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 multi-drug 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 cellular envelope, 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, Pal in the outer membrane, and TolB in the periplasm. (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 detergents 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*. *gadBC* encodes glutamate decarboxylase which converts glutamate into GABA and CO_2 (2).

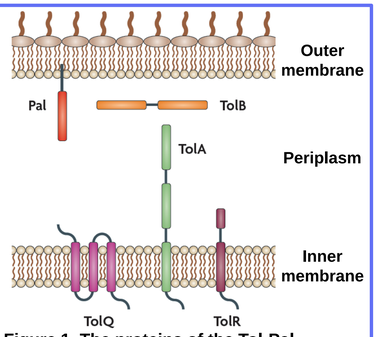

Figure 1. The proteins of the Tol-Pal system. TolB floats freely in the periplasm and interacts with Pal and TolA. Pal is anchored to the outer membrane while TolQ, TolR, and TolA all have trans-membrane helices spanning the inner membrane (3).

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 at pH 2 or pH 10 and control tubes buffered at pH 7 (20 mM MOPS) or pH 8 (20 mM TAPS), respectively. Exposure tubes were rotated at 37°C for 2 hours. Control tubes were immediately diluted into M63A media and plated. All exposure tubes were then diluted and plated in the same manner. 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.

Growth assay. Overnight cultures were grown at pH 7 for 16-18 hours at 37°C, rotating. Overnight cultures were then diluted in triplicate 100-fold into 10 ml LBK with 100 mM of the appropriate buffer (pH 5.0: HOMOPIPES, pH 5.5–6.0: MES, pH 6.5: PIPES, pH 7.0–7.5: MOPS, pH 8.0–8.5: TAPS). Cultures were grown at 37°C, rotating at 260 rpm. Culture OD_{600} was recorded every 30 minutes after dilution. Growth rates were calculated at similar OD_{600} values in early log-phase for each strain and mean population doublings/hour were determined.

Fluorescence microscopy. *E. coli* W3110 and JLS1054 (W3110 *pal*::Km) carrying plasmid pGFPR01 were observed at excitation wavelengths of 425 nm and 465 nm. Excitation was from a xenon lamp (Sutter) on an Olympus BX61WIF-5 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 FCS3 flow cell chamber (Bioptechs) perfused with M63A minimal medium supplemented with casein hydrolysate (0.4 g/L KH_2PO_4, 0.4 g/L K_2HPO_4, 2 g/L $(NH_4)_2SO_4$, 2 g/L casein hydrolysate, 7.45 g/L KCl) 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. Kitko and J.P. Mershon, unpublished).

Survival

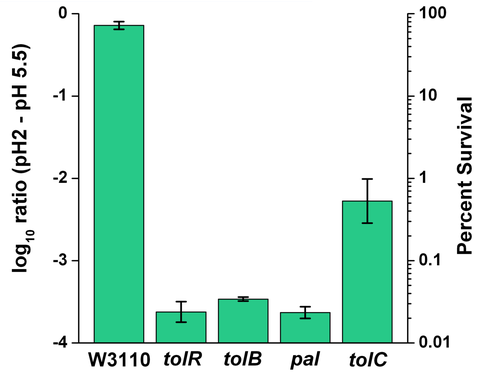

Figure 2. TolR, TolB, and Pal are required for survival in extreme acid. Strains were exposed to pH 2 media for two hours. For all survival assays, error bars = SEM, n = 6.

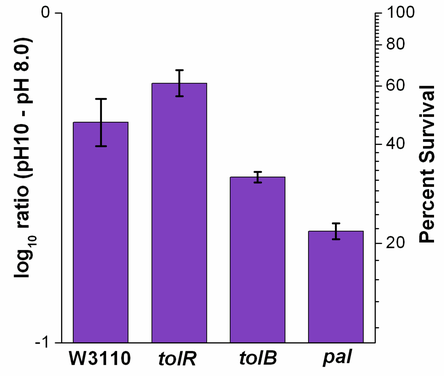

Figure 3. TolB and Pal are required for survival in extreme base. Strains were exposed to pH 10 media for two hours.

