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Review Article

Production strategies of Amylase Enzymes from Microbial Strains

Aratrika Chatterjee[1], Manikantan Pappuswamy[2], Arun Meyyazhagan[3], Murugesh Easwaran[4], Balamuralikrishnan Balasubramaniam[5]

[1&2]Department of Life Sciences, CHRIST (Deemed to be) University, Bangalore, Karnataka-560029, India
[3]Euroespes Biomedical Research Centre, International Centre of Neuroscience and Genomic Medicine, 15165 Corunna, Spain
[4]Nutritional Improvement of Crops, International Centre for Genetic Engineering and Biotechnology, New Delhi-110067, India.
[5]Department of Food Science and Biotechnology, College of Life Science, Sejong University, Seoul, 05006, Republic of Korea.

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ABSTRACT

Amylase enzymes produced from different microbial strains is reviewed in this work. Amylases are the enzymes which break down starch or glycogen into sugar. Amylases can be derived from plants, animals and microorganisms. The advantage of using a microbial strain for amylase production is the large-scale production capacity and easy microbial manipulation to get the amylase enzymes with most desirable characteristics Amylase is an associate accelerator that breaks down starch into sugar. Enzymes are biochemical substances, naturally secreted in the digestive system, where they begin the natural action of digestion tics. Foods like rice, potato, contain lot of starch but very little sugar. When such foods are chewed, they taste sweet, because our saliva has amylase in it and when it converts starch into sugar, it tastes sweet. Plants and a few microorganisms conjointly turn out amylase

INTRODUCTION

Amylase is an associate accelerator that breaks down starch into sugar. Enzymes are biochemical substances, naturally secreted in the digestive system, where they begin the natural action of digestion [2]. Foods like rice, potato, contain lot of starch but very little sugar. When such foods are chewed, they taste sweet, because our saliva has amylase in it and when it converts starch into sugar, it tastes sweet. Plants and a few microorganisms conjointly turn out amylase [4]. As diastase, enzyme was the primary accelerator to be discovered and isolated by Anselme Payen in 1833. All amylases are the most naturally occuring hydrolases and act on the α-1,4-glycosidic bonds in polysaccharides resulting in formation of simple sugars [5,6]. Amylases accelerate the process of industrial starch conversion method [7]. The enzymes act on starch and connected oligo- and polysaccharides bonds. Within the food industry chemical process enzymes have an oversized scale of applications, like the assembly of aldohexose syrups, high fruit sugar corn syrups, malt sugar sirup, reduction of consistency of sugar syrups, reduction of turbidity to provide processed potable for extended shelf-life, solubilization and saccharification of starch in the production business [12,18]. The baking industry uses amylases to delay the staling of bread and alternative baked products; the paper business uses amylases for the reduction of starch consistency to attain the appropriate coating of paper. The enzyme, chemical process accelerator is employed in the textile business for warp filler of textile fibers, and used as a organic process aid within the pharmaceutical industry [19,26]. Fungi, genus Aspergillus are most

commonly used for the assembly of amylase [11,14,16]. Production of enzymes by solid-state fermentation (SSF) here these molds are turned into a cost-effective production technique. (S. Shivaramakrishnan, 2006) [26,38]. Among the assorted kinds of amylases made, commercially thermostable amylases are gaining much more benefits compared to alternative sorts (Popovic et al., 2009) [12]. Other microorganisms manufacturing considerable quantity of different quantity of enzyme protein are Escherichia sp. Micrococcus sp. Pseudomonas spp. Proteus sp., bacteria genus sp. Candida, Cephalosporium, Mucor, Penicillium fungus genus etc [11,16,19,15,30]. In recent years the potential of victimization microorganisms as biotechnological sources of industrially relevant enzymes has stirred interest within the exploration of extracellular accelerator activity in many microorganisms (Akpan et al., 1999; Pandey et al., 2000; Abu et al., 2005) [15]. Amylases are necessary enzymes used within the starch process industries for the chemical reaction of polysaccharides like starch into monosaccharides constituents (Akpan et al., 1999; Mitchell and Lonsane, 1990) [12,15]. In storage tissues like seeds, starch, a saccharide of aldohexose is hydrolyzed by growing seed plant to satisfy its energy demand. today the new potential of using microorganism as biotechnological sources of industrially relevant enzymes has stirred interest in studies and exploration of additional cellular enzymatic activities in many microorganisms [19]. These enzymes are found in animals, plants, microorganism and molds (Abu et al., 2005). Sources of amylases in yeast, microorganism and molds are according and their properties are described (Akpan et al., 1999; Buzzini and cocktail, 2002). The enzyme of plant life origin was found to be a lot of stable than the microorganism enzymes on a billboard scale, several tries are created to optimize culture conditions and appropriate strains of fungi (Abu et al., 2005) [22,21]. Few tries are created to elucidate the management mechanisms concerned in formation and secretion of additional cellular enzymes [33,36,37]. Molds are capable of manufacturing high amounts of amylase; genus Aspergillus niger is employed for business production of α-amylase [12,16]. Studies on plant life amylases particularly in developing countries have focused mainly on genus Aspergillus niger, most likely thanks to their omnipresent nature and non-fastidious organic process requirements of those organisms (Abu et al., 2005) [2,19,35]. It is attainable to enlist the utilization of amylases underneath extreme condition of pH scale and temperature victimization thermo-acidophilic and basic amylases [1,3]. Since the foremost effective preparation of some applications contain alternative enzymes, particularly amyl-glucosidases and submerged strategies give a slim spectrum of further enzymes and it's worthy to isolate appropriate strains of genus Aspergillus niger for economical mechanism [22,38]. Solid substrate fermentation (SSF) has gained revived interest thanks to its potential to supply higher yields of flora metabolites than submerged fermentation (SmF) [15,22,35]. Many employees have obtained enzyme in SSF of wheat bran (Saha&Zeikus 1989, Pandey 1990, Ramash&Losane 1990). Recently, SSF of rice bran was developed to switch wheat bran within the tropics (Akpan et al. 1999). Despite the potential of SSF, accelerator utilization within the developing countries of the tropics is restricted, thanks to storage issues associated with the elevated temperatures found within the tropics. Enzymes are stabilized for long storage by refrigeration or freeze (Geisow 1991) [12,17]. However, in most tropical developing countries, these facilities are either lacking or the technology is just too high-ticket to amass. Several reports have shown that soluble enzymes fortified with their substrates/substrate analogues have improved thermal and storage stability (Geisow 1991) [38].

