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学 位 論 文 要 旨
Dissertation Abstract

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Title of Dissertation

Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) and Crude Oil by Saline-Alkaline Tolerant Fungi
(海水およびアルカリ性耐性菌による多環芳香族炭化水素(PAHs)および原油の生分解)

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One major problem facing the industrialized world today is the contamination of environments (soil, ground water, sediment, surface water, and air) by hazardous and toxic chemicals. Modern society relies on a striking array of organic chemicals, and the quantities used are staggering. Polycyclic aromatic hydrocarbons (PAHs) are one of the most toxic and persistent organic chemicals in the environment. PAHs are high-risk pollutants that affect human health because of their carcinogenic and mutagenic effects. The United States Environmental Protection Agency (USEPA) designated 16 PAH compounds as priority pollutants to be measured in environmental samples. PAHs are a group of toxic compounds that are formed when organic materials are burned; the amount of PAHs in soils coming from atmospheric fall-out rose steadily during the twentieth century. They appear in particularly high concentrations at many industrial sites, particularly those associated with petroleum and gas production. They are also found in high concentrations in the wood-preserving industry as wood protectants in creosote and anthracene oil (creosote contains 85% PAHs by weight). The main sources of PAH contamination are natural oil seeps, refineries, oil storage, accidental oil spills, and municipal and urban waste water. These processes generate large volumes of unwanted sludge that contains PAHs and is detrimental to the surrounding ecosystems, especially marine environments and coastal areas. Recent studies on PAH contamination have focused on these areas.

Bioremediation shows greater potential as a solution to PAH contamination than physical or chemical treatments. It is inexpensive, completely destroys organic pollutants (via mineralization to produce carbon dioxide and water as the final products), and can be used as an in situ treatment. Bioremediation is also supported by the biodiversity of microbes in nature, such as white rot fungi (WRF), which can degrade many hazardous xenobiotic compounds. WRF are well-known ligninolytic enzyme producers [laccase (Lac), manganese peroxidase (MnP), and lignin peroxidase (LiP)], which can degrade many pollutants such as PAHs. However, most studies have focused on the degradation of PAHs by WRF under non-saline rather than saline conditions because limited numbers of saline-tolerant basidiomycetes have been identified and examined. On the other hand, saline-tolerant WRF and their extracellular ligninolytic enzymes potentially have environmental applications in coastal areas because they degrade a broad array of environmental pollutants such as high molecular weight PAHs (HMW-PAHs).

In a pre-screening of 82 fungal strains using Remazol brilliant blue R for the degradation of PAHs, *Bjerkandera adusta* SM46 exhibited the highest tolerance to saline-alkaline stress. Moreover, a *B. adusta* culture grown in a liquid medium containing BaP (benzo[a]pyrene, 5 ring PAH) exhibited resistance to salinities up to 20 g l^{-1}. These conditions did not inhibit fungal growth or the expression of MnP or LiP. The degradation rate also rose as salinity increased to 20 g l^{-1}. Fungal growth and

enzyme expression were inhibited at a salinity of 35 g l^{-1}. These inhibitory effects directly decreased the degradation rate (>24%). $MnSO_4$ as an inducer improved the degradation rate and enzyme expression. MnP and LiP activity also increased 7-fold and 5-fold, respectively. SM46 degraded BaP (38–89% over 30 days) in an acidic environment (pH 4.5) and under saline-alkaline stress conditions (pH 8.2). Investigating the metabolites produced revealed BaP-1,6-dione as the main product, indicating the important role of ligninolytic enzymes in initializing BaP cleavage. The other metabolites detected (naphthalene acetic acid, hydroxybenzoic acid, benzoic acid, and catechol) may have been ring fission products. Based on our present results and previous findings, we propose that ligninolytic enzymes are key enzymes in the degradation of BaP by *B. adusta* SM46 under saline-alkaline stress conditions because they initialize the formation of BaP-1,6-dione. Although this fungus is a terrestrial-derived fungus, it performed and adapted well in a more complex environment (saline-alkaline stress), which suggests its potential as a candidate for bioattenuation and bioremediation processes.

In the present study, we used an enhanced culture system (ECS) for *B. adusta* SM46 on BaP degradation under acidic (pH 4.5) and saline conditions (pH 8.2). The ECS using Tween 80 (T-80) and $MnSO_4$ enhanced both the ligninolytic enzyme activities and degradation rate by the fungus. Based on contour plot analysis, the highest degradation was achieved with $MnSO_4$ higher than 0.4 mM and Tween 80 of 0.4–0.7% at pH 4.5. Applying the optimum condition to the fungal culture system under saline-alkaline stress could increase the degradation efficiency to more than 44%. MnP and LiP activity was higher at pH 4.5 (110.9 and 79.9 U/L, respectively). Moreover, Lac and MnP activity was higher (75.6 and 130.0 U/L, respectively) at saline pH 8.2, indicating stress condition stimulation.

Using lignocellulosic materials (wood meal, kapok fibre, rice straw, and pulp waste) as a solid support medium for WRF was also found to be effective in enhancing PAH degradation in sea water and sea media by *B. adusta* SM46. Rice straw was selected as the most suitable support based on fungal growth, ligninolytic enzyme production, and degradation rates of PAHs after inoculation with *B. adusta* SM46. Rice straw-immobilized *B. adusta* (RSIB) showed faster growth and colonization, and increased Lac, MnP, and LiP activity. The optimum granule size of rice straw as an immobilizing agent for *B. adusta* was 840 µm. Lac, MnP, and LiP activities were monitored for 15 and 30 d. Low molecular weight PAHs (LMW-PAHs, 2–3 rings) were the most extensively degraded by RSIB. When grown on high molecular weight PAHs (HMW-PAHs, 4–5 rings), degradation rates varied between 16 and 63% on contaminated sea sand and between 22 and 61% on contaminated sea water. The mean degradation rates for cellular and enzymatic degradation differed among the PAHs tested in the order of naphthalene > phenanthrene > benzo[a]pyrene > chrysene. RSIB may also be used as an alternative method to more effectively and efficiently produce ligninolytic enzymes than the submerged culture method.

To degrade crude oil by bioremediation of crude oil-contaminated sea sand, five fungi including *B. adusta* SM46, capable of growing on the BaP-containing saline malt agar medium (pH 8.2), were selected. A co-culture of *Pestalotiopsis* sp. NG007 and *Polyporus* sp. S133 at a ratio of 25/75 was found to be the most suitable fungal co-culture for both degrading crude oil and enzyme activities. The crude oil (C-heavy oil, asphalt, A-heavy oil) in the sea sand was degraded efficiently by the fungal co-culture. Periodically adding malt extract (10%) as a nutrient source, Tween 80 (0.5%) as a surfactant, and a mineral mixture of $MnSO_4$ and $CuSO_4$ (1 mM) as enzyme inducerss to the crude oil-contaminated sea sand enhanced enzymatic activities during bioremediation even after 120 d, by contributing to a new generation of mycelia during bioremediation. The nutrient biostimulation enhanced enzymatic activities, leading to the greater degradation of total petroleum hydrocarbon (TPH) than the control treatment (without nutrient biostimulation).