Salmonella stress response mediates growth repression in succinate media

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Abstract

Bacteria have evolved to sense and respond to their environment by altering gene expression and metabolism to promote growth and survival. In this work we demonstrate a novel phenotype wherein *Salmonella* actively represses its growth when using dicarboxylates such as succinate as the sole carbon source. This repression is mediated by RpoS, the RssB anti-adaptor IraP, and to a lesser degree the stringent response. We also show that small amounts of proline or citrate can act as inducers of growth in succinate media. Ultimately this regulatory cascade represses *dctA*, encoding the primary dicarboxylate importer, and constitutive expression of *dctA* induced growth. Additionally, we show that this phenotype diverges between *Salmonella* and its close relative *E. coli*, and replacing the *Salmonella dctA* promoter with that of *E. coli* was sufficient to abolish growth repression. We hypothesized that this divergence might reflect an adaptation to *Salmonella*'s virulent lifestyle including survival in macrophage where levels of succinate increase in response to bacterial LPS. We found that impairing *dctA* repression had no effect on *Salmonella*'s survival in acidified succinate or in macrophage but propose alternate hypotheses of fitness advantages acquired by repressing dicarboxylate uptake. In summary we identify a novel *Salmonella* phenotype and insight into its regulation. This phenotype is divergent from *E. coli* and may represent an adaptation to *Salmonella*'s virulent lifestyle.

Importance

Bacteria have evolved to sense and respond to their environment to maximize their chance of survival. By studying differences in the responses of pathogenic bacteria and closely related non-pathogens, we can gain insight into what environments they encounter inside of an infected host. Here we demonstrate that *Salmonella* diverges from its close relative *E. coli* in its response to the metabolite succinate and other dicarboxylates. We show that this is regulated by stress response proteins and ultimately can be attributed to *Salmonella* repressing its import of dicarboxylates. Though this exclusion of dicarboxylates did not influence *Salmonella*'s survival in macrophage, we propose other advantages that this trait may provide *Salmonella* within an infected host. Understanding this phenomenon may reveal a novel aspect of the *Salmonella* virulence cycle, and our charcterization of its regulation yields a number of mutant strains that can be used to further study it.

Introduction

Bacteria must adapt to changing environmental conditions by sensing their surroundings and integrating signals to initiate rapid growth in nutrient rich situations or instigate defence mechanisms and metabolic hibernation in response to stress (1). Pathogenic bacteria have further adapted their mechanisms of sensing and reacting to their environment such that they are especially equipped to survive and replicate within their particular host niche. A useful approach to study differences in pathogenic and commensal bacteria is comparing the genomes and phenotypes of two well characterized enterobacteria, *E. coli* and *Salmonella*. These bacteria are closely related, yet *Salmonella* has acquired a number of adaptations that accommodate its virulent lifestyle; for example allowing *Salmonella* to invade tissues and survive within host cells such as macrophage, which is important for *Salmonella* virulence (2-6).

Metabolic modulation is important during conditions of stress as exemplified by the bacterial stringent response, wherein the second messenger molecule, guanosine 5'-disphosphate 3'-diphosphate (ppGpp) is produced by RelA or SpoT in response to amino acid starvation or other cellular stress cues (7-10). Bacteria can also alter gene expression using the general stress response sigma factor RpoS (σ^S), which has been linked to virulence in a number of pathogenic bacteria by contributing to virulence gene expression and survival within an infected host (11-13). RpoS can be activated in response to a variety of conditions including starvation, hyper-osmolarity and oxidative stress, and can be regulated at all levels of synthesis from transcription to protein degradation where it is recognized by the adaptor RssB (also known as MviA, SprE, or ExpM) and chaperoned to the ClpXP protease (14-18). In response to specific stresses, the anti-adaptors IraP, RssC (IraM in *E. coli*) and IraD can be induced, which impair RssB and thereby rapidly stabilize RpoS (19-21). Strains with reduced *rpoS* activity have been demonstrated to grow faster than wild-type *E.* coli when grown using the weak carbon source succinate,

suggesting that relying on succinate can induce RpoS and repress growth (22-24). Furthermore, aerobic growth using succinate relies on the dicarboxylate importer, DctA, which is known to be regulated by the DcuSR two-component system in response to dicarboxylates, as well as by DctR (YhiF) in *E. coli* lacking ATP Synthase activity (25-27).

Our previous studies of *Salmonella* Elongation Factor P (*efp*) mutants (which have reduced expression of ATP Synthase genes) employed phenotype microarrays that led us to find that, like *rpoS* mutants, deletion of *efp* results in improved growth using succinate as a carbon source (28-31). Here we demonstrate that in response to dicarboxylic acids as a sole carbon source, wild-type *Salmonella* shuts down its growth for an extended yet consistent length of time and that this phenotype diverges between *Salmonella* and *E. coli*. We go on to characterize this phenotype, examinine the underlying regulatory mechanism, and propose evolutionary advantages it may offer *Salmonella*.

Results

Salmonella delays its growth using dicarboxylic acids as a sole carbon source

Our previous work investigating *Salmonella* with impaired EF-P activity demonstrated that these mutants display a hyper-active metabolism relative to wild-type when grown under specific nutrient limited conditions (28, 29). Phenotype microarrays (31) conducted in these works appeared to show a lack of growth for wild-type *Salmonella* when using succinate as the sole carbon source. To recapitulate this phenotype, we tested the growth of *Salmonella* in minimal media containing succinate as the sole carbon source. We found that the *efp* mutant, as well as an *rpoS* mutant used as a positive control, was able to grow readily (Figure 1). In contrast, wild-type *Salmonella* exhibited an extended lag phase for over 30 hours before initiating logarithmic growth. These data suggest that *Salmonella* is capable of growing on

succinate yet makes a regulatory decision not to using a mechanism involving RpoS and some protein(s) requiring *efp* for its efficient translation. The dicarboxylic acid transporter, DctA, was also required for growth and an isogenic *dctA* deletion strain showed no sign of growth by 48 hours. Moreover, the extended lag of wild-type *Salmonella* occurred during growth on two other dicarboxylic acids, fumarate and malate (Figure 1C). Consistent with succinate, the *efp* mutant grew significantly earlier using these compounds as a sole carbon source. This suggests that the growth repression instigated by wild-type *Salmonella* is not specific to succinate but also occurs during growth using other dicarboxylic acids.

