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# Sociality in *Escherichia coli*: Enterochelin Is a Private Good at Low Cell Density and Can Be Shared at High Cell Density

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**ABSTRACT**

**Many bacteria produce secreted iron chelators called siderophores, which can be shared among cells with specific siderophore uptake systems regardless of whether the cell produces siderophores. Sharing secreted products allows freeloading, where individuals use resources without bearing the cost of production. Here we show that the *Escherichia coli* siderophore enterochelin is not evenly shared between producers and nonproducers. Wild-type *Escherichia coli* grows well in low-iron minimal medium, and an isogenic enterochelin synthesis mutant (Δ*entF*) grows very poorly. The enterochelin mutant grows well in low-iron medium supplemented with enterochelin. At high cell densities the Δ*entF* mutant can compete equally with the wild type in low-iron medium. At low cell densities the Δ*entF* mutant cannot compete. Furthermore, the growth rate of the wild type is unaffected by cell density. The wild type grows well in low-iron medium even at very low starting densities. Our experiments support a model where at least some enterochelin remains associated with the cells that produce it, and the cell-associated enterochelin enables iron acquisition even at very low cell density. Enterochelin that is not retained by producing cells at low density is lost to dilution. At high cell densities, cell-free enterochelin can accumulate and be shared by all cells in the group. Partial privatization is a solution to the problem of iron acquisition in low-iron, low-cell-density habitats. Cell-free enterochelin allows for iron scavenging at a distance at higher population densities. Our findings shed light on the conditions under which freeloaders might benefit from enterochelin uptake systems.**

**IMPORTANCE**

**Sociality in microbes has become a topic of great interest. One facet of sociality is the sharing of secreted products, such as the iron-scavenging siderophores. We present evidence that the *Escherichia coli* siderophore enterochelin is relatively inexpensive to produce and is partially privatized such that it can be efficiently shared only at high producer cell densities. At low cell densities, cell-free enterochelin is scarce and only enterochelin producers are able to grow in low-iron medium. Because freely shared products can be exploited by freeloaders, this partial privatization may help explain how enterochelin production is stabilized in *E. coli* and may provide insight into when enterochelin is available for freeloaders.**

Iron is an essential nutrient for the vast majority of bacteria (1, 2). However, under aerobic conditions at neutral pH, iron is found predominantly in insoluble $Fe^{3+}$ compounds, resulting in extremely low iron concentrations in water (about $10^{-18}$ M) (3). Within a host, available iron is also limited, as many host organisms produce iron-binding molecules, which sequester iron away from bacteria (4). Thus, bacteria have evolved a variety of mechanisms to acquire this resource. One mechanism is to secrete molecules, called siderophores, that bind and solubilize extracellular iron. Different bacteria produce different high-affinity secreted siderophores and there are specific high-affinity uptake systems for the siderophores (5, 6).

Siderophores, by necessity, are secreted, and there has been interest in and debate over the cooperative nature of siderophore production (7–17). In the simplest case, a siderophore, once secreted, is available to all members of a community. Consequently, a freeloading cell, which uses siderophores but does not produce them, may have a fitness advantage over a producing cell, as it does not incur the metabolic cost of siderophore production. Freeloading should destabilize siderophore production. However, competition for siderophores is often much more complex, as evidenced by numerous studies and the continued existence of siderophore-producing bacteria. The availability of siderophores and other extracellular products for use by freeloaders depends on siderophore diffusion as well as population density and structure (18–20). Some bacteria have also evolved mechanisms that appear to prevent or minimize siderophore freeloading, including maintaining variability in structure of siderophore-receptor pairs (diversifying selection) (21, 22) and privatizing siderophores through high hydrophobicity or association with membrane-bound receptors (23–26). We are interested in siderophore maintenance through privatization, for which there is little direct evidence in microbes (24).

