*, ferrous iron is taken up by a high-affinity transport system encoded by *feoABC* (10, 25). In addition, *E. coli* can use more than 10 different siderophores as carriers for ferric iron and can transport these complexes via seven TonB-dependent siderophore receptors in the outer membrane. TonB mutants are unable to use siderophore-bound iron efficiently, and *aroB* (unable to synthesize 2,3-dihydroxybenzoate and enterochelin, the cognate siderophores of *E. coli*), *tonB*, and *feo* triple mutants are still able to grow on rich media (10). The question arose as to how these cells satisfy their iron needs. Evidence is presented here that magnesium transport systems might contribute to the iron supplies of the cells.

Ferrous iron uptake. Uptake studies with ferrous iron in the presence of 1 mM magnesium have shown that *E. coli feoB* mutants take up less ferrous iron than the parent strain does (10). The *feoABC* genes encode the only defined ferrous iron transport system in *E. coli*. In contrast, ferrous iron was enriched in an *E. coli feoB* mutant when the magnesium concentration in the medium was lowered (Fig. 1A). Part of the ferrous iron taken up was released from the cells by the addition of Mg^{2+} (Fig. 1B). In the transport experiment, the cells were always washed twice with a mixture of EDTA (0.2 mM) and nitrilotriacetic acid (0.1 mM) after being applied to the filter membrane. EDTA permeabilizes the outer membrane (5, 12) and, together with nitrilotriacetic acid, should complex all loosely bound iron in the outer membrane and the periplasm. Therefore, most of the radioactive iron accumulated should be in the cytoplasm of the cells.

Uptake experiments with ferrous iron were always performed in the presence of 1 mM sodium ascorbate to keep iron in a reduced state. Oxidation of iron in the medium leads to rapid precipitation of iron on the filter and results in a high background in liquid scintillation counting.

Since the magnesium uptake systems in *S. typhimurium* LT2 are characterized better than those in *E. coli*, similar experiments were performed with strain LT2. In principle, the same results were obtained (Fig. 1C and D). An eightfold increase in the uptake of ferrous iron was measured at 25 μM ferrous iron and low magnesium concentrations (Fig. 1C). The high iron uptake at low Mg^{2+} concentrations was independent of *feo*, since it occurred in *feoB* and *feoB*$^+$ strains (Fig. 1C). CorA has been described as the major magnesium transporter in *E. coli* and *S. typhimurium* (4, 16). Ferrous iron uptake was approximately 30% lower in the *corA* mutant than in the parent strain (Fig. 1D). Similar results were obtained with *E. coli* (data not shown). Some of the ferrous iron taken up could be chased by magnesium (Fig. 1D), as was observed in *E. coli* (Fig. 1B).

Siderophore-mediated ferric iron uptake. In the ferrichrome-ferric iron uptake system, the iron-free siderophore is rapidly released from the cell after the uptake of ferrichrome (7) and there is additional evidence that the ferric iron taken up with the help of a siderophore is reduced to ferrous iron (13). It is assumed that reduction is an essential step in the removal of Fe^{3+} from siderophores; Fe^{2+} has a much lower

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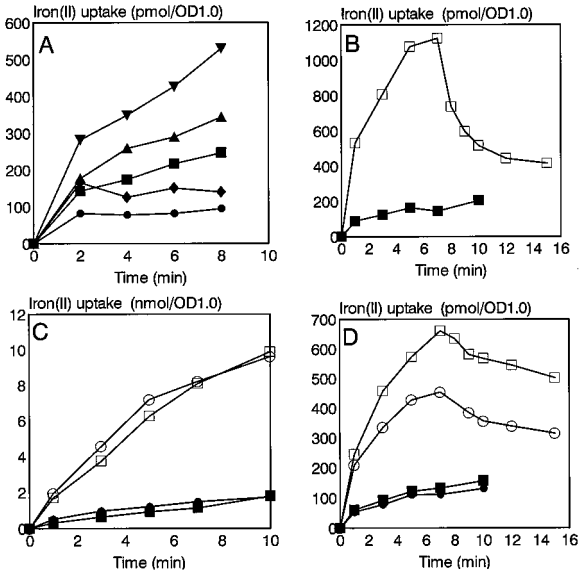