Many *Salmonella* but few *E. coli* strains delay growth using succinate

To address whether the delayed growth of *Salmonella* using dicarboxylic acids as a carbon source is a genus-specific or more common phenomenon, we compared growth of *Salmonella* to the closely related bacterium, *E. coli*. We found that a lab strain of *E. coli* grew more readily in minimal media using succinate as a sole carbon source (Figure 2A). To assess a more comprehensive number of strains, we tested growth using succinate for all 105 non-typhoidal strains in the *Salmonella* Genetic Stock Centre (SGSC) collection, as well as all 72 strains of the *E. coli* Reference (ECOR) collection. Though there are exceptions, the majority of *E. coli* strains grew more readily in succinate media compared to most *Salmonella* strains, which displayed extended lag phases (Figure 2B-D). Once logarithmic growth was initiated, *Salmonella* also appeared to trend towards a slightly longer doubling time than the majority of *E. coli* strains (Figure 2C and E). To ensure that the observed effects were not due to variations in RpoS activity, each strain was also screened for catalase activity as an analog of functional RpoS. Regardless of catalase activity, the trend was maintained that *E. coli* strains in general showed a shorter lag phase than *Salmonella* when using succinate as the sole carbon source (Figure S1).

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116 **IraP contributes to growth repression in succinate media**

117 Since mutation of the *rpoS* gene resulted in early growth on succinate, we investigated
118 how regulators of *rpoS* could be involved in sensing succinate media as a stress and instigating
119 growth shutdown via RpoS. In response to specific stressors, the RpoS protein can be stabilized
120 by three known anti-adaptor proteins, IraP, RssC and IraD, which inhibit the adaptor RssB to
121 prevent the degradation of RpoS (14, 19, 20, 32). Similar to the *ΔrpoS* strain, targeted deletion
122 of the *Salmonella iraP* gene led to drastically earlier growth in minimal media with succinate as
123 the sole carbon source and this phenotype could be partially complemented by expressing IraP
124 from its native promoter on a plasmid (Figure 3). In contrast, deletion of the other anti-adaptors,
125 RssC and IraD, had only minor effects. Deletion of the *rssB* gene itself yielded inconsistent
126 results, likely due to suppressor mutations in *rpoS* arising to compensate for the lack of RssB-
127 mediated RpoS degradation. These findings demonstrate that IraP plays a role in repressing
128 *Salmonella*'s growth using succinate as the sole carbon source.

129

130 **Proline and citrate induce growth in succinate media**

131 To examine how *iraP* may be induced and whether *Salmonella* requires additional
132 nutrients to grow, we supplemented succinate media with various compounds. We initially
133 noticed that supplementation with small amounts of LB media could induce earlier growth
134 (Figure S2). Further investigation demonstrated that addition of minute amounts of either proline
135 or citrate induced growth in succinate media in a manner resembling a diauxy wherein growth is
136 repressed again following depletion of the proline or citrate (Figure 4A and C). This suggests
137 that these compounds can provide *Salmonella* with a metabolite that is either limiting with
138 succinate as a sole carbon source or can act as a regulatory signal to alleviate growth repression.

Interestingly, the inducing metabolite does not appear to be proline itself as growth induction by proline (but not citrate) required the enzyme PutA, which degrades proline to glutamate.

We further examined proline- and citrate-induced growth using a *Salmonella relA spoT* double mutant that cannot produce the stringent response secondary messenger ppGpp. This $ppGpp^0$ strain showed slightly earlier growth in succinate than wild-type *Salmonella* but moreover did not repress growth following proline or citrate stimulation (Figure 4B and D). This suggests that following induction by proline or citrate, ppGpp plays a significant role in restoring *Salmonella's* repressed growth state and this signal may contribute to growth shutdown in the absence of these inducers.

Repression of succinate import accounts for growth lag

We hypothesized that RpoS may repress the expression of the *dctA* gene, encoding the primary dicarboxylate transporter, and thereby restrict *Salmonella* from taking up dicarboxylates such as succinate for consumption. To test if such a repression accounts for why wild-type *Salmonella* does not grow on succinate, we constitutively expressed *dctA* from a plasmid. Indeed, constitutive expression of *dctA* (but not *lacZ*) resulted in earlier growth in succinate media suggesting that the limiting factor in *Salmonella*'s growth was synthesis of the dicarboxylate importer DctA (Figure 5A and C). It therefore appears that, in *Salmonella*, RpoS represses *dctA* expression and restricts the uptake of dicarboxylic acids.

The *E. coli dctA* promoter is sufficient to induce *Salmonella* growth using succinate

In light of the finding that the *Salmonella dctA* gene is repressed in succinate media, we examined the role of the *dctA* promoter (P_{dctA}). Since the growth phenotype appears to be divergent between *Salmonella* and the closely related species *E. coli*, we compared the P_{dctA} from

these bacteria (Figure S3). It is possible that *Salmonella* contains a transcriptional repressor that is not present in *E. coli*, so we generated transcriptional fusion plasmids and tested the two *dctA* promoters when expressed in *E. coli*. We found that the *dctA* promoter from *Salmonella* was expressed to a lower degree (Figure S4), suggesting that it contains a distinctive region that is recognized by a common regulator that is also present in *E. coli*.

To further examine the impact of the *dctA* promoter, we swapped the *E. coli* P_{dctA} into the *Salmonella* chromosome using an upstream chloramphenicol resistance cassette to select for successful recombination. Replacing the 500bp upstream of the *Salmonella dctA* start codon with those of *E. coli* was sufficient to abolish *Salmonella*'s ability to repress its uptake of dicarboxylic acids and this strain grew readily in succinate media (Figure 5B and D). As a control, using the same method to insert *Salmonella*'s native *dctA* promoter yielded no difference from wild-type *Salmonella*. Of note, reducing the swapped region to 200bp maintained the full effect, but swapping only 54bp (constituting the 5' untranslated region) resulted in only a slight restoration of growth.

Restricting succinate import does not influence survival in macrophage cell lines

To probe the question of why *Salmonella* may have acquired the trait of blocking dicarboxylate utilization and what evolutionary advantage it may gain by it, we considered that succinate levels increase significantly in activated macrophage, an environment that *Salmonella* (but not *E. coli*) has adapted to survive in effectively (5, 6, 33). In the *Salmonella*-containing vacuole, the pH reaches approximately 5.0 and the lower estimates reach pH 4.4, which is comparable to the acid dissociation constants of succinate ($pK_{a1,2}$ = 4.2, 5.6) (34, 35). This suggests that in the acidified phagosome, succinate may become protonated and act as a proton

shuttle to acidify the bacterial cytoplasm. The ability of *Salmonella* to restrict its uptake of succinate could therefore provide a survival advantage in this environment.