The *Escherichia coli* siderophore enterochelin (Ent) has been studied in detail. It is a catecholate produced not only by *E. coli* but also by several other *Enterobacteriaceae* (27–29). Ent synthesis and uptake genes are under the control of the ferric uptake regulator (Fur) protein (30, 31). Ent is produced in the cytoplasm by-prod-

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ucts of the *ent* genes via nonribosomal peptide synthesis (32–35). Following synthesis, Ent is secreted via EntS (36) and released from the cell. Upon binding $Fe^{3+}$, ferric enterochelin (FeEnt) enters the periplasm via the TonB-dependent receptor FepA (37, 38). In the periplasm, FeEnt binds FepB, a periplasmic binding protein (39), and then moves into the cytoplasm through an ABC transporter (40, 41). Finally, FeEnt is hydrolyzed by the cytoplasmic enterochelin esterase (Fes) and free iron is released (42). Each Ent molecule can only be used once; however, the linear Ent breakdown products can be repurposed as lower-affinity siderophores (43).

It is of interest that *Pseudomonas aeruginosa* possesses the complete machinery for FeEnt uptake and utilization (44) but it cannot produce Ent itself. The general belief is that this enables *P. aeruginosa* to steal iron from Ent producers; it is capable of freeloading on enteric bacteria like *E. coli*. In fact, a variety of microbes possess Ent uptake systems but not *ent* biosynthesis genes (45). Here we present evidence that Ent is a partially privatized *E. coli* product. Although Ent mutants are unable to grow independently in iron-limited media, they can grow when cocultured with Ent producers. However, growth of Ent mutants in these cocultures is dependent on the overall frequency and density of Ent producers. Additionally, when an Ent producer is alone, its growth in low-iron media is independent of cell density. Our results are consistent with a model in which some fraction of Ent must remain associated with the cells that produce it.

## MATERIALS AND METHODS

**Bacterial strains and strain construction.** All bacteria used were *E. coli* BW25113 (46) derivatives. We obtained an *entF*::*kan* mutant from the Keio collection (47) and moved the mutation into *E. coli* BW25113 by using P1 transduction. The kanamycin resistance marker was removed by using Flp recombinase methods (48) to create *E. coli* RS100, which has a nonpolar scar in place of *entF*. We constructed a Δ*entCEBAH* mutant by using the method of Datsenko and Wanner (46). Briefly, a kanamycin marker flanked by Flp recombination target (FRT) sites was PCR amplified from pKD4. The amplification primers included DNA homologous to the regions upstream of *entC* and downstream of *entH*. The PCR product was introduced into the *E. coli* BW25113 chromosome by λ red recombination to yield a Δ*entCEBAH* operon mutant with a deletion extending from the *entC* translation start codon to the *entH* stop codon. We used pKD46 to express the λ red genes and removed the kanamycin marker as described above. Where noted, strains also carried either kanamycin or chloramphenicol resistance markers. These markers were PCR amplified from pKD4 or pKD3, respectively, before insertion into the arsenate transporter (*arsB*) gene in the opposite orientation as described elsewhere (46). Again, we used pKD46 to express the λ red genes. The markers replaced most of *arsB*, leaving only the start codon, codons for the 6 C-terminal residues, and the stop codon. Previous work has shown that inserting a marker into this gene does not noticeably affect growth under normal laboratory conditions (47, 49, 50).

**Bacterial growth media.** We use Luria-Bertani broth (LB) with added kanamycin sulfate (30 μg/ml), chloramphenicol (25 μg/ml), or ampicillin sodium salt (100 μg/ml) as appropriate for routine bacterial growth. The low-iron minimal medium for our experiments consisted of 50 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS; pH 7), 50 mM NaCl, 40 mM $NH_4Cl$, 5 mM $KH_2PO_4$, 5 mM $Na_2HPO_4$, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 1% glycerol. Where noted, $FeCl_3$ was added to a final concentration of 5 or 50 μM, apoenterochelin to 5 μg/ml (Sigma Chemicals or Genaxxon BioScience), human apotransferrin to 100 μg/ml (Sigma Chemicals), and sodium bicarbonate to 20 mM. In some experiments we included siderophore extracts of *E. coli* culture fluid prepared as described below.

**Preparation of siderophore extracts.** Extracts from *E. coli* BW25113 growth medium were prepared as described previously (36). Briefly, spent medium from stationary-phase cultures grown in low-iron medium was centrifuged and filtered to remove cells. The pH of the culture fluid was then reduced to about 2.5 with 12 N hydrochloric acid, and the siderophores were extracted with two equal volumes of ethyl acetate. The ethyl acetate extract was dried under a steady stream of nitrogen, and the residue was dissolved in ethyl acetate. This extraction method should result in a solution containing Ent and recycled Ent degradation products (36, 51). Extracted siderophores were added to growth medium as follows: the ethyl acetate preparation was added to sterile 18-mm tubes at the desired level, and the ethyl acetate was removed by evaporation under a stream of nitrogen gas. After removal of ethyl acetate, the siderophores were dissolved in low-iron medium.