FIG. 1. Ferrous iron uptake by cells grown in TY medium under oxic conditions. Cells were washed twice and suspended at about 10^9 cells per ml in a HEPES-buffered medium (30 g of HEPES/liter, 4.65 g of NaCl/liter, 1.5 g of KCl/liter, 1 g of NH_4Cl/liter, and 0.425 g of Na_2SO_4/liter adjusted to pH 7.2 and sterilized), with 0.2% glucose and magnesium added as indicated. (A) *E. coli* SO74 *feoB* in the presence of 3 (●), 1 (◆), 0.3 (■), 0.1 (▲), and 0.03 mM (▼) $MgCl_2$. The $^{55}Fe^{2+}$ concentration was 3 μM. (B) *E. coli* AB2847 in medium without magnesium. After 7 min, 3 mM $MgSO_4$ was added (□). ■, uptake in the presence of 3 mM $MgSO_4$. The $^{55}Fe^{2+}$ concentration was 3 μM. (C) *S. typhimurium* LT2 (○) and H5337 *feoB*::*tet* (□) in medium without Mg^{2+} and LT2 (●) and H5337 (■) in the presence of 3 mM Mg^{2+}. The $^{55}Fe^{2+}$ concentration was 25 μM. The strains were grown with 50 μM ethylenediamine-di(*o*-hydroxyphenylacetic acid) added to derepress the iron transport systems. (D) *S. typhimurium* LT2 (□) and H5333 *corA* (○) without added magnesium. After 7 min, 3 mM $MgSO_4$ was added. Uptake by LT2 (■) and H5333 (●) in the presence of 3 mM $MgSO_4$. The $^{55}Fe^{2+}$ concentration was 3 μM. Iron(II), ferrous iron; OD1.0, optical density of 1.0 at 578 nm.

affinity than Fe^{3+} to the siderophores, which are highly specific in complexing ferric iron. An experiment was performed to determine whether ferrous iron released from ferrichrome can be chased from the cell by high magnesium concentrations. Transport was determined in *E. coli* MC4100, grown with 50 μM ethylenediamine-di(*o*-hydroxyphenylacetic acid) to derepress iron uptake, and in *E. coli* H1941 Δ*fur*, in which siderophore uptake is constitutive. The experiment was performed in a medium without added magnesium. The addition of 3 mM Mg^{2+} after 7 min had no influence on the uptake kinetics at 1 μM [^{55}Fe]ferrichrome (data not shown), and a small reduction in uptake was observed at 25 μM [^{55}Fe]ferrichrome (Fig. 2). The same results were obtained in the *fur* mutant strain (data not shown). These results indicated that the iron taken up with the siderophore ferrichrome was tightly bound in the cell and not as easily accessible for an exchange with magnesium ions as ferrous iron taken up by CorA and other divalent ion carriers.

Killing of cells by Fe^{2+}. To test the hypothesis that a magnesium uptake system is used by Fe^{2+}, the sensitivity of *E. coli* MC4100 and H5329 *corA* to Fe^{2+} at low Mg^{2+} concentrations was determined. Cells were grown in complex medium (tryptone-yeast extract [TY]) and suspended in a HEPES-buffered medium because HEPES does not have metal-complexing activities as strong as that of phosphate, which is often used in minimal media. As can be seen in Fig. 3A, MC4100 was much more sensitive to Fe^{2+} than the mutant H5329 *corA*. This

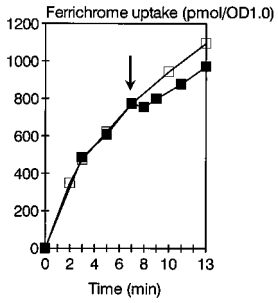

FIG. 2. Ferrichrome uptake into *E. coli* strain MC4100 in a HEPES-buffered medium (see legend to Fig. 1) with 0.2% glucose. The uptake was studied in two samples; the arrow indicates the addition of 3 mM magnesium to one sample (■). The $^{55}Fe^{3+}$-ferrichrome concentration was 25 μM. OD1.0, optical denisty of 1.0 at 578 nm.

indicated that CorA contributes significantly to the sensitivity of the cells to Fe^{2+}.

Similar experiments were performed in the better-characterized *S. typhimurium* LT2. High magnesium concentrations protected cells against the toxicity of ferrous iron (Fig. 3B). The *corA* mutant was more resistant than the parent strain to Fe^{2+} (Fig. 3B). Introduction of an additional *feo* mutation did not change the number of cells killed (data not shown), which indicates that uptake of ferrous iron via the Feo system did not contribute to the killing of the cells. As shown in Figure 1C, Feo did not contribute to iron uptake under the conditions used.

A dilute tryptone medium has been reported to be low in magnesium (~35 μM) (18). *S. typhimurium corA* was grown in this medium to induce *mgtA* and *mgtB*. However, no increased sensitivity to ferrous iron was observed. A slight increase in sensitivity was observed with a HEPES-buffered synthetic medium when no magnesium was added. This indicated that MgtA and MgtB did not contribute to the ferrous iron sensitivity of *S. typhimurium corA*. These transport systems may be more specific for magnesium and might not contribute much to the magnesium-suppressible ferrous iron uptake.

