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Isolation, Characterization and Identification of Thermo-Stable Amylase Enzyme Producing Bacteria from Compost Production Sites

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Abstract

Thermophilic bacteria have altered to thrive and function in relatively hot environments. They can produce thermo-stable enzymes that can survive under high temperatures. Among other enzymes, amylase plays an important role in food, paper and textile industries. Amylase enzyme production has reached up to 30% of the global enzyme market. Therefore, this study has focused on thermo-stable amylase enzymes producing bacteria. The samples were collected from compost production sites at *Govijanaseva Department, Kadawatha* (sample 01) and *Seethawakapura compost site, Awissawella*, (sample 02). The standard pour plate method and streak plate method were carried out using Nutrient Agar plates at room temperature to observe bacterial colonies. Bacterial isolates were screened for the production of amylase enzymes using a starch hydrolysis test. The secondary screening for the enzyme activity was conducted using Di-nitro salicylic acid (DNS) assay and the effect of temperatures and pH levels on crude enzymes of amylase producing bacterial isolates were determined. The genomic DNA of the most promising bacteria was extracted and the 16S rRNA gene sequencing was performed for molecular identification. The soil samples were collected at 61℃ (sample 01) and 65℃ (sample 02) of temperature. Ten and seven morphologically different bacterial colonies were observed from sample 01 and sample 02 respectively. Out of ten morphologically different bacterial colonies, 04 isolates (*GV5, GV6, GV9* and *GV10*) and out of seven bacterial colonies 04 colonies (*SW1, SW2, SW3* and *SW4*) were positive for the production of thermo-stable amylase enzyme. *GV2* was shown to have the highest optimum temperature of 70℃ and optimum pH 8 for amylase enzyme activity from the sample 01. The optimum temperature and pH for amylase activity was recorded as 80 ℃ and pH 6 respectively from the sample 02 (*SW2*). The molecular identification was carried out only for the sample 02 and *SW2* was identified as *Bacillus subtilis.* As per the results, the bacterial isolate: *GV9, SW2* were identified as the most potential bacteria for biotechnological processes and industrial uses under high temperatures.

Keywords: *Biotechnology, Compost, Thermo-stable amylase, Thermophilic bacteria*