Japan-ASEAN Science, Technology and Innovation Platform (JASTIP)
Report of JASTIP-Net Activity

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<table>
<tr><td rowspan="5">1. Research partner</td><td colspan="3">Affiliated Organization:
1. Research Center for Biomaterials-LIPI,
2. Chulalongkorn University, Thailand,
3. RISH – Kyoto University, Japan.</td></tr>
<tr><td>Name</td><td colspan="2">Dr. Dede Heri Yuli Yanto</td></tr>
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<tr><td>2. Collaborative research</td><td>Collaborative research theme</td><td colspan="2">Headquarters
☐ To Develop Operational Linkages and Human Resources among Academic Sector, Government Agencies, and Private Sectors in ASEAN countries and Japan.
☐ To Introduce Various STI Collaborations for Effectively and Efficiently into the Society based on the three joint laboratories' activities.

Energy & Environment Joint Lab
☐ Studies on Rural/Community Renewable Energy.
☐ Development of Renewable Energy Technology adapted to the ASEAN region.
☐ Studies on Energy Policy/Security in the ASEAN region.

Bioresources & Biodiversity Lab
☐ Studies on Biodiversity in the ASEAN Region Contributing to the Improvement of Identification, Collection and/or Information.
☑ Sustainable Utilization of Bioresources for Biorefinery,</td></tr>
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		Bioremediation, Wood Construction, Food or Medicine. ☐ Plant Improvement for Agroforestry Systems and Carbon Sequestration Contributing to the Mitigation of and/or Adaptation to Climate Change. **Disaster Prevention Joint Lab** ☐ Innovative Ideas on Disaster Prevention, Mitigation and Recovery Technologies and Policies Peculiar to Each ASEAN Country. ☐ How to Cope with Trans-Boundary Disasters in the ASEAN Region Such as Tsunami, Flood, Drought and Haze. ☐ Understanding and Quantitative Evaluation of Disaster Risks Peculiar to ASEAN Countries.
	Collaborative research title	Bioremediation of synthetic dyes, polycyclic aromatic hydrocarbons (PAHs) and crude oil by tropical fungi from Indonesia and Thailand
	Host core-researcher	Prof. Dr. Takashi Watanabe RISH – Kyoto University, Japan

3. Members

Name	Dr. Dede Heri Yuli Yanto		
Affiliation	Research Center for Biomaterials - LIPI	Position	Researcher, Dr.
Name	Prof. Takashi Watanabe		
Affiliation	RISH, Kyoto University	Position	Professor
Name	Professor Dr. Hunsa Punnapayak		
Affiliation	Chulalongkorn University, Thailand	Position	Professor
Name	Associate Professor Dr. Sehanat Prasongsuk		
Affiliation	Chulalongkorn University, Thailand	Position	Researcher, Dr.
Name	Dr. Wichanee Bankeeree		
Affiliation	Chulalongkorn University, Thailand	Position	Researcher, Dr.
Name	Dr. Hiroshi Nishimura		
Affiliation	RISH, Kyoto University, Japan	Position	Assistant Professor, Dr.
Name	Dr. Chen Qu		
Affiliation	RISH, Kyoto University, Japan	Position	Postdoctoral Researcher, Dr.
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Name	Sita Heris Anita		
Affiliation	Research Center for Biomaterials - LIPI	Position	Researcher
Name	Maulida Oktaviani		
Affiliation	Research Center for Biomaterials - LIPI	Position	Researcher

4. Report of activities

4.1 Bioremediation of dye-contaminated wastewater

Among several methods used for synthetic dye decolorization, enzymatic treatment seems to be the most efficient due to its complete degradation and non-toxic byproduct formation. The major problem of this treatment is the high investment cost since the enzymes could not be reused. The new technique for laccase immobilization was developed in this research by using expanded perlite, glutaraldehyde and 3-aminopropyltriethoxysilane (APTES) as the supporter and cross-linker, respectively. Crude laccase produced from a white rot fungus *Trametes prolyzona* CU-16, isolated in Thailand, presented the highest ability to decolorize reactive black 5 (RB5; 50 ppm) at 98.45 ± 5.25 % compared to those from other 25 fungal isolates. Therefore, laccase from this isolate was used as the model enzyme in this study. The appropriated ratio for enzyme immobilization was investigated according to the experimental design of Box-Behnken (1960) with three different concentrations of laccase, glutaraldehyde and APTES. The maximum percentage of RB-5 decolorization was achieved at 97.34 ± 2.54% when the compositions for laccase immobilization were 5 g expanded perlite, 3% (v/w) APTS, 5% (w/v) glutaraldehyde and 20 U/mL crude laccase. The immobilized laccase obtained in this experiment was found to be active over 16 cycles of dye decolorization. In the sixteenth cycle, the reused perlite was able to decolorize 49.25 ± 3.66 % the original mixture. Moreover, the immobilized enzyme extended the optimum pH range of laccase activity from 6 to 10 and tolerated a temperature up to 10 °C higher than that of the free enzyme. These results suggest that the expanded perlite has a great potential as the matrix for enzyme immobilization, which has applications in wastewater treatment.

