Brief Report

Phenotypic characteristics contributing to the enhanced growth of *Escherichia coli* bloom strainsBuddhie S. Nanayakkara,^{1,2} Claire L. O'Brien^{3,4} and David M. Gordon ^{1*}¹Division of Ecology and Evolution, Research School of Biology, The Australian National University, RN Robertson Building, 46 Sullivans Creek Road, Canberra, ACT, 2601, Australia.²Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya, 20400, Sri Lanka.³Medical School, The Australian National University, Canberra, ACT, 2601, Australia.⁴Gastroenterology and Hepatology Unit, Canberra Hospital, Canberra, ACT, Australia.

Summary

During bloom events, *Escherichia coli* cell counts increase to between 10,000 and 100,000 cfu/100 ml of water. The strains responsible for bloom events belong to *E. coli* phylogenetic groups A and B1, and all have acquired a capsule from *Klebsiella*. A pan-genome comparison of phylogroup A *E. coli* revealed that the ferric citrate uptake system (*fecIRABCD*) was overrepresented in phylogroup A bloom strains compared with non-bloom *E. coli*. A series of experiments were carried out to investigate if the capsule together with ferric citrate uptake system could confer a growth rate advantage on *E. coli*. Capsulated strains had a growth rate advantage regardless of the media composition and the presence/absence of the *fec* operon, and they had a shorter lag phase compared with capsule-negative strains. The results suggest that the *Klebsiella* capsule may facilitate nutrient uptake or utilization by a strain. This, together with the protective roles played by the capsule and the shorter lag phase of capsule-positive strains, may explain why it is only capsule-positive strains that produce elevated counts in response to nutrient influx.

Introduction

Escherichia coli bloom events have been reported from freshwater reservoirs and recreational lakes across Australia (Power *et al.*, 2005). During bloom events, *E. coli* cell counts increase between 10,000 and 100,000 cfu/100 ml of water, well above the 'safe' cut-off level of 235 cfu/100 ml in a single sample of recreational water (Ishii and Sadowsky, 2008; U.S. EPA, 2012). Thirty-five isolates representing eight different strains have been isolated from bloom events that occurred at different time periods (2002–2015) in multiple localities across Australia. Three strains are from the east coast of Australia, and two of these belong to the *E. coli* phylogenetic group A, while the other belongs to group B1. Five strains have been isolated from Western Australia and four of these belong to phylogroup A, while one strain belongs to phylogroup B1. The phylogroup A strains represent the majority of bloom strains isolated to date (Power *et al.*, 2005; Nanayakkara *et al.*, 2019).

Common to all bloom strains is the presence of a capsule originating from *Klebsiella* (Power *et al.*, 2005; Nanayakkara *et al.*, 2019). The *Klebsiella* capsule is an outermost layer of polysaccharide, of approximately 160 nm in thickness, which covers the bacterial cell surface (Amako *et al.*, 1988; Whitfield and Roberts, 1999). In the external environment, the capsule is believed to enhance cell persistence by providing protection from adverse conditions, including ultraviolet (UV) radiation, desiccation, osmotic stress, predation by protozoa, and bacteriophage infection (Weiner *et al.*, 1995; Rendueles *et al.*, 2017).

During non-bloom periods, bloom strains occur in water bodies at low densities (Power *et al.*, 2005). Nutrient influx events such as dust storms and the autumn die off of aquatic vegetation are known to precede elevated counts of bloom strains, where they outcompete all other co-occurring *E. coli* (Littlefield-Wyer, 2006; Water Research Australia, 2019). Given that the capsule enhances cell persistence, capsulated strains are more likely to be present in water than non-capsulated strains when a nutrient influx event occurs. A typical water sample, however, contains multiple

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genotypes of *E. coli* (Casarez *et al.*, 2007; Higgins *et al.*, 2007), and it remains to be investigated if the presence of a capsule alone is sufficient to explain why only capsulated strains, and not all co-occurring *E. coli*, increase in number in response to nutrient influxes.