Constitutive overexpression of *dctA* but not *lacZ* led to decreased survival in both acidifed succinate media and in the human monocyte THP-1 cell line (Figure S5). However, overexpression of *dctA* has been demonstrated to be toxic to *E. coli* and we found that survival in acidified succinate was just as low for a *dctA* point mutant (N301A) that is defective for succinate transport (36). This implicated that the reduced survival was not due to succinate uptake but rather was an artifact of *dctA* overexpression to toxic levels. To bypass this artifact, we tested the *Salmonella* strain containing the chromosomal *dctA* promoter from *E. coli,* which grows readily in succinate media (Figure 5) yet does not constitutively overexpress *dctA* from a plasmid and so does not exhibit the associated toxic effects. Using this strain we found no decrease in survival relative to wild-type *Salmonella* in acidifed succinate media or in human (THP-1) or mouse (J774) macrophage cell lines (Figure 6). As well, deletion of *dctA* or *iraP* genes did not appear to significantly influence *Salmonella* survival in THP-1 macrophage.

Discussion

In this work we demonstrate an uncharacterized *Salmonella* phenotype wherein it diverges from *E. coli* and restricts its growth using dicarboxylates as a sole carbon source. This phenotype does not reflect a metabolic inability of *Salmonella* to utilize dicarboxylates as we show multiple mutations in regulatory genes that allow the cells to grow early in succinate media. Rather it appears that *Salmonella* employs RpoS, IraP and to some degree RelA or SpoT to sense this environment as a stress condition and shut down expression of *dctA* to restrict its import of dicarboxylic acids. Interestingly, growth was stimulated by the addition of small amounts of citrate or proline, suggesting that these supplements can alleviate the repression. As

well, growth repression following proline or citrate depletion required ppGpp. Since the stringent response and ppGpp can impact the expression of *rpoS* and RssB anti-adaptors including *iraP*, RelA or SpoT may be involved in the initial sensing of succinate media as a stress and activating the IraP- and RpoS-mediated shutdown of growth (14, 37-39). These findings propose a working model of the mechanism of *Salmonella* growth repression in succinate media (Figure 7).

The growth repression phenotype described here is divergent between *Salmonella* and the closely related species, *E. coli*, as evidenced by sampling a range of the genetic diversity of these bacteria using the SGSC and ECOR collections. Yet there are exceptions, including a number of *Salmonella* strains that grew early despite having a catalase positive phenotype. In all instances where multiple strains from the same *Salmonella* serovar were tested, at least one exhibited an extended lag phase (Dataset S1). Thus the earlier growth does not appear to be a trait of particular serovars but rather may reflect individual strains having lost (or never acquired) the delayed growth phenotype. This could occur by mutations in genes other than *rpoS*, such as *efp* or *iraP*, that grant early growth in succinate while remaining catalase positive. Other exceptions include multiple *E. coli* strains that show an extended lag in their growth using succinate. While it is possible that these have mutations in genes required for the uptake of succinate (such as *dctA* or *dcuSR*), these may be genuine variations in how *E. coli* strains respond to succinate.

Replacing the *Salmonella dctA* promoter with that of *E. coli* was sufficient to abolish *Salmonella*'s ability to repress its uptake of succinate. This suggests that since diverging from *E. coli Salmonella* has obtained a regulatory element in its *dctA* promoter that allows it to be repressed under these conditions. Our data demonstrate that RpoS is involved in this regulation, yet RpoS is a transcriptional activator. The lack of growth on dicarboxylates suggests that *dctA* is tightly repressed, suggesting against solely sigma factor competition for RNA polymerase, but rather that RpoS likely acts via an intermediate and yet undetermined transcription factor (Figure

7). While it remains possible that this factor could involve a small RNA, our finding that swapping just the 5' untranslated region of *dctA* (P_{dctA} 54) was insufficient to reverse growth repression suggests a protein factor acting on the promoter at the transcriptional level.

The difference in the response of *Salmonella* and *E. coli* to dicarboxylic acids may offer important clues to identifying the evolutionary advantage conveyed by this adaptation. Since many of the traits that *Salmonella* has acquired since their divergence are related to its pathogenic lifestyle it follows that this phenotype may reflect a situation that *Salmonella* encounters during infection of a host. Interestingly, it has recently been shown that *Salmonella* utilizes microbiota-derived succinate in the lumen of the inflamed gut (40). This suggests that the uptake of succinate in the gut employs anaerobic dicarboxylate transporters rather than DctA, or that an inducing compound such as proline or citrate is present in this environment and can stimulate succinate uptake.

The recent finding that succinate accumulates to high levels in activated macrophage suggests that *Salmonella*'s intracellular survival may represent the crucial selective environment that has led to the *dctA*-repression phenotype (33). It is conceivable that *Salmonella* recognizes the succinate produced by activated macrophage and restricts its uptake of this dicarboxylate in response. Our examination of *Salmonella* strains that are impaired in their regulation of succinate uptake identified no survival defect in acidifed succinate or in macrophage cell lines. However, it remains possible that this repression phenotype is related to other aspects of the *Salmonella* virulence cycle beyond survival. For instance, *Salmonella* may restrict its import and consumption of succinate in order to maximize macrophage succinate levels leading to the production of the pro-inflammatory citokine IL-1β (33). This would not grant *Salmonella* an advantage for survival in macrophage *per se*, but would give *Salmonella* remaining in the gut lumen a growth advantage by maximizing the immune system's oxidative burst and subsequent

production of tetrathionate, a compound that *Salmonella* is distinctively equipped to use as a terminal electron acceptor (41-43). Thus if *Salmonella* were to import and consume succinate in macrophage, the inflammatory response may be deterred, restricting the ability of *Salmonella* to outcompete the gut microbtioa during infection.

In summary, we demonstrate a divergent phenotype between *Salmonella* and *E. coli* involving regulation by RpoS, IraP and the stringent response to repress *dctA* expression and succinate uptake. This repression of dicarboxylate uptake may reflect an adaptation for *Salmonella* virulence, however its specific evolutionary benefit remains to be elucidated.

