2003 URE OMSS

Undergraduate Research Experience in Ocean, Marine and Space Science

ONR
NOAA
MU-SPIN

ECSU
NIA

Home | Staff | Teams | Photos | Schedule | Travel Guidelines

The Purification of Sortase, University of Alabama

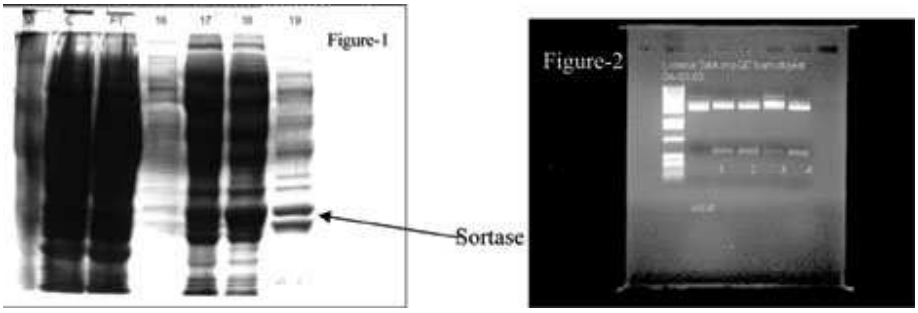
Mentors: Dr. Ramarao Chodavarapu, PhD and Dr. Suresh Kuman Muthuvel, PhD
University of Alabama at Birmingham's Center for Biophysical Science and Engineering: Division of Molecular Biology

Infectious bacteria, such as Listeria monocytogenes, are composed of a thick cell wall dotted with surface proteins designed to interact specifically with human cells as a first step towards establishing infections. These pathogens anchor themselves to the cell membrane of our body's cells and Sortase is a gram-positive bacterial enzyme that aids in the anchoring of bacteria to the cell wall of human cells. Without this outer weaponry, gram-positive bacteria could not invade people's throats, skin and other susceptible tissues.

Sortase works by activating a five-amino acid tag present on many of the proteins localized on the bacterial cell wall, abbreviated LPXTG, where L is Leucine, P is Proline, X is any amino acid, T is Threonine, and G is Glutamine. It is this tag that is hooked directly into the cell wall of human cells, allowing infection. Sortase effectively cleaves the LPXTG motif near the middle before linking the remainder of the protein to the cell wall. Hypothetically speaking, if the cleaving and "sorting" actions of sortase could be restricted, then scientists would have a modern weapon in the fight against bacterial infection.

We sought out to purify sortase in order to make crystals for research. From these crystals, researchers will attempt to produce a working model of a sortase inhibitor. This inhibitor would revolutionize the way in which we view bacterial anchoring. A fully-functional working model could lead to the global eradication of gram-positive bacterial infections, including Diphtheria, Listeria, Tuberculosis, Scarlet Fever, and Leprosy.

Results



After viewing the PAGE results of the first gradient elution, it was decided that further purification was needed. Thus, we dialyzed fractions 17-19 overnight. All three of these fractions possessed the dark band around 30 kilo Daltons, which is the enzyme Sortase. After the second purification, the resulting "pure" fractions, as measured by the ÄKTA-explorer system, were collected and given to Dr. Ynong Jhong for crystallization.

Box 672 1704 Weeksville Road, Elizabeth City State University, Elizabeth City, NC 27909
(252) 335-3696 voice (252) 335-3790 fax