Iron is a limited nutrient and is fundamentally important for bacterial growth (Neilands, 1981; Braun, 2003; Leiby *et al.*, 2012; Braun and Hantke, 2013; Westrich *et al.*, 2016). Iron received via Saharan dust has been implicated in causing up to a 30-fold increase in culturable *Vibrio* in the Caribbean and sub-tropical Atlantic waters (Westrich *et al.*, 2016). A pan genome analysis of phylogroup A *E. coli* strains revealed that genes of the ferric citrate uptake system; *fecIRABCDE* (Staudenmaier *et al.*, 1989), were present in 100% of the phylogroup A bloom strains compared with non-bloom phylogroup A *E. coli* (<39%) (Nanayakkara *et al.*, 2019). The ferric citrate uptake system operates aerobically for the uptake of iron via a citrate chelator, as dinuclear ferric citrate (Frost and Rosenberg, 1973; Braun, 2003; Grass, 2006). The contribution of this citrate-mediated iron uptake system and the co-occurring *Klebsiella* capsule to the overall growth rate of *E. coli* has not been investigated. The combination of this iron uptake system and capsule may confer bloom strains a growth advantage, thereby leading to the elevated cell counts observed during bloom events.

Experiments were undertaken with the objective of exploring phenotypic attributes and growth characteristics that may contribute to the elevated cell counts observed in bloom events.

Results

The effect of capsule, fec operon, iron, and citrate on growth rate

The media were selected so as to monitor the effect of the *fecIRABCDE* operon on growth through the availability of iron and citrate. The assumption was that in the presence of both iron and citrate, the *fecIRABCDE* operon would be functional for iron uptake. In the absence of either one or both components, the *fecIRABCDE* operon is presumed not to be functional. Overall, the mean growth rate was significantly higher in media with both iron and citrate [iron+cit+], and neither iron nor citrate [iron-cit-], compared with media having only one component. The enhanced growth of strains in the absence of both iron and citrate imply that the *fecIRABCDE* operon had not played a role in enhancing growth. The capsulated strains had a mean growth rate that was significantly higher than that of both capsule-negative genotypes [cap-fec+ and cap-fec-], and grew better than the capsule-negative strains in all media (analysis of variance [ANOVA]: genotype, $p < 0.0001$; media, $p < 0.0001$; genotype $\times$ media, $p = 0.002$) (Fig. 1).

Fig. 1. Growth rate of *E. coli* strains representing the four capsule/fec genotypes in four different media with or without iron and/or citrate.

However, the growth rate of the cap+fec+ strains was more or less similar independent of media, and this caused the significant interaction effect. Overall, the results indicated that the dominant growth response was caused by the capsule.

2,2'-Dipyridyl was added to the same media to eliminate the availability of iron, and thereby to infer if any growth advantage is mitigated in the absence of iron. When dipyridyl was added to the media an overall decline in the growth rate was observed, with the exception of cap+fec- strains (genotype, $p < 0.0001$; media, $p = 0.01$; genotype $\times$ media, $p = 0.60$) (Fig. 2). Across media, the cap+fec- strains had a significantly higher growth rate compared with the other genotypes. On average, growth was better in media with iron present than when it was absent, indicating that dipyridyl had not completely eliminated iron availability. As was observed in the first experiment, the growth rate of the cap+fec+ strains did not vary with media.

Presence/absence of other major iron uptake systems

All strains were positive for the Fe(II) uptake systems encoded by *feoABC* and *efeUOB*. The enterobactin biosynthesis and uptake system were present in all capsulated strains, and 92% (11 out of 12) of capsule-negative strains. Aerobactin was present in 17% of both capsulated and non-capsulated strains. Salmochelin was present in 17% of capsulated strains and absent from non-capsulated strains. Yersiniabactin was present in 25% of capsulated strains and 33% of non-capsulated strains (Supporting Information Table S1).