Materials and Methods

Bacterial strains and plasmids

As described previously, lambda red recombination (28, 44) and subsequent P22 phage transduction (45) was used to generate all of the gene knockout mutants in *Salmonella enterica subsp. enterica* serovar Typhimurium (*S.* Typhimurium) strain 14028s. *E. coli* mutants were obtained from the Keio collection in the K12 BW25113 strain background (46). To sample the genetic diversity of *Salmonella* and *E. coli* isolates, the *Salmonella* genetic stock centre (SGSC) SARA (47), SARB (48), and SARC (49) collections were employed and compared to the *E. coli* reference (ECOR) collection (50).

The full length *DctA* ORF was expressed from the pXG10sf plasmid under the control of the constitutively active PLtet0-1 promoter (51, 52). The IraP complementation plasmid was generated by inserting the *iraP* ORF and the upstream 300bp into pXG10sf. For promoter expression, the *dctA* promoter (500bp upstream of the *dctA* start codon) was inserted into pXG10sf to drive expression of superfolder GFP(52, 53). To generate the chromosomal *dctA* promoter swap strain, 500bp upstream of the *E. coli dctA* start codon, along with a

chloramphenicol resistance cassette for selection, was inserted into the corresponding location of the *Salmonella* chromosome.

Growth using dicarboxylates as a sole carbon source

Overnight LB cultures inoculated from single colonies were resuspended in MOPS minimal media with no carbon source to an optical density (OD_{600}) of approximately 1.75. This suspension was used to inoculate (1/200 dilution) MOPS minimal media containing 0.2% carbon source (succinate unless otherwise indicated). Growth was conducted in a TECAN Infinite M200 plate reader at 37ºC with shaking and OD_{600} was read every 15 minutes. For salts and hydrates of carbon sources the final concentration reflects the percent of the carbon source itself (e.g., 0.2% succinate was made as 0.47% sodium succinate dibasic hexahydrate).

For the SGSC and ECOR collections screen, 47 strains were assessed in duplicate per run in a 96-well plate. Wild-type and *rpoS* mutant *Salmonella* were included on every plate as quality controls. Each strain was tested on at least three separate days.

Catalase assay

For each replicate of the SGSC and ECOR collections screen, each strain was tested for catalase activity as an analog for RpoS function (54). In parallel to the LB overnight cultures used as inoculum, 10µl of each culture was spotted onto an LB plate. The next day the spots were tested for catalase activity by the addition of 10µl hydrogen peroxide. Bubbling was scored compared to wild-type (catalase positive) and *rpoS* mutant (catalase negative) *Salmonella*.

Acid survival

LPM media was made as described previously (55) and succinate or itaconate were added to either 0.2% or 0.4% as indicated in figures. The pH of the media was then adjusted to 4.4. LB overnight cultures were resuspended to an OD of 0.1 in acidified media and incubated in a 37ºC water bath. At time points, samples were taken, serial diluted and plated for colony forming units (CFU).

Intra-macrophage survival

The THP-1 human monocyte cell line and the J774 mouse macrophage cell line were maintained in RPMI Medium 1640 (with L-glutamine) supplemented with 10% FBS and 1% Glutamax, and grown at 37ºC and 5% CO_2. For infection assays, THP-1 cells were seeded in 96-well plates at 50,000 per well with 50nM PMA (phorbol 12-myristate 13-acetate) added to the media to induce differentiation to adherent macrophage. After 48h, the media was replaced with normal growth media (no PMA) overnight. For infections with J774 macrophage the cells were seeded in 96-well plates at 50,000 per well overnight. *Salmonella* in RPMI were added onto seeded cells at a multiplicity of infection (MOI) of approximately 20 bacteria to 1 macrophage and centrifuged for 10 minutes at 1000rpm for maximum cell contact. After centrifuging the plate was placed at 37ºC (5% CO_2) and this was called ‘time 0’. After 30 minutes, non-adherent *Salmonella* were washed off by three washes with PBS followed by replacement with fresh media containing 100 µg/ml gentamicin to kill extracellular *Salmonella*. At 2 hours the media was replaced with media containing gentamicin at 10 µg/ml. At timepoints, intracellular bacteria were recovered using PBS containing 1% Triton X-100 and vigorous pipetting. Samples were serially diluted and five 10µl spots were plated for CFU counting. Each sample included three separate wells as technical replicates (a total of 15 x 10µl spots counted per biological replicate).

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Figure Legends

Figure 1: *Salmonella* displays an extended lag phase using dicarboxylic acids as a sole carbon source but *efp* and *rpoS* mutants do not. **A)** Representative growth curve of *Salmonella* Typhimurium 14028s strains grown in MOPS minimal media with 0.2% succinate as the sole carbon source. Data shows optical density at 600nm (OD_{600}) for 48 hours from the time of inoculation. The data shown is representative of greater than three biological replicates. An OD_{600} of 0.1 is emphasized by a dashed line. **B)** Graphs showing the average time in hours that wild-type (WT) or mutant *Salmonella* takes to reach an OD of 0.1 as an analog of the length of lag phase using succinate as the sole carbon source. The data shows the average of at least three biological replicates and error bars show one standard deviation. >48h indicates the strain did not grow by 48 hours. **C)** As in (B) but comparing the use of three different dicarboxylic acids as the sole carbon source.

Figure 2: *Salmonella* grows significantly later than *E. coli* using succinate as the sole carbon source. Growth in MOPS minimal media with 0.2% succinate as the sole carbon source. Growth was conducted in a TECAN Infinite M200 plate reader and reads were taken every 15 minutes. **A)** Representative growth curve. An OD_{600} of 0.1 is emphasized by a dashed line. **B)** Percentage of SGSC *Salmonella* (green line) or ECOR *E. coli* (blue line) strains that (on average) had surpassed an OD_{600} of 0.1 by indicated times post inoculation. Red line is for *Salmonella* but excludes the 14 tested strains of the SARC collection that do not belong to subspecies *enterica*. **C)** Overview of 105 *Salmonella* strains (SGSC collection) and 72 *E. coli strains* (ECOR collection). Each strain is plotted by average time it takes to reach OD 0.1 (x-axis) compared to doubling time during growth from OD 0.1 to OD 0.2. All *Salmonella* are coloured black and all *E. coli* strains are coloured red. Strains with at least one replicate that did not grow by 72 hours

are shown as triangles; these values are the average of the remaining replicates. Inset at top right shows zoomed out view to include outliers. **D)** Average time in hours for strains to reach an OD of 0.1. Data compares all 105 *Salmonella* strains to all 72 *E. coli* strains tested. Line indicates the median, boxes show the 25th to 75th percentiles, and whiskers show the 10th to 90th percentiles. An unpaired t-test with Welch's correction indicated a p-value < 0.0001. **E)** As in (D) but comparing doubling time in hours. An unpaired t-test with Welch's correction indicated a p-value < 0.05 with all data points included or p-value < 0.0001 when excluding slow growing outliers (doubling time > 5h).