Conclusions. Ferrous iron was taken up in large amounts by *E. coli* and *S. typhimurium* when the medium contained a low concentration of magnesium. This ferrous iron uptake was independent of the ferrous iron transport system Feo (Fig.

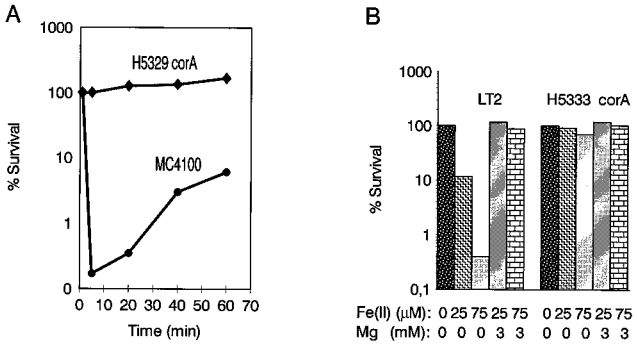

FIG. 3. Killing of cells by ferrous iron in a HEPES-buffered medium (see legend to Fig. 1) with 0.2% glucose and 10 μM $MgSO_4$. (A) *E. coli* MC4100 and H5329 *corA* were incubated with 30 μM Fe^{2+} and 1 mM sodium ascorbate. The cell density was about 4×10^8/ml. (B) Killing of *S. typhimurium* LT2 and H5333 *corA* at a density of 2×10^3 to 4×10^3 cells/ml with various concentrations of Fe^{2+} after addition of magnesium sulfate as indicated. After 15 min cells were plated on TY medium.

1C), which is found in *E. coli* and *Salmonella* (10, 25). Moderately high concentrations of magnesium (1 to 3 mM) suppressed this *feo*-independent ferrous iron uptake. The difference in iron uptake between *feoB*$^+$ and *feoB* was small (Fig. 1C), since the iron concentration used in the transport experiment was 25 μM, which is much higher than the K_m of the Feo transport system (approximately 0.8 μM).

Some of the iron was taken up by the CorA magnesium transport system, as was shown by uptake experiments with *S. typhimurium* and a *corA* mutant (Fig. 1D). The same has been observed in *E. coli* (data not shown). In addition, *corA* mutants were more resistant to killing by ferrous iron, which again reflected the lower uptake of ferrous iron (Fig. 3B).

However, other divalent ion uptake systems also seemed to accept ferrous iron, as could be seen by the magnesium dependence of ferrous iron uptake in a *S. typhimurium corA* mutant (Fig. 1D). These other divalent ion uptake systems seem not to be MgtA or MgtB. In *corA* mutants grown with high magnesium, MgtA and MgtB are repressed (20). Induction of *mgtA* and *mgtB* by growth in a low-magnesium medium did not increase the ferrous iron sensitivity of a *S. typhimurium corA* mutant (data not shown). This suggested that other divalent ion transport systems contribute to the ferrous iron sensitivity of this strain.

A significant part of the iron taken up by these divalent ion uptake systems was not tightly bound to the cell and could be released by a chase with high concentrations of magnesium. In contrast, iron taken up via ferrichrome was only chased in small amounts by magnesium (Fig. 2). This may indicate that the intracellular metabolism of the iron taken up by these systems differs and that it depends on the uptake route of the iron. The *corA* mutants of *E. coli* and of *S. typhimurium* lost nearly as much iron as the parent strains by a chase with magnesium. CorA in *S. typhimurium* has also been implicated in the efflux of magnesium (4), while MgtA and MgtB do not show this activity. The observed efflux of iron after the addition of magnesium did not depend on CorA (Fig. 1D). We concluded that efflux of ferrous iron does not occur through CorA but occurs through other unknown systems.

Iron taken up by ordinary iron transport systems is usually not toxic for the cells even after a shift from anoxic to oxic conditions. Only certain mutants have been shown to be sensitive to iron taken up by Feo or a siderophore, such as nucleotide reductase mutants (6) and *recA* Δ*fur* double mutants (23). In both cases, the undefined free iron inside the cell seems to be the toxic species. In addition iron taken up via a magnesium transport system has also been shown by the Ames test to be mutagenic in *S. typhimurium*, as one would expect for a system generating DNA damaging radicals (15).

