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Comparison of Benzo[a]pyrene-Degrading Activities between Olleya sp. ITB9 Isolated from Tokyo Bay and Other Type Strains of the Genus Olleya

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Benzo[a]pyrene (BaP) is one of the strongest carcinogenic compounds among polycyclic aromatic hydrocarbons (PAHs). We previously identified the ITB9 strain of Olleya species, which shows BaP-degrading activity; our report was the first about BaP degradation by the genus Olleya. In this study, BaP-degradation efficiency by ITB9 was about 50% when the strain was suspended in 20 ml of L9 liquid medium with 100 µg/ml BaP and 0.2 M NaCl, with pH 8.0, and incubated at 25°C for 5 days. Under the same conditions, all four type strains (O. marilimosa CIP108537, O. aquimaris KCTC22661, O. namhaensis KCTC23673, and O. algicola KCTC22024) also showed BaP-degrading activities, at efficiencies ranging from 49% to 63%. Olleya sp. ITB9 and O. aquimaris KCTC22661 were found to be in the same clade in the phylogenetic tree of the genus Olleya, given that the homology of 16S rRNA gene sequences between ITB9 and KCTC22661 was 99.77%.

Key words: Polycyclic aromatic hydrocarbons / Benzo[a]pyrene / Biodegradation / Genus Olleya.

Polycyclic aromatic hydrocarbons (PAHs) are chemicals that are seriously detrimental to human health due to their carcinogenicity and teratogenicity in addition to their persistence in the environment and accumulative properties in living bodies (Lawal, 2017). The United States Environmental Protection Agency has designated 16 kinds of PAHs as priority pollutants, based on concerns that they might cause cancer in animals and humans (Keith and Telliard, 1979). Benzo[a]pyrene (BaP), which has five benzene rings, is a particularly strong carcinogenic compound among PAHs and is metabolized by both cytochrome P450 and epoxide hydrolase to BaP-7,8-diol-epoxide, which binds to DNA in live hosts (Baird et al., 2005). On March 11, 2011, the Great East Japan earthquake was followed by various kinds of oil spill in the Tokyo Bay area (Krausmann and Cruz, 2013). We collected environmental waters from oil pollution zones of Tokyo Bay in April-August 2011 and isolated 1,096 bacterial strains from the samples. And we determined BaP degrading activities of 9 strains (Sphingobium amiense ITB2, Mycobacterium fluoranthenivorans ITB3, Herbiconiux ginseng ITB4, Mesorhizobium septentrionale ITB5, Mycobacterium brisbanense ITB6, Vibrio rumoiensi ITB7, Bacillus megaterium ITB8, Olleya sp. ITB9, and Mesoflavibacter zeaxanthinferiens ITB11) from the strains. They degraded 60-86% BaP after 42 days incubation at 25°C using 50 µg/ml BaP. Among them, Olleya sp. ITB9 degraded about 80% BaP in the condition and was found to have no reports on the BaP degradation by Olleya sp. Therefore, we thought Olleya sp. ITB9 might be a new candidate bacterium for bioremediation of BaP in the environments (Okai et al., 2015a). We studied the genome sequence of Olleya sp. ITB9, which consists of 58 contigs corresponding to 3.4 Mb, and a

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G+C content of 31.2% (Okai et al., 2015b); the BaP-degrading enzymes in ITB9 are under investigation.

