

CELLULASE-PRODUCING BACTERIA ISOLATED FROM PHILIPPINE MANGROVES

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Abstract:

Two-hundred and twenty-five (225) mangrove bacterial isolates from different mangrove areas in the Philippine archipelago were qualitatively screened for cellulase production. Qualitative enzyme activity was determined following the method described by Laurent et al. 2000. Pure cultures were inoculated on plates containing (g L⁻¹): Sodium Carboxymethyl Cellulose 1.2, Yeast Extract 0.5, K₂HPO₄ 1.0, MgSO₄·7H₂O 0.5, KCl 0.5, NaNO₃ 0.3, agar 15.0, supplemented with 1.5% marine salts. The plates were incubated at 30°C for 5-10 days, stained with Congo red solution (0.1 g L⁻¹) for 15 min then washed with 1 M NaCl to reveal an orange halo surrounding the colony against a red media background indicative of cellulase production. One hundred fifty four isolates (154) were found to produce cellulase. Ten strains showed very strong activities (halo diameter >10 mm surrounding the colony), which were then quantified. Five with the statistically highest activities: BBCS-11, BBCS-14, BBoB2L2-2, BOrMGS-2, BOrMGS-3, and *Cellulomonas sp. BIOTECH 1240*, were further subjected to partial optimization studies. The effects of substrate, pH, incubation temperature and time were determined. With some modifications, cellulase activity was assayed following the protocol described by FAO www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/Additive-222.pdf. The peak cellulase activities of the strains using CMC as substrate were significantly higher than those obtained using Avicel. Majority of the strains had peak cellulase activities at basic pH (pH 9), decreasing as pH approaches neutral or acidic. Only isolate BBoB2L2-2 had its peak cellulase activity at neutral pH (pH 7) while BOrMGS-3 registered its peak cellulase activity at an acidic pH (pH 5). All of the strains had their peaks on the 6th day except for BBCS-11, which was on the first day. The cellulase activities (U mL⁻¹) of the strains under optimized conditions were: 56.60 (BBCS-11); 66.50 (BBCS-14); 50.33 (BBoB2L2-2); 51.04 (BOrMGS-2); and 48.70 (BOrMGS-3) and 54.80 (Control). The five promising cellulase producers were characterized and identified using conventional methods and with the aid of the API CH-50. They were identified as follows: BBCS-11 as *Bacillus cereus*; BBCS-14 as *Bacillus licheniformis*; BOrMGS-2 and BOrMGS-3 as *Bacillus pumilus*; and BBoB2L2-2 as *Bacillus sp.*

Key words: cellulase-producing, bacteria, mangroves