Growth rate in different carbon sources

The four sugars selected differ in their uptake mechanism through the bacterial inner and outer membrane. The growth

Fig. 2. Growth rate of *E. coli* strains representing the four capsule/*fec* genotypes in media with reduced iron. DP = 2,2'-dipyridyl.

rate of the strains was highest in trehalose, followed by maltose, with the exception of cap-fec- strains. The strains exhibited the lowest growth rates in mannitol and glycerol ($p < 0.0001$). Except for mannitol, the capsulated strains grew better compared with the capsule-negative strains (Fig. 3). In trehalose, the maximum growth rate of the capsule-positive strains was markedly higher compared with the capsule-negative strains, suggesting that harbouring the capsule increases the growth rate in trehalose. This suggests that the capsulated strains might have an enhanced LamB and/or the bacterial phosphotransferase system (PTS)-mediated nutrient acquisition system.

Growth at low and high glucose concentrations

At both low (0.01 mM) and high (10 mM) glucose concentrations, the cap+fec+ strains grew better than the other genotypes (Table 1). At both glucose concentrations, the genotype effect was significant, while the initial cell density N_0 did not have a significant effect on the outcomes (0.01 mM glucose: analysis of covariance [ANCOVA]: genotype, $p = 0.03$; N_0 , $p = 0.2$; genotype $\times N_0$, $p = 0.3$; 10 mM glucose: ANCOVA genotype, $p = 0.03$; N_0 ,

Fig. 3. Growth rate of strains of the four capsule/*fec* genotypes in four different carbon sources.

$p = 0.4$; genotype $\times N_0$, $p = 0.4$). In 0.01 mM glucose, the cap+fec+ strains grew significantly better than the cap-fec- strains, but the growth of the cap+fec- and cap-fec+ strains did not differ significantly from the other genotypes. In 10 mM glucose, the cap+fec+ strains grew significantly better than the cap-fec+ strains, yet the growth of the cap+fec- and cap-fec- strains did not differ significantly from the others. Overall, the cap+fec+ strains grew better than the others, regardless of the glucose concentration.

Length of lag phase in 10 mM glucose

The capsulated strains had a lag phase that was shorter than that of the capsule-negative strains (Table 1). The genotype effect was significant ($p < 0.0001$). The cap+fec+ genotype had the shortest lag phase followed by the cap+fec- strains, while the cap-fec- genotype had the longest lag phase.

Discussion

We show that capsulated strains are at a growth rate advantage relative to non-capsulated strains regardless of the media composition, and that they have a shorter lag phase.

Whole genome sequence comparisons revealed that the ferric citrate uptake system *fecIRABCDE* is overrepresented in the phylogroup A bloom strains (100%) compared with phylogroup A non-bloom *E. coli* (<39%) (Nanayakkara *et al.*, 2019). The ferric citrate system acquires Fe(III) that is bound to a citrate chelator, which shuttles iron to the FecA receptor without citrate entering the cell (Staudenmaier *et al.*, 1989; Braun, 2003; Grass, 2006). Hence, the functioning of the *fec* operon requires both Fe(III) and citrate to be present in the culture media. Citrate on its own is presumed to have no effect on growth as most *E. coli* are unable to utilize citrate aerobically as a sole carbon source due to the absence of an

Table 1. Growth characteristics of *E. coli* strains with respect to possession of a *Klebsiella* capsule and *fec* operon.

Genotype	Yield in 10 mM glucose (cfu/ml), mean $\pm$ SE	Yield in 0.01 mM glucose (cfu/ml), mean $\pm$ SE	Lag phase duration (min)
			in 10 mM glucose, mean $\pm$ SE
Capsule+/fec operon+	706 $\pm$ 84.3	4949 $\pm$ 549	26 $\pm$ 5.5
Capsule+/fec operon-	462 $\pm$ 66.8	2977 $\pm$ 575	36 $\pm$ 5.2
Capsule-/fec operon+	338 $\pm$ 65.3	3442 $\pm$ 529	48 $\pm$ 5.2
Capsule-/fec operon-	498 $\pm$ 78.7	2212 $\pm$ 617	88 $\pm$ 5.4

aerobically functioning transport system (Koser, 1924; Lara and Stokes, 1952; Hall, 1982).