The genus Olleya was first proposed by Nicols et al. (2005) to accommodate strictly aerobic, Gram-negative, rod-shaped cells in gliding and orange-yellow colonies in marine bacteria affiliated with the family Flavobacteriaceae. The strain isolated from the Southern Ocean as the sole recognized species was placed in a novel taxon as Olleya marilimosa gen. nov., sp. nov. based on phylogenetic, phenotypic, chemotaxonomic, and genotypic analysis. Next, Lee et al. (2010) reported that a bacterial strain from seawater in Korea was Olleya aquimaris sp. nov., which emended the description of the genus Olleya by Nicols et al. (2005). Lee et al. (2013) also reported Olleya namhaensis sp. nov., from wood falls in the South Sea of Korea, which emended the genus Olleya in Lee et al. (2010). Nedashkovskaya et al. (2017) then reported that the marine bacterium isolated from the green alga Ulva fenestra was Olleya algicola sp. nov., which emended the description of the genus Olleya in Lee et al. (2013). Thus, to date, the genus Olleya consists of four species; Olleya marilimosa, Olleya aquimaris, Olleya namhaensis, and Olleya algicola. The physiological activities of the genus Olleya have been extensively characterized in the above literatures, but to the best of our knowledge, no data have yet been reported about their BaP-degradation activities. In this study, we compared the benzo[a]pyrene-degrading activities of Olleya sp. ITB9 isolated from Tokyo Bay and that of the other type strains of the genus Olleya.

The bacterial strains used for the present study were as follows: Olleya sp. ITB9 came from our microbial library, a type strain of the genus Olleya; Olleya marilimosa CIP108537 was gifted from Dr. Carol Mancuso Nichols, University of Tasmania, Australia, together with three kinds of type strain; and Olleya aquimaris KCTC22661, Olleya namhaensis KCTC23673, and Olleya algicola KCTC22024 were from the Korean Collection for Type Culture (KCTC). The five strains were cultured in L9 liquid medium (Na2HPO4 7.1 g, KH2PO4 3.0 g, NH4Cl 1.1 g, MgSO4 7H2O 0.247 g, ZnCl2 0.131 mg, CoCl2 6H2O 0.99 mg, Na2MoO4 2H2O 0.078 mg, H3BO4 0.076 mg, MnCl2 4H2O 0.074 mg, CuSO4 5H2O 0.049 mg, NiCl2 6H2O 0.131 mg, and CuCl2 0.028 mg/l water) or 20 ml L9 solid medium containing L9 liquid medium plus 2% (w/v) agar at 25°C.

Each cryopreserved strain from our bacterial library was inoculated onto the L9 solid medium followed by incubation at 25°C for 3 d. Each colony grown on the agar plate was picked up, inoculated to the L9 liquid medium, and cultured at 25°C for 1 d. The cells were collected by centrifugation at 2,136 × g for 20 min and washed three times in physiological saline. Then, each 20 mg (wet weight) of the rinsed cells was suspended in 20 ml of L9 liquid medium with 100 µg/ml BaP in a 50-ml Erlenmeyer flask at 25°C for 5 d with shaking at 120 rpm under dark conditions, and its BaP-degrading activity was measured. The bar graph values are the means of triplicate trials, with their standard deviation (SD) values.

FIG. 1. Comparison of BaP-degrading activities between Olleya sp. ITB9 and the other type strains of the genus Olleya Olleya sp. ITB9, O. marilimosa CIP108537, O. aquimaris KCTC22661, O. namhaensis KCTC23673, or O. algicola KCTC22024 was incubated in 20 ml of L9 liquid medium with 100 µg/ml BaP in a 50-ml Erlenmeyer flask at 25°C for 5 d with shaking at 120 rpm under dark conditions, and its BaP-degrading activity was measured. The bar graph values are the means of triplicate trials, with their standard deviation (SD) values.
On the other hand, all type strains of the genus Olleya – *O. marilimosa* CIP108537, *Olleya aquimaris* KCTC22661, *Olleya namhaensis* KCTC23673, and *Olleya algicola* KCTC22024 – were also found to have BaP-degrading activities, at efficiencies of about 49-64%, with *O. marilimosa* CIP108537 having the highest efficiency at about 64%. As mentioned above, the physiological activities of the genus *Olleya* had been partially characterized previously, but this is the first report that the genus *Olleya* has BaP-degrading activity.

Figure 2 shows the phylogenetic tree of the genus *Olleya*. *Olleya* sp. ITB9 is in the same clade as *O. aquimaris* KCTC22661 because of the homology of the 16S rRNA gene sequences between the two was 99.77%. Therefore, species of *Olleya* sp. ITB9 are thought to be very closely related to those of *O. aquimaris*. For further study, genome DNA-DNA hybridization between the two strains will elucidate ITB9 species.