**Growth and competition experiments.** For starter cultures, bacteria were first grown overnight in LB broth, washed once in an equal volume of low-iron medium, and then used as an inoculum (1%, vol/vol) in low-iron medium containing siderophore extract prepared as described above. The extract used was equivalent to the material extracted from a 1% volume of *E. coli* BW25113 culture fluid. The bacteria were then grown in 5 ml of medium in 18-mm tubes with shaking at 37°C. When cultures reached mid-logarithmic phase (optical density at 600 nm of 0.2 to 0.3), cells were washed twice in low-iron medium, diluted to an optical density at 600 nm of 0.1, mixed at desired starting ratios for competition experiments, and then used as inocula for growth or competition experiments. The addition of siderophore extract allows equal growth of the Δ*entF* mutant and the wild type in the starter cultures. For growth curves, bacteria were grown in 2.5 ml of medium in 18-mm tubes with shaking at 37°C.

To determine cell numbers in single-strain growth experiments, we performed plate counts on LB agar. For competition experiments, cells were grown for 14 to 16 h. The frequencies of competing strains were measured before and after growth by plate counting on LB agar with kanamycin or chloramphenicol, and total cells per milliliter were calculated as the sum of the number of cells resistant to each antibiotic.

Our detection threshold for colony counting was $10^2$ cells per ml. The counts for the Δ*entF* mutant at the lowest starting density in the density-dependent fitness experiments occasionally fell below this threshold. When this happened, we assigned a value equal to one-half of the detection threshold, or 50 cells per ml, to these cultures. The competitive index (CI) is defined as the ratio of mutant to wild type in the output divided by the ratio of mutant to wild type in the input (52). To calculate generations, we used the following formula: number of generations $= \ln(N_{final}/N_{initial})$, where $N$ is the measured cells per milliliter. We used GraphPad Prism software to generate graphs and nonlinear semilog fit curves for the wild type-to-wild type competitions. To test whether slopes were significantly different from 0, we used a sum-of-squares F test with a $P$ value of $<0.05$.

## RESULTS

**Growth of the Δ*entF* mutant in low-iron medium with and without added enterochelin.** As expected, our Δ*entF* mutant showed very limited growth in low-iron medium compared to that of the parent (Fig. 1). The final growth yield was <1% of the parent strain. The addition of 5 or 50 μM $FeCl_3$ to cultures failed to rescue Δ*entF* mutant growth. In the presence of added apo-Ent (5 μg/ml), however, growth of the Δ*entF* mutant was equivalent to growth of the wild type (Fig. 1). This is consistent with the general view that Ent can serve as a shared resource, which can be used by any cell with an Ent receptor system whether or not that cell produced Ent. To show that iron limits growth in unsupplemented low-iron medium, we measured growth yields. Without added enterochelin, yields were $2.6 \times 10^9$ cells per ml without added iron and $6.7 \times 10^9$ cells per ml with 50 μM $FeCl_3$. With enterochelin,

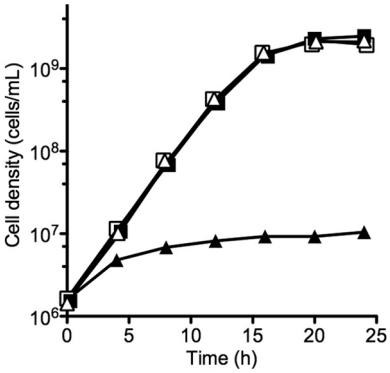


**FIG 1** Growth curves of wild-type *E. coli* BW25113 grown with (open squares) or without (closed squares) added Ent and the Δ*entF* mutant grown with (open triangles) and without (closed triangles) added Ent. We used low-iron medium for these experiments and supplemented with 5 μg/ml of Ent where indicated. Cultures were run in duplicate, and ranges are within the symbols.

yields were $2.3 \times 10^9$ cells per ml without added $FeCl_3$ and $8.6 \times 10^9$cells per ml with 50 μM $FeCl_3$. Wild-type growth rates, however, were indistinguishable between the conditions of no addition of iron and addition of 50 μM $FeCl_3$ ($0.45 \pm 0.02$ generations per hour). These growth curve data indicate that Ent production is relatively inexpensive.

