RECENT RESEARCH ACTIVITIES

Vanillin-induced cellular response in the white rot fungus, *Ceriporiopsis subvermispora*

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White-rot fungi can mineralize lignin in wood. Some of fragments generated during lignin degradation are putative signal molecules that regulate the mechanism of wood decay. In the well-known white-rot fungus, *Phanerochaete chrysosporium*, its cellular response to vanillin has been analyzed in detail [1]. Vanillin is one of the key intermediates found during lignin biodegradation. *P. chrysosporium* exposed to vanillin drastically changes the metabolic flux from the glyoxylate cycle to the tricarboxylic acid cycle and then activates the heme biosynthesis pathway. Moreover, one of the lignin-degrading enzymes, manganese peroxidase, which contains a heme, is highly expressed in the presence of vanillin. Therefore, it is likely that the effective heme biosynthetic system, branching the tricarboxylic acid cycle, contributes not only to supply a manganese peroxidase with a heme but also to promote lignin degradation in *P. chrysosporium*.

Based on these observations in *P. chrysosporium*, we now focus on the cellular response of a white-rot fungus, *Ceriporiopsis subvermispora*, to vanillin. *C. subvermispora* has very different characteristics from *P. chrysosporium*: i) selective ligninolysis without serious damage to cellulose; ii) secretion of large amounts of fatty acids and their peroxidation at an early stage of wood decay; iii) high resistance to growth inhibition by vanillin; iv) no activity of lignin peroxidase; v) possession of a suppression mechanism of cellulolytic hydroxyl radical; vi) no activity of cellobiohydrolase. Therefore, *C. subvermispora* would behave differently in response to vanillin from *P. chrysosporium*. Indeed, we have demonstrated that a gene involved in the biosynthesis of linoleic acid is inducibly transcribed in the presence of vanillin [2]. This observation is not reported in *P. chrysosporium*.

In order to investigate the cellular response of *C. subvermispora* to vanillin, we quantitatively analyzed fungal growth, glucose consumption, production of lipid-related metabolites, and activities of lignin-degrading enzymes using cells grown on a synthetic liquid medium supplemented with vanillin. Unlike *P. chrysosporium*, no growth inhibition was detected in *C. subvermispora* exposed to vanillin. The production of lipid-related metabolites was remarkably increased in culture supernatant fluid of *C. subvermispora* exposed to vanillin. Moreover, we successfully obtained many gene clones that are up-regulated in the presence of vanillin by suppression subtractive hybridization. We are now trying to predict the function of these genes using various genome databases.

Using two intracellular proteins from *C. subvermispora* grown with and without vanillin, on the other hand, we are also performing fluorescence two dimensional difference gel electrophoresis (2D-DIGE). In this method, we can co-separate and visualize two different protein samples on a single two-dimensional gel. Because the expression ratio from each of the protein spots obtained in 2D-DIGE are quantitatively calculated, we can easily select the protein spots that are positively or negatively expressed in the presence of vanillin. If 2D-DIGE works well, these proteins of interest will be identified by mass spectrometry. Furthermore, we will be able to compare the cellular response of *C. subvermispora* with that of *P. chrysosporium* against exogenous addition of vanillin.

References

[1] Shimizu, M., Yuda, N., Nakamura, T., Tanaka, H., and Wariishi. (2005) Metabolic regulation at the tricarboxylic acid and glyoxylate cycles of the lignin-degrading basidiomycete *Phanerochaete chrysosporium* against exogenous addition of vanillin. *Proteomics* **5,** 3919-3931.

[2] Watanabe, T., Tsuda, S., Nishimura, H., Honda, Y., and Watanabe, T. (2010) Characterization of a Δ12-fatty acid desaturase gene from *Ceriporiopsis subvermispora*, a selective lignin-degrading fungus. *Appl. Microbiol. Biotechnol.* **87,** 215-224.