

**Title: Identification of Four Bacterial Isolates from Residues of Native Wheat that Antagonize Wheat Pathogenic Fungi**

**Author:** Nichole Baye

**Abstract:**

Four bacterial strains isolated from native wheat residues that antagonize wheat pathogenic fungi have been maintained in the Soil Microbiology Laboratory at South Dakota State University. These organisms have been studied for potential use as biocontrol agents. Several different methods were employed to ascertain the identity of these four organisms. The methods used incorporated membrane fatty acid methyl ester analysis (FAME), partial 16S ribosomal RNA (rRNA) sequencing, cellular and colonial morphological study, and physiological tests. FAME analysis concluded that strains 1BA and 1D3 were *Bacillus lentimorbus*, and that strains 1BC and 1BE were *Bacillus subtilis*. All four strains had identical partial 16S rRNA sequences with the best match for *Bacillus amyloliquefaciens*. Colonial and cellular morphology along with biochemical tests indicated that all four organisms most closely matched *Bacillus firmus*. The conflicting result, among these tests, implies that these organisms may belong to a new but related taxon to *Bacillus subtilis*, *Bacillus lentimorbus*, *Bacillus amyloliquefaciens*, and *Bacillus firmus*. Further studies with these organisms will involve assaying for their mode of action against fungi.

## **Title: Protective effects of Nicotinamide in Human Cortical Neurons**

**Author:** Suraj Bhansali

### **Abstract:**

Neurodegenerative diseases are quite prevalent in United States, especially in older people and are the third highest natural cause of death in USA. These diseases include ischemia, Alzheimer's disease and Parkinson's disease among others. Previous studies have shown that cell death (apoptosis) in neurodegenerative diseases occurs because of oxidative stress of free radicals in the brain. The oxygen radical generating toxin tertiary butylhydroperoxide (t-BuOOH), which induces DNA fragmentation and apoptosis in neuronal cells, was used to mimic the oxidative injury that has been implicated in these diseases. It was observed that t-BuOOH induces DNA fragmentation and apoptosis in all regions of mouse brain, and that it also causes significant cell death in human cortical neurons (HNC1-A and HNC2) in culture. In addition, previous studies have also shown that the poly (ADP ribose) polymerase (PARP) inhibitor nicotinamide is able to prevent DNA fragmentation and apoptosis induced by t- BuOOH in mouse brain and in human neuronal cells HCN1-A and HCN2. Evidence indicate that nicotinamide is able to prevent the up-regulation of the pro-apoptotic proteins p53 and p21/WAF-1, and the down regulation of the anti-apoptotic protein bcl-2 that is induced by t-BuOOH in HCN1-A and HCN2 cells, though exact molecular mechanism at cellular level is not known. My research involves delineation of molecular mechanism(s) by which nicotinamide is able to protect HCN1-A and HCN2 cells in presence of free radical generating toxin. This project applies the techniques of genomics to examine the molecular mechanism underlying neuronal protection. The central hypothesis of my project is that nicotinamide protects human brain cells from the toxic effects of free radical generating toxin by regulating the levels of various pro- and anti-apoptotic proteins and that this protection can be enhanced in combination with other neuro-protective agents.

Significance of this project includes evaluation of two human cortical neuronal cell lines, HCN1-A and HCN2, which can be used as cell culture model systems to evaluate various potential neuro-protective agents. This study will provide insight into the effect that PARP inhibitors can have in preventing neuronal death and hence can be potential therapeutic agents against neurodegenerative diseases. It will also help in better understanding of the underlying molecular mechanism(s) involved in the protective effects of PARP inhibitors in the brain by studying the effects of PARP inhibitors on regulation of pro-and anti-apoptotic proteins. Once a clear mechanism is established, therapeutic agents with different mechanism of action will be used in combination with nicotinamide to observe synergistic effect in protecting human neurons.

The experimental techniques involve cell culture of human cortical neuronal cells, extraction and purification of RNA from these cells, RNA gel electrophoresis, DNA microarray to compare up- and down-regulation of various genes. Enzyme Linked Immuno Sorbent Assay (ELISA) and Western immunoblot assays will be performed to determine the levels of various proteins. The results from the DNA microarray will be corroborated with RT-PCR. Here at SDSU, I have successfully learned cell culture laboratory techniques and have been able to extract and purify RNA from human brain

cells. I also have done RNA gel electrophoresis. In near future I will be doing all the aforementioned tests and assays.

