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A Study on the Biological Decolorization by *Geotrichum candidum* Dec 1

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A fungus, *Geotrichum candidum* Dec 1, newly isolated from soil as a dye-decolorizing microorganism, decolorized 18 kinds of reactive, acidic and dispersive dyes and 3 model compound on a solid medium, showing a broad spectrum of decolorization. Reactive blue 5 of 12 g/l was degraded without significant decline of activity. An energy source and oxygen were essential for the expression of decolorizing activity. Some extracellular peroxidases are responsible for dye-decolorization. A peroxidase (DyP) involoved in the decolorization was purified from culture broth of *G. candidum* Dec 1. DyP, a glycoprotein, is glycosylated with N-acetylglucosamine and mannose (17%) and has a molecular mass of 60 kDa, and an isoelectric point (pI) of 3.8, and Soret band at 406 nm corresponding to a hemoprotein in which $Na_2S_2O_4$-reduced form of peak at 556 nm indicates the presence of a protoheme as its prosthetic group. Nine of the 21 types of dyes that were decolorized by Dec 1 cell were decolorized by DyP. The optimal temperature for DyP activity was 30, and its activity was stable even after incubation at 50°C for 11 h.

Aryl alcohol oxidase (AAO) produced by *G. candidum* Dec 1 was purified by ultrafiltration and by using diethylaminoethyl (DEAE) Sephacel, Butyl-Toyopearl and Mono-Q columns. H_2O_2 produced by concomitant AAO oxidation of veratryl alcohol (VA) to veratraldehyde was consumed by a peroxidase (DyP) purified from Dec 1 culture, the existence of H_2O_2 and veratraldehyde was confirmed during cultivation, when dye-decolorization and AAO activities were maintained. This indicates that VA produced by Dec 1 was oxidized by AAO to veratraldehyde, generating H_2O_2, which supported dye-decolorizing activity of Dec 1 *in vivo*. The prevention of polymerization of DyP oxidation products of a dye in the presence of AAO was shown.

An anthraquinone model dye, AQ-2 (sodium, 1-amino-4-methylamino- anthraquinone-2-sulfonate), an identical degradation product by the whole culture of *G. candidum* Dec 1, was employed for analysis of a decolorization mechanism in microbial and enzymatic system. AQ-2 was firstly transformed to compound I (sodium, 1-aminoanthraquinone-2-sulfonate) and II (sodium, 1,4-diamino- anthraquinone-2-sulfonate) by peroxidases of Dec 1. Compound I was then split to two benzaldehydes, compound III (3-chloro-4-methoxybenzaldehyde) and IV (3,5-dichloro-4-methoxybenzaldehyde) by ring opening by Dec 1 attack and sequent reactions as follow : chlorination, hydroxylation, deprotonation and methoxylation. Opening of anthraquinone bone was progressed only by the whole culture of Dec 1, and the decolorizing enzymes, DyPs, of Dec 1 was certainly involved in initial degradation step of AQ-2, even if it could not open anthraquinone bone.

Decolorization of crude molasses which was also used as an energy source of Dec 1, was conducted in a jar fermenor system having a fan-type propellers and a pressure swing adsorption oxygen

generator. The oxygen-fortified air supply was effective not only to obtain a highest decolorization degree of 80% molasses but also the highest peroxidase activity which is responsible for the decolorization of the dyes.

In a repeated batch cultivation, an eight % decolorization of molasses and a stable peroxidase activity were maintained for approximately four weeks, after which, both activities deteriorated significantly. Therefore, a refill and draw culture method using immobilized cell on polyurethane foam was employed to solve aforementioned problems and consequently resulted stable decolorization of molasses as well as stable peroxidase activity for more than eight weeks.