**The Δ*entF* mutant can grow in low-iron medium when cocultured with the Ent-producing wild type.** Coculture with wild-type *E. coli* BW25113 supported growth of the Δ*entF* mutant (Fig. 2). When the starting ratio of Δ*entF* mutant to wild type was 1:1 and the starting density was $10^5$ cells per ml, the two strains grew equally well: the final cell yield was comparable to that of the wild type in pure culture, and the CI was about 1. These findings indicate that the Δ*entF* mutant can freeload; it can use Ent produced by the wild type to acquire iron. The CI is a measure of the relative fitness of a mutant strain, with a CI of >1 indicating an advantage and a CI of <1 indicating a disadvantage. A CI of about 1 indicates that the cost of Ent production is low under the conditions of this experiment.

**Enterochelin mutants have a negative frequency-dependent fitness in competition with the wild type.** To more thoroughly investigate whether production of Ent confers a burden to the wild type in coculture with the Δ*entF* mutant, we measured the CI in

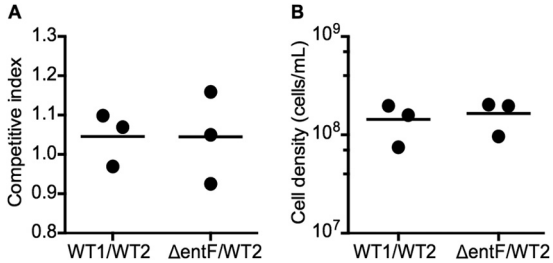


**FIG 2** The Δ*entF* mutant can grow in low-iron medium when in coculture with the wild type. *E. coli* BW25113 *arsB*::*kan* (WT1) or Δ*entF arsB*::*kan* (Δ*entF*) was mixed at a 1:1 ratio with *E. coli* BW25113 *arsB*::*cat* (WT2). (A) Competitive index; (B) final cell density. The initial cell density was about $10^5$ cells per ml. Total cells per milliliter and strain frequencies were measured at the start of the experiment and after 14 h. The competitive index was calculated as described in Materials and Methods. Results from three independent experiments are shown, and the bars indicate the means.

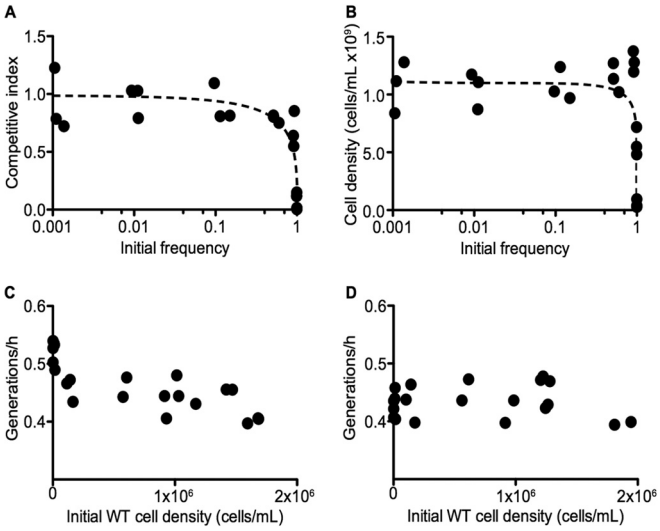


**FIG 3** Negative frequency-dependent fitness of the Δ*entF* mutant. Competition experiments were conducted with WT1 or Δ*entF* against WT2. Initial ratios ranged from 1,000:1 to 1:1,000, and initial cell densities were about $10^6$ cells per ml. Cells were grown in low-iron medium. (A) CI of competitions between the Δ*entF* mutant and WT2; (B) total yield of the cultures. At high frequencies of the Δ*entF* mutant, its fitness and total culture yield fall off. Controls with WT1 and WT2 show a CI of about 1 regardless of initial frequency, and total yields also remain constant. Frequencies were measured at the start of the experiment and after 14 to 16 h of growth. Also shown are growth of WT2 in culture with the Δ*entF* mutant (C) or with WT1 (D). Generations per hour were calculated as generations over the growth period divided by the hours of growth.

