Degradation of recalcitrant pollutants in sediment by halotolerant white-rot fungus *Phlebia* sp. MG-60

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Marine fungi are regarded as major decomposers of woody and herbaceous substrata entering marine ecosystem, and they have been identified from substrata containing lignocelluloses. Most of the studies, however, were concentrated on biodiversity and classification of marine fungi. Marine fungi grow on marine and estuarine environments, so these white-rot fungi were isolated from decayed mangrove and sea grass, and their lignin-modifying enzymes might have certain hypersaline ability and could be applied to the salt environment. *Phlebia* sp. MG-60 is a ligininolytic basidiomycete isolated from mangrove stands in Japan based on its lignin degradative ability. This fungus can grow and exhibit high lignin degrading ability under the culture condition with high salt concentration. In this study, therefore, we investigated the production of manganese peroxidase (MnP) by MG-60.

The strain *Phebia* sp. MG-60 grew faster and produced more MnP activity in the medium containing 3% sea salts. When 3% sea salts were added to the culture of *Phebia* sp. MG-60 at the 10-day of the incubation without sea salts, the MnP activity also largely increased. The increase of MnP activity was observed within 48 hours after the sea salts addition.

We sequentially harvested the crude culture fluid from the culture of *Phebia* sp. MG-60, 0, 12, 24, 48 hours after sea salts addition, and ran SDS-PAGE of them to see the secreted protein profiles. A 45 kDa protein was the only major protein during 24 hours, thereafter, this protein disappeared instead, 47 kDa and 50 kDa proteins were produced until 48 hours. This striking change was well consistent with the increase of MnP activity. These results suggest that these newly produced proteins (47 kDa and 50 kDa) are sea salts-responsive MnP isoformes. Thus, *Phlebia* sp. MG-60 changes MnP isoforms as the response to sea salts.

The RT-PCR and 3'-RACE technique resulted in cloning two partial MnP cDNA, *MGmnp1*(750 bp), *MGmnp2*(1100 bp). These partial MnP cDNAs, *MGmnp1* and *MGmnp2*, are less similar (ca. 50%) each other. The *MGmnp2* was extensively expressed in the culture containing sea salt and was also clearly induced within 48 h after the addition of sea salts to the culture without sea salts. However, *MGmnp1* mRNA was not induced or enhanced in the sea salt containing culture. These results indicate that *MGmnp2* is a cDNA encoding sea salts-inducible MnP isoform of *Phlebia* sp. MG-60.