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Cellulase Activity of the Halophilic Fungal Degraders

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Abstract

Cellulases are pivotal enzymes involved in cellulose degradation making them highly valuable in industries such as biofuel, paper, textile, brewery and in waste management. Due to the recalcitrant nature of cellulose and the low efficiency of available enzymes, exploring novel cellulases from the underexplored environments is crucial. Mangroves are an ecologically unique environment characterized by dynamic temperature, salinity, and tidal fluctuations, which are responsible for recycling organic matter accumulation in their sediments. Although mangroves house many detritivores, fungi are known to be more efficient biomass degraders than bacteria. Salt-tolerant cellulases are of particular interest compared to ordinary cellulases. Cellulases have a great application prospect in the improvement of saline-alkali soil. Lignocellulosic biomass in saline-alkali soil is difficult to be degraded due to high salinity and water-logged conditions, leading to poor circulation of organic material. Hence, this study delves into the cellulolytic potential of halophilic fungi isolated from leaf litter. The fungi were isolated from mangrove ecosystems neighbouring the NARA Regional Research Centre in Kalpitiya (8.25° or 8°15' North latitude, 79.77° or 79°46'15" East longitude). For the isolation and growth of fungi, media were prepared using the aged and filtered water from the same mangrove. Putative isolates were obtained by (i.) directly plating litter fragments in Water Agar and (ii.) incubating the litter in a moist chamber. Isolates were transferred to 2% malt extract agar (MEA) plates using the hyphal tip method. Of the 87 putative halophytic fungi isolated, 34 different morphotypes were identified based on detailed macroscopic and micromorphological features. The morphotypes were screened preliminary on carboxymethyl cellulose (CMC) plates. The hydrolysis rate of CMC served as the indicator, and only 23 of the isolates (26.42%) displayed positive cellulolytic results. From the pool of 23 promising isolates, the best 10 producers were coded and used for further investigation through the standard filter paper assay (FPA). These isolates were cultivated in Cellulase Basal Media (CMB) and subjected to an 8-day incubation in a shaking water bath. Whatman no. 1 filter paper strips were used as the primary source of cellulose. Crude enzymes were prepared through centrifugation, appropriately diluted, and then used for the FPA. The study also examined the impact of temperature (37° C, 50° C, and 60° C) and pH (pH 4, pH 5, and pH 6) on the cellulolytic activity of the crude enzymes. Notably, isolates A001, A030, B041, B047 and E082 showed promising results and isolate B047 showed good thermal stability in all three temperatures tested. Furthermore, the research extended to the extraction and assessment of the genomic DNA of the promising isolates. The identification of the promising isolates is underway.

Keywords: Cellulase activity, FPA, Halophilic fungi, CMB, Lignocellulosic