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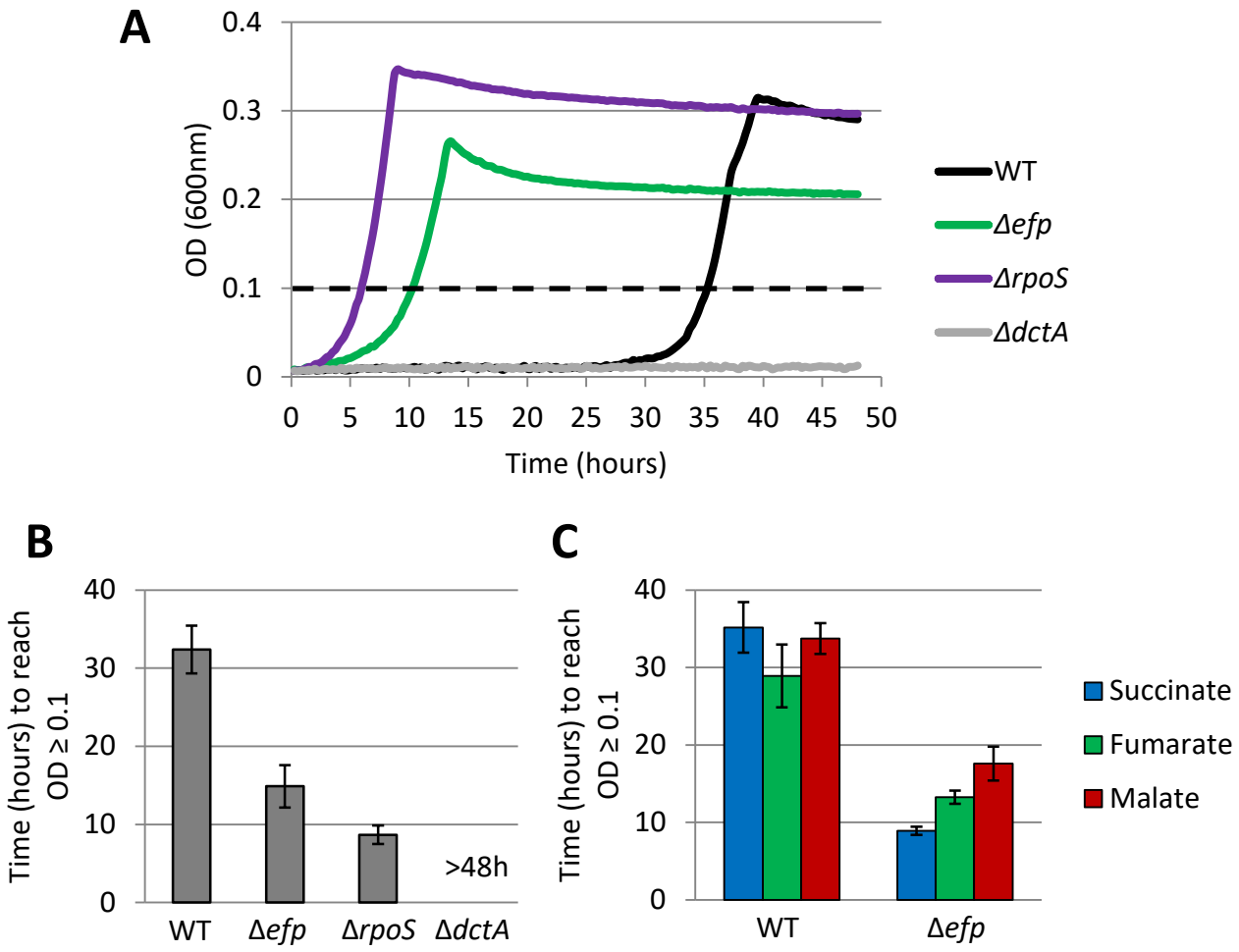

