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Production of thermolabile and thermostable α -amylase using *Bacillus* species

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α -Amylases are enzymes that catalyze the hydrolysis of internal α -1, 4-glycosidic linkages in starch. These enzymes are widely used in industry. The entire α -amylase requirement of Sri Lanka is imported, resulting in a loss of foreign exchange. In this study we compared the production of thermolabile α -amylase from *Bacillus subtilis* and thermostable α -amylase from *Geobacillus stearothermophilus*. Previous studies show cassava root extract to be a potential fermentation medium to produce α -amylase using *B. subtilis* (personal communication by S. L. Dalpatadu). Therefore, cassava was ground, mixed with deionised water (20% w/v), filtered and supplemented with yeast extract (0.5% w/v) and used as a fermentation medium. The fermentation medium was incubated for 72 hours after inoculating with respective bacterial strains. Both bacterial strains secrete α -amylase to the fermentation medium. Therefore, the supernatant resulting from centrifuging the fermentation medium was used as an enzyme extract and was purified using a Sephadex G50 column. Dinitrosalicylic (DNS) assay was used to compare α -amylase activity. One unit (U) of α -amylase activity was defined as the amount of glucose released in 1 ml of final assay solution in 1 minute (1 unit = 1 μ moles/ ml⁻¹ min⁻¹). The data are expressed as mean \pm standard error of the mean (SEM) and analysis of difference between two groups was done using student t-test. In order to express thermostable α -amylase in a eukaryotic system, PCR primers were designed based on α -amylase sequence from *G. stearothermophilus* (NCBI accession - AY705090.1) and analyzed using the OligoAnalyzer 3.1 online software. The 1.5 kb PCR amplified product was cloned in pGEM®-T easy vector. Recombinants were identified by colony PCR, restriction analysis and sequencing. The enzyme activity of *B. subtilis* and *G. stearothermophilus* α -amylase was 219.8 \pm 7.1 U and 31.4 \pm 3.9 U respectively, indicating that the α -amylase production by *G. stearothermophilus* was significantly less than by *B. subtilis*. The DNA sequence of a putative clone (GSA23) showed 99.9% similarity at nucleotide level while translated amino acid sequence and the amino acids of the active site showed similarities of 99.2% and 100% respectively. The presence of the signal peptide within the region was confirmed by using signal P 4.1 server online software. This study indicates that the level of enzyme production by *G. stearothermophilus* is relatively low compared to *B. subtilis*. Therefore, the gene for thermostable α -amylase production in *G. stearothermophilus* was successfully cloned and will be used to enhance α -amylase production by using recombinant protein expression techniques, with a view to locally produce α -amylase at an industrial scale.

Keywords: Amylase, cassava, thermostable