Environmental Microbiology Research Section

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1. Introduction

There is a very close relationship between energy resource consumption and environmental protection, becoming an essential research issue for developing a sustainable society. We still heavily rely on fossil energy, and there is concern that emitted greenhouse gases break the harmony of the global environment. Besides, we need a great deal of energy to fix environmental pollution that continues to be the shadow of civilization's progress due to the energy consumption of fossil fuels. As one of the solutions, we will develop a practical method using 'enzymes' derived from environmental microorganisms with high energy utilization efficiency in catabolism. Also, we are remarking on sustainable food production methods, which are the energy of life. We are globally working with academics, biotechs, and university start-ups to network research toward the social implementation of our technologies.

2-1. Two-compositely microbial catalyst efficiently degraded polychlorinated biphenyls.

Polychlorinated biphenyls (PCBs) are well-known environmental pollutants broadened in all living environments. Biphenyl dioxygenase (BDO) plays a crucial role in the degradation of PCBs. BDO catalyzes the incorporation of two oxygen atoms into the aromatic ring of PCB, which induces the aromatic ring cleavage. Significantly, we developed the composite type of catalytic enzyme consisting of the two BDOs

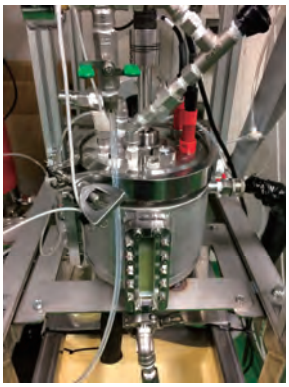

Figure 1. The composite BDOs-microbial catalyst was evaluated in the dedicated experimental bioreactor with the device of oxygen microbubble generation.

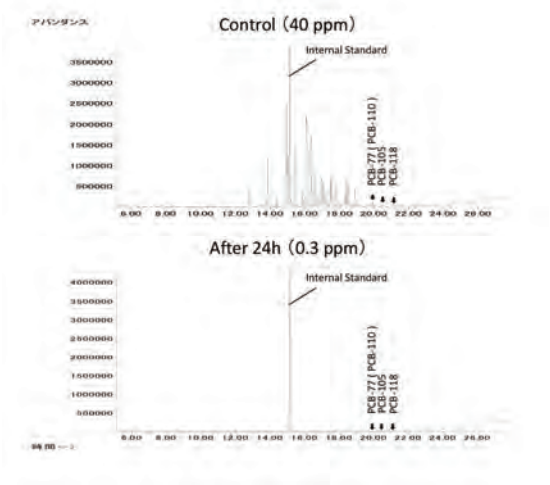

Figure 2. The data from the gas chromatography-quadrupole mass spectrometer showing the PCBs degradation by the composite BDOs-microbial catalyst.

with different substrate specificities; moreover, we developed the bioreactor for generating oxygen microbubbles that enhance the enzymatic activities BDOs (Figure 1). As a result, we succeeded in constructing the practical system that degraded 99.3% of 40 mg L^{-1} of major commercial PCBs (Kenechrol KC-300 and KC-400) in 24 hours (Figure 2). Moreover, this result achieved the waste disposal standard defined by the Ministry of the Environment Government of Japan.

2-2. Several bacterial species associated with PCBs dechlorination were genetically identified on PCBs contaminated sites.

To extend further the composite degrading reaction of PCBs, we have been trying to create a unique artificial enzyme that dechlorinates PCBs by two-electron reduction. Here, we collected fresh-water sediments from the contaminated site with PCBs in the Osaka area and investigated whether the bacteria associated with PCBs dechlorination exist. As a result, it was estimated that *Dehalobacter* sp. and *Desulfitobacterium* sp. by 16S rRNA gene phylogenetic analysis. Wang and He (Environ Sci Technol, 2013) reported that '*Deharobacter*' dechlorinates penta-/hexachlorinated biphenyls and '*Desulfitobacterium*'

dechlorinates tetra-chlorinated biphenyls hydroxylated at the para position. We succeeded in preparing the media for growing these particular bacterial species and their cultivation method. Besides, we also observed that these two bacterial species reduce PCBs in the artificial model of the polluted environment. Even today, repeated long-term observation is being made to confirm whether the result is correct.

3-1. Discovery of a novel anti-filamentous fungal protein secreted by *Rhizoctonia solani*.

