Microorganisms relevant to bioremediation

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Introduction

Bioremediation is a technology that utilizes the metabolic potential of microorganisms to clean up contaminated environments. One important characteristic of bioremediation is that it is carried out in non-sterile open environments that contain a variety of organisms. Of these, bacteria, such as those capable of degrading pollutants, usually have central roles in bioremediation, whereas other organisms (e. g. fungi and grazing protozoa) also affect the process. A deeper understanding of the microbial ecology of contaminated sites is therefore necessary to further improve bioremediation processes.

In the past two decades, molecular tools, exemplified by rRNA approaches, have been introduced into microbial ecology; these tools have facilitated the analysis of natural microbial populations without cultivation. Microbiologists have now realized that natural microbial populations are much more diverse than those expected from the catalog of isolated microorganisms. This is also the case for pollutant-degrading microorganisms, implying that the natural environment harbors a wide range of unidentified pollutant-degrading microorganisms that have crucial roles in bioremediation. This article summarizes the results of recent studies of microbial populations that are relevant to bioremediation.

Molecular ecological information is thought to be useful for the development of strategies to improve bioremediation and for evaluating its consequences (including risk assessment). Molecular tools are especially useful in bioaugmentation, in which exogenous microorganisms that are introduced to accelerate pollutant biodegradation need to be monitored. This article

discusses recent examples of the successful application of molecular ecological tools to the study of bioremediation.

Microorganisms relevant to methane oxidation

Traditionally, studies on pollutant biodegradation have been initiated by the isolation of one or more microorganisms capable of degrading target pollutants; however, conventional isolation methods have resulted in the isolation of only a fraction of the diverse pollutant-degrading microorganisms in the environment. In addition, most isolated organisms have shown pollutant-degradation kinetics that differ from those observed in the environment. For example, laboratory-cultivated methanotrophs exhibit half-saturation constants for methane oxidation which are one to three orders of magnitude higher than those observed in soil. Using molecular phylogenetic analyses of isotope-labeled DNA, (Radajewski et al.) successfully identified two novel methanotrophs that actively degrade methane under environmental conditions. Molecular approaches that target the 16S rRNA gene (16S rDNA) and genes encoding enzymes involved in key metabolic steps (e. g. those encoding particulate methane monooxygenase) have been applied to the analysis of methanotrophs in rice field soil, lake sediments and forest soil. Methanotrophs are considered to be important for reducing the emission of methane, a greenhouse gas, from soil and sediment. In addition, methanotrophs co-metabolize trichloroethylene (TCE); therefore, TCE bioremediation often employs methane injection as a means to stimulate the TCE-degrading activity of indigenous methanotrophs (i. e. methane biostimulation). Methanotrophs which occurred at a methane biostimulation site were recently analyzed using denaturing gradient gel electrophoresis

(DGGE) of polymerase chain reaction (PCR)-amplified 16S rDNA and soluble methane monooxygenase gene fragments.

Marine petroleum hydrocarbon degradation

Molecular ecological approaches have also been used to analyze bacterial populations that occur in petroleum-contaminated marine environments. Spilled-oil bioremediation experiments conducted at a sandy beach found that phylotypes affiliated with the subclass of Proteobacteria appeared in the DGGE fingerprints obtained for oiled plots but not in those for unoiled plots, suggesting their importance in spilled-oil bioremediation. Another oil-spill experiment conducted at a beach in the Norwegian Arctic showed that 16S rDNA types affiliated with the Î³-Proteobacteria, especially those belonging to the Pseudomonas and Cycloclasticus groups, were abundant in fertilized oil sands. Microbial populations which occurred in seawater after supplementation with petroleum and inorganic fertilizers have been analyzed using rRNA approaches; it was reported that bacterial populations belonging to the Proteobacteria and the genus Alcanivorax showed accelerated growth. These studies have indicated that some groups of bacteria commonly occur in oil-contaminated marine environments, although other populations change under different environmental conditions.

