

**PHYTOGENETIC ANALYSIS BY 16S rRNA GENE SEQUENCE OF A PROTEASE-
SECRETING THERMOPHILIC BACTERIAL ISOLATE KITNT-3 FROM KITEEZI
LANDFILL**

BY

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ABSTRACT

A thermophilic protease-secreting bacterial isolate KITNT-3 from Kiteezi landfill was characterized phylogenetically by determining its relationship with other existing known bacterial strains. Genomic DNA was isolated from the cells and the 16S rRNA gene amplified using universal primers. The amplified PCR product was purified using an elaborate ethanol/EDTA precipitation method and subjected to sequence analysis using an ABI 3130XL genetic analyzer. In order to determine the phylogenetic position of the isolate, the partial 16S rRNA gene sequence was compared with other known 16S rRNA gene sequences of related taxa from the GenBank. A phylogenetic tree was constructed using the *MEGA* v4 program. The analysis showed that the isolate clusters with members of the genus *Bacillus* and was closest to *Bacillus thuringiensis* strain T13001 (ACNC01000278), sharing 99.8 % nucleotide sequence similarity. The crude enzyme was obtained from the culture filtrate of the isolate after an overnight incubation at 55⁰C in a shaking incubator at 80 rpm and the extract was characterized for its caseinolytic activity. The morphological and growth characteristics showed that isolate KITNT-3 is an aerobic, Gram-positive, endospore-forming, thermophile with rod-shaped cells measuring 3.2 µm in length and 0.8 µm in width respectively. The crude enzyme was found to exhibit a specific caseinolytic activity of 0.0065 µmoles tyrosine equivalents/min/mg protein at optimal assay conditions of pH 6 and temperature of 55⁰C. Further purification and characterization of the crude enzyme solution is deemed necessary for a comprehensive enzyme classification.