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Native expression and in silico characterization of chitinases from bacterial isolates with potential for sustainable biocontrol

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Chitinases catalyze the degradation of chitin, a major structural component of fungal cell walls and insect exoskeletons, and are considered promising tools for sustainable agricultural applications. This study aimed to investigate the native production of these enzymes in bacterial isolates by integrating experimental evidence with in silico approaches. Computational analyses of *Chitinophaga* sp. CB10 and *Bacillus bombycepticus* JAB01 included homology searches, physicochemical predictions, and conserved domain identification. Proteins showing high similarity to members of the glycosyl hydrolase family 18 (GH18) were detected, displaying structural features typical of chitinases and, in some cases, predicted signal peptides indicating extracellular secretion. For experimental validation, isolates were cultivated in Luria-Bertani medium supplemented with 1% colloidal chitin under agitation (150 rpm) at 30 °C for 24 h. After centrifugation, the supernatant was subjected to ammonium sulfate precipitation (15–70% saturation), followed by dialysis. Extracellular protein concentration was quantified using the Bradford assay over time, showing a progressive increase with a peak at 8 h (≈11.3–11.4 µg/mL), followed by stabilization. Samples were analyzed by SDS-PAGE (10%), revealing bands consistent with the expected molecular weights. Chitinolytic activity was further assessed on agar plates containing 1% colloidal chitin by applying 50 µL of dialyzed supernatant (70% fraction) into wells. The formation of degradation halos confirmed enzymatic activity. Together, these findings demonstrate that the evaluated isolates are capable of producing functional chitinolytic enzymes under native conditions, with experimental results supporting computational predictions. These microorganisms represent promising candidates for sustainable biocontrol strategies.

KEYWORDS: chitinase, bacterial isolates, enzyme expression, sustainable agriculture

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