Anaerobic petroleum hydrocarbon degradation

As petroleum hydrocarbons are persistent under anaerobic conditions, their contamination of groundwater is a serious environmental problem. The microbial diversity in a hydrocarbon- and chlorinated-solvent contaminated aquifer undergoing intrinsic bioremediation was assessed by cloning and sequencing bacterial and archaeal 16S rDNA fragments. This study detected

phylotypes that were closely related to Syntrophus spp. (anaerobic oxidizers of organic acids with the production of acetate and hydrogen) and Methanosaeta spp. (aceticlastic methanogens), suggesting their syntrophic association. Phylotypes affiliated with candidate divisions (that do not contain any isolated organisms) were also obtained in abundance from the contaminated aquifer, although their physiology is completely unknown. A similar syntrophic association of bacteria and archaea has also been reported in a methanogenic enrichment that slowly degrades hexadecane. Likewise, a toluene-degrading methanogenic consortium was characterized by rRNA approaches. The consortium comprised two archaeal species related to the genera Methanosaeta and Methanospirillum, and two bacterial species, one related to the genus Desulfotomaculum and the other unrelated to any previously described genus. Fluorescence in situ hybridization (FISH) with group-specific rRNA probes was used to analyze a denitrifying microbial community degrading alkylbenzenes and n-alkanes; the Azoarcus/Thauera group was found to be the major bacterial group. Bacteria affiliated with the Îµ-Proteobacteria were found to grow in petroleum-contaminated groundwater which accumulated at the bottom of underground crude-oil storage cavities. Microbial communities associated with anaerobic benzene degradation under Fe(III)-reducing conditions in a petroleumcontaminated subsurface aquifer were also analyzed by DGGE analysis, and it has been suggested that Fe(III)- reducing Geobacter spp. have an important role in the anaerobic oxidation of benzene. The available electron acceptors are the principal determinants for the types of microorganisms that occur in anaerobic environments, and microbial populations identified in the above papers are considered important for petroleum hydrocarbon degradation in

subsurface environments under the respective conditions. On the basis of these results, future developments in anaerobic hydrocarbon bioremediation are anticipated. It is noteworthy that phylotypes that are only distantly related to known genera are often detected as major members of the anaerobic communities, suggesting that parts of anaerobic hydrocarbon biodegradation processes remain unidentified.

Polycyclic aromatic hydrocarbon degradation

Polycyclic aromatic hydrocarbons (PAHs) are compounds of intense public concern owing to their persistence in the environment and potentially deleterious effects on human health. A soil-derived microbial consortium capable of rapidly mineralizing benzo[a]pyrene was analyzed by DGGE profiling of PCR-amplified 16S rDNA fragments The analysis detected 16S rDNA sequence types that represented organisms closely related to known high molecular weight PAH-degrading bacteria (e. g. Burkholderias, Sphingomonas and Mycobacterium), although the degradation mechanisms have yet to be resolved. In soil environments, the reduced bioavailability of PAHs due to sorption to natural organic matter is an important factor controlling their biodegradation. Friedrich et al. reported that different phenanthrene-degrading bacteria occurred in soil enrichments when different sorptive matrices were present. It has also been shown that the application of surfactants to soil enrichments that degrade phenanthrene and hexadecane altered the microbial populations responsible for the degradation. These results have common implications for bioremediation; that is, nature harbors diverse microbial populations capable of pollutant

degradation from which a few pollutant-degrading populations are selected according to bioremediation strategies.

Metal bioremediation

Because of its toxicity, metal contamination of the environment is also a serious problem. Recent studies have applied molecular tools to the analysis of bacterial and archaeal populations that are capable of surviving in metal-contaminated environments. Bacterial communities in soil amended for many years with sewage sludge that contained heavy metals were assessed using rRNA approaches, including FISH and cloning and sequencing. The study found that two sequence groups affiliated with the Proteobacteria and Actinobacteria were frequently obtained from clone libraries from the metal-contaminated soil, although most Actinobacteria sequences showed low similarity (<85%) to the sequences of any hitherto cultured actinomycete. The detoxification machineries that some of these organisms may have are considered useful for metal bioremediation, and comparisons with the machineries of previously isolated metal-resistant bacteria may yield interesting results. Recently, heavy-metal-tolerant Ralstonia eutropha was genetically engineered to express mouse metallothionein on the cell surface. It was demonstrated that the inoculation of Cd2+-polluted soil with the genetically engineered Ralstonia significantly decreased the toxic effects of the heavy metal on the growth of tobacco plants.