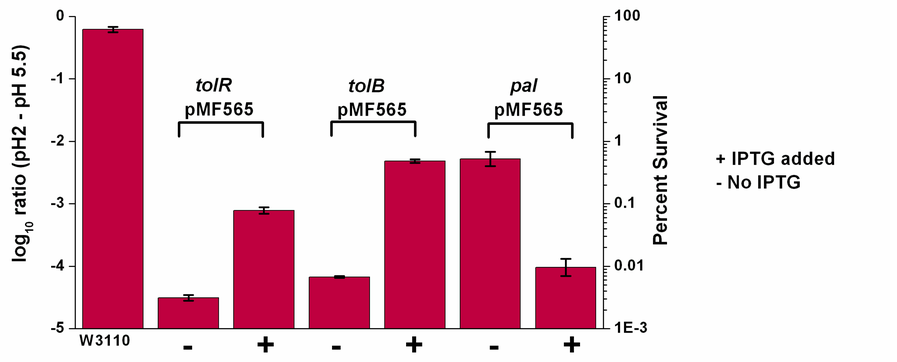

Figure 4. *gadBC* expression does not restore the survival of *tol-pal* mutants to that of W3110. pMF565 is a plasmid that encodes GadBC. pMF565 expression is induced with IPTG. IPTG was either added (+) or not added (-) to overnight cultures.

Fluorescence Microscopy

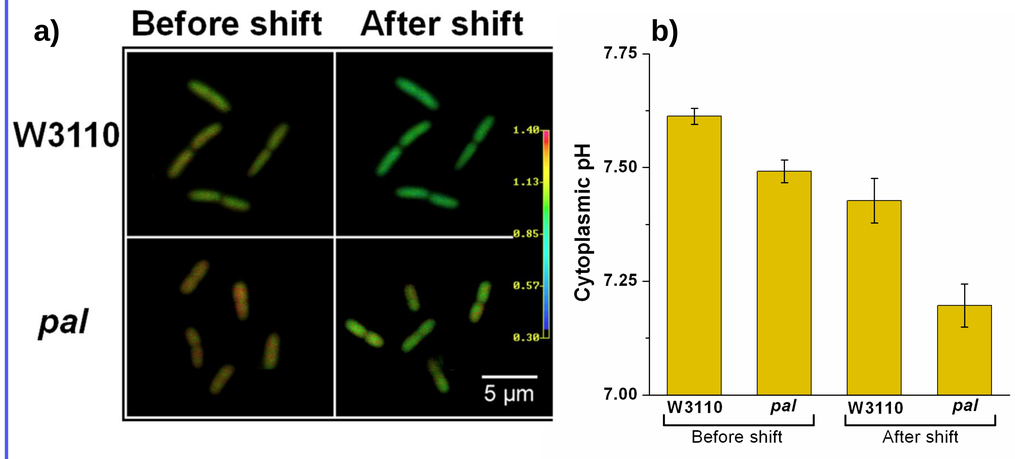

Figure 5. pH-dependent ratiometric GFP fluorescence microscopy with W3110 and *pal* strains. a) Microscopy images of representative cells before (pH 7.5) and after (pH 5.5) shift. **b)** Average cytoplasmic pH values of cells (n ≈100 for each strain at each pH) calculated using the standard curve of fluorescence ratios.

Growth

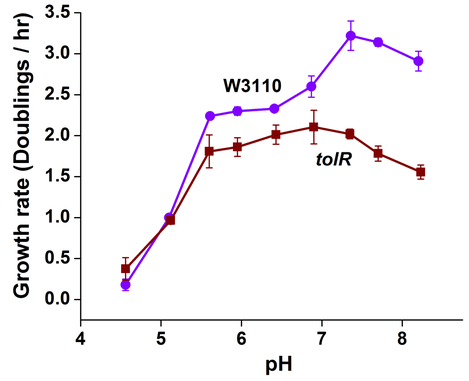

Figure 6. Growth rates of the *tolR* strain over a range of pH 4.5 to pH 8.5. Cultures were grown overnight in LBK media and diluted 200-fold into flasks containing LBK media buffered at the appropriate pH. Each point on the curve represents a biological replicate. Doublings/hour were calculated using OD_{600} values from the log phase of growth.

Conclusions

- TolR, TolB, Pal, and TolC 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 *tolR and tolB*) 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 *tolR* to have a lower growth rate compared to that of W3110 at all pH values measured. Unlike the *tolC* mutant, we believe the *tolR* mutant lacks a pH-dependent growth phenotype (4).

Acknowledgements

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