4.2 Biosurfactant synthesis from industrial waste for remediation of oil spill in the ocean

Xylan is the most abundant heteropolysaccharide in plant biomass that commonly found as organic waste especially in black liquor from the pulp and paper industry. The utilization of this polysaccharide to produce the value-added products including xylooligosaccharide, xylose, xylitol and alcohol has gained an attention for several decades while the reports of surfactant production from xylan were rarely found. Alkyl xylosides are nonionic surfactant that normally synthesized by chemical reaction while some microbial β-xylosidases also exhibit transxylosyl reaction to synthesize these compounds from xylan. In this research, several types of alkyl xylosides were successfully synthesized by the reactions between black liquor xylan, crude β-xylosidase from *Aureobasidiun pullulans* and primary alcohols (C1-C10). The reactions were performed at high temperature (70 °C) and high concentration of alcohols (60 % (v/v)) due to the optimum condition of this enzyme. The structures of obtained compounds were revealed by ^{1}H and ^{13}C-NMR spectroscopy and were found to be methyl xyloside, ethyl xyloside, propyl xyloside, butyl xyloside, pentyl xyloside, hexyl xylobioside, heptyl xyloside, octyl xyloside, nonyl xyloside and decyl xyloside with the production yields of 740 ± 23, 683 ± 11, 662 ± 64, 571 ± 20, 500 ± 12, 545 ± 10, 454 ± 23, 429 ± 26, 372 ± 18 and 244 ± 27 mg/g xylan, respectively. The properties of these compounds were also analyzed. All alkyl xylosides are biodegradable and display

little-to-no toxicity to living cells. The obtained alkyl xylosides with the short hydrophobic tail lower than eight carbons presented the properties of wetting agents that could be used in paper impregnation and other wood product industries due to their critical micelle concentration (CMC) values (> 100 mM). Methyl xyloside was also found to effectively induce xylanase production in many microorganisms. For long chain, nonyl and decyl xyloside, they presented the CMC values at 18 and 10 mM, respectively. These CMC values were comparative with those of the commercial surfactants including sodium cholate (14 mM), SDS (7-10 mM), and C_{12}TAB (35 mM). In addition, these compounds were found to reduce the surface tension of engine oil and to create small droplets of oil in water. This phenomenon could make engine oil dispersed through the water very well and easier to degrade by both physical forces and microbes. These results suggest that the nonyl and decyl xyloside synthesized from black liquor xylan could be efficiently used in remediation of oil spill in the ocean.

4.3. Biodegradation of crude oil and PAHs mixture by newly isolated tropical fungi

Spills, blowouts, and malfunctions may occur during any offshore oil and gas exploration activity. Spilled oil can be rapidly lethal to fish, birds, mammals, and shoreline organisms due to the readily dissolved components of oil. Polycyclic aromatic hydrocarbons (PAHs) is a hydrocarbon class that is generally more toxic than saturates. Therefore, it needs to do environmental bioremediation. This technique utilizes and manipulates the detoxification abilities of living organisms to convert hazardous organic wastes into harmless products. White rot fungus WM 01 identified as *Cymatoderma dendriticum* WM01 based on DNA sequence analysis was newly isolated from Laiwangi Wanggameti National Park, Sumba, Nusa Tenggara Timur, Indonesia. It has the ability to produce enzymes including manganese peroxidase (MnP) and laccases to degrade the oil and PAHs. It was reported that biodegraded the aliphatic and aromatic fractions and also PAHs in 45 d were 70, 62.50, and 43.90%, respectively. The biodegradation of PAHs indicated laccases and MnP activities of 42.99 and 7.38 U/L, respectively, when incubated for 45 d in liquid medium. Degradation products of PAHs mixture were analyzed by TMS derivatization using GCMS. These result indicated that fungi WM 01 has the ability to degrade oil and PAHs.

5. List of publications

Wichanee Bankeeree, Takashi Watanabe, Dede Heri Yuli Yanto, Satoshi Oshiro, Ruibo Li, Pongtharin Lotrakul, Sehanat Prasongsuk and Hunsa Punnapayak, “Immobilization of laccase from tropical isolate *Trametes polyzona* on expanded perlite for wastewater treatment,” Manuscript in preparation

Wichanee Bankeeree, Takashi Watanabe, Dede Heri Yuli Yanto, Satoshi Oshiro, Ruibo Li, Rico Ramadhan, Pongtharin Lotrakul, Sehanat Prasongsuk and Hunsa Punnapayak, “Alkyl β-D-xyloside synthesis from black liquor xylan using immobilized β-xylosidases from *Aureobasidium pullulans* CBS 135684,” Manuscript in preparation.