Waste treatment

Microbial consortia involved in wastewater treatment have been a major subject of microbial ecology, and many papers have been published in which molecular tools were used for community analyses. Bacterial community

structures and physiological states within an industrial phenol bioremediation system were recently analyzed. Comparisons made between the amounts of group-specific rRNAs and the process chemistry enabled the authors to identify some phylogenetic groups of bacteria important for the process performance. The phylogenetic diversity of bacterial communities supported by a seven-stage, fullscale bioreactor used to treat pharmaceutical wastewater was studied using PCR-based techniques (i. e. DGGE fingerprinting and cloning of 16S rDNA fragments). These two techniques detected similar phylotypes, although they failed to concede on their relative distribution, suggesting difficulties in quantitative interpretation based on these methods. A combination of 16S rDNA cloning, hybridization with oligonucleotide probes for ammonia-oxidizing bacteria (AOB) and sequencing of the hybridization-positive clones suggested that novel Nitrosospira-like populations were the major AOB in a rhizosphere zone used to treat wastewater (rhizoremediation). To identify microbial populations responsible for phosphorus removal in activated-sludge, the structure of the bacterial population was analyzed by FISH during the operation of a laboratory-scale reactor with various phosphorus removal rates. FISH has also been used to analyze microbial populations in mesophilic and thermophilic sludge granules, foaming activated-sludge and bulking activated-sludge.

Temperature-gradient gel electrophoresis (TGGE) of PCR-amplified 16S rDNA fragments was used to identify the major phylotypes in phenol-digesting activated-sludge. Physiological characterization of isolated bacteria corresponding to these phylotypes identified microbial transition that caused

a failure in the phenol treatment. The ecological information obtained in this study was successfully used to develop a countermeasure against the failure in the phenol treatment. These papers present successful examples which showed the utility of molecular ecological approaches for manipulating microbial consortia for bioremediation.

Bioaugmentation

The introduction of exogenous microorganisms into environments (bioaugmentation) has been used in an attempt to accelerate bioremediation. It is desirable that the fate of an introduced organism be monitored in order to prove its contribution to pollutant degradation and to assess its influence on the ecosystem. Molecular tools have been used for this purpose. DGGE/TGGE fingerprinting of 16S rDNA fragments has been used to examine the effects of bioaugmentation on indigenous bacterial community structures in a range of situations: a laboratory-scale semicontinuous activated-sludge system loaded with 3 chloroaniline; experimental model sewage plants subjected to shock loads of chlorinated and methylated phenols; and in 2, 4-dichlorophenoxyacetic-acidcontaminated soil horizons. Quantitative PCR assays targeting catabolic genes and gyrB (the gene coding for the subunit B protein of DNA gyrase) have successfully been used to monitor the fates of introduced bacteria in complex microbial communities (e. g. those in activated-sludge and in soil). In some cases, where genetically modified organisms were utilized, bioaugmentation improved pollutant-biodegradation rates in the environment due to the establishment of transconjugants capable of

degrading the pollutants rather than the direct contribution of the inoculated organisms.

Conclusions

Bioremediation is still considered to be a developing technology. One difficulty is that bioremediation is carried out in the natural environment, which contains diverse uncharacterized organisms. Most pollutant-degrading microorganisms isolated and characterized in the laboratory are now thought to make a minor contribution to bioremediation. Another difficulty is that no two environmental problems occur under completely identical conditions; for example, variations occur in the types and amounts of pollutants, climate conditions and hydrogeodynamics. These difficulties have caused the bioremediation field to lag behind knowledge-based technologies that are governed by common rationales.

As summarized in this review, information on microbial populations relevant to bioremediation is accumulating rapidly with the aid of molecular ecological approaches. Although our knowledge is not yet complete, it is time to initiate more comprehensive approaches to find common rationales in bioremediation. In some cases, for example, marine petroleum bioremediation, we have already found that similar bacterial populations occur even at geographically distant sites. Understanding the physiology and genetics of such populations may prove very useful to assess and improve bioremediation. Most importantly, we need to identify general aspects in certain types of bioremediation. For this purpose, I wish to propose the construction of a database that collects the results of molecular ecological assessments of contaminated and bioremediated sites. The database would

provide bioremediation with ecological backgrounds and, in concert with currently available databases relevant to bioremediation, would facilitate the development of commonly applicable schemes for certain types of bioremediation.