RECENT RESEARCH ACTIVITIES

Screening and identification of useful enzymes from biphenyl/PCB-degrading bacteria that metabolize lignin-derived aromatic compounds

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A variety of microorganisms play a significant role in the mineralization of plant lignin. The lignin-derived aromatic compounds are further degraded by soil bacteria. So far, a number of biphenyl/polychlorinated biphenyls (PCB)-degrading bacteria have been isolated from various environmental samples and characterized in terms of biochemical and genetic aspects [1]. Recently, the genome analyses of ten biphenyl/PCB-degrading bacteria, isolated from biphenyl-contaminated soil in Kitakyushu, Japan, were performed [2-3]. Interestingly, some of these biphenyl/PCB-degrading bacteria grew well on lignin-derived aromatic compounds as the sole sources of carbon and energy, as well as xenobiotic compounds like PCB. This finding implies that biphenyl/PCB-degrading bacteria may be a type of terminal lignin-degrading bacterium.

To globally screen and identify expressed proteins involved in the metabolism of lignin-derived aromatic compounds from these biphenyl/PCB-degrading bacteria, a proteomic approach is needed. On the other hand, I have already optimized a fluorescence two-dimensional difference gel electrophoresis (2D-DIGE) method for a white-rot fungus surrounded by polysaccharide sheath [4]. Therefore, I tried to modify this method for biphenyl/PCB-degrading bacteria and to perform 2D-DIGE using intracellular proteins prepared from *Pseudomonas furukawaii* (formerly *P. pseudoalcaligenes*) KF707 [5]—one of the best-characterized biphenyl/PCB-degrading bacteria—grown in the presence and absence of biphenyl. As a result, I successfully detected the difference in protein expression under the two culture conditions and also identified up-regulated, constantly expressed, and down-regulated proteins by peptide mass finger printing. These results show that using proteomic and genomic approaches, I can efficiently screen and identify candidate proteins and genes involved in the metabolism of lignin-derived aromatic compounds from biphenyl/PCB-degrading bacteria. These approaches can also lead to the production of useful aromatic compounds from wood biomass.

References

[1] Furukawa, K., Fujihara, H., "Microbial degradation of polychlorinated biphenyls: biochemical and molecular features". *J. Biosci. Bioeng.* **105,** 433-449 (2008).

[2] Suenaga, H., Fujihara, H., Kimura, N., Hirose, J., Watanabe, T., Futagami, T., Goto, M., Shimodaira, J., Furukawa, K., "Insights into the genomic plasticity of *Pseudomonas putida* KF715, a strain with unique biphenyl-utilizing activity and genome instability properties". *Environ. Microbiol. Rep.* **9,** 589-598 (2017).

[3] Hirose, J., Fujihara, H., Watanabe, T., Kimura, N., Suenaga, H., Futagami, T., Goto, M., Suyama, A., Furukawa, K., "Biphenyl/PCB degrading *bph* genes of ten bacterial strains isolated from biphenyl-contaminated soil in Kitakyushu, Japan: comparative and dynamic features as integrative conjugative elements (ICEs)". *Genes* **10,** 404 (2019).

[4] Watanabe, T., Yoshioka, K., Kido, A., Lee, J., Akiyoshi, H., Watanabe, T., "Preparation of intracellular proteins from a white-rot fungus surrounded by polysaccharide sheath and optimization of their two-dimensional electrophoresis for proteomic studies". *J. Microbiol. Methods* **142,** 63-70 (2017).

[5] Kimura, N., Watanabe, T., Suenaga, H., Fujihara, H., Futagami, T., Goto, M., Hanada, S., Hirose, J., "*Pseudomonas furukawaii* sp. nov., a polychlorinated biphenyl-degrading bacterium isolated from biphenyl-contaminated soil in Japan". *Int. J. Syst. Evol. Microbiol.* **68,** 1429-1435 (2018).