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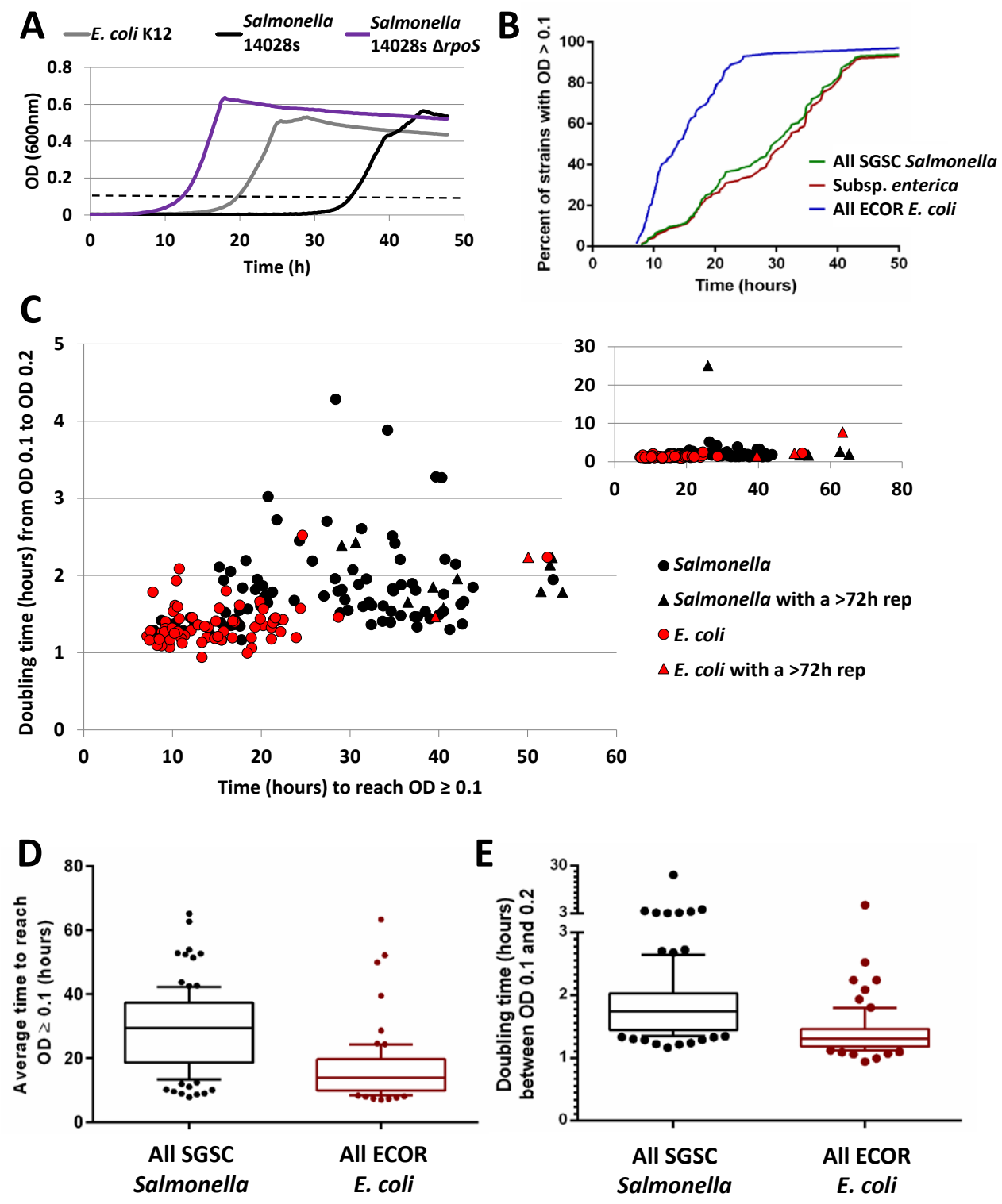

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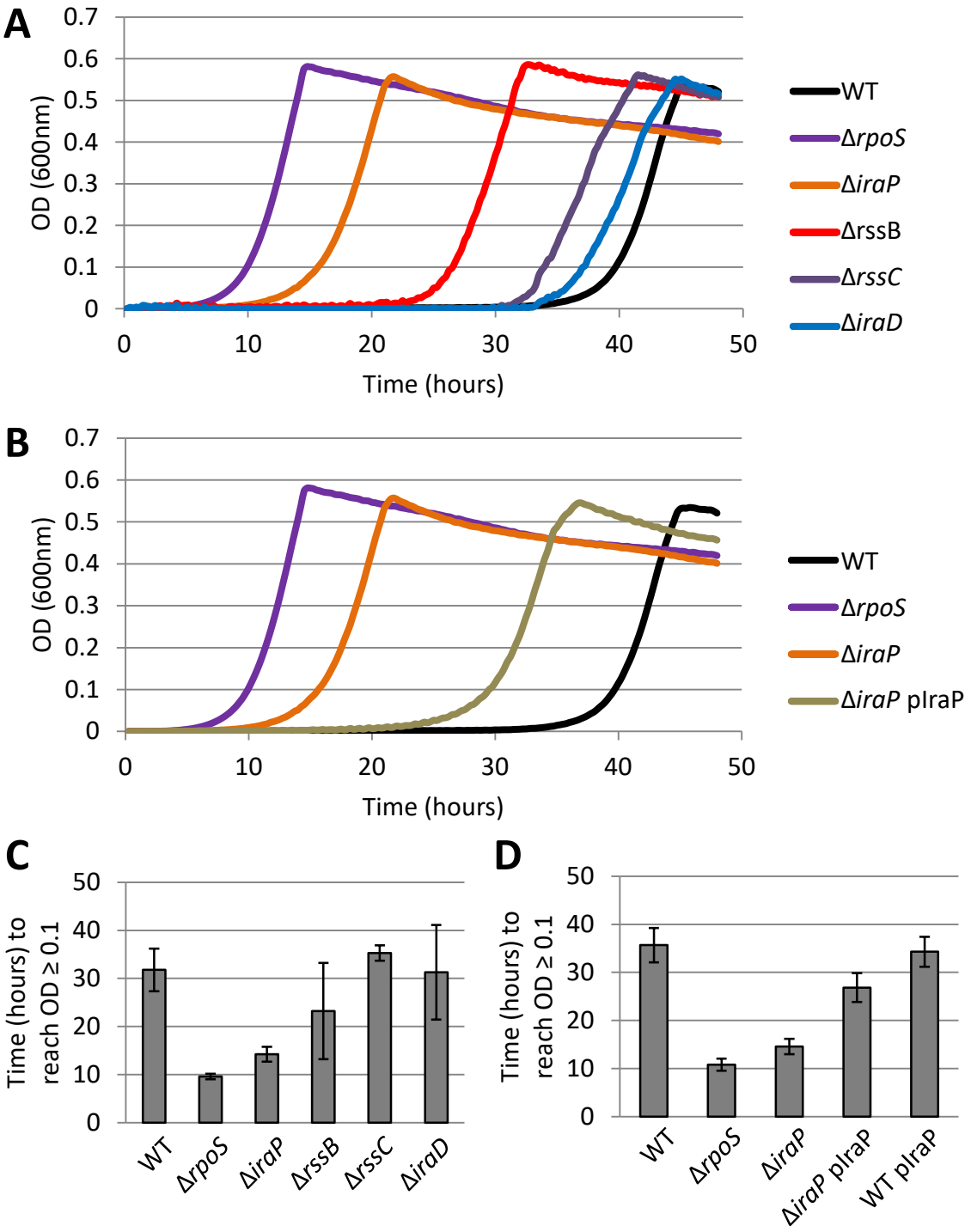

