Environmental Microbiology Research Section

T. Hara, Program-Specific Professor
Y. Takatsuka, Program-Specific Associate Professor

1. Introduction

The relationship between energy resource consumption and environmental protection is crucial to developing a sustainable society. Despite our heavy reliance on fossil energy, there is concern that greenhouse gas emissions are disrupting the harmony of the global environment. Additionally, environmental pollution continues to be the shadow of civilization's progress due to the energy consumption of fossil fuels. One of the solutions is to develop a practical method that uses 'enzymes' derived from environmental microorganisms with high energy utilization efficiency in catabolism. Meanwhile, we are also working on sustainable agricultural techniques, which are the source of life energy. We are collaborating with academics, biotechs, and university start-ups globally to network research towards the social implementation of our technologies.

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

We have developed a composite microbial catalyst that can efficiently degrade polychlorinated biphenyls (PCBs), well-known pollutants found widely in the environment. Biphenyl dioxygenase (BDO) plays a crucial role in the degradation of PCBs. It incorporates two oxygen atoms into the aromatic ring of PCB, which then induces aromatic ring cleavage. In more technological detail, our laboratory has designed a composite catalytic enzyme consisting of two BDOs with different substrate specificities. We have also forged a bioreactor that generates oxygen microbubbles to enhance the enzymatic activities of BDOs. With these innovations, we have constructed a practical system that degrades 99.3% of 40 mg L^{-1} of domestic major commercial PCBs (Kanechrol KC-300 and KC-400 from KANEKA Corp.) in 24 hours. This result meets the waste disposal measure defined by the Ministry of the Environment Government of Japan (Figure 2).

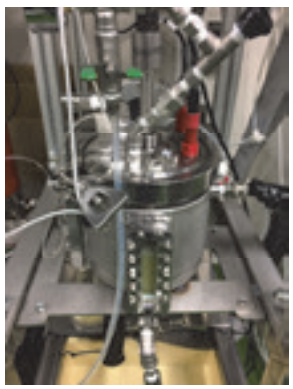

Figure 1: The composite BDOs-microbial catalyst was tested in a dedicated experimental bioreactor with an oxygen microbubble generation device.

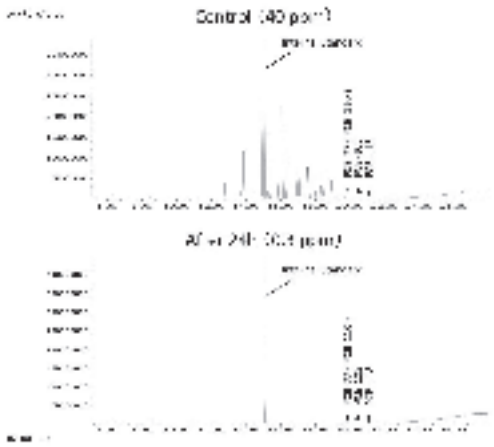

Figure 2: Gas chromatography-quadrupole mass spectrometer data show the degradation of PCBs by the composite BDOs-microbial catalyst.

2-2. Several bacterial species associated with PCB dechlorination were genetically identified at PCB-contaminated sites.

We have been developing an artificial enzyme that can dechlorinate PCBs by two-electron reduction. To do this, we collected sediments from freshwater pit pools contaminated with PCBs in the Yodogawa riverside in the Osaka area and investigated whether bacteria associated with PCB dechlorination exist.

According to 16S rRNA gene phylogenetic analysis, it has been observed that *Dehalobacter* sp. and *Desulfitobacterium* sp. are present in that specific location. Wang and He (Environ Sci Technol, 2013) have reported that "*Deharobacter*" can dechlorinate penta- and hexa-chlorinated biphenyls while

"*Desulfitobacterium*" can dechlorinate tetra-chlorinated biphenyls that are hydroxylated at the para position.

We successfully prepared the medium for growing two specific bacterial species and developed a suitable cultivation method. In addition, we observed that these bacterial species can reduce PCBs in an artificial model of a polluted environment. Even today, after a decade of starting this investigation, we continue to observe their long-term effects to confirm the accuracy of our results.

3. A new protein secreted by *Rhizoctonia solani* suppresses filamentous fungi growth.

Rhizoctonia solani is a filamentous fungus belonging to the phylum Basidiomycota. This fungus is well-known for its ability to infect and cause severe diseases in many crops, such as rice sheath blight, which significantly impacts paddy-rice production. The exact mechanism by which this phytopathogenic fungus infects plants has yet to be fully understood. However, studies have shown that when wheat bran is added to the growth medium, this fungus secretes glycosidases that digest plants and fungi cell walls. Our team has discovered a new protein secreted from *R. solani* when cultured with wheat bran as a solid medium. This protein has a molecular weight of approximately 10 kDa and exhibits antifungal properties against filamentous fungi. This protein inhibited the growth of *Fusarium fujikuroi*, another phytopathogenic filamentous fungus belonging to the phylum Ascomycota. It was suggested that this protein also inhibited conidium formation and germination of *F. fujikuroi*.