Following the completion of the project, I should be able to elicit the molecular mechanism(s) of protective effects of nicotinamide and identification of other potential drug candidates, which will show synergistic effect in combination with nicotinamide. It will open new horizons in the research field of neurosciences.

## **Title: Glycosylphosphatidylinositol-anchored Protein from Winter Wheat**

**Author:** Dong He

### **Abstract:**

Proteins containing glycosylphosphatidylinositol (GPI) anchors are widespread in animal cells, yeast and parasitic cells, where they are highly localized on the outer face of the plasma membrane. Recently, their existence has been confirmed in higher plant tissues. This report describes the possible existence of GPI-anchored protein in the plant winter wheat ( *Triticum aestivum* L.cv.Winoka ). A wheat cDNA clone (pTACR7) was isolated by RT-PCR. Based on the deduced amino acid sequence, TACR7 is highly hydrophobic, with only one major hydrophilic region, however, no helices were observed. Furthermore, western blot analyses were performed with affinity purified anti-peptide TACR7 antibody or pre-immune serum. One band of about 33KD was observed in the membrane fraction but not in the soluble fraction of wheat leaf tissue with anti TACR7 antibody TACRT7, another of about 17 KD was observed in the soluble fraction with anti TACRT7 antibody. These results suggest that there is a TACR7 like species in the membrane fraction and the soluble fraction. This HMW species maybe represent the GPI-anchored TACR7 protein. To determine the relation between these two species and the deduced amino acid sequence, we will determine the sequences of the 33 KD and the 17 KD species by Mass spectrometry. The western blotting of the membrane fractions treated with phosphatidylinositol specific phospholipase C (PI-PLC) will be performed to determine if this 33 KD protein is GPI-anchored. Finally we will compare this protein with other proteins in the available protein database to aid in determining its biologic function.

**Title: Understanding the role of starter cultures in Mozzarella cheese functionality.**

**Author:** Sumita Chanda

**Abstract :**

Mozzarella cheese has shown unprecedented growth among the cheese varieties in the US. It is estimated that approximately 70% of Mozzarella cheese is used as an ingredient on pizza. Mozzarella cheese functionality has been studied for for 40 years by researchers. However, a better understanding of the basic chemical, biological and processing governing melting characteristics and functionality is required to enable manufacturers to keep pace with the increasing demand by the food service industry for cheeses with functional properties tailored for specific applications viz. pizza, poppers, Mexican cuisine, nachos or as Mozzarella sticks.

The avenues for this study includes a multi dimensional approach to study the contribution of selected strains of lactobacilli starter culture proteinases in melt and microstructure of Mozzarella cheese brought about by proteolysis; the interaction between the type of coagulant and lactic cultures and relation to cheese functionality ; the role of total starter bacterial numbers, their arrangement in cheese protein matrix and relationship of these to cheese functionality in terms of meltability , rheology and flowability.

This study aims to identify and develop new starter cultures and finally develop protocols for the manufacture and storage of Mozzarella cheese using the new cultures.

**Title: Isolation and Characterization of the agrp Gene in Domestic and Wild Pigs.**

**Author:** Juanita Perera.

**Abstract:**

The agrp gene plays an important role in energy homeostasis. Its gene product, agouti-related protein (AGRP), antagonizes the action of the melanocortin-4 receptors (MC4Rs) in the hypothalamus and exhibits potent appetite stimulant activity. Alleles of agrp are likely to produce proteins with different structural and functional properties. Such properties could be highly beneficial in breed improvement. Studies on genes regulating energy balance will provide the information necessary for improving the production efficiency in livestock. Kent Donelan, a former graduate student in our lab, sequenced the agrp gene in 16 breed panels of domestic pigs. Unexpectedly he did not find polymorphisms in the agrp gene. It may be that agrp is highly conserved over evolutionary time due to the critical and essential role it plays in energy homeostasis. Alternatively, intensive selective breeding of pigs for thousands of years may have resulted in the disappearance of low frequency polymorphisms in agrp. We predict that polymorphisms may still exist in pigs that have not undergone intensive selective breeding, such as pigs that live in the wild. Therefore, we intend to isolate and sequence the agrp genes in the wild relatives of domestic pigs. Desirable agrp alleles (those that promote positive production traits) present in wild pigs may also exist within untested domestic stocks. It may also be possible to introduce favorable agrp alleles into domestic pigs via conventional breeding and/or transgenesis. We believe that this study has the potential to significantly improve the pig production efficiency.