In situ bioremediation by microorganisms is thought to be an efficient approach to alleviate cancer risks associated with BaP-contaminated soils and waters, and several studies have reported BaP-degradation by fungi or bacteria (Ping et al., 2014; Zafra et al., 2015; Cao et al., 2020). Therefore, the genus *Olleya* is a novel candidate for the bioremediation of BaP-polluted environments.

**REFERENCES**

Baird, W. M., Hooven, L. A., and Mahadevan, B. (2005) Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. *Environ. Mol. Mutagen.*, **45**, 106-114.

Cao, H., Wang, C., Liu, H., Jia, W., and Sun, H. (2020) Enzyme activities during benzo[a]pyrene degradation by the fungus *Lasiodiplodia theobromae* isolated from a polluted soil. *Sci. Rep.*, **10**, 865.

Felsenstein, J. (1993) PHYLIP (Phylogeny Inference Package). Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Keith, L., and Telliard, W. (1979) Priority pollutants 1-a perspective view. *Environ. Sci. Technol.*, **13**, 416-423.

Krausmann, E., and Cruz, A. M. (2013) Impact of the 11 March 2011, Great East Japan earthquake and tsunami on the chemical industry. *Nat. Hazards*, **67**, 811-828.

Lawal, A. T. (2017). Polycyclic aromatic hydrocarbons. A review. *Cogent Environ. Sci.*, **3**, 1339841.

Lee, S. Y., Park, S., Oh, T. K., and Yoon, J. H. (2010) Description of *Olleya aquimaris* sp. nov., isolated from seawater, and emended description of the genus *Olleya* Mancuso Nichols et al. 2005. *Int. J. Syst. Evol. Microbiol.*, **60**, 887-891.

Lee, S. Y., Jung, Y. T., Park, S., and Yoon, J. H. (2013) *Olleya namhaensis* sp. nov., isolated from wood falls, and emended description of the genus *Olleya* Mancuso Nichols et al. 2005 emend. Lee et al. 2010. *Int. J. Syst. Evol. Microbiol.*, **63**, 1610-1615.

Nedashkovskaya, O. I., Kim, S. G., Zhukova, N. V., and Mikhailov, V. V. (2017) *Olleya algicola* sp. nov., a marine bacterium isolated from the green alga *Ulva fenestra*.* Int. J. Syst. Evol. Microbiol.*, **67**, 2205-2210.

Nicols, C. M., Bowman, J. P., and Guezennec, J. (2005) *Olleya marilimosa* gen. nov., sp. nov., an exopolysaccharide-producing marine bacterium from the family *Flavobacteriaceae*, isolated from the Southern Ocean. *Int. J. Syst. Evol. Microbiol.*, **55**, 15571661.

Okai, M., Kihara, I., Yokoyama, Y., Ishida, M., and Urano, N. (2015a) Isolation and characterization of benzo[a]pyrene-degrading bacteria from the Tokyo Bay area and Tama River in Japan. *FEMS Microbiol. Lett.*, **362**, fnv143.

Okai, M., Watanabe, A., Ishida, M., and Urano, N. (2015b) Draft genome sequence of a benzo[a]pyrene-degrading bacterium, *Olleya* sp. ITB9. *Genome Announce.*, **3**, e-01328-15.

Ping, L., Zhang, C., Zhang, C., Zhu, Y., He, H., Wu, M., Tang, T., Li; Z., and Zhao, H. (2014). Isolation and characterization of pyrene and benzo[a]pyrene-degrading *Klebsiella pneumoniae* PL1 and its potential use in bioremediation. *Appl. Microbiol. Biotechnol.*, **98**, 3819-3829.

Zafra, G., Moreno-Montano, A., Absalon, A. E., and Cortés-Espinosa, D. V. (2015) Degradation of polycyclic aromatic hydrocarbons in soils by a tolerant strain of *Trichoderma asperellum*. *Environ. Sci. Pollut. Res.*, **22**, 1034-1042.