Isolation of Lignin-Degrading Microorganisms from Landfill Sites and Plastic-Contaminated Soils

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In response to global carbon neutrality initiatives and the promotion of sustainable forest resource utilization, microbial lignin degradation has emerged as a promising strategy for simultaneously expanding renewable energy use and reduce greenhouse gas emissions. Lignin-degrading bacteria offer a biologically efficient and environmentally low-impact alternative to conventional chemical pretreatment methods. However, current strains often exhibit limitations in degradation rate and environmental adaptability, necessitating the discovery of novel strains capable of stable and rapid lignin decomposition under diverse conditions.

In this study, we aimed to isolate lignin-degrading bacteria from decayed wood and plastic-contaminated soil collected from a landfill site in Muroran City and a polluted area in Sapporo City, Hokkaido, respectively. Initially, 20 g of each sample was suspended in 100 mL of phosphate-buffered saline (PBS) to remove soil particles adhering to microbial cells. To increase biomass, 20 mL of the PBS suspension was inoculated into 100 mL of Nutrient Broth (NB) and incubated with shaking for 12 hours. The resulting pre-culture was serially diluted from the original concentration to 10^{-6} and screened on glucose–yeast extract–peptone (GYP) agar medium supplemented with Remazol Brilliant Blue R (RBBR). Colonies that decolorized the blue dye to white were further inoculated into alkaline lignin liquid media at concentrations of 1.0, 2.0, 3.0, and 5.0 g/L to evaluate the degree of decolorization.

Samples exhibiting a shift from the lignin-derived dark brown coloration to light brown or colorless were subjected to adsorption assays. Cultures grown under static conditions in lignin medium for five days were centrifuged, and the wet weight of the resulting cell pellets was measured. Equal amounts of biomass were then inoculated into fresh lignin medium, and their adsorption behavior was assessed at 30, 60, and 90 minutes.

Screening on RBBR agar yielded approximately 400 decolorizing strains from both wood and soil samples. Among these, only five strains demonstrated decolorization in

alkaline lignin medium. Adsorption tests confirmed that the decolorization was not due to physical adsorption, as no lignin-derived coloration was observed on the cell pellets. These five strains were designated CTU1 through CTU5. After five days of incubation, CTU1 exhibited a decolorization rate of 2.17%, CTU2 of 56.2%, CTU3 of 54.9%, CTU4 of 1.00%, and CTU5 of 79.9%.
These findings enhance our understanding of bacterial lignin degradation and provide a foundation for the development of low-environmental-impact pretreatment technologies. Future work will focus on the taxonomic identification of the CTU strains and detailed characterization of their enzymatic systems. This study suggests that bacterial approaches may complement or even surpass existing fungal-based methods in lignin bioconversion.

Keywords : lignin degradation, Lignin-degrading bacteria, low-environmental-impact pretreatment, bioconversion, decolorization rate