<Laboratory for Complex Energy Processes> Environmental Microbiology Research Section

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Environmental Microbiology Research Section

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1. Introduction

There is a very close relationship between energy resources consumption and environmental protection, becoming an important 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 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 utilisation efficiency in catabolism. Also, we are remarking on the sustainable methods of food production, which is the energy of life. We are globally working with academisms, biotechs and university start-ups to aim for networking of researches toward social implementation of our technologies.

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

Polychlorinated biphenyls (PCBs) are well known environmental pollutants and dispersed in all our living environments. Biphenyl dioxygenase (BDO) plays a crucial role in the degradation of PCBs. BDO catalyses the incorporation of two oxygen atoms into the aromatic ring of PCB, which induces the aromatic ring cleavage. Importantly, we developed the composite type of catalytic enzyme that consists of the two BDOs

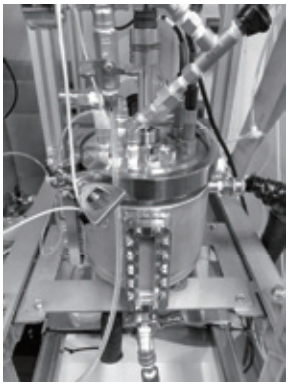

Figure 1. The composite BDOs-microbial catalyst was evaluated in the dedicated experimental bioreactor with the device of oxygen microbubble generation.

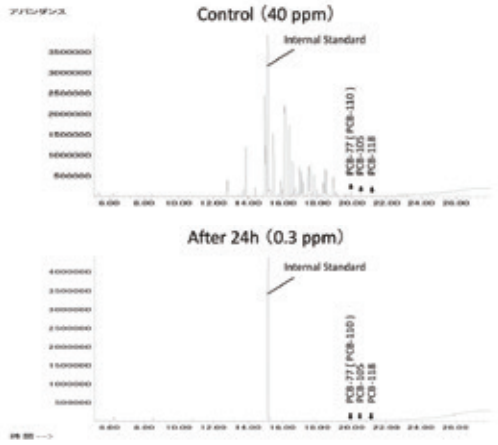

Figure 2. The data from the gas chromatography-quadrupole mass spectrometer showing the PCBs degradation by the composite BDOs-microbial catalyst.

with different substrate specificities; moreover, we developed the bioreactor for generating oxygen microbubbles that enhances the enzymatic activities of 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 of Japan.

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

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 *Dehalobacter* sp. and *Desulfitobacterium* sp. by 16S rRNA gene phylogenetic analysis. Wang and He (*Environ Sci Technol*, 2013) reported that '*Dehalobacter*' dechlorinates penta-/hexa-chlorinated 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 so far. Besides, we also observed that these two bacterial species reduce PCBs in the artificial model of the polluted environmental. Even today, repeated long-term observation is being made to confirm whether the result is correct.

3-1. The biological enzymatic pesticide may become a new pesticide with a new sterilizing mechanism to replace organic synthetic chemicals.

Many plant diseases are generally caused by either ascomycetes or basidiomycetes that are belonging to filamentous fungi. 'Filamentous fungi' are hyphae and proliferate to mycelia. The cell wall is a peculiar composite material that incorporates a mix of cross-linked fibres and matrix components. The fibrous components of the cell wall are glucan, chitin, and mannan, and these sugar chains contribute to form a supple and solid filiform microfibril wall. Glycosidase is one of the hydrolases that catalyse the hydrolysis of glycosidic bonds in complex sugars. We are developing a new bio-macromolecular type of fungicide utilizing the hydrolysis reactions of glycosidases against the fungal microfibril wall. So far, our composite type of bacterial catalyst composed of 5 strains from class *Bacilli*, which produce and secrete various glycosidases, controlled 99.3% of a tomato-Pestalotia disease with *Pestalotiopsis* sp. (Figure 3). Glycosidases are classified into approximately 130 families, and their catalytic reactions are roughly divided into anomeric inversion and/or anomer retention and exoglycosidase or endoglycosidase. Given, the classification of glycosidase can be understood as diverse. We have considered that it is possible to efficiently digest fungi cell wall by compositely capably using these diversities of enzyme activities.