In both experiments involving iron and citrate, on average the capsulated strains grew better compared with the capsule-negative strains, regardless of the presence or absence of the *fec* operon. The growth rate of the cap⁺fec⁺ strains did not vary with the media (Figs 1 and 2). The cap⁺fec⁻ strains and the capsule-negative genotypes grew better when both iron and citrate were present and when neither iron nor citrate was present compared with when one component was absent (Fig. 1). The results of these experiments show that the subtraction of iron using dipyrrolyl reduces the growth rate across genotypes except for cap⁺fec⁻, proving that iron enhances bacterial growth, as reported previously (Leiby *et al.*, 2012; Braun and Hantke, 2013; Westrich *et al.*, 2016); however, the results do not suggest that the presence of the *fec* operon plays a key role in determining the growth rate. Rather, the dominant growth response appears to have been caused by the capsule. The overrepresentation of the *fecIRABCDE* genes in the phylogroup A bloom strains might merely be due to chance, and the absence of the *fec* operon in the phylogroup B1 bloom strains further validates this.

The strains used in the iron/citrate assays carry genes for several iron uptake systems including the siderophore, enterobactin. Multiple iron uptake systems can operate simultaneously (Frost and Rosenberg, 1973; Braun and Hantke, 2013), and certain proteins are shared among different iron uptake systems, for instance, the degraded iron–salmochelin complex can be transported via the enterobactin-related ABC transporter FepBDGC (Hantke *et al.*, 2003). The variable presence of multiple other iron uptake systems in the strains complicates this study, which focuses solely on the ferric citrate system. Despite the presence of other iron uptake systems, the capsule-positive strains were shown to have a growth rate advantage relative to the capsule-negative strains, and that was largely independent of the presence or absence of iron.

Capsulated strains had an enhanced growth rate in trehalose compared with non-capsulated strains. The induction of the outer membrane porin LamB (λ receptor) is crucial for the efficient uptake of trehalose into the cell (Klein and Boos, 1993), which subsequently passes through the PTS system in a phosphorylated form, facilitated by the transporter protein EIIBC^{Tre} (Boos *et al.*, 1990; Travisano and Lenski, 1996). Maltose uptake also occurs via LamB and may enter the trehalose PTS system, and diffusion is facilitated by EIIC^{Tre}, and it is transported in an unphosphorylated form (Nikaido and Vaara, 1985; Benz *et al.*, 1986; Decker *et al.*, 1999; Steen *et al.*, 2014). Capsulated strains did not outperform the capsule-negative strains to the same extent in maltose

as they did in trehalose. We speculate that the enhanced growth in trehalose may be due to efficient diffusion of trehalose via LamB and the PTS system in its phosphorylated form. However, it is not known if PTS transport of phosphorylated carbohydrates is more efficient than non-phosphorylated carbohydrates. Overall, our data suggest that capsulated strains have an enhanced trehalose-related nutrient uptake and/or utilization system, but further studies are required to establish how this occurs.

During non-bloom periods, the water bodies in which *E. coli* bloom events occur are presumably oligotrophic, where sugars and amino acids are at very low concentrations, yielding very low bacterial growth rates (Hobbie and Hobbie, 2013). Bloom strains occur at low densities during non-bloom periods and elevated counts are triggered by nutrient influx events such as dust storms (Water Research Australia, 2019). Glucose concentrations of 1 mM resulted in cell densities of around 10⁸ cells/ml, whereas *E. coli* counts during bloom events are 10²–10³ cells/ml of water. Therefore, the nutrient concentrations needed to obtain the cell densities observed in bloom events would be very low. This suggests that bloom strains grow better than non-bloom strains at low nutrient concentrations. As the nutrients that trigger bloom events are not yet defined (Water Research Australia, 2019), there were constraints in designing artificial media to mimic bloom waters. A comprehensive chemical analysis of bloom water samples is warranted to determine what nutrients promote *E. coli* blooms. As bloom events are rare in occurrence, there are significant practical difficulties in using actual bloom water samples for experimentation.