experiments started at a wide range of Δ*entF* mutant-to-wild type ratios. Previous work with *P. aeruginosa* showed that a siderophore mutant can have negative frequency-dependent fitness in competition with a wild type: the siderophore mutant showed greater fitness when at low relative abundance than it did at higher relative abundance (11). We tested whether initial frequencies affect the competitive fitness of the Δ*entF* mutant. The starting cell densities for these experiments were about $10^6$ cells per ml of low-iron medium, and the starting ratios ranged from 1,000:1 to 1:1,000 for the Δ*entF* mutant to the wild type. As a control, we performed a similar experiment in which the wild type with a kanamycin resistance marker was cocultured with the wild type with a chloramphenicol resistance marker. The control provides a measure of any burden from the antibiotic resistance marker. The control showed little effect of the antibiotic resistance markers and did not show frequency dependence. At low initial frequencies of the Δ*entF* mutant, the CI was about 1, indicating that neither strain has a significant growth advantage over the other. This supports the view that Ent production is inexpensive and therefore does not significantly reduce the growth rate of the Ent producers (Fig. 3A). In contrast, at high Δ*entF* mutant starting frequencies, the CI was <1. That is, the wild type was more fit than the Δ*entF* mutant. The antibiotic markers are not responsible for this result, as the CIs in wild-type control experiments remained about 1.

We performed similar experiments with a Δ*entCEBAH* mutant. This mutant is defective in conversion of chorismic acid to the aromatic substrate for Ent synthesis, whereas EntF is required for incorporation of serine in Ent biosynthesis. Our results with the Δ*entCEBAH* mutant were consistent with those obtained with

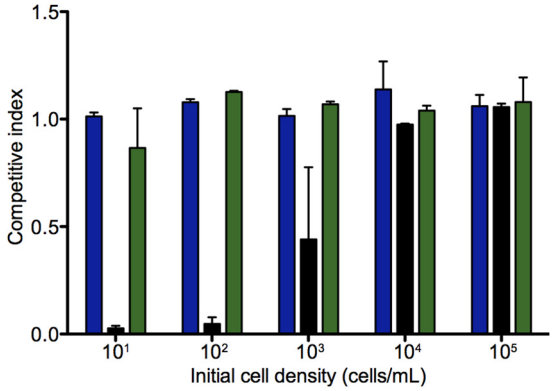


**FIG 4** Fitness of the Δ*entF* mutant versus the wild type depends on initial cell density. The *E. coli* BW25113 *arsB*::*cat* strain was grown together with the *E. coli* BW25113 *arsB*::*kan* strain or the Δ*entF* mutant (*E. coli* Δ*entF arsB*::*kan*) in low-iron medium with or without supplements as indicated. Starting ratios were 1:1, and initial cell densities are shown. Blue bars represent the two Ent wild-type strains in unsupplemented low-iron medium. The CI was about 1 regardless of inoculum size. Black bars represent the Δ*entF* mutant and the wild type in unsupplemented medium. The Δ*entF* mutant is competitive only at initial cell densities of $10^3$ to $10^4$ or greater. Green bars represent the Δ*entF* mutant and wild type in medium supplemented with Ent (5 μg/ml). The mutant is competitive regardless of inoculum size. Cell numbers and frequencies were determined immediately after inoculation and at 14 h. The bars represent results from two independent experiments, and error bars show ranges.

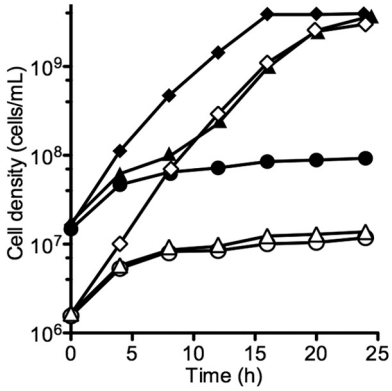


**FIG 5** Growth of wild-type *E. coli* in the presence of transferrin is dependent on inoculum size. *E. coli* BW25113 and the Δ*entF* mutant were grown in low-iron medium with human apotransferrin and sodium bicarbonate. Open circles represent the Δ*entF* mutant started from low density and closed circles the Δ*entF* mutant started from high density. Open triangles represent the wild type started from low density and closed triangles the wild type started from high density. Open diamonds represent the wild type started from low density with Ent added, and closed diamonds represent the wild type started from high density with Ent added. Cultures were run in duplicate, and ranges are within the symbols.

the Δ*entF* mutant. For the Δ*entCEBAH* mutant, the competitive index is close to 1 at high wild-type frequencies and rapidly decreases as the starting mutant frequency increases above 10%.

