

F214 Molecular Characterization of putative *nifA* clones from *Frankia* EulK1; a symbiont of *Elaeagnus umbellata*

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To identify *nifA* homologous gene from *Frankia* EulK1, degenerate PCR primers based on conserved central domain of *nifA* was used to amplify *nifA* homologous sequence on the genome of *Frankia* EulK1. The subclone of amplified PCR product was sequenced and showed homology with other *nifA*-like genes. Putative *nifA* clones were selected from the cosmid library of *Frankia* EulK1 using the subclone of PCR product as a probe. The putative *nifA* clones were divided into four groups based on restriction digestion and hybridization patterns. The subclones of restriction fragments hybridized with the probe were partially sequenced and showed homology with transcriptional activators of other microbial organisms. Molecular characterization of four putative *nifA* clones will be discussed on the basis of nucleotide sequence and genomic Southern hybridization.

F215 Molecular Cloning and Characterization of *nifS* from *Frankia* EulK1, a symbiont of *Elaeagnus umbellata*

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It has been shown that major *nif* gene cluster of *Frankia* EulK1 strain from *nifH* to part of *nifS* was contained in a cosmid genomic clone, pEuNIF. To clone rest of *nifS* and other putative *nif* genes, another genomic clone, pEuNIF2, was selected by screening the library using the partial *nifS* as a probe and its overlapping nature with the pEuNIF was confirmed by partial restriction mapping and hybridization experiments. The total size of the pEuNIF2 was estimated to be about 44 kb and 7.2 kb *Bam*HI fragment was hybridized with the previous probe. For determination of nucleotide sequence, 7.2 kb *Bam*HI fragment was subcloned as two fragments; 4.2 kb *Eco*RI/*Bam*HI and 3.0 kb *Eco*RI/*Bam*HI fragments. Partial nucleotide sequence of the two fragments showed homology with *nifS* and *fix* from other nitrogen-fixing microorganisms, respectively. Structure and function of *nifS* and other putative *nif* genes located on the 7.2 kb *Bam*HI fragment and organization of entire *nif* genes in EulK1 strain will be discussed.