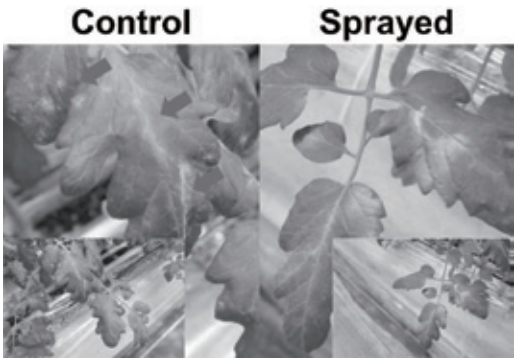

Figure 3. The glycosidase secreted type of the composite microbial catalyst inhibited tomato-*Pestalotia disease*.

3-2. Phytopathogenic filamentous fungi that secrete various glycosidases kill hostile phytopathogenic filamentous fungi for their survival.

We investigated the fungicidal properties of glycosidases produced by a phytopathogenic filamentous strain belonging to basidiomycetes. This filamentous strain secretes enzymes when grown in a bran medium and exhibits various glycosidase activities. The crude enzyme fraction showing such composite glycosidase activities digested 3 out of 6 wet-rice-specific epidemically filamentous fungi (Figure 4). There are not so many enzymes showing high digesting activity against multiple strains of phytopathogenic filamentous fungi. Single glycosidase activity, however, digested only 2 strains. These results suggest that the compositive glucosidase has a highly fungicidal activity rather than the individual glycosidase. In fact, we try to purify the components of this crude enzyme.

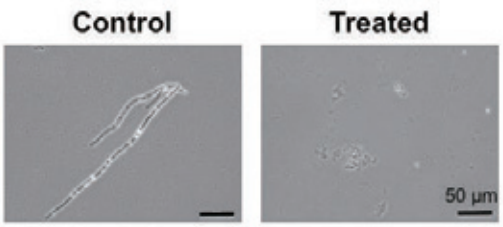

Figure 4. The crude enzyme fraction secreted from the phytopathogenic filamentous fungus digested a wet-rice specific epidemically filamentous fungal strain.

Soon, we will be able to clarify the effectively fungicidal mechanism of this crude enzyme by definite the type of the enzyme(s), the amounts of the secretion, and the specific activities.

4. Pigmented and non-pigmented *Bacillus* spores work together to improve growth, quality and health of shrimp.

The collaborative research with Vietnam National University found that two strains of *Bacillus* isolated from the intestinal tract of white-leg shrimp showed excellent health-improving effects (1). This *Bacillus* probiotic avoids using antibiotics and synthetic chemicals in feeding, enhances shrimp health and growth efficiency, and reduces the energy consumption in white-leg shrimp cultivation. This cultivates industry has been recently growing in Japan, but there is really almost no appropriate feed. To meet that market demand, we have begun considering the possibility of feed registration with the Ministry of Agriculture, Forestry and Fisheries of Japan in collaboration with the company in Vietnam and Japan.

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Collaboration Works

原富次郎，髙塚由美子，Lamont Doherty Earth Observatory-Clumbia 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

Financial Support

1. Grant-in-Aid for Scientific Research

原富次郎，挑戦的研究（萌芽），嫌気的自然環境で起こる有機汚染物質の脱塩素化反応を好気条件下で実現させる（分担金）

髙塚由美子，挑戦的研究（萌芽），嫌気的自然環境で起こる有機汚染物質の脱塩素化反応を好気条件下で実現させる

2. Others

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）

原富次郎，（株）竹中工務店，環境微生物学研究部門

原富次郎，東洋ガラス（株），環境微生物学研究部門

原富次郎，とつかわ水産（株），環境微生物の調査研究のため

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