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Extended Abstract

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A dimeric and Ca2+ independent ÃŽÂ±-amylase from a newly isolated B. subtilis US572 strain: biochemical and molecular characterization

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New amylase (AmyKS) was purified from a newly isolated Bacillus subtilis US572 strain. The optimum pH and temperature recorded for enzyme activity were 6 and 60 °C, respectively. It displayed a marked thermostability with a half-life of 10 min at 70 °C. AmyKS is a Ca2+ independent enzyme and able to hydrolyze soluble starch into single sugars with predominant proportions of maltose and maltotriose. It presents a high affinity towards soluble starch with a Km value of 0.252 mg mL-1. The analysis of the enzyme in native and denaturing conditions suggests that it has a dimeric form (140 kDa). This is the first report on the purification and characterization of a nonmaltogenic Bacillus α-amylase with a dimeric structure. A 3D model and a dimeric model of AmyKS were constructed and accordingly evidence was found about the high substrate affinity and the high catalytic efficiency of this enzyme. A study was carried out with a newly isolated bacterial strain yielding extracellular amylase. The phylogenetic tree constructed on the basis of 16S rDNA gene sequences revealed this strain as clustered with the closest members of *Bacillus* sp. and identified as *Bacillus subtilis* BI19. The effect of various fermentation conditions on amylase production through shake-flask culture was investigated. Rice flour (1.25%) as a cheap natural carbon source was found to induce amylase production mostly. A combination of peptone and tryptone as organic and ammonium sulfate as inorganic nitrogen sources gave highest yield. Maximum production was obtained after 24 h of incubation at 37°C with an initial medium pH 8.0. Addition of surfactants like Tween 80 (0.25 g/L) and sodium lauryl sulfate (0.2 g/L) resulted in 28% and 15% increase in enzyme production, respectively. Amylase production was 3.06 times higher when optimized production conditions were used. Optimum reaction temperature and pH for crude amylase activity were 50°C and 6.0, respectively. The crude enzyme showed activity and stability over a fair range of temperature and pH. These results suggest that *B. subtilis* BI19 could be exploited for production of amylase at relatively low cost and time. Amylase represents a group of extracellular enzymes (consisting of α-amylase, β-amylase, and glucoamylase) that act on starch or oligosaccharide molecules in a random manner and hydrolyze into diverse products including dextrins and progressively smaller polymers composed of glucose units. They have most widely been reported to occur in micro-organisms (fungi, yeast, bacteria, and actinomycetes), although they are found in plants and animals. In present day they have found applications in all the industrial processes such as in food, detergents, textiles, pharmaceutical, paper and fine chemical industries for the hydrolysis of starch. Amylase has great significance in present-day biotechnology having approximately 25–30% of the world enzyme market. These extensive potentials of amylase to be used in broad range of industries have placed greater stress on researchers to search for more efficient amylase production. The genus Bacillus has been becoming a reliable option to find out novel and promising bacteria for the production of amylase and other extracellular enzymes. Different species of Bacillus, most notably, B. subtilis, B. licheniformis, B. amyloliquefaciens, and B. stearothermophilus, are reported to produce approximately 60% of commercially available enzymes. Short fermentation cycle, capacity to secrete proteins into the extracellular medium, safe handling, eco-friendly behavior, easy manipulation to obtain enzymes of desired characteristics, high enzymatic activity in a wide range of conditions (extreme pH, temperature, osmolarity, pressure, etc.), and simple and cost effective production have made this genus as bacterial workhorses for the production of a variety of enzymes as well as fine biochemicals for decades. Different Bacillus species have similar growth patterns and enzyme profiles but depending upon the strain their general properties (thermostability, pH profile, pH stability, etc.) and optimized fermentation conditions may vary. Thus it is really challenging to obtain a strain that can produce amylase meeting specific industrial demands. There is no local production and thereby availability of amylase in Bangladesh. As a result, most of the existing and growing starch based industries are using expensive chemicals for starch hydrolysing based purposes. Keeping in mind the growing demand of amylases by different industrial sectors this study was carried out to obtain laboratory scale fermentation of amylases in shake flask culture by newly isolated B. subtilis BI19 along with optimization of medium components and culture conditions for enhanced production, thereby to understand its potential for biotechnological application.

Bottom Note: *This work is partly presented at Joint Event on Biotechnology, Biochemistry and Aquaculture* August 08-09, 2019 | Paris, France