Environmental Microbiology Research Section

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1. Introduction

There is a very close relationship between energy resources consumption and environmental protection, becoming essential research issues 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 utilization efficiency in catabolism. Also, we are remarking on sustainable food production methods, which is the energy of life. We are globally working with academisms, 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

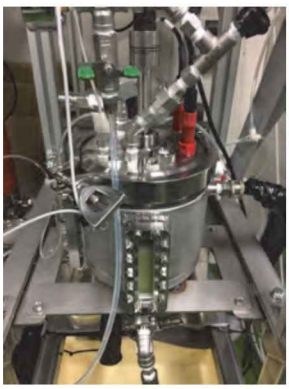

Figure 1. The composite BDOs-microbial catalyst was evaluated in the dedicated experimental bioreactor with the device of oxygen microbubble generation.

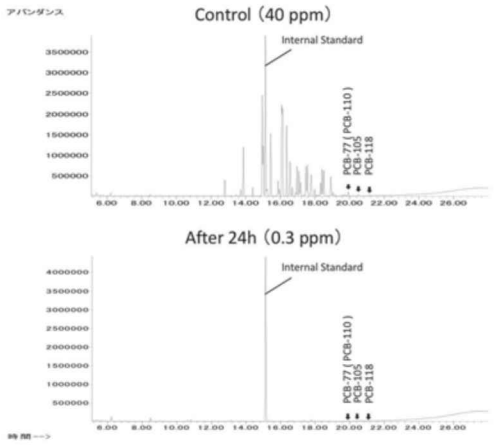

Figure 2. The data from the gas chromatography-quadrupole mass spectrometer showing the PCBs degradation by the composite BDOs-microbial catalyst.

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 of Japan. These technical foci were reported in the international journal published this fiscal year.

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-/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. 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. 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 belong 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 fibers and matrix components. The fibrous components of the cell wall are glucan, chitin, and mannan, and these sugar chains contribute to forming a supple and solid filiform microfibril wall. Glycosidase is a hydrolase that catalyzes 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 exo-glycosidase or endo-glycosidase. Given that, the classification of glycosidase can be understood as diverse. We have considered it possible to efficiently digest fungi cell walls by compositely capably using these diversities of enzyme activities.

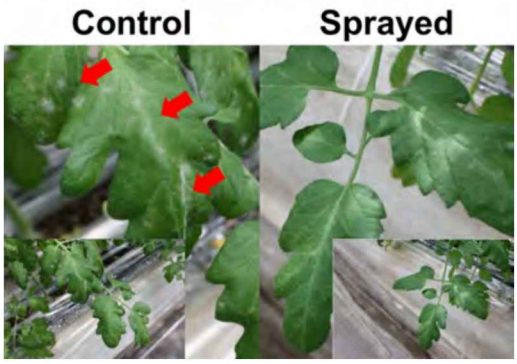

Figure 3. The glycosidase secreted type of the composite microbial catalyst inhibited tomato-*Pestalotia disease*.

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*. When grown in a bran medium, this filamentous strain secretes enzymes and exhibits various glycosidase activities. This crude enzyme fraction showing composite glycosidase activities digested 3 out of 6 wet-rice-specific epidemically filamentous fungi (Figure 4). There are not almost enzymes showing high digesting activity against multiple strains of phytopathogenic filamentous fungi. In the case of single glycosidase activity, on the other hand, digested only 2 strains. These results suggested that the compositive glucosidase has a more fungicidal activity than the individual glycosidase. We try to purify the components of this crude enzyme. Soon, we may 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.

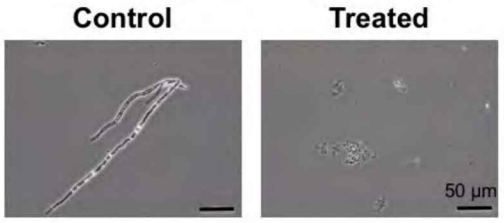

Figure 4. The crude enzyme fraction secreted from the phytopathogenic filamentous fungus digested a wet-rice specific epidemically filamentous fungal strain.

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

Our collaborative research with Vietnam National University revealed that two strains of *Bacillus* isolated from the intestinal tract of white-leg shrimp had excellent health-improving functions to the same class's shrimps. 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 cultivated industry has been recently growing in Japan, but there is almost no appropriate feed. Therefore, we are collaborating with academic institutions specializing in crustaceans to verify their usefulness in detail.

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

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，東洋ガラス（株），環境微生物の探索と機能解明の研究のため

原富次郎，（株）竹中工務店，環境微生物の探索と機能解明の研究のため

原富次郎，（株）オーガニック・ソリューションズ・ジャパン，抗食品危害真菌物質の探索

Publications

Hara T, Takatsuka Y. Aerobic polychlorinated biphenyl-degrading bacteria isolated from the Tohoku region of Japan are not regionally endemic. Canadian Journal of Microbiology. 2022:1-12.

T. Hara, Y. Takatsuka, Y. Shiwa, K. Yokota, Draft Genome Sequence of the Polychlorinated Biphenyl Degrader *Comamonas testosteroni* Strain YAZ2 Isolated from a Natural Landscape in the Tohoku Region of Japan, Microbiol Resour Announcements, 11, 1, e00806-21, 2022

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Presentations

山岸純一，髙塚由美子，川端千翔，上野 誠，松本慎吾，原富次郎，*Rhizoctonia solani* D138 株分泌酵素群による薬用ニンジン病害性糸状菌の成長阻害，第 73 回日本生物工学会大会，オンライン開催，2021.10.27-29