RHIZOBIUM LEGUMINOSARUM BV *VICIAE* STRAIN LC-31: ANALYSIS OF NOVEL BACTERIOCIN AND ACC DEAMINASE GENE(S)

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Rhizobium spp., in addition to symbiotic nitrogen fixation, utilizes a variety of mechanisms, both direct and indirect, to stimulate the growth of plants and/or to compete in nodulation. Ethylene is a gaseous phytohormone produced by the plant during normal growth conditions. But, under biotic and abiotic stresses, the synthesis of ethylene increases and results in stunted root growth and low nodulation in legumes. The bacteria with ACC-deaminase gene activity convert the precursor of ethylene, ACC (1-Amino cyclopropane-1-carboxylic acid), into NH_3 and alpha-ketobutyric acid.

The amplified ACC-deaminase gene from bacterial strain LC-31 showed 99% homology with *Rhizobium leguminosarum* bv. *viciae* (Wenbo et al., 2003). A probe based on the LC-31 ACC-deaminase gene was synthesized and used for screening 15 rhizobial strains by the dot-blot technique. All but one strain showed the presence of the ACC-deaminase gene on the chromosomal DNA, whereas two strains showed its presence on both plasmid and chromosomal DNA. Expression profiling of the ACC-deaminase gene was studied by RT-PCR and differential display PCR. The gene was up-regulated at 1 mM ACC. In another experiment, LC-31 was supplemented with ACC (1 mM) and IAA (5–50 mM) to determine any correlation between IAA and ACC-deaminase gene activity. The ACC-deaminase gene is down regulated at 50 mM. *R. leguminosarum* LC-31 exhibited both properties, i.e., IAA and ACC-deaminase activity. The regulation of different genes, after ACC treatment of LC-31, is being investigated by differential PCR. cDNA was constructed by random hexamer primers using cDNA single strand kit. Differentially expressed genes were cloned and sequenced and data analysis is in progress.

In addition to ACC-deaminase activity, *R. leguminosarum* bv. *viciae* strain LC-31 also showed a typical narrow spectrum activity. It was more effective against the most closely related *R. leguminosarum* strains, but less effective against *Agrobacterium* and *Bradyrhizobium* strains. A 40-kDa protein band was sequenced and submitted to Swiss Prot Database under the accession number P84703. MALDI-TOF analysis of the 40-kDa

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protein gave a molecular weight of 37.6 kDa, which correlated with SDS-PAGE results (Hafeez et al., 2005). The bacteriocin gene has three encoding regions; *rzcA*, for bacteriocin, and *rzcB and rzcD*, which are required for bacteriocin secretion. The sequence of accession number AF273216 was retrieved from gene bank and used to design random primers. An 82-bp *rzcA* fragment of the bacteriocin gene was amplified. The protein sequence of the *rzcA* fragment showed 88% homology with the protein sequence of *rzcA* from the gene bank. It also showed 58% similarity with protease-associated peptidase of *Bacillus cereus* sub sp. *cytotoxis* NVH, which has a molecular weight of 2,961.3 Da.

R. leguminosarum bv *viciae* LC-31 is an efficient nodulating N_2-fixing (182 ± 9.50 nmole C_2H_4 produced $h^{-1}g^{-1}$ nodule dry weight) and IAA-producing strain (3.40 mg L^{-1}). Moreover, it is potent bacteriocin producer and has ACC-deaminase activity. Therefore, LC-31 is a novel *Rhizobium* strain having competitive ability that can be used as a good inoculant.

References

Hafeez FY et al. (2005) Environ. Exp. Bot. 54, 142–147.
Wenbo M et al. (2003) Appl. Environ. Microbiol. 69, 4396–4402.