Studying livestock manure and stream water, Cook *et al.* (2011) showed that *E. coli* strains with group II capsules were less capable of surface attachment compared with those without a capsule. Schembri *et al.* (2004) have also demonstrated that the capsule shields the cell surface adhesion structures and thereby hinder biofilm formation by *E. coli*. It is speculated that non-adherence promotes the transport of *E. coli* cells in the environment (Cook *et al.*, 2011). Rendueles *et al.* (2017) also revealed that capsules are more likely to occur in free-living species than in pathogens, suggesting a significant role of capsules in bacterial survival outside a host. However, apart from the Australian bloom strains (Power *et al.*, 2005; Nanayakkara *et al.*, 2019), solid evidence for a direct involvement of capsules in the survival of *E. coli* in the environment is scant.

All *E. coli* strains associated with bloom events are *Klebsiella* capsule positive, while the frequency of *Klebsiella* capsules in *E. coli* overall is only 7% (Nanayakkara *et al.*, 2019). This means that the *Klebsiella* capsule is a vital attribute of bloom strains. From an ecological perspective, the possession of a capsule is believed to enhance strain persistence by providing protection from

desiccation, osmotic stress, UV radiation and protozoal predation (Weiner *et al.*, 1995; Rendueles *et al.*, 2017). Due to the protective features of the bacterial capsule, it is probable that capsulated strains, compared with non-capsulated strains, are more likely to be present in water when nutrient influx events occur. However, a typical water sample contains multiple genotypes of *E. coli* (Casarez *et al.*, 2007; Higgins *et al.*, 2007), and it might be expected that all strains present should respond to a nutrient influx event. Bacteria in oligotrophic waters are on a starvation-survival lifestyle, and are generally quick to respond when substrate is added (Hobbie and Hobbie, 2013). However, it is only the capsule-positive strains that produce blooms in response to nutrient influxes. The results of the present study indicate that the capsule-positive strains are better able to take advantage of nutrient influxes because they have shorter lag phases, which means that they start dividing earlier, and likely have an enhanced nutrient uptake system, leading to higher growth rates than non-capsulated strains.

The *Klebsiella* capsule region is made up of approximately 14–25 genes, but none of these genes are thought to be actively involved in metabolism. In addition to shorter lag phase, other factors such as efficient nutrient uptake and faster cell division together might drive the elevated counts of capsulated strains. The capsule synthesis regulator *rscB* is implicated in the activation of *ftsZ* which is involved in forming the cell division apparatus in *E. coli* (Gervais *et al.*, 1992; Carballès *et al.*, 1999), and this might yield higher growth rates. Furthermore, the capsule core gene product GalF via its interaction with GalU, leads to increased cellular levels of uridine diphosphate (UDP)–glucose (Marolda and Valvano, 1996), which is a precursor for carbohydrate biosynthesis and hence may lead to the production of energy (Marolda and Valvano, 1996; Berbís *et al.*, 2015; Ebrecht *et al.*, 2015). Structurally, the *Klebsiella* capsule is thicker (160 nm) than the *E. coli* K1 capsule which is less than 10 nm in thickness, and the *Klebsiella* capsule has a denser arrangement of fine fibres compared with the *E. coli* K1 capsule (Amako *et al.*, 1988). It may be that the structure of the *Klebsiella* capsule drives the growth advantage, by being more conducive to nutrient concentration and uptake. It might also be that nutrients are stored in the capsule. Further studies are required to clarify these speculations and determine the role of the *Klebsiella* capsule towards growth promotion.

Experimental procedures

Growth rate in the presence/absence of iron and/or citrate

Strain selection. Assuming that the *fecIRABCDE* operon in conjunction with the *Klebsiella* capsule might confer a

growth advantage on *E. coli*, a collection of 24 phylogroup A *E. coli* strains that were either *Klebsiella* capsule positive or negative (cap+/cap–) and either did or did not carry genes for the *fec* operon (fec+/fec–) were used for the experiments described below. The strains represented four 'genotypes', referred to as cap+fec+, cap+fec–, cap–fec+, and cap–fec–, where each genotype was represented by six strains, and whole genome data were available for each strain. The selected strains have a diversity of sequence-type and capsule-type combinations and were isolated from a variety of sources (Supporting Information Table S2).

