STRESS-TOLERANCE PROTEINS IN DEINOCOCCUS HOPIENSIS

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Deinococcus hopiensis is a bacterium that was recently discovered in the Sonoran desert of Arizona. Very little is known about D. hopiensis and only a handful of articles exist referencing the existence of the bacteria. (Bagaley, et al., 2005). Deinococcus radiodurans, however, is a well characterized bacteria found in a wide variety of environments, that has shown to be resistant to environmental stressors such as radiation and desiccation.(Gao et al., 2003, Hua et al., 2003, Ohba et al., 2009) The ability of D. radiodurans to withstand such extreme conditions is attributed to a DNA repair mechanism which involves several genes including, ppri, ppra, pprm, and reca. (Gao, et al., 2003; Hua et al., 2003; Kota & Misra, 2006; Lu et al., 2009; Ohba et al., 2009). Sequence database searches have revealed that D. hopiensis also contains ppri, ppra, pprm, and reca genes. Both D. radiodurans and D. hopiensis also contain a Late Embryogenesis Abundant (lea) protein, which is known to confer salt tolerance in plants and marine bacteria. (Liu et al., 2009)

The goals of our project are to characterize the effects of biological stressors on D. hopiensis and D. radiodurans, and to transform the genes of interest from both species into E. coli to determine how the same genes from different bacteria affect the ability of this commonly studied bacterium to withstand the environmental stresses.

In this study, we have focused on the late embryogenesis protein in D. hopiensis. We designed PCR primers to amplify the coding sequence of this gene from D. hopiensis genomic DNA. These primers were designed based on the Biobrick standard (Shetty et al. 2008), which facilitated the construction of an expression construct composed of a LacI-sensitive promoter, a ribosome binding site, the lea coding sequence, and a terminator sequence. This construct was transformed into competent E. coli (New England Biolabs 10-beta) cells for physiological testing. The expression construct allows us to control the expression of the gene of interest by induction with IPTG or lactose. Salt tolerance will be determined by measuring the growth rate of lactose-induced E. coli carrying the lea expression construct under different NaCl concentrations. Growth will be measured using a spectrophotometer to assess increased culture turbidity over time.

Currently, we have completed our first expression construct that contains the lea gene and successfully transformed this construct into E. coli. Following validation of our expression construct, we will test the effects of this gene on salt tolerance in E. coli. The next steps in this project include repeating the assays described above with the lea gene from D. radiodurans, and expanding our testing to include other genes implicated in resistance to desiccation and other environmental stressors. Ultimately, we hope to compare the stress-tolerance effects of genes from other species in the genus Deinococcus that have been discovered in Arizona soils.