epiphytic bacteria in the liberating leaf solution were well observed on filters stained by both types of fluorochrome, although DAPI showed better fluorescent images than AO and not necessarily required a washing step of the filters stained. The optimum conditions of the DAPI stains were 5 mg ml⁻¹ for 5 min both for leaves and for filters of the liberating solution. It was confirmed that a critical step in the epifluorescence microscopy of leaf surfaces was to minimize release of water from the leaf. For this, the stained leaf samples were put on a filter paper, kept in a dry oven at 70°C for 2 min instead of air-drying, and then immediately observed by epifluorescence microscopy. The established technique was applied to enumerate epiphytic bacteria on oak tree leaf surfaces.

B307

Isolation and Phenotypic
Characterization of
2-(2,4-Dichlorophenoxy)-Propionic
Acid Degrading Bacteria from Soils

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dominant Eight numerically 2-(2,4-dichlorophenoxy)-propionic acid(2,4-DP)-degrading bacteria and four pairs of bacteria showing syntrophic metabolism of 2,4-DP were isolated from soils, and their phylogenetic and phenotypic characteristics were investigated. The isolates were able to utilize the herbicide 2,4-DP as a sole source of carbon and energy and their 2,4-DP degradative enzymes were induced by the presence of 2,4-DP. Analysis of 16SrDNA sequences indicated that the isolates were related to members of the genera, Sphingomonas, Herbaspirillum, and Afipia. The chromosomal DNA patterns of the isolates obtained by polymerase chain reaction(PCR) amplification of repetitive extragenic palindromic(REP) sequences were distinct from each other. Nine of the isolates were observed to have plasmids, but none of them was transmissible. Many of the isolates degraded 2,4-D, MCPP, and MCPA as well as 2,4-DP. Oxygen uptake experiments indicated that most of the isolates degraded 2,4-DP through 2,4-dichlorophenol.

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Catabolic Potential of *Pseudomonas*rhodesiae Strain KK1 Isolated from
PAH-Contaminated Soil at a Former
Manufactured Gas Plant (MGP)
Facility

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Catabolic potential of strain KK1 from polycyclic aromatic hydrocarbons (PAH)-contaminated soil at a former manufactured gas plant (MGP) facility was analyzed using dioxygenase clonal library radiospirometry. The tar-contaminated soils at the MGP site had experienced significant PAH contamination for at least 100 years. Strain KK1 was identified as Pseudomonas rhodesiae using BIOLOG analysis system, fatty acid analysis, and 16S rDNA sequencing, and designated Pseudomonas rhodesiae KK1. To evaluate catabolic potential of Pseudomonas rhodesiae KK1, total DNA was extracted from KK1 cell and dioxygenase genes were amplified using the PCR process with random dioxygenase

primers. PCR products were cloned and 50 randomly selected clones were sequenced. Comparative sequence analysis indicated that dioxygenase clones from strain KK1 were divided into 6 groups. Radiospirometric analysis for the substrates anthrancene, naphthalene, phenanthrene, pyrene, and benzo(a)pyrene revealed that strain KK1 has the catabolic potential for anthracene, naphthalene, and phenanthrene.

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자외선 광촉매 장치를 이용한 미생물 살균효과 측정

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두 가지 형태의 UV-TiO₂ 반응기를 이용하 여 Escherichia coli와 Saccharomyces cerevisiae의 살균효과를 측정하였다. 첫 번째 형태는 반응 기를 한 개 사용했을 때의 경우이며 또 다른 형 태는 반응기를 하나 더 설치하여 두 개의 반응 기를 이용할 경우이다. 254 nm에 최대 39 watt 의 자외선 방출량을 내는 램프를 원형 Pyrex 유리관 중앙에 설치하였고 TiO2는 석영관에 박막증착된 형태, Glass bead에 박막증착된 형 태와 Alginate bead에 박막증착된 형태로 나 누어 회분식으로 살균정도를 측정하였다. 반 응기를 하나만 사용했을 때 E. coli에 대한 1분 동안의 살균력은 TiO2로 박막증착된 석영관을 사용했을 경우 33.5%의 살균효과를 나타냈으 며 Glass bead에 박막증착하였을 경우에는 89.9 %의 살균효과를 나타내었다. Glass bead 를 이용한 반응기에 기포를 주입하였을 경우 초기 균의 개수가 7.1 × 10³ cells/mL에서 시 작하여 1분 동안 95%의 살균효과를 보인 반면 기포를 주입하지 않을 경우 90.6%의 살균효과 를 나타내었다. TiO2로 박막증착된 Alginate bead에 기포를 주입하였을 때 1분 동안 86%의 살균 효과를 보여 Glass bead를 사용하였을 때 보다 살균효과가 떨어지는 것으로 관찰되었 다. 살균제인 과산화수소를 첨가하였을 경우 1 분 동안에 99.9%의 살균효과를 보였으며 이때 과산화수소의 농도는 25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, 500 mg/L 이였다. Glass bead를 이용하고 폭기를 시켜면서 반응기를 하나 더 설치하였을 경우의 살균효과는 96.4%로 반응기가 하나일 때보다 조금 더 높은 살균효과를 나타내었다. S. cerevisiae와의 비교실험에서는 E. coli에 대한 살균력이 1분 동안에 좀더 높게 나타났다. 살균제인 NaClO를 첨가하였을 경우 유효염소 농도로서 0.5 mg/L, 1 mg/L, 1.5 mg/L의 저농도 주입시 두 개의 반응기를 이용하여 Glass bead에 폭기를 시켰을 경우와 비교했을 때 살균효과가 없었으며 10 mg/L, 30 mg/L, 50 mg/L의 농도로 주입하였을 경우 1분 동안에 최고 99.6%의 살균효과를 나타내었고 살균 후 균체들의 재활성은 거의 없었다.

B310

Aerobic Reduction Mechanism of Manganese Oxide by a Bacterial Strain MR4

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Aerobic reduction mechanism manganese oxide was investigated with a soil bacterium designated as strain MR4. Based on the examination with either dialysis bag or spent medium, organic acid production was found to be the most probable mechanism for Mn (IV) reduction by this strain. Using HPLC and other specific determination methods available, organic acids generated by glucose oxidation were positively identified as pyruvate and oxalate in the spent medium of MR4 cultures grown on AMR medium supplemented with 100 μg MnO₂/ml. In AMR medium, an evolution of pyruvate was maximal at 4 hr (0.2 mM) of incubation. An oxalate concentration was gradually increased to 12 hr (0.2 M) and maintained this concentration to 48 hr. Time versus oxalate production curve corresponded to the pattern of MnO2