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The Role of Multidrug Resistance Efflux Pumps in pH Homeostasis in Escherichia Coli

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Abstract

In the Gram-negative bacteria *Escherichia coli*, certain channels in the cell membranes help the cell to maintain internal homeostasis. Multidrug resistance efflux pumps are complexes that cross both the outer membrane and the inner membrane and use the cell's proton motive force to remove toxins from the cytoplasm to the outside environment. The proton current flows through both the outer membrane complex and the inner membrane complex in exchange for the toxin. Many multidrug efflux pumps share the same outer membrane complex, TolC, but have different inner membrane complexes, such as AcrAB, EmrAB, and MdtABC. TolC and certain TolC-associated inner membrane complexes EmrB and MdtB contribute to acid growth and survival. Both EmrB and MdtB were required for extreme aerobic acid survival. TolC was required for aerobic extreme acid and anaerobic acid/base survival. In addition, TolC was required for growth at acidic conditions. The acid growth phenotype was restored when TolC was reintroduced to W3110 Δ*tolC* on the low copy plasmid pMX. An acid growth defect with a *tolC* mutant suggests that these multidrug resistance efflux pumps evolved as a mechanism to maintain pH homeostasis within the cell but were co-opted for drug resistance. Strains that select for such multidrug resistance efflux pumps may be able to survive extreme acid conditions, such as the stomach environment, with more success compared to typical *Escherichia coli* cells.

Introduction

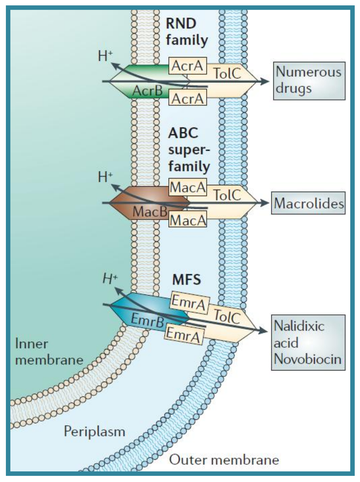

Figure 1: Different families of TolC associated MDR efflux pumps in gram negative bacteria. The drug or toxin inside of the cell is effluxed out of the cell in exchange for a proton (4).

- Acid and base resistance mechanisms are necessary for survival in a changing environment to enteric bacteria like *Escherichia coli*. En route to the intestines, *E. coli* must be able to grow in both extreme acid and extreme alkaline environments (1).
- Certain genes in *Escherichia coli* code for a group of trans-membrane efflux pumps that provide the cell with antibiotic resistance. Specific pumps, such as the AcrAB-TolC and MdtEF-TolC efflux pumps function as proton antiporters which exchange protons with the exported material across the membrane (2,3).
- A previous study discovered a multidrug resistance (MDR) pump that provided *Escherichia coli* with alkalitolerance which showed a physiological function for MDR pumps aside from providing drug resistance (2).
- This project explores the physiological role of several TolC associated MDR pumps in pH homeostasis.

Materials and Methods

Aerobic and Anaerobic Survival Assay. For extreme acid and base survival, strains were grown at pH5.5 and pH8.5 respectively overnight for 16-18 hours at 37°C, rotating. Overnight cultures were diluted into exposure at pH2 or pH10 and control tubes at pH7. Exposure tubes rotated at 37°C for 2 hours. Control tubes were diluted and plated. All exposure tubes were then diluted and plated. Colonies were counted as viable cells. Percent survival was assessed by subtracting the log number of viable exposure colonies from the log number of control colonies. A phenotype was observed through a decrease in survival compared to W3110, the background strain for all mutants constructed (5).

