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PCR and immunological detection methods for the rapid identification of *Paenibacillus larvae* in honeybee larvae.

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American foulbrood(AFB) is a disease of larval honeybees caused by the bacterium *Paenibacillus larvae*. For the rapid identification of this pathogen, PCR primers were designed on the basis of the 16SrRNA gene of *P. larvae* and the unique 405-bp PCR products was amplified by PCR using specific primer-pair. For the immunological detection of *P. larvae*, specific antibody against whole bacterium was produced from guineapig and tested. Polyclonal antibody specifically reacted with various *P. larvae* subsp. on ELISA. Both rapid detection method will be useful to confirm AFB and monitering of existence and quantity of *P.larvae* in field.

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Monitoring of Released Microorganisms into Oil-Contaminated Sand Microcosms Using Terminal-Restriction Fragment Length Polymorphism (T-RFLP) Analysis

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Terminal-restriction fragment length polymorphism (T-RFLP) has been applied to monitor inoculated oil-degrading microorganisms into sand microcosm. For the simultaneous detection of two bacterial strains, *Corynebacterium* sp. IC10 and *Sphingomonas* sp. KH3-2, and a yeast strain, *Yarrowia lipolytica* 180, an universal primer pair, fluorescently labelled 521F and 1392R, was adapted based on SSU (small subunits) rDNA sequences. Digestion of the 5'-end fluorescently labelled PCR products with restriction enzymes produced specific terminal-restriction fragments (T-RFs) corresponding to each strain.The *Hha*I produced specific termirtal-restriction fragments (T-RFs) of 185 and 442 bases corresponding to *Corynebacterium* sp. IC10 and *Yarrowia lipolytica* 180, respectively. The enzyme, *Nru*I, produced a specific T-RF of 338 base for *Sphingomonas* sp. KH3-2. The detection limit of the inoculated oil-degrading microorganisms into natural environments was determined to be 0.01% of the total microbial populations regardless of different background environments. In the monitoring of released three oil-degrading microorganisms into oil-contaminated sand microcosms, the strain IC-10 and strain 180 survived for 35 days after inoculation. The strain KH3-2 was found at 8 days but not at 35 days. This result showed that T-RFLP could be a useful tool for monitoring the survival and relative abundance of specific inoculated microbial strains into environments.

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The genetic diversity and similarity analysis of bacteria community in groundwater by denaturing gradient gel electrophoresis(DGGE)

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The community structure of bacteria in groundwater was examined by PCR amplified 16S rDNA-denaturing gradient gel electrophoresis(DGGE). DGGE is attractive technique, as it separate same length dsDNA according to sequence variation typical 16S rDNA genes. The genetic diversity and similarity of bacterial community in groundwater was analyzed by GC341f and PRUN518r primer sets for amplification of V3 region of eubacteria 16S rDNA.

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Eubacterial Diversity as Determined by 16S rRNA Gene with Depth in Lake Soyang

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The phylogenetic relationship of the domain bacteria with the depth was investigated by performing a comparative sequence analysis of PCR-amplified 16S ribosomal DNAs. Water and sediment samples were collected with the depth (0, 10, 20, 50, 80, 100 and sediment) in front of Soyang dam. DNA extraction was performed to identify members of the domain eubacteria which inhabit such an aquatic environment, we used PCR to construct a library of 16S rDNA genes cloned from DNA extracted from the waters of Lake Soyang. The 16S rDNAs were amplified by PCR by using oligonucleotide primers (27F-1492R) complementary to 16S rRNA genes and gel-purified PCR products were cloned into vector pGEM-T. Clone libraries (501 clones) of PCR-amplified archaeal rRNA genes were constructed with samples from 0 m to 100 m depth. A restriction fragment length polymorphism (RFLP) analysis of the 16S rDNAs was performed with MspI and AluI. Partial sequencing of the cloned 16S rDNAs revealed an extensive amount of phylogenetic diversity within this system. Sixty-one 16S rDNA clones were partially sequenced. The estimated values of richness in the SY6 (14.76) clone library was much higher than other sites. By comparative sequence analyses, the majority of the examined clones could be affiliated with the Verrucomicrobiales in upper depths and Proteobacteria in lower depths.

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Relationship of the Eubacterial Communities by DGGE Profiles in Lake Soyang

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The community structure of eubacteria in mesotrophic Lake Soyang was examined by PCR amplification of the V3 region of 16S rRNA from microbial communities recovered from various depths in the water column and sediment. Amplified DNA fragments were resolved by denaturing gradient gel electrophoresis (DGGE), and the resulting profiles were reproducible and specific for the communities from different depths. Eubacterial diversity estimated from the number and intensity of specific fragments in DGGE profiles. The similarities of DGGE profiles were determined by UPGMA. SY1 and SY2 of the DGGE profiles were similar over 95%. The similarity between SY4 and SY5 of the DGGE profiles was over 82%. Several dominant fragments in the DGGE profiles were compared with environmental clones. Among the dominant populations were representatives related to methanotroph, sulfate-reducer and so on.

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