Dede Heri Yuli Yanto[1*], Dwi Sriyani[2], Takashi Watanabe, Hunsa Punapayyak, Sehanat Prasongsuk, Wichanee Bankeeree, Satoshi Oshiro, Suminar Setiati Achmadi[2], Ajeng Arum Sari, and Maulida Oktaviani[1]. Biodegradation of crude oil and polycyclic aromatic hydrocarbons by a newly isolated *Cymatoderma dendriticum* WM01. Manuscript in preparation.

Dede Heri Yuli Yanto[1,*], Wichanee Bankeeree[2], Takashi Watanabe[3], Hunsa Punnapayak[2], Sehanat Prasongsuk[2], Sita Heris Anita, Maulida Oktaviani[1], Fahriya Puspita Sari[1], and Raden Permana Budi Laksana[1]. Decolorization and detoxification of syntetic dyes by laccase from *Trametes hirsuta* D7 immobilized on perlite. Manuscript in preparation.

Dede Heri Yuli Yanto[1,*], Wichanee Bankeeree[2], Takashi Watanabe[3], Hunsa Punnapayak[2], Sehanat Prasongsuk[2], Sita Heris Anita, Maulida Oktaviani[1], Fahriya Puspita Sari[1], and Raden Permana Budi Laksana[1]. Formulation of perlite activation and utilization of activated perlite – enzyme for textile dye decolorization. Under submission process for Indonesian Patent.

6. List of oral presentations

Prasongsuk P, Bankeeree W, Lotrakul P, and Punnapayak H. Potential of plant biomass and plant biomass-degrading microbes in Thailand for bioenergy and biorefineries. International Conference on Biosciences & Medical Engineering, Johor, Malaysia, 10-11 November 2016

Dede Heri Yuli Yanto[1,*], Wichanee Bankeeree[2], Takashi Watanabe[3], Hunsa Punnapayak[2], Hiroshi Nishimura[3], Satoshi Oshiro[3], Ruibo Li[3], Chen Qu[3], Maulida Oktaviani[1], Sehanat Prasongsuk[2], and Sita Heris Anita[1]. Decolorization and detoxification of synthetic dyes and PAHs by tropical fungi from Indonesia and Thailand. Third JASTIP Symposium, Bangkok, February 5th, 2017.

Punnapayak H, Bankeeree W, Prasongsuk P, and Lotrakul P. Bioremediation of wastewater in Thailand. German Academic Exchange Service (DAAD) Regional Alumni Conference in Environment and Health, Hanoi, Vietnam, 25-28 May 2017

Dede Heri Yuli Yanto[1*], Dwi Sriyani[2], Takashi Watanabe, Hunsa Punapayyak, Sehanat Prasongsuk, Wichanee Bankeeree, Satoshi Oshiro, Suminar Setiati Achmadi[2], Ajeng Arum Sari, and Maulida Oktaviani[1]. Biodegradation of crude oil and polycyclic aromatic hydrocarbons by white rot fungu. The 4th International Symposium on Science, Bogor, October 19 – 20, 2017.

Punnapayak H, Watanabe T, Yanto DHY, Bankeeree W, Prasongsuk P, and Lotrakul P. Bioremediation of contaminated water with oil and toxic dyes. The 3rd JASTIP Bioresources and Biodiversity Workshop "Synergy of ASEAN Countries and Japan for Sustainable Development", Bogor, Indonesia, 3 November 2017

Prasongsuk P, Bankeeree W, Lotrakul P, and Punnapayak H. β-Xylosidase from *Aureobasidium pullulans* CBS 135684 and its application for alkyl-D-xyloside synthesis. The 7th International Symposium for Sustainable Humanosphere, Bogor, Indonesia, 3 November 2017

Bankeeree W, Watanabe T, Yanto DHY, Prasongsuk P, Lotrakul P, and Punnapayak H. Decolorization of synthetic dyes by immobilized laccase produced from tropical fungi *Trametes polyzona* on waste perlite. The 7th International Symposium for Sustainable Humanosphere, Bogor, Indonesia, 3 November 2017

Prasongsuk P, Bankeeree W, Lotrakul P, and Punnapayak H. β-Xylosidase from *Aureobasidium pullulans* CBS 135684 and its application for alkyl-D-xyloside synthesis. The 13th Young Scientist Seminar, Fukuoka, Japan, 18-19 November 2017