Davis minimal media without citrate was prepared using $K_2HPO_4 \cdot 3H_2O$ (7 g/l), KH_2PO_4 (2 g/l), $(NH_4)_2SO_4$ (1 g/l), $MgSO_4$ (0.1 g/l) and thiamine (0.002 g/l) (Leiby *et al.*, 2012). Glucose (1 mM) was used as the carbon source. $C_6H_5Na_3O_7 \cdot 2H_2O$ equivalent to 1.7 mM disodium citrate was added to prepare minimal media containing citrate (cit+). Fe(II) was supplemented immediately before the experiment, from $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ to achieve a final Fe(II) concentration of 10 μ M (iron+). Four different media; iron+cit+, iron+cit–, iron–cit+ and iron–cit– were prepared.

The strains were dilution streaked on MacConkey agar from –80°C freezer cultures and incubated overnight at 37°C. An isolated colony was inoculated into lysogeny broth, incubated overnight at 37°C with shaking at 170 r.p.m., then diluted in sterile normal saline (0.85% wt/vol). For acclimation, an aliquot (100 μ l) was inoculated into Davis minimal media without citrate and incubated for 24 h at 37°C with shaking. A 10 μ l aliquot of the diluted acclimation culture (10^4 cells/ml) was added to the wells of a microtitre plate, each containing 190 μ l of one of the four media, so as to achieve a starting cell density of approximately 500 cells/ml. These cultures were incubated at 37°C in a PowerWave_x 340 (Bio-Tek Instruments) plate reader, which automatically records the optical density (OD) at 600 nm every 10 min, with the plate being shaken at high intensity for 1 min prior to every read. Incubation was carried out until stationary phase was reached. In a microtitre plate, each media sample contained one example of each strain and the experiment was repeated four times. The maximum growth rate was estimated by regressing the natural log of the OD readings against time. The five to six consecutive data points that gave the maximum slope were used to calculate the maximum growth rate.

The same experiment described above was carried out after removing iron from the media, by adding the iron chelator 2,2'-dipyridyl (250 μ M) (Massé and Gottesman, 2002). This was to determine if any growth rate advantage would be mitigated in the absence of iron, or persist.

Presence/absence of genes involved in other major iron uptake systems

E. coli strains carry several other iron uptake systems (Grass, 2006) including enterobactin (Langman *et al.*, 1972; Ozenberger *et al.*, 1987; Braun, 2003; Grass, 2006), aerobactin (de Lorenzo *et al.*, 1986; Braun, 2003), salmochelin (Hantke *et al.*, 2003), yersiniabactin (Braun, 2003; Bultreys *et al.*, 2006), and Fe(II) uptake systems (Braun, 2003; Lau *et al.*, 2015). The presence/absence of

these iron uptake systems in whole genomes sequences of the strains used for the iron/citrate assays was investigated using progressiveMauve genome alignments (Darling *et al.*, 2004) and the MicroScope platform (<https://www.genoscope.cns.fr/agc/microscope/home/>).