Additionally, we found that although the overall culture growth was reduced with high Δ*entF* mutant starting frequencies (Fig. 3B), the approximate generations of the competing wild type per hour were unaffected by Δ*entF* mutant frequency (Fig. 3C and D). Therefore, the reduced growth yield in these cultures is due solely to the reduced growth of the Δ*entF* mutant. We believe that the results of the frequency dependence experiments indicate that Ent is not a freely shared public good. Rather, it is partially privatized by the wild type, and when wild-type cells are scarce, they make sufficient Ent to support themselves but there is insufficient free Ent for iron acquisition by the Δ*entF* mutant.

**Fitness of the Δ*entF* mutant depends on the initial cell density.** Privatization of bacterial extracellular products has been demonstrated by showing density-dependent fitness during competition, although previous work has been done on surfaces where population structure plays a large role (12). By measuring density-dependent fitness, we can determine how wild-type cell density affects fitness of the Δ*entF* mutant. For these experiments, the initial ratios of the wild type to the Δ*entF* mutant were 1:1, and the initial cell densities were varied between $10^1$ and $10^5$ per ml (Fig. 4). The Δ*entF* mutant showed density-dependent fitness in low-iron medium. The greater the initial cell density, the greater the fitness of the Δ*entF* mutant. These data, in agreement with the data on frequency-dependent fitness, indicate that Ent is partially privatized by Ent-producing *E. coli*. We interpret the results to mean that at sufficiently high densities of wild-type *E. coli*, the fraction of Ent in the culture fluid is sufficient for growth of the mutant. Cell-free Ent concentrations are too low to be of value at lower cell densities where only the wild type with cell-associated Ent is capable of growth in low-iron medium. When Ent is added to the culture medium, the mutant and wild type grow well together regardless of the starting cell density. Thus, the inability of the Ent mutant to compete with the wild type when the initial cell density is low is a direct consequence of the lack of sufficient cell-free Ent.

One possible explanation for our results is that a significant fraction of iron-free Ent partitions to the periplasm of producing cells and diffusion of $Fe^{3+}$ into the periplasm allows growth on low-iron medium when cell density is low. We do not have conclusive evidence for or against this hypothesis, but it is worth noting that inclusion of the iron chelator transferrin (with bicarbonate) in the low-iron medium restricts the growth of the wild type at low starting cell densities but not at higher starting densities (Fig. 5). Exogenous enterochelin can support wild-type growth in the presence of transferrin even at low starting cell densities, and the Δ*entF* mutant does not grow in the presence of transferrin regardless of starting density. One interpretation of these findings is that cell-associated Ent is within the periplasm, where it cannot obtain iron bound to transferrin. At high cell densities there is sufficient cell-free Ent for iron acquisition from transferrin.

**Growth of Ent-producing wild-type *E. coli* in low-iron medium is not influenced by cell density.** The ability of Ent-producing wild-type *E. coli* to grow at low cell density and the inability of the Δ*entF* mutant to grow together with the wild type at low cell density indicate that some fraction of Ent remains with producing cells. At low cell density there is insufficient public Ent for cell growth. To obtain further evidence that the growth rates of the wild type are similar at very low and higher cell densities, we serially diluted the wild type in low-iron medium and followed growth. Growth was observed even from starting densities of one or a few cells per milliliter (Fig. 6). Previous work on yeast invertase has shown that for predominantly shared products, growth rate is severely limited at low starting densities, as products rapidly diffuse away from producers (53). Therefore, our finding that growth of *E. coli* in low-iron medium is unaffected by cell density