Growth Curve. Overnight cultures were grown at pH7 for 16-18 hours at 37°C, rotating. Overnight cultures were then diluted 1:100 into 10mL LBK 100mM HOMOPIPES, pH5 for growth at pH5 and 10mL LBK 100mM MES pH5.5 for growth at pH5.5. Cultures were grown at 37°C, rotating. OD_{600} was taken every 20 minutes after dilution to measure cell growth. Each strain had three replicates. Growth rates were calculated at similar OD_{600} for each strain and mean population doublings/hour were determined. A phenotype was observed through a decrease in survival compared to W3110.

Acid Shift. Overnight protocol was similar to growth curve. Overnight cultures were diluted 1:100 into 10mL LBK 100mM MOPS pH7 and grown to OD_{600} of ~0.4. Strains were then diluted 1:20 into 9.5mL LBK 100mM HOMOPIPES pH5 for exposure and LBK 100mM MOPS pH7 for control. OD_{600} was taken every 20 minutes after shift to measure cell growth. Each strain had three replicates. Growth rates were calculated at similar OD_{600} for each strain and mean population doublings/hour were determined. A phenotype was observed through a decrease in survival compared to W3110.

Results

Multidrug Resistance Efflux Pumps	Proton-Dependent Component	Observed Phenotype(s)
AcrAB-TolC	AcrB	No observed phenotype. Tested only in pH2 extreme acid survival (Figure 2B)
EmrAB-TolC	EmrB	Required for pH 2 extreme acid survival. (Figure 2B)
EmrKY-TolC	EmrY	No observed phenotype. Tested only in pH2 extreme acid survival (Figure 2B)
MdtABC-TolC	MdtB	Required for pH 2 extreme acid survival (Figure 2B).
MdtEF-TolC	MdtF	No observed phenotype. Tested only in pH2 extreme acid survival (Figure 2B)
TolC	TolC	Required for acid growth and survival (Figures 2A, 3).

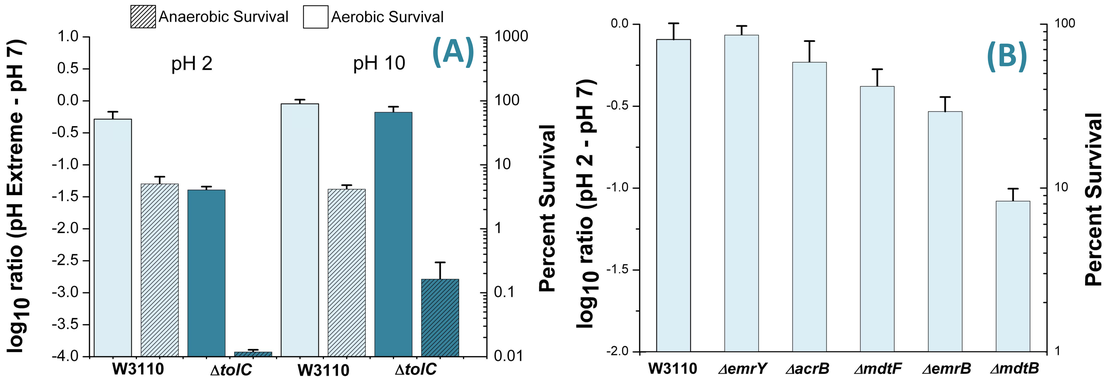

Figure 2: Certain MDR efflux pumps are required for survival in extreme conditions. (A) Δ*tolC* was exposed to both pH 2 and pH10 aerobic and anaerobic conditions for two hours. (B) Pumps from the table above were exposed to pH2 aerobic conditions for two hours. Overnight cultures were diluted into exposure tubes and control tubes. Control tubes were immediately diluted and plated. After two hours, exposure tubes were diluted and plated. Colonies were counted as viable cells. Percent Survival was assessed by comparing the experimental cells to the control cells as a difference between the log number of viable exposure cells and the log number of control cells. Error bars=SEM.