The low-magnesium conditions used in this study may have two effects in the presence of Fe^{2+}: (i) nonspecific uptake of high amounts of ferrous iron via magnesium transport systems is stimulated, and (ii) ferrous iron may occupy sites in the cell at the DNA or at membranes, which normally bind magnesium; during oxidative stress, the radicals would be generated at the highly sensitive sites of the cell, and the DNA or membranes would be damaged by the radicals. This second point seems plausible, but is not covered by the experiments presented here. One can calculate from the transport data that, depending on the conditions, roughly 3×10^6 to 1×10^7 iron ions have to be taken up per cell to kill 90% of the cells. This is about 30 to 100 times more than a cell needs for growth (1). Ferrichrome-bound iron at the same concentration in the medium is not toxic for the cells. Compared to ferrous iron, 10 times less iron is taken up into the cells by the TonB-dependent route (Fig. 2), and this iron is tightly bound in the cell.

Ferrous iron may also be taken up by magnesium transport systems in other organisms. In *Bifidobacterium thermophilum*, Mg^{2+} inhibits ferrous iron uptake, and it has been speculated that Mg^{2+} and ferrous iron share a permease (11). In vertebrates, a Mg^{2+}-Na^+ antiporter in erythroid cells allows the uptake of iron (21). The uptake route via nonspecific magnesium transporters may be identical to the often-described nonspecific, low-affinity iron uptake systems that are independent of transferrin (reviewed in reference 22).

Why do many bacteria have this Achilles heel? They are able to discriminate between Fe^{2+} and Mg^{2+} during transport, as the existence of Feo and MgtA and MgtB shows, even though the ion radii are similar (Fe^{2+}, 0.76×10^{-10} m; Mg^{2+}, 0.65×10^{-10} m [for comparison, the radius of Mn^{2+} is 0.8×10^{-10} m, that of Ca^{2+} is 0.99×10^{-10} m, and that of Fe^{3+} is 0.64×10^{-10} m] [9]). Under most circumstances, the low specificity of CorA may be advantageous for the cells, since metals such as Co, Mn, and Ni are relatively rare elements and are needed in very small amounts by the cell. Fe is also needed in amounts 1,000 to 10,000 times smaller than those of Mg in the cell. Near-neutral-pH Fe^{3+} does not occur in amounts that support bacterial growth, since it is rapidly hydrolyzed to iron hydroxide and precipitates. Fe^{2+} may be present under anoxic conditions, which are readily obtained in densely populated media by respiratory consumption of oxygen by aerobic bacteria. Therefore, it might be beneficial in most cases for the cells to have only one transport system, like CorA, for divalent cations that discriminates against Ca^{2+} and allows the uptake of rare and seldom-needed divalent cations in addition to magnesium. The low specificity of CorA becomes a problem only in habitats normally not colonized by *E. coli*, such as in certain well waters that are toxic for *E. coli* and *S. typhimurium* (17). These well waters are from anoxic regions below the earth and contain relatively large amounts of ferrous iron (up to 500 μM [17]). When bacteria are suspended in these fresh well waters, they are killed by taking up too much ferrous iron in the presence of oxygen, which results in the high hygienic quality of these groundwaters (17).

I thank Stefanie Kurtz for expert technical assistance, Volkmar Braun for helpful suggestions and discussions, Claudia Schön for reference 17, and Karen A. Brune for reading the manuscript.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 323) and the Fonds der Chemischen Industrie.

REFERENCES

1. **Braun, V., K. Hantke, and W. Köster.** Bacterial iron transport: mechanisms, genetics and regulation. *In* A. Siegel and H. Siegel (ed.), Iron transport and storage in microorganisms, plants and animals, in press. Marcel Dekker, Inc., New York, N.Y.
2. **Burland, V., G. Plunkett, H. J. Sofia, D. L. Daniels, and F. R. Blattner.** 1995. Analysis of the Escherichia coli genome. VI. DNA sequence of the region from 92.8 through 100 minutes. Nucleic Acids Res. **23:**2105–2119.
3. **Daniels, D. L., G. Plunkett III, V. Burland, and F. R. Blattner.** 1992. Analysis of the Escherichia coli genome: DNA sequence of the region from 84.5 to 86.5 minutes. Science **257:**771–778.
4. **Gibson, M. M., D. A. Bagga, C. G. Miller, and M. E. Maguire.** 1991. Magnesium transport in Salmonella typhimurium: the influence of new mutations conferring Co^{2+} resistance on the CorA Mg^{2+} transport system. Mol. Microbiol. **5:**2753–2762.
5. **Hancock, R. E. W.** 1984. Alterations in outer membrane permeability. Annu. Rev. Biochem. **38:**237–264.
6. **Hantke, K.** 1988. Characterization of an iron sensitive Mud1 mutant in E. coli lacking the ribonucleotide reductase subunit B2. Arch. Microbiol. **149:** 344–349.
7. **Hartmann, A., and V. Braun.** 1979. Uptake and conversion of the antibiotic albomycin by *Escherichia coli* K-12. Eur. J. Biochem. **99:**517–524.
8. **Hmiel, S. P., M. D. Snavely, C. G. Miller, and M. E. Maguire.** 1986. Magnesium transport in *Salmonella typhimurium*: characterization of magnesium influx and cloning of a transport gene. J. Bacteriol. **168:**1444–1450.
9. **Hughes, M. N., and R. K. Poole.** 1989. Metals and micro-organisms, p. 4–9. Chapman and Hall, London, England.