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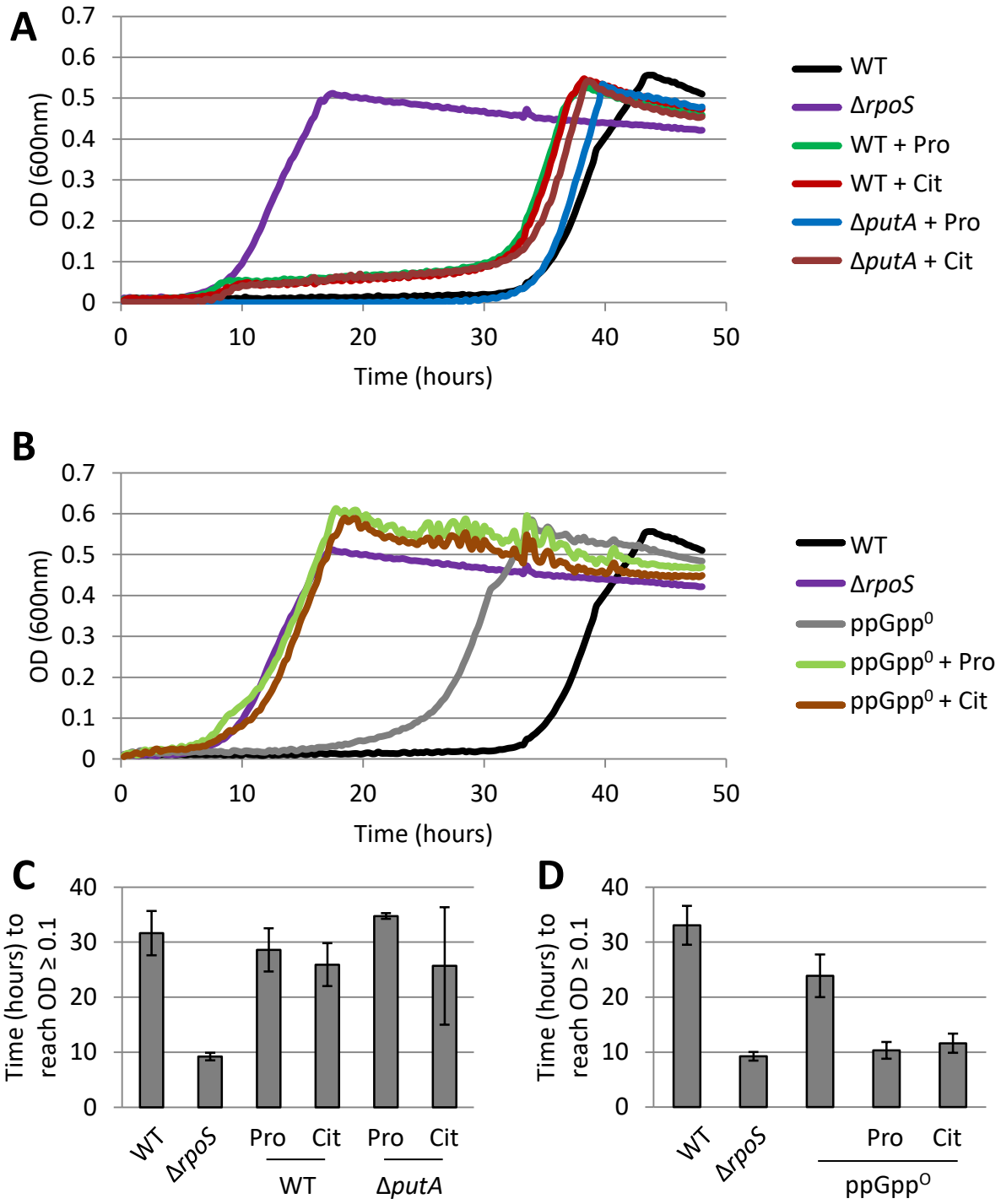

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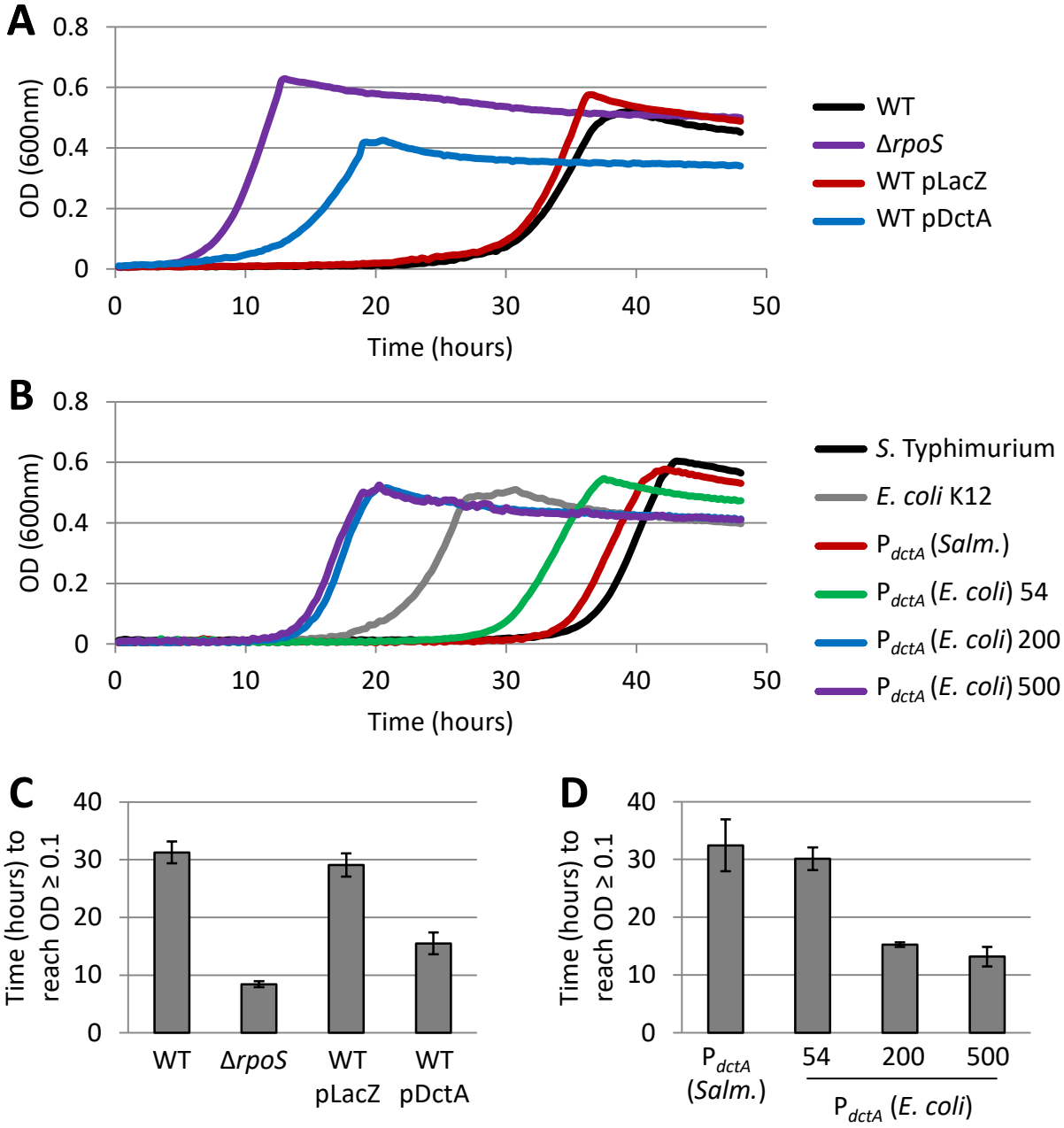

