The Tol-Pal trans-envelope complex is important for acid survival of Escherichia coli
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Abstract

The Gram-negative opportunistic pathogen Escherichia coli is able to cause infection by surviving passage through the extremely acidic stomach environment and into the small intestine. TolC, the major efflux protein used for colicin transport into cells, is required for E. coli cells to survive in extreme acid. The Tol-Pal system, a group of five proteins located in the outer membrane, is also required for colicin uptake. Here, we demonstrated the importance of the Tol-Pal proteins TolR, TolB, and Pal for E. coli survival in extreme acid. Less than 0.1% of cells from TolR, TolB, and Pal survived when exposed for two hours in media at pH 2, whereas approximately 1% of cells from a tolC strain survived under similar conditions. TolB and Pal were also required for survival in extreme base (pH 10); however, the survival phenotype observed at pH 10 was less severe than that seen at pH 2. Despite the presence of survival phenotypes, there was no pH-specific growth defect associated with tolR. tol-pal mutants transformed with a plasmid encoding GadBC did not show increased survival relative to non-transformed strains. pH-dependent ratiometric GFP fluorescence microscopy showed that the pal strain maintains a lower cytoplasmic pH than that of the wild type. The severe acid and base survival phenotypes observed in the Tol-Pal mutants are consistent with the requirement of the Tol-Pal proteins for membrane stability.

Background

• The Tol-Pal system is composed of five proteins: TolR, TolQ, and TolA in the inner membrane, TolB in the periplasm, and Pal in the outer membrane. (Fig. 1)

• The Tol protein complexes are used by colicins to gain access to the interior of the cell (1).

• Mutants lacking one of the five proteins are also highly susceptible to detergent and bile salts (1), and resistant to infection by filamentous bacteriophages (1).

• The Gad glutamate decarboxylase system is one of the primary acid resistance mechanisms of E. coli. gadBGC encodes glutamate decarboxylase which converts glutamate into GABA and CO₂ (2).

Materials and Methods

Survival Assay. For extreme acid and base survival, strains were grown at pH 5.5 and pH 8.5, respectively, overnight for 16-18 hours at 37°C, rotating. When necessary, IPTG (0.5 mM) was added to overnight cultures to induce the expression of pMF565 (For more on pMF565 and overnight growth conditions, see reference 4). Overnight cultures were diluted into exposure tubes containing LBK media buffered at the appropriate pH. Cultures were grown at 37°C, rotating at 200 rpm. Culture OD₆₀₀ was recorded every 30 minutes after dilution. Growth rates were calculated at similar OD values in early log phase for each strain and mean population doublings/hour were determined.

Fluorescence Microscopy. E. coli W3110 and JLS104 (W3110 pal::Km) carrying plasmid pGPFR01 were observed at excitation wavelengths of 425 nm and 465 nm. Excitation was from a xenon lamp (Sutter) on an Olympus BX61WI microscope (100X oil-immersion objective).

Bacteria were cultured to early log phase in LBK 100 mM MOPS pH 7.5 at 37°C, then spotted on cover slips coated with 0.01% poly-L-lysine (1). Cells were observed in a FC31 flow cell chamber (Biotoph). Periplasmic fluid was aspirated with M3A minimal medium supplemented with casamino acids (0.4 g/L KNO₃, 0.4 g/L KH₂PO₄, 2 g/L NaH₂PO₄, 7.45 g/L KCl, 2 g/L casein hydrolysate, 7.45 g/L KClo) buffered at pH 7.5 (100 mM MOPS or pH 5.5 (100 mM MES)). Cytoplasmic pH values were calculated using a standard curve of fluorescence ratios (R.D. Kirov and J.P. Marshon, unpublished).

Conclusions

• TolR, TolB, and Pal were required for survival in extreme acid, however the TolR, TolB, and Pal deficient strains had much lower survival rate than that of the TolC deficient strain (Fig. 1).

• tol-pal strains carrying pMF565 (gadBC) had virtually no increase in survival relative to the non-transformed mutant strains (Fig. 4). We therefore believe that unlike TolC, which was found to play a role in the Gad system (4), the Tol-Pal proteins are not involved with the Gad system.

• Relative to the WT, the pal mutant has an impaired ability to maintain cytoplasmic pH (Fig. 5). This is consistent with the low survival rate of the pal (and tolB and tolC) strain in extreme acid.

• W3110 growth rates at pH 4.5 and 5.0 (Fig. 6) were unusually low compared to other growth experiments (4). Therefore, we would expect tolB to have a lower growth rate compared to that of W3110 at all pH values measured. Unlike the tolC mutant, we believe the tolB mutant lacks a pH-dependent growth phenotype (4).

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References


Figure 1. The proteins of the Tol-Pal system. TolB floats freely in the periplasm and interacts with Pal and TolD. Pal is anchored to the outer membrane while TolQ, TolR, and TolA all have trans-membrane helices spanning the inner membrane (3).

Figure 2. TolR, TolB, and Pal are required for survival in extreme acid. Strains were exposed to pH 2 media for two hours. For all survival assays, error bars = SEM, n = 6.

Figure 3. TolB and Pal are required for survival in extreme base. Strains were exposed to pH 10 media for two hours. No IPTG added. Pal expression is induced with IPTG. IPTG was either added (+) or not added (-) to overnight cultures.

Figure 4. gadBC expression does not restore the survival of tol-pal mutants to that of W3110. pMF565 is a plasmid that encodes GadBC. pMF565 expression is induced with IPTG. IPTG was either added (+) or not added (-) to overnight cultures.

Figure 5. pH-dependent ratiometric GFP fluorescence microscopy with W3110 and pal strains. a) Microscopy images of representative cells before (pH 7.5) and after (pH 5.2) shift. b) Average cytoplasmic pH values of cells (n=100 for each strain at each pH) calculated using the standard curve of fluorescence ratios.

Figure 6. Growth rates of the tolR strain over a range of pH 4.5 to pH 8.5. Cultures were grown overnight in LBK media and diluted 600 fold into flasks containing LBK media buffered at the appropriate pH. Each point on the curve represents a biological replicate. Doublings/hour were calculated using OD₆₀₀ values from the log phase of growth.