10. **Kammler, M., C. Schön, and K. Hantke.** 1993. Characterization of the ferrous iron uptake system of *Escherichia coli*. J. Bacteriol. **175:**6212–6219.
11. **Kot, E., and A. Bezkorovainy.** 1993. Effects of Mg^{2+} and Ca^{2+} on Fe^{2+} uptake by *Bifidobacterium thermophilum*. Int. J. Biochem. **25:**1029–1033.
12. **Leive, L.** 1968. Studies on the permeability change produced in coliform bacteria by ethylenediaminetetraacetate. J. Biol. Chem. **243:**2373–2380.
13. **Matzanke, B. F., I. Berner, E. Bill, A. X. Trautwein, and G. Winkelmann.** 1991. Transport and utilization of ferrioxamine-E-bound iron in Erwinia herbicola (Pantoea agglomerans). Biol. Met. **4:**181–185.
14. **Nelson, D. L., and E. P. Kennedy.** 1972. Transport of magnesium by a repressible and a nonrepressible system in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **69:**1091–1093.
15. **Pagano, D. A., and E. Zeiger.** 1992. Conditions for detecting the mutagenicity of divalent metals in Salmonella typhimurium. Environ. Mol. Mutagen. **19:** 139–146.
16. **Park, M. H., B. B. Wong, and J. E. Lusk.** 1976. Mutants in three genes affecting transport of magnesium in *Escherichia coli*: genetics and physiology. J. Bacteriol. **126:**1096–1103.
17. **Schulze, E.** 1995. Bakterizide Eigenschaft von Grund- und Mineralwasser. Bundesgesundheitsblatt **11:**438–440.
18. **Silver, S., P. Johnseine, E. Whitney, and D. Clark.** 1972. Manganese-resistant mutants of *Escherichia coli*: physiological and genetic studies. J. Bacteriol. **110:**186–195.
19. **Smith, R. L., and M. E. Maguire.** 1995. Distribution of the CorA Mg^{2+} transport system in gram-negative bacteria. J. Bacteriol. **177:**1638–1640.
20. **Snavely, M. D., S. A. Gravina, T. T. Cheung, C. G. Miller, and M. E. Maguire.** 1991. Magnesium transport in Salmonella typhimurium. Regulation of mgtA and mgtB expression. J. Biol. Chem. **266:**824–829.
21. **Stonell, L. M., D. L. Savigni, and E. H. Morgan.** 1996. Iron transport into erythroid cells by the Na^{+}/Mg^{2+} antiport. Biochim. Biophys. Acta **1282:**163–170.
22. **Theil, E. C., and P. Aisen.** 1987. The storage and transport of iron in animal cells, p. 491–520. *In* G. Winkelmann, D. van der Helm, and J. B. Neiland (ed.), Iron transport in microbes, plants and Animals. VCH Verlag, Weinheim, Germany.
23. **Touati, D., M. Jacques, B. Tardat, L. Bouchard, and S. Despied.** 1995. Lethal oxidative damage and mutagenesis are generated by iron in Δ*fur* mutants of *Escherichia coli*: protective role of superoxide dismutase. J. Bacteriol. **177:** 2305–2314.
24. **Townsend, D. E., A. J. Esenwine, J. George III, D. Bross, M. E. Maguire, and R. L. Smith.** 1995. Cloning of the *mgtE* Mg^{2+} transporter from *Providencia stuartii* and the distribution of *mgtE* in gram-negative and gram-positive bacteria. J. Bacteriol. **177:**5350–5354.
25. **Tsolis, R. M., A. J. Bäumler, F. Heffron, and I. Stojiljkovic.** 1996. Contribution of TonB- and Feo-mediated iron uptake to growth of *Salmonella typhimurium* in the mouse. Infect. Immun. **64:**4549–4556.