

Expanded Salt Tolerance of *Escherichia coli* Through Adaptive Laboratory Evolution

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Halophiles have a vast range of uses, from biofuel production to pharmaceutical applications. *Escherichia coli* has one of the fastest duplication times of any organism, as low as twenty minutes in optimal conditions. By utilizing adaptive laboratory evolution (ALE), *E. coli* can be evolved to be more salt tolerant, giving rise to possible applications in various industrial settings. Six *E. coli* populations, using parallel batch cultures, and grown in LB broth for 101 days. Throughout the period, the NaCl concentration of the broth was increased to a maximum of 20.00%. Three of the populations had an additional KCl concentration of 4.00% m/v to facilitate the influx of K⁺ ions. All populations had very high densities in salt concentrations up to 15.00%. The culture densities at 20% NaCl, though lower, were still vastly improved over the mother culture's growth in NaCl. A t-test for two dependent means was conducted and demonstrates that the rate of the increase in salt concentration was higher in the second half of experimentation ($p < 0.05$). The ability of all populations to grow optimally in NaCl, as well as in the absence of salt, classifies all experimental populations as facultative halophiles, or alternatively, haloversatile. The doubling time of the evolved *E. coli* was drastically lengthened, though significantly shorter than the majority of halophiles from previous literature. The ability of *E. coli* to synthesize glycine betaine, its drastically improved salt tolerance, and relatively short replication time demonstrates the need for further research to be completed.

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