Cloning of Urea Amidolyase from *Saccharomyces cerevisiae*, *Wolinella succinogenes*, and *Pseudomonas syringae*

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Biotin-dependent enzymes are metabolically important enzymes distributed across all forms of life and pertinent to a range of human health problems including diabetes and metabolic disorders. These enzymes are considered biotin-dependent because they require a mobile biotin "arm" to transfer substrates between active sites. With few biotin-dependent enzyme structures solved to date, little is known about how the active sites are regulated. One set of biotin-dependent enzymes is used in bacteria, yeast, and algae to break down urea for a nitrogen source. In eukaryotes, this two-step process is performed by a single multifunctional enzyme, urea amidolyase (UAL). In prokaryotes, this process is accomplished through the coordination of two separate enzymes, urea carboxylase (UC) and allophanate hydrolase (AH). Determining the structure of UAL is an excellent opportunity to study the interactions between different regions in multifunctional enzymes, specifically the regulation of active sites.

This study represents the initial steps in a project designed to characterize the structure and function of UAL from *Saccharomyces cerevisiae* by X-ray crystallography. The immediate goal was to clone the UAL gene from yeast into an *E. coli* expression vector. Because UAL's large size presents challenges at many steps of the crystallization process, work was also done towards determining the structures of UC and AH from the prokaryotes *Wolinella succinogenes* and *Pseudomonas syringae*. Due to the high degree of homology between UC/AH and UAL, the structures of UC and AH will offer some structural insights into the structure and function of UAL.

Sequences coding for UAL, UC, and AH were amplified from all three organisms by PCR. The inserts were ligated into a modified pET28a vector, electroporated, and transformed. Colonies that tested positive by colony PCR for the insert were confirmed by restriction digest. UC and AH from *W. succinogenes* were sent for sequencing. AH was successfully cloned and expression testing was initiated, but UC was found to have incorporated two potential point mutations. UC and AH from *P. syringae* and UAL await final confirmation by sequencing.