Rhizoctonia solani is a filamentous fungus belonging to the phylum Basidiomycota and displays a polyxeny plant-necrotizing pathogenicity that is well known to cause severe diseases in many crops, including rice sheath blight which seriously damages paddy-rice production. The infection mechanism of this phytopathogenic filamentous fungus has not yet been fully elucidated, but when wheat bran is added to the growth medium, it is characterized by the secretion of glycosidases that lyse the cell walls of plants and fungi. We discovered a novel protein with a molecular weight of approximately 10 kDa that exhibits anti-filamentous fungal activities in the secretion of *R. solani* cultured with wheat bran as a solid medium. The results were reported for the first time this year at the 2023 Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry[1]. This protein inhibited the growth of *Fusarium fujikuroi*, another phytopathogenic filamentous fungus belonging to the phylum Ascomycota; moreover, it was suggested that this protein also inhibited conidium formation and germination of *F. fujikuroi*. However, this protein did not inhibit the growth of *Saccharomyces cerevisiae*, a yeast that belongs to the same phylum Ascomycota as *F. fujikuroi* but is different from filamentous fungi. A BLAST search of this protein's partial amino acid sequence revealed by amino acid sequence analysis suggested that it is a protein of unknown function. Likewise, the whole genome sequence of the *R. solani* strain producing this protein was analyzed, and a BLAST search of the deduced full-length amino acid sequence also suggested that it is a protein of unknown function. Based on the above process and sequence analysis of the cDNA encoding this protein, this protein's complete amino acid sequence was 122 residues, and the amino acid sequence of the mature protein, excluding the presumed signal peptide sequence, was 88 residues. As a result, the molecular weight of this protein was estimated to be 9648.24. This mature protein's primary amino acid sequence has a unique structural feature that has never been reported. Specifically, this protein consisting of 88 residues had a double-repeat structure of 41 residues with extremely high homology (92%) across the central 6 amino acid residues. The functional role of such repetitive sequences is generally still poorly understood. We named this anti-filamentous fungal protein "**Double-Repeating Homologous Sequence Anti-Fungal Polypeptide (DRHS-AFP)**."

3-2. Preparation of a recombinant DRHS-AFP and evaluation of its anti-filamentous fungal spectrum.

We constructed a genetically recombinant protein expression system for DRHS-AFP. The DRHS-AFP gene encoding the 88 residues of the mature protein with the additional initiation methionine was amplified by using PCR. Then, the DRHS-AFP expression plasmid, pEp10, was constructed by inserting the DRHS-AFP gene into the *E. coli* expression vector pET-15b and *E. coli* host strain BL21(DE3) was transformed with pEp10. The anti-filamentous fungal activity of the recombinant DRHS-AFP was evaluated against some plant pathogenic filamentous fungi, *F. fujikuroi* (Figure 3), *Fusarium solani*, *Pyricularia oryzae*, *Trichoderma viride* (from the phylum Ascomycota), *Pythium* sp. (the phylum Oomycota), *Rhizoctonia solani* (the phylum Basidiomycota), and *Rhizopus microsporus* (the phylum Zygomycota). As a result, it was shown that the genetically recombinant DRHS-AFP had a growth suppressive effect only on 4 strains of filamentous fungi belonging to the phylum Ascomycota and that the genetically recombinant DRHS-AFP had an anti-filamentous fungal activity comparable to that of the native DRHS-AFP.

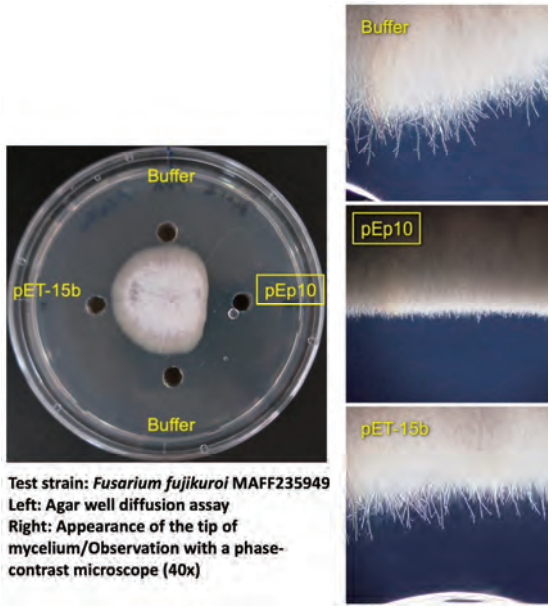

Figure 3. The anti-filamentous fungal activity of genetically recombinant DRHS-AFP.

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Collaboration Works

原富次郎，髙塚由美子， Lamont Doherty Earth Observatory-Columbia University（アメリカ），ポリ塩化ビフェニル類を分解する微生物とその由来酵素

原富次郎，髙塚由美子， Department of Civil and Environmental Engineering-National University of Singapore（シンガポール），ポリ塩化ビフェニルを脱塩素化する細菌

原富次郎，髙塚由美子，東京農業大学（日本）微生物生産物質による抗菌性評価試験の委託

原富次郎, 髙塚由美子, 大阪大学産業科学研究所（日本），DRHS-AFP の薬剤排出ポンプに対する阻害性調査

Financial Support

1. Grant-in-Aid for Scientific Research

原富次郎，基盤研究(C)，高塩素置換型ポリ塩化ビフェニル類の還元的脱塩素化を大気下で実現させる（分担金）

髙塚由美子，基盤研究(C)，高塩素置換型ポリ塩化ビフェニル類の還元的脱塩素化を大気下で実現させる

2. Others

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原富次郎，日本医療研究開発機構，新メソッドによる薬用ニンジンの品質評価を軸とした伝統的栽培法数値化と効率的生産法の開発(AMED 原資）

原富次郎，日本医療研究開発機構，新メソッドによる薬用ニンジンの品質評価を軸とした伝統的栽培法数値化と効率的生産法の開発（企業原資）

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