MOLECULAR CLONING AND CHARACTERIZATION OF LEA GENES FROM DEINOCOCCI

Erica Soboslay, Esther Juárez, Robert Ziemba, James Tuohy
Glendale Community College, Glendale, AZ

Bacteria in the genus *Deinococcus* are known for their ability to resist extreme environmental challenges such as radiation, oxidation, and desiccation. Resistance to desiccation, in *Deinococcus radiodurans* is known to be attributed, in part, to the expression of group 3 Late Embryogenesis Abundant Protein (LEA). Little is known about group 3 LEA proteins in *D. radiodurans,* but their function may be related to the hydrophilic structure of the protein and changes to that structure during desiccation.

Several new species of *Deinococcus* have been discovered in the Sonoran Desert. The functional role of LEA proteins in these species is unknown. However, the extreme nature of their habitat suggests these species may share similar desiccation resistance strategies to that of *D. radiodurans.*

This study is aimed at cloning and characterizing LEA proteins from *D. radiodurans* and *D. hopiensis* as well as other desert *Deinococcus* species. In order to investigate the role of LEA proteins in the desiccation tolerance of *Deinococci*, the *lea1* gene was isolated and cloned into an expression vector and transformed into *Escherichia coli.*

Sequencing results have verified the successful cloning of lea1 from both *D. radiodurans* and *D. hopiensis*. Sequence analysis and expression studies of the LEA protein are in progress to determine the role of the protein in desiccation resistance in the *Deinococci*.

STRESS-TOLERANCE PROTEINS IN DEINOCOCCUS HOPIENSIS

Erica Soboslay, Esther Juárez, Fern Van Vliet and James Tuohy
Glendale Community College

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 *Deinococcus radiodurans* and *Deinococcus 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 *Deinococcus hopiensis* and *Deinococcus radiodurans*, and to transform the genes of interest from both species into *Escherichia 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 *Deinococcus 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.