Energy Transduction and Nitrogen Metabolism

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Characterization of Transgenic *Lotus japonicus* Containing Sense and Anti-sense Uricase Genes.

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Legume plants can grow under poor nitrogen conditions because bacteroids can transfer di-nitrogen to ammonia. To translocate nitrogenous compounds some legumes form amides and others form ureides. Uricase is an enzyme that catalyzing the degradation of uric acid to form allantoin (ureide). In nodules forming ureides uricase gene has been reported to be over-expressed, and the gene was designated as nodulin-35.

To elucidate the physiological role of uricase gene expression, we employed an anti-sense RNA experiment using transgenic *Lotus japonicus* plants. Although *Lotus japonicus* translocates fixed nitrogen as amides, the uricase gene expression has been reported to be observed in uninfected cell of the nodules. In this experiment we introduced an anti-sense strand of *Lotus japonicus* cDNA into transgenic *Lotus japonicus* , and surveyed the changes of phenotype in addition to Southern and Northern blot.

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Characterization of Uricase Proteins Expressed Using pET32 and Soybean Nodule cDNA Clones

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Legume plants can utilize atmospheric nitrogen with the help of Rhizobia in a symbiotic tissue, nodules. Uricase is an enzyme that catalyzing the degradation of uric acid to form allantoin(ureide). In soybean nodules fixing di-nitrogen, two uricase cDNA genes (*UR2* and *UR9*) have been cloned and *UR9* was expressing in nodules dominantly and *UR2* expression was constitutive.

To elucidate the characteristics of these uricase proteins, we employed pET32 system.After pET32:uricase construct was introduced in *E.coli*(BL21), the culture containing IPTG was incubated.Fused uricase protein was purified the enzyme characterization was surveyed after deleting the tag by enterokinase. Optimum pH, Km values and effect of various inhibitor were analyzed using the purified protein.

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DISTRIBUTION OF HYDROGENASE IN SEVERAL CYANOBACTERIAL STRAINS AND THEIR ROLES IN HYDROGEN PRODUCTION MEDIATED BY NITROGENASE

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The enzymes directly involved in hydrogen metabolism in cyanobacteria are hydrogenases and nitrogenase. Functionally, two types of hydrogenase are known: uptake hydrogenase (Hup) and bidirectional hydrogenase (Hox). Distribution of hydrogenases among 12 filamentous cyanobacteria was studied by heterologous Southern hybridizations with *hupL* and *hoxH* probes from Anabaena PCC7120 and by direct assay of in vitro hydrogenase activities. All 12 strains were positive for the *hupL* probe. Two strains were negative with the *hoxH* probe and had no *in vitro* Hox activity. The *hupL*$^-$ and *hupL*$^-$*hoxH*$^-$ mutants exhibited H_2 producing activity about five times as high as that of the wild type under N_2-fixing condition. The activity of the *hoxH*$^-$ mutant was about the same with that of the wild type strain.

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EXPRESSION OF GENES ENCODING NODULINS CONTAINING CONSERVED CYSTEINE CLUSTERS IN PEA NODULES

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Several nodulin genes have been isolated from pea nodules by the subtraction procedures and their expression were compared between effective nodules and ineffective nodules induced on the pea mutant E135 (*sym*13). Nodulin genes whose expression were reduced in E135 nodules were leghemoglobin and 5 distinct genes characterized by cysteine clusters. The latter genes were homologous to nodulin genes, *PsENOD3*, *PsENOD14* and *PsNOD6*, and they all encoded for small peptides that contained putative signal peptides and two pairs of cysteine clusters such as Cys-X5-Cys and Cys-X4-Cys. They were expressed in a similar way to leghemoglobin during nodule development and in early symbiotic zone of nodules, suggesting that functions of those nodulin genes are closely related to nitrogen fixing activity.