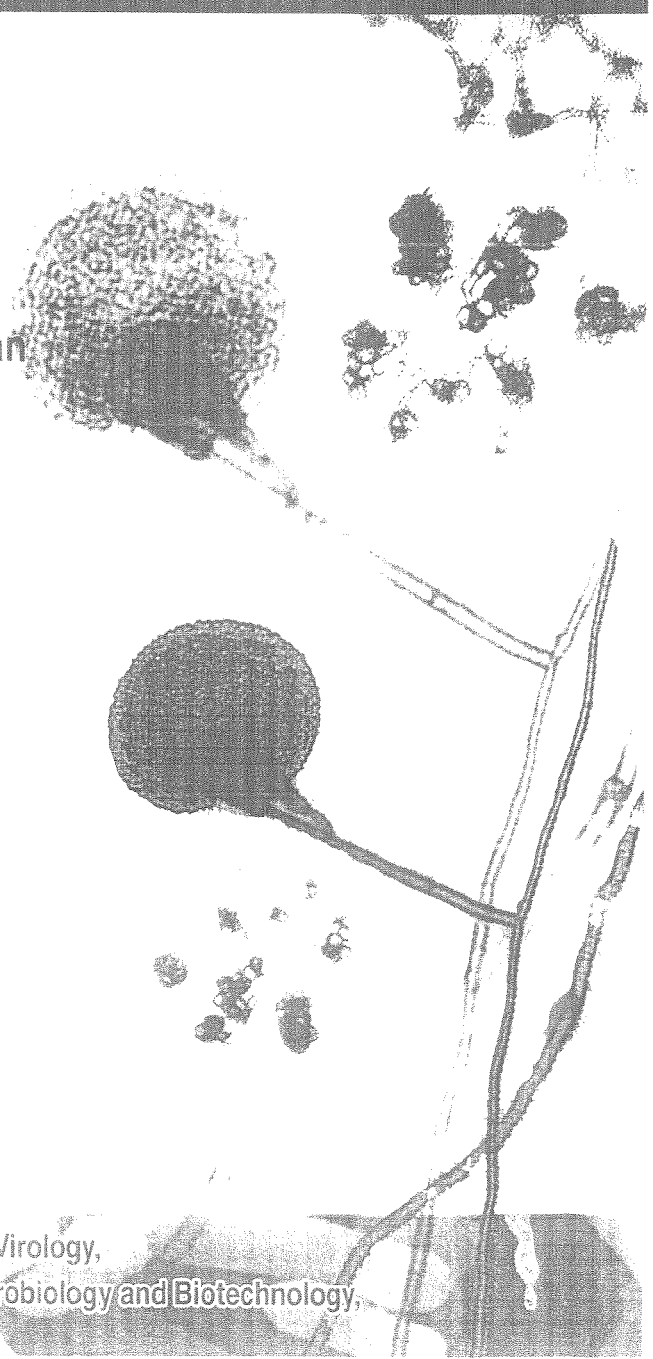




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B017

Use of Stable Isotope Probing to Explore Time-Dependent Dynamics of PCB-Degradative Population Dynamics in Biphenyl-Fed Soil Microbial Communities

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Very little is known about the influence of pollutant exposure history on community structure and population dynamics among biodegraders in soil. One of such reasons may be the currently limited capability of microbial ecology tools for community functioning, i.e., functional community analysis. Here, we used stable isotope probing (SIP) in exploring time-dependent dynamics of biodegradative populations in biphenyl-fed soil microbial communities. After a clean soil from PCB contaminated area (Picatinny, NY) was fed with isotopic carbon labeled biphenyl (¹³C-biphenyl), only biphenyl- and PCB-degrading populations grew and their biomass was incorporated with the heavy carbon. At different periods of incubation (7, 14, and 28 days), DNA and RNA were extracted, and heavy (¹³C-labeled) nucleic acids were isolated using ultracentrifugation and fractionization. The following 16S rRNA gene amplification and clone-sequencing provided results to link phylogenetic with functional (biphenyl- and PCB-degradation) information. Although *Actinobacteria* were the predominant in the soil, *proteobacteria* populations became enriched in the biphenyl-fed microbial communities. The SIP results revealed that beta-*Proteobacteria* were not only early-time but also late-time specialists. Probably because of this heterogeneous nature, the biphenyl-degradative beta-*Proteobacteria* could become the predominant as a group.