After conducting an amino acid sequence analysis, it was found that this protein's partial amino acid sequence suggests an unknown function. Additionally, the full-length amino acid sequence of the protein was deduced after investigating the whole genome sequence of the *R. solani* strain that produces it. The BLAST search results on this sequence also suggested that the protein has an unknown function.

According to the sequence analysis of the cDNA that encodes this protein, the complete amino acid sequence of the protein was found to be 122 residues. The mature protein's amino acid sequence, which excludes the assumed signal peptide sequence, was 88 residues in length. Based on this information, the estimated molecular weight of this protein was 9648.24. Furthermore, this antifungal protein has made us aware of unique structural features in its primary amino acid sequence that have never been reported. The protein consists of 88 residues and has a double-repeat structure of 41 residues, with a high homology of 92% across the central 6 amino acid residues. The functional role of such repetitive sequences still needs to be better understood. We have named this antifungal protein "**Double-Repeating Homologous Sequence Antifungal Polypeptide (DRHS-AFP)**." We have already succeeded in producing recombinant DRHS-AFP (Figure 3) and are conducting detailed investigations into its functions, focusing on its antifungal activity.

4. Research into preventing coffee tree fungal diseases that may be induced by global warming is underway.

The "2050 problem" refers to the impending challenges faced by the global supply of coffee beans. The region best suited for coffee cultivation, known as the "coffee belt," lies around the equator between 25 degrees north and 25 degrees south latitude. It is projected that up to 50% of this coffee belt could be lost due to plant diseases worsened by climate change. Coffee arabica is highly sensitive to these changes. It is significantly impacted by a disease called Coffee Leaf Rust, which is caused by the fungus *Hemileia vastatrix*.

As of 2022, the Lao People's Democratic Republic is the 14th largest coffee-producing country in the world. Arabica coffee is primarily grown on the Bolaven Plateau, which is around 1,200 meters above sea level in Champasak Province, accounting for 60% of the nation's total coffee production.

To address these challenges, we have formed an international collaborative research team that includes the National University of Laos (NUOL), the Laos National Forestry Research Institute (NAFRI), Chiang Mai University, and Pibulsongkram Rajabhat University (PSRU). Our research focuses on controlling *H. vastatrix* in the Bolaven Plateau. This fiscal year, we have provided internship research guidance to NUOL faculty staff and, in collaboration with PSRU faculty, established a genetic identification system for *H. vastatrix* at NAFRI on the Bolaven Plateau.

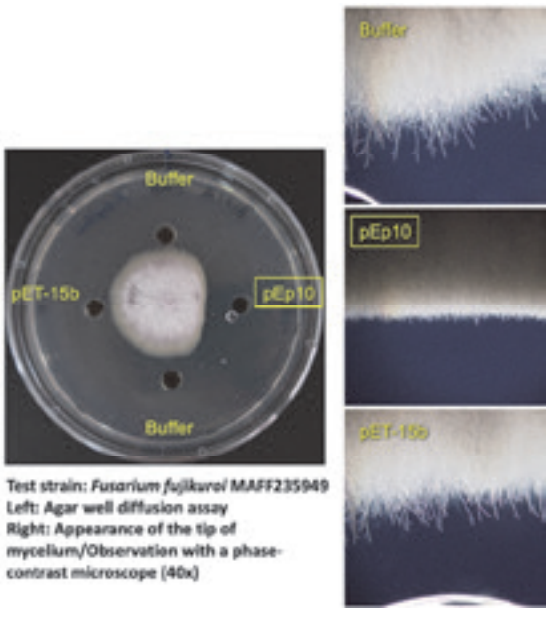

Figure 3. The anti-filamentous fungal activity of genetically recombinant DRHS-AFP.

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

髙塚由美子，原富次郎，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，Faculty of Engineering, National University of Laos（ラオス），ラオス国コーヒー伝染病観測所の設置と病害対策

原富次郎，髙塚由美子，Pibulsongkram Rajabhat University（タイ），コーヒーノキさび病真菌のゲノム解析と培養方法の開発

原富次郎, 髙塚由美子, 大阪ハイテクノロジー専門学校, DRHS-AFP を用いた細胞毒性評価

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

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

Presentations

R. Kodsueb, R. Cheewangkoon, S. Haituk, Y. Takatsuka, T. Hara, Whole genome analysis and culture method development of Thai coffee leaf rust fungus, The 15th International Symposium of Advanced Energy Science, Uji Campus, Kyoto University, 2024. 12. 10-13

K. Khounvilay, S. Singharaj, Y. Takatsuka, T. Hara, B. Phengphachanh, S. Orlavanh, H. Sengthong, T. Sibounheung, D. Phengphachanh, H. Ohgaki, Achieving carbon-neutral organic coffee cultivation through biocontrol, The 15th International Symposium of Advanced Energy Science, Uji Campus, Kyoto University, 2024.12.10-13

肥後佑衣, 髙塚由美子, 山口琳花, 上野誠, 原富次郎, *Rhizoctonia solani* 分泌性 DRHS-AFP の菌糸融合群および亜群別における抗糸状菌特異性の調査, 日本農芸化学会 2025 年度大会（札幌）, 札幌コンベンションセンター, 2025. 3. 4-8