Abstract

_**Escherichia coli** was evolved for 1,000 generations in the presence of the proton uncoupler carbonyl cyanide m-chlorophenylhydrazone (CCCP). Half of the 48 populations were evolved at a low pH condition (6.5) and the other half were evolved in a high pH condition (8.0). After 1,000 generations all populations could survive in 150μM CCCP, an increase from a starting concentration of 20μM for the low pH and 50μM for the high pH.

Selected populations from this evolution were sequenced using Illumina MiSeq next generation sequencing. The sequences were re-annotated against the ancestral genome. The mutations were then evaluated using the computational pipeline bresseq. The most prevalent mutations by far were found in the mprA, emrA, and emrB multidrug efflux complex. The emrA and emrB genes form a multidrug efflux pump and mprA codes for a repressor of the emrA gene. All evolved strains except for one, B1-1, had one or more mutations in the mprA operon. Although we knew the locations and prevalence of these mutations we couldn’t be sure what the mutations in the mprA and emrA efflux complex were responsible for in terms of growth difference. Growth curve analyses were run to determine the growth effects of the removal of the emrA gene. In concentrations of CCCP that the ancestor could survive its high pH, the high pH strains were equally fit with or without an intact emrA gene (Figure 1A). In low pH strains, those with emrA present performed better than those with emrA removed, but even the mprA knockouts did better than the ancestor indicating mutations outside of emrA that are of importance to low pH survival. When low pH strains were grown in high pH all emrA knockouts grew as poorly as the ancestor except for A3-1 (Figure 1B). When high pH strains were grown in low pH all strains, with or without emrA, died except for D11-1. These findings suggest that mutations outside of the emrA operon are conferring fitness to strains.

When mprA was removed from the ancestral strain, the growth differences were minimal in the high pH condition, but in low pH D13C with no mprA gene could survive in levels of CCCP beyond which the ancestral strain with mprA in tact could (Figure 2). This leads us to believe that the emrA operon is much more important to strains facing the stress of low pH than those at high pH.

**Escherichia coli** are a unique specimen for study because of their position in the human body and the many stresses they face while moving through the gastrointestinal tract. The pH stress in particular allows for advantageous mutations to become prevalent in the populations that survive pH stress as long as is found in our bodies. _E. coli_’s pH stress mitigation finds its roots in the ability of the bacteria to expel protons through the cell membrane to maintain a survivable internal pH [2]. The difference between the internal pH of the bacteria and the external pH of the environment allows for an energetic potential to be formed which can be harnessed by the E. coli to synthesize energy. This potential is known as proton motive force or PMF, and it is key to survival of _E. coli_ in varying pHs [3].

Our choice of CCCP as the stressor molecule in this evolution relies on the fact that uncouplers by nature break down the PMF and create an energy stress for the _E. coli_ in the face of pH stress. CCCP accomplishes this by possessing a hydrophobic region that can pass through the cell membrane whether it is protonated or not [4]. This allows the internal pH of the cell to be maintained as the CCCP shuttles protons into the cell at an unregulated rate. This pressure over the evolution allowed for interesting mutations to sustain certain populations in the face of the uncoupler.

**Methods**

**Evolution:** 24 populations of _E. coli_ K-12 W3110 were grown in medium buffered at pH 6.5, and 24 populations were grown at pH 8.0 for 1000 generations. These 48 populations were cultured to stationary phase, then were diluted 1:100 daily into a new microwell plate containing concentrations of CCCP that increased over the course of the evolution (20μM - 150μM).

**Transduction:** Bacteriophage was allowed to infect a Keio strain with a kanamycin deletion insertion at emrA. The lysate this formed was then added to the chosen strains which then gained the kanR deletion at emrA gene. The same methods were used for mprA knockouts.

**Growth Curves:** Sterile 96-well plates were inoculated with 200μL of LB 100mM PIPES or TAPS (pH 6.5 and 8.0, respectively), CCCP, and 1μL of the strain being tested. OD values were taken every 15 minutes for 22 hours. Growth was read kinetically in a SpectraMax Spectrophotometer.

**Results**

**Conclusions**

In low pH strains it is apparent that the emrA operon is necessary to the survivability of those populations. Figure 2A shows that in the low pH evolved strains with emrA removed could not survive as well as those strains with the mutated emrA gene in tact. This is true for both their evolved pH (6.5) and the inverse pH growth curves (8.0). An important finding in Figure 1A is the emrA knockouts grow significantly better than the ancestor, W3110D13. This suggests that there are mutations outside of the emrA complex that are necessary to the fitness of the populations. The _emrA_ complex appears to be more important to strain maintenance in a low pH stress than those in a high pH environment. Out of seven strains of major interest to the post-evolution CCCP project, only one did not possess any mutation to _emrA_ or _mprA_ and that strain (B1-1) was grown in a high pH environment. Furthermore, as displayed in Figure 1A, the high pH strains grew as well with or without an emrA gene present. Figure 2B supports this claim because the _mprA_ knockouts in low pH have much greater fitness than the ancestor, whereas for the high pH strains the difference is not nearly as drastic.

In future research, these strains, through recombining, a method of gene editing using red lambda phage and genetic homologs[1], will be engineered to possess complete, ancestral forms of the entire _emrA_ operon. This will allow for us to determine what growth differences _emrA_ and _mprA_ are accountable for and move forward with other novel mutations that could be responsible for other discrepancies.

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**References**