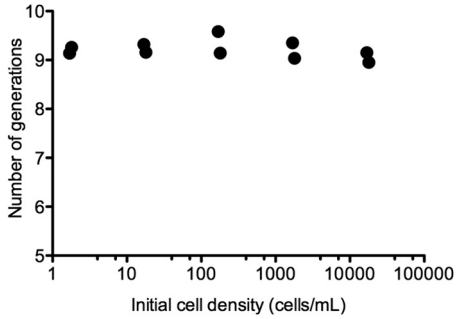


**FIG 6** Growth rate of wild-type *E. coli* in low-iron medium is not affected by inoculum size. *E. coli* BW25113 was grown in low-iron medium starting at various densities as indicated (initial cell density). Cell numbers were determined by plate counting at the time of inoculation and at 18 h. In all cases, growth was in logarithmic phase at 18 h. The number of generations is the number of doublings in 18 h. Cultures were run in triplicate. One of the lowest-density starting cultures failed to grow, presumably because this culture did not receive any cells during inoculation; this culture was excluded from analysis.

is further evidence that a fraction of this siderophore remains bound to producing cells.

## DISCUSSION

We tested the hypothesis that the *E. coli* siderophore Ent is a secreted public good. Our results indicate that sufficient Ent or Ent breakdown products are released from cells to support the growth of non-siderophore-producing mutants, but only at relatively high cell densities. At low densities Ent producers grow well, but they cannot support growth of nonproducers. This leads to an alternate hypothesis that only a fraction of the Ent produced by wild-type cells is public, and the public Ent is not sufficient to support growth in low-iron medium when *E. coli* is at low population densities. Furthermore, we did not find that Ent mutants were cheating. Under any condition we tested, they did not have a fitness advantage over the wild type. Thus, we conclude that EntF expression and Ent production are relatively inexpensive.

The apparent partial privatization of Ent contrasts with findings for the *P. aeruginosa* siderophore pyoverdine. Pyoverdine nonproducers arise both *in vivo* and *in vitro*, and they are social cheats; they can have a fitness advantage over pyoverdine producers in low-iron media (7, 13, 54). However, there are indications that, as we have found with Ent, pyoverdine too is somewhat privatized (14, 24). Mathematical models indicate that social cheating can occur even in the face of significant privatization, so the differences in competition between siderophore-producing and nonproducing *E. coli* and *P. aeruginosa* may be due to siderophore cost and degree of privatization (15, 18–20).

Although it has long been suspected that many siderophores might remain cell associated after secretion (25, 55–57), evidence for this notion is limited and restricted to a few bacterial species. Furthermore, aside from the few studies on amphiphilic siderophores and pyoverdine, the mechanisms for privatization appear to be largely unexplored (23, 24, 26). Apo-Ent might be retained in the periplasm, it might bind to FepA, and it might adhere to the cell surface nonspecifically. Evidence indicates that adherence of FeEnt to the surface of *E. coli* is almost entirely mediated by FepA (58), but the mechanism for apo-Ent adherence is unclear, as studies typically measure adsorbance and transport of radiolabeled iron-siderophore complexes into cells, not aposiderophores (59). Due to the hydrophobicity of apo-Ent, it is possible that it remains associated with the cell surface after secretion (25, 56). We have not addressed the mechanism of Ent privatization, but we have found that iron chelated with transferrin supports growth of *E. coli* only when cells are at sufficient population densities or if exogenous enterochelin is added. This result suggests that Ent may be sequestered in the periplasm. By studying salmochelins, which are a family of glycosylated enterochelins, it may be possible to assess the importance of siderophore hydrophobicity to privatization. Salmochelin glycosylation results in siderophores that are more hydrophilic than enterochelin (60).

Many microbes produce FeEnt uptake receptors despite their lack of Ent biosynthesis genes. The general view is that this allows these microbes to acquire iron at the expense of Ent-producing enteric bacteria. Our experiments suggest that this occurs only when the density of Ent-producing cells is sufficiently high. Thus, Ent production is stabilized in at least two ways: Ent production is inexpensive and nonproducers should have little to no growth advantage over producers, and Ent producers will always have an initial head start over nonproducing cells when growing from low densities, such as after a dispersal event. What might be the advantage of making Ent publicly available at high cell densities? One might imagine that as cell density rises, local iron concentrations become increasingly limited. In this situation iron will come in contact with cells less often than at low cell densities. Here iron scavenging by diffusible Ent might provide a benefit to any cell in the group with an Ent uptake system. Ent may also serve purposes other than iron acquisition. For example, it has been suggested that cell-free siderophores may be beneficial to producers because they can sequester iron from competitors or possibly to support mutualists (27, 61, 62).

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