Growth rate in carbon sources that differ in their uptake mechanism

The results of the growth experiments indicated that the presence of the capsule enhanced growth. We next wanted to investigate if this growth promotion was due to an enhanced mode of nutrient uptake possessed by the capsulated strains. For this, two mechanisms by which nutrients enter the cell through the bacterial outer membrane (porins OmpF and LamB), and two mechanisms by which nutrients subsequently cross the inner membrane (phosphoenolpyruvate: carbohydrate phosphotransferase system–PTS and non-PTS) were selected. Accordingly, an experiment was performed using four sugars that differ from each other in their uptake mechanism through the bacterial outer membrane (porins OmpF and LamB) and inner membrane (PTS and non-PTS) (Travisano and Lenski, 1996). Mannitol and glycerol diffuse through the outer membrane porin OmpF (Travisano and Lenski, 1996), while maltose and trehalose enter through the porin LamB (Nikaido and Vaara, 1985; Benz *et al.*, 1986; Klein and Boos, 1993). Subsequently, mannitol and trehalose cross the inner membrane and enter the cytoplasm via the PTS system (Erni, 1989; Boos *et al.*, 1990; Deutscher *et al.*, 2006), while glycerol and maltose enter the cytoplasm via the non-PTS system (Postma *et al.*, 1993; Deutscher *et al.*, 2006). A close association between the transport of trehalose and maltose is indicated, and evidence suggests that maltose may also be transported via the trehalose PTS system (Decker *et al.*, 1999; Steen *et al.*, 2014). Though the four sugars do not represent the nutrient sources *E. coli* encounters in non-enteric habitats, they were selected because their uptake systems are well established (Travisano and Lenski, 1996), and because the primary focus of the experiment was to explore if any of the selected nutrient uptake systems was more enhanced in capsulated strains compared with non-capsulated strains, based on their growth in the sugars selected. It was hypothesized that an enhanced uptake would contribute to produce better growth. A constraint in selecting more relevant sugars was that the nutrients that promote *E. coli* blooms are not yet defined (Water Research Australia, 2019).

A single colony was taken from MacConkey agar and inoculated into Davis minimal media (10.6 g/l) having glucose (1 mM) and thiamine (0.002 g/l). These acclimation cultures were incubated for 24 h at 37°C with shaking at 170 r.p.m. Davis minimal media each containing one of

the four sugars; mannitol, glycerol, maltose, or trehalose (180 mg/l), was added to wells of a microtitre plate in 190 µl aliquots. The acclimation cultures were diluted to a cell density of 10⁴ cells/ml in sterile normal saline (0.85% wt/vol) and 10 µl was used to inoculate the media in the microtitre plate, so as to have a starting cell density of approximately 500 cells/ml. Incubation was done in an automated PowerWave_x 340 plate reader at 37°C where the OD of the cultures was measured at 600 nm every 10 min. The plate was shaken at high intensity before each read and incubation continued until stationary phase was reached. The maximum growth rates were calculated as explained previously.

Growth at low and high glucose concentrations

The following experiment was conducted to determine if capsulated strains have a growth advantage in either low (0.01 mM) or high (10 mM) glucose concentration. Strains were grown for a prescribed amount of time and then cell counts were determined.

Minimal media containing a low glucose concentration (0.01 mM) was used for inoculating the strains at time (*t*) = 0, and cell densities were determined. The cultures were incubated for 5.5 h at 37 °C with shaking, then the final cell densities were calculated. A lag phase of 0.5 h was assumed when determining the incubation time. The incubation time was adjusted to 3 h for high glucose concentration (10 mM) inoculum, taking the faster growth rate into account. The final cell density (*N_f*) was compared between genotypes, using the starting cell density (*N₀*) as a covariate.

Length of lag phase in 10 mM glucose

The lag time of the strains in 10 mM glucose was determined as follows: Davis minimal media with glucose (1 mM) was inoculated with a single colony followed by incubation for 24 h at 37°C with shaking. A 10 µl aliquot of the 24 h culture was used to inoculate each well of a microtitre plate containing 190 µl of Davis minimal media having glucose (10 mM). The microtitre plate was incubated in an automated PowerWave_x 340 plate reader at 37 °C and OD readings at 600 nm were taken every 5 min. The plate was shaken at high intensity for 1 min before every read. The logarithm of the OD readings was plotted against time to obtain the growth curves. The lag time was the time interval before the first two consecutive increases in OD, as obtained from the growth curve. The experiment was replicated four times.

Statistical analysis

Statistical analyses were performed using JMP 12.2.0 software (2015 SAS Institute, Inc.). ANOVA was conducted

with a full factorial model, with genotype, media, and interaction as model effects, and technical replicates were incorporated as a random effect. The final cell density (N_t) at low (0.01 mM) and high (10 mM) glucose concentrations were analyzed by genotype, using the starting cell density (N_0) as a covariate.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Iron uptake systems present in the *E. coli* strains used in the present study.

Table S2. Characteristics of the *E. coli* strains used for the growth experiments.