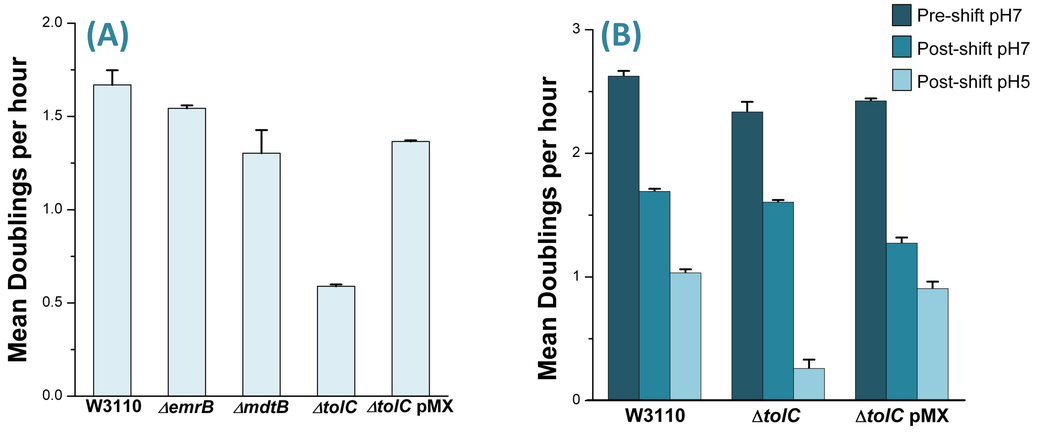

Figure 3: Certain MDR efflux pumps are required for growth in acidic conditions. (A) Strains were grown in LBK 100mM HOMOPIPES pH5 until they reached stationary phase. OD_{600} was taken at regular time intervals to assess cell growth. Growth rates were calculated from equivalent OD_{600} readings across each strain and mean doublings per hour were determined. (B) Strains were grown in LBK 100mM MOPS pH7 until the reached an OD_{600} of ~0.40 then diluted into LBK 100mM HOMOPIPES pH5 as the exposure and LBK 100mM MOPS pH7 as a control. OD_{600} was taken at regular time intervals to assess cell growth. Growth rates were calculated using the same methods as described in the growth curve assay. Plasmid pMX, carrying a low-copy number of *tolC* was used to restore the acid growth phenotype to Δ*tolC* (6). Error bars=SEM.

Discussion

- TolC was required for aerobic extreme acid survival and anaerobic extreme acid and base survival (Fig. 2A). EmrB and MdtB were also required for aerobic extreme acid survival (Fig. 2B).
- Mutants lacking the *tolC* gene were less able to grow in acidic conditions compared to the wild type W3110 (Fig. 3A). When the *tolC* gene was reintroduced on a low-copy vector pMX, the acid growth phenotype was restored (Fig. 3A,B).
- Δ*tolC* mutants grew more slowly after an acid shift from pH7 to pH5 compared to the wild type W3110 (Fig. 3B). When the *tolC* gene was reintroduced on a low-copy vector pMX, the growth rate after an acid shift was increased.
- Plasmid complementation of pMX to Δ*tolC* restored all acidic growth abilities of the mutant Δ*tolC* strain (Fig. 3A,B). Growth of Δ*tolC* pMX was similar to the growth of W3110, suggesting that TolC provides the cell with the ability to grow in acidic conditions and that the observed phenotype(s) were not a result of experimental error.
- The determined need for TolC in growth and survival in acidic conditions suggests that TolC plays a physiological role in pH homeostasis in addition to its function as an outer membrane component in several multidrug resistance efflux pumps. Other multidrug resistance pumps, such as MdfA, have been observed to provide *Escherichia coli* with alkalitolerance (2).
- Future work could investigate the result of over-expressing *tolC* and whether or not over-expression of *tolC* exaggerates the acid growth phenotype and allows *Escherichia coli* to grow at low pH values. In addition, further work could investigate the role of *tolC* in rapid internal pH recovery after an acid shift using fluorescence at the pH values from previous experiments where the acid growth phenotype has been observed.

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