Keywords: Stable isotope probing, Polychlorinated biphenyl (PCB), Functional community analysis

B018

Molecular Analysis of Denitrifying Bacterial Communities in Paddy Soils Planted with Transgenic Rice Varieties

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The diversity and seasonal variations of denitrifying bacterial communities in rice field soils planted with genetically-modified (GM) rice varieties were studied by using both culture-dependent and DNA-based analyses. Most probable number (MPN) estimation showed that the seasonal levels of denitrifying bacterial populations ($4.8 \times 10^4 \sim 2.5 \times 10^5$ cells/g soil in GM subplots and $8.1 \times 10^4 \sim 5.8 \times 10^5$ cells/g soil in non-GM subplots) were similar to each other between GM and non-GM subplots. Both in the GM and non-GM subplots, dominant denitrifying bacteria isolated from the paddy soils over the year were the *nirS* type denitrifiers and belonged to the *Pseudomonas* and *Bacillus* species. Sequence diversity analysis of the *nirS* and *nirK* genes cloned from soil DNAs revealed that the community structures of the denitrifying bacteria were similar to each other between the GM and non-GM subplots in a given month, suggesting that there were no significant differences in the structures of denitrifying microbial populations between GM and non-GM rice paddy soils during the experiment. However, the denitrifying bacterial community structures appeared to change seasonally as shown by different DGGE DNA banding patterns over the year. The results of this study suggested that the denitrifying bacterial community structures of the experimental rice field were changed with time, but that they were not significantly affected by cultivation of GM rice plants.

Keywords: denitrifying bacteria, denitrification, *nir* gene, rice field, transgenic plants, DGGE

B019

Cultivation of 19 Novel Bacterial Species from a Eutrophic Freshwater Pond, Inkyong

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Although a dilution-to-extinction culturing has shown a great potential to isolate previously uncultured bacteria, it has a little shortcoming in the typical bacterial classification system because standard biochemical characterization requires much quantity of biomass. To isolate and classify previously uncultured freshwater bacteria, a standard dilution plating method was applied to a eutrophic freshwater, Inkyong pond, located within Inha University. A total of 60 strains, 15 strains per each culture medium, were obtained using four different kinds of culture media, including R2A, 1/10R2A, PCA, and 1/10PCA. Among the 60 strains isolated, 27 strains showed less than 97% 16S rRNA gene sequence similarities to validly published species, and thus they are considered to comprise 19 novel species. Of the 27 strains assigned to the novel species, the majority of the strains (20 strains) were affiliated with the *Alphaproteobacteria* and *Betaproteobacteria*. The remaining 7 strains were affiliated with the *Gammaproteobacteria*, *Firmicutes*, *Actinobacteria*, and *Deinococci*. Remarkably, 11 novel strains assigned to the *Betaproteobacteria* comprised 9 novel species. Because we have isolated 19 novel species from a usual freshwater pond, using a conventional culturing technique, our results suggest that an unexplored ecosystem, even if it looks like a common ecosystem found elsewhere, harbors diverse unidentified microbes, which will be definitely further characterized.

Keywords: cultivation, freshwater, novel species, 16S rRNA gene, bacterial diversity

B020

Effects of pH on Microbial Communities of Activated Sludge Performing Enhanced Biological Phosphorus Removal in a Sequencing Batch Reactor

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The effects of pH on microbial communities of activated sludge performing enhanced biological phosphorus removal (EBPR) were investigated in an anaerobic/aerobic sequencing batch reactor (SBR) supplied with acetate as a sole carbon source. Almost complete P-removal could be achieved at all pH, meaning that pH changes did not effect on phosphorus removal efficiencies. However, terminal restriction fragment length polymorphism (T-RFLP) and 16S rRNA gene sequencing analyses showed that microbial communities were changed dramatically by pH changes. At high pH (No pH control, ~ pH 8.4) *Pseudomonas*-related bacteria were present predominantly, but *Rhodocyclus*-related bacteria that have been known as typical phosphorus accumulating organisms (PAOs) were absent. The population of *Pseudomonas*-related bacteria decreased by pH decrease and at low pH (pH 6.5) *Rhodocyclus*-related bacteria became a major group. This suggested that *Pseudomonas*-related bacteria group and *Rhodocyclus*-related group might switch their roles as Polyphosphate Accumulating Organisms (PAO) at different pH in an anaerobic/aerobic sequencing batch reactor (SBR) supplied with acetate. We isolated *Pseudomonas*-related bacterium and confirmed that the isolate contained polyphosphate kinase (ppk) and polyhydroxy alkanic acid synthase (phaC). Additionally in this study sequence analysis of *ppk* and *phaC* genes and possibility of *Pseudomonas*-related bacteria as a PAO candidate will be discussed.

Keywords: Enhance biological phosphorus removal, Microbial community, pH