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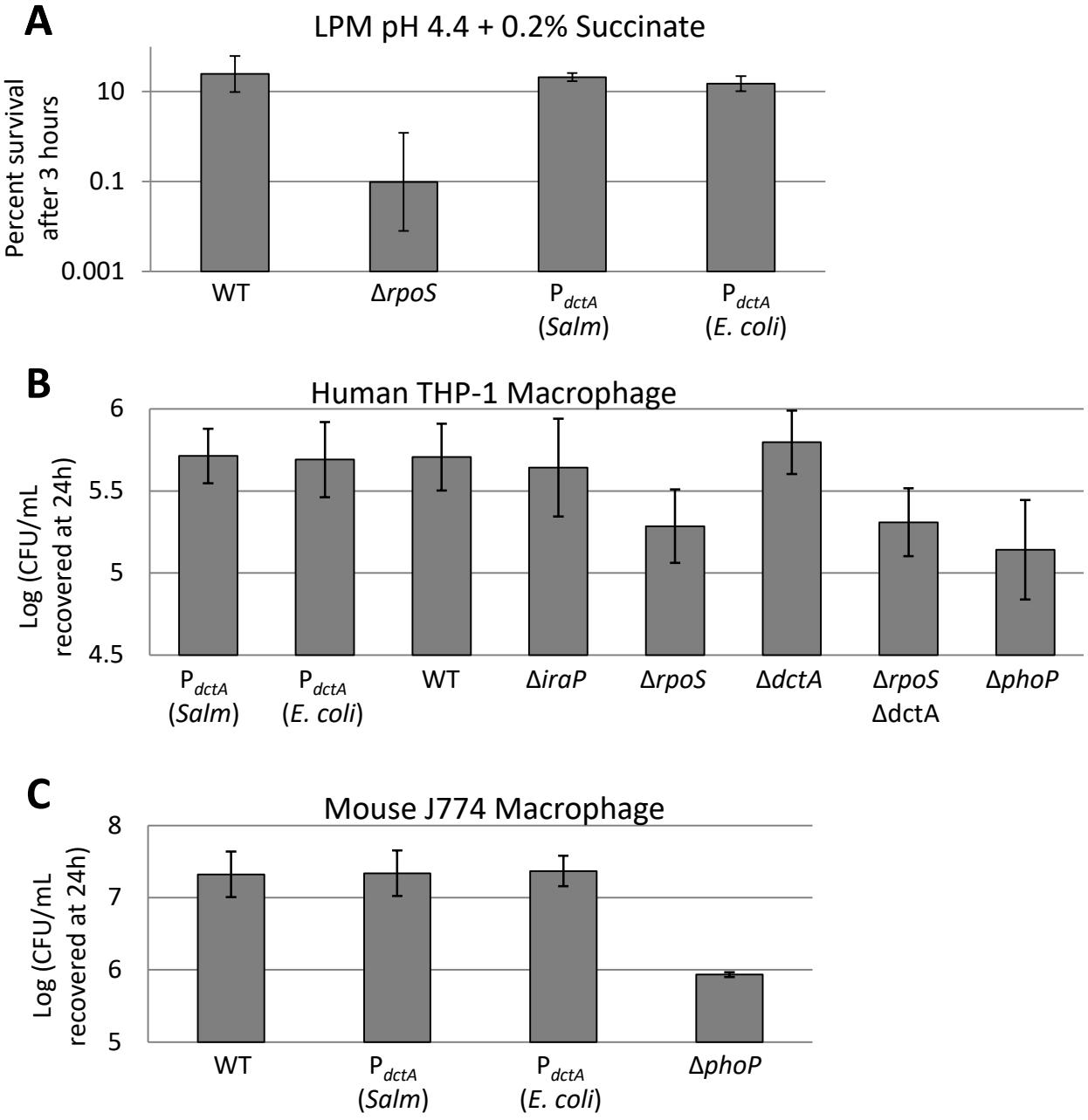

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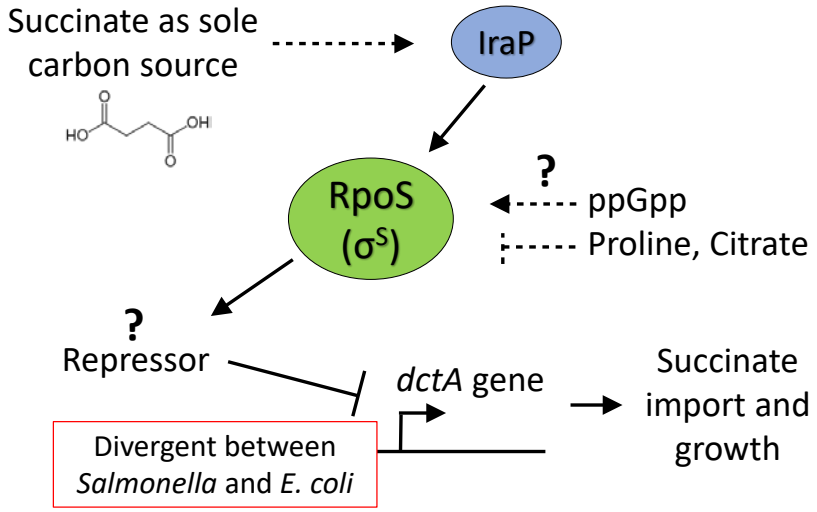

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