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Oral Public Examination For the Degree of Doctor of Philosophy

Functions of *mid* and *pyd* genes required for mimosine degradation by *Rhizobium* sp. strain TAL1145

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2:15 PM October 13, 2005 Agricultural Science Building, Room 219

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Committee Members

Dr. Dulal Borthakur (Chair) Dr. Sean Callahan Dr. Tung Hoang Dr. John Hu Dr. Qing Li

Publications

- 1. Awaya JD, Fox PM, Borthakur D (2005) *pyd* genes of *Rhizobium* sp. strain TAL1145 are required for degradation of 3-hydroxy-4-pyridone, an aromatic intermediate in mimosine metabolism. J. Bacteriol. 187 (13): 4480-4487.
- 2. Awaya J, Fox PM and Borthakur D (2003) Genes encoding a fructose-1,6-bisphosphate aldolase and a fructose-1,6-bisphosphatase are present within the gene cluster for mimosine degradation in *Rhizobium* sp. strain TAL1145. Plant Soil 257: 11-18.
- 3. Awaya J, Walton C and Borthakur D. The *pydA-pydB* fusion gene produces an active dioxygenase-hydrolase protein in *Rhizobium* and *Escherichia coli* that degrades 3-hydroxy-4-pyridone, an intermediate of mimosine metabolism (manuscript in preparation).

Future plan

Postdoctoral research at Notre Dame starting on February 1, 2006

ABSTRACT

Mimosine and 3-hydroxy-4-pyridone (HP) are toxic aromatic compounds produced in treelegume leucaena(Leucaena leucocephala). These can be degraded by some leucaena-nodulating Rhizobium strains, such as TAL1145. Previously, a cosmid clone, pUHR263, containing the mid and *pyd* genes for mimosine and HP degradation, was isolated from a clone library of TAL1145. The aim of this project was to identify genes for mimosine and HP degradation in pUHR263 and determine their functions. Mimosine degradation by *Rhizobium* involves at least two major steps; in the first step mimosine is degraded to HP, which is then converted to pyruvate, formate and ammonia in the second step. Two structural genes, *pydA* and *pydB*, encode a *meta*-cleavage dioxygenase and a hydrolase, respectively. *pydA* and *pydB* are required for degradation of HP, and *pydC*, *pydD* and *pydE* encode proteins of an ABC-transport system involved in the uptake of HP by TAL1145. *pydA*, *pydB*, *pydC*, *pydD*, and *pydE* are induced by HP, although *pydA* and *pydB* show low levels of expression in the absence of HP. *pydA* and *pydB* are cotranscribed while *pydC*, *pydD*, and *pydE* are each transcribed from separate promoters. *pydR* is located upstream of the *pyd* genes and encodes a transcriptional regulator for the activation of *pvdA and pvdB* in the presence of HP. Elucidation of the HP degradation pathway in *Rhizobium sp.* strain TAL1145 may provide a useful strategy to genetically engineer leucaena and rhizosphere bacteria to disrupt the biosynthesis of mimosine and for bioremediation of aromatic toxins, respectively.