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### Bacterial Diversity in Rhizosphere Forest Soil Compared by Cultivation and 16S rRNA Gene Cloning

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In order to investigate the diversity of bacterial community in the *Quercus* and *Conifereus* rhizosphere forest soil were examined 16S rDNA gene. We cultured a collection of 44 isolates by using agar media. Clone libraries were constructed, 108 clones from *Quercus* soil and 111 clones from *Conifereus* soil were clustered based on restriction patterns using computer program, GelCompar II.

Twenty-six different RFLP types were detected from 108 clones (*Quercus* soil) and 30 different RFLP types were detected from 111 clones (*Conifereus* soil). In the case of *Quercus* soil, 20 isolates and 26 selected clones sequenced fell into five bacterial phyla; alpha-, beta-Proteobacteria, *Acidobacteria*, *Verrucomicobia*, *Actinobacteria*, and *Fermicutes*. Twenty-four isolates and 30 clones from *Conifereus* soil fell into six bacterial phyla; alpha-, gamma-, delta-Proteobacteria, *Microbacteriaceae*, *Flavobacteria*, *Plactomycetes*, *Acidobacteria*, and *Actinobacteria*. Thirty four of the isolates belonged to unidentified or uncultured family level groupings.

A018

### MIDI, PCR-based Method for the Comparison of Dominant Members of Fast Growing Copiotrophic Bacteria from Forest Soil.

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The process of colony formation soil bacteria on agar plates was simulated by a colony forming curves (CFCs). Three CFCs were recognized during an incubation period of 1,200h. We report here on the results of grouping of the isolates from the colonies appearing from CFC-I (up to 288h) Soil bacteria isolated on agar plate were divided into two categories according to the capability of growth on NB medium those capable of growing on NB were referred to as copiotrophs. Among 191 isolates from CFC-I were selected 76 isolates as fast growing copiotrophs. The cellular fatty acid of 42 fast growing copiotrophic isolates were analyzed. All isolates were grouped into 5 clusters and we made further 9 subclusters. About 40% of the total isolates belong to cluster III-1 contained C16:0, C17:0 cyclo, C18:1 w7c identified to *Burkholderia*. These isolates were assigned to 32 groupings by using amplified ribosomal DNA restriction analysis (ARDRA) Forty percentage of the isolates belonged to *Bacillus*. It considered that cellular fatty acid analysis is unstable for differentiation and grouping belong to family-level phylogenetic groups

A019

### Purification and Characterization of Protease Producing Hyperthermophilic Bacterium *Geobacillus thermolevorans* C-2

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The new isolate, strain C-2, is the strictly aerobic organism grew well between 60 and 70°C, and cell division still occurred at 120°C. No growth was observed at 35°C. The number of living cells by direct viable count (DVC) comprised 15% of the total direct count (TDC) at 90°C. Hydrolysis of protein was tested on agar plates containing 10g skim milk l<sup>-1</sup>, protease activity largely increased at 90°C.

Cells are spore forming rod (0.5 to 1.0 μm in diameter), Gram-positive. Colonies are spindle, convex and opaque. The major isoprenoid quinone was menaquinone-7; cellular fatty acid profiles consisted of significant amounts of iso-15:0, iso-16:0 and iso-17:0 fatty acids (84.6% of the total). The phylogenetic analysis based on 16S rRNA gene indicates that strain C-2 is a member of *Geobacillus*. The sequence of their 16S rRNA genes were found to be 99% to *Geobacillus thermolevorans* DSM53366<sup>T</sup>, which their closest related type strain.

A020

### Polyphasic Studies of *Geobacillus* Strains Isolated from Soils and Composts

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Forty-three thermophilic endospore-forming *Geobacillus* isolates were obtained from soils and composts from different areas. Polyphasic analyses were performed to elucidate taxonomic relationships among 16 reference and 43 environmental *Geobacillus* isolates. Genotypic tests included single-locus analyses (16S rDNA and GyrB sequencing), genomic DNA profiling (repetitive DNA element-based PCR), and phenotypic analyses encompassed fatty acid methyl ester (FAME) analysis, API galleries (50CHB, ZYM and 20E strips) and 12 other routine phenotypic tests. The information obtained with these techniques was processed with the BioNumerics software in order to analyse taxonomic relationships existing between isolated strains and various reference species of the genus. The usefulness of the techniques will be discussed in term of identification and/or typing of *Geobacillus* spp.