Production of Amylase enzymes from Microbial strains:

Aspergillus oryzae was obtained from the University Culture assortment Center [1,3,11] . The strain was fully grown on potato glucose agar (PDA) at 30º C, maintained on organizer slants at about 4º C and sub-cultured twice for a month. The fungal spores on agar slants were used as matter [12,13,14]. The bacteria species was isolated from setting and maintained on medium slants and sub cultured for each ten days. a bit of bread was unbroken in a very damp condition at temperature in dark for two days [15,20,35]. The bread sample was serially diluted and totally different dilutions were plated on potato glucose agar (PDA) medium. The Petri plates were incubated at temperature for around 4-5 days [20,21]. The fungal cultures were ascertained on organizer medium. Around 5 totally different fungous cultures were selected and sub cultured on organizer slants. All the five fungal

strains were subjected to lactophenol cotton blue staining for learning the morphology [16,19,22]. All the fungal cultures were confirmed as genus Aspergillus niger by learning the morphology and also the reproductive structure color [1,3,11,14]. Bacillusspp was which was isolated from the environment was maintained on nutrient agar slants and sub cultured for every 10 days [15,35]. About 1 gram of soil sample was taken in 9 ml of sterile distilled water. This was placed in water bath and maintained at 90º C for about an hour. After performing the serial dilution 1 ml of the mixture was poured into a sterile petri dish using the pour plate method which had cool molten agar with 1 % starch [13]. The poured plates were then incubated at 37º C for around 24 hours. Sub culturing was then done into fresh nutrient agar plates. Screening of the isolated pure strains was done for the production of extracellular amylase production (Bertrand et al., 2004) [11,13,20,35]. The pure cultures were streaked at the sterile starch agar plates and incubation was done at 37º C for around 24 hours. About 1% iodine solution was then over layered on the incubated plates and the substrate utilized zones were observed around the colonies. According to Bergey's manual of systematic bacteriology (Garrity et al., 2001), the isolated bacterial strain was identified as Bacillus megaterium [20,21,23].

Methods Administered in Microbial strain isolation and Amylase production:

Lactophenol Cotton Blue Staining

A rectangular PDA slab was prepared and kept on a clean glass slide [16,17]. The fungal culture was inoculated and another glass slide was kept on it forming a sandwich [20]. The "sandwiched" setup was kept for incubation for 3 days at room temperature. A loop full of fungal cultures such as (A. nigerBAN 1E, BAN 2E, BAN 3E, BAN 4E, BAN 5E) were kept on clean glass slides and a drop of lactophenol cotton blue stain was mixed with the culture [20,22,24,25]. After keeping a clean coverslip, the culture was observed under microscope and the fungal morphology was observed [16,17,28,33,37,38].

Fermentation

A changed rice bran solid medium of Akpan et al. (1999) was used. Rice bran (10 g) was moistened with tap water within the magnitude relation 1:1.5(w/w) to fifty fifth wet content [17,18]. Numerous ingredients were added as supplements to moistened rice bran in numerous ratios and mixed to obtain six differing types of media [21]. Each medium contained in the flask (pH adjusted to 4.0–4.3 with 0.1 M H2SO4) was sterilized for twenty minutes and inoculated with 1ml spore suspension of a 72 hour recent culture of A. oryzae[25]. Incubation was at 30º C for 72 hour beneath stationary conditions [25,27]. In some studies, after incubation of bacteria, the fermentation medium was harvested [23,23,27]. Further centrifugation was done at 5000 rpm for about 20 minutes at 4º C [18,20,21]. The supernatant obtained after centrifugation was subjected to the amylase activity [16,17,20,23].

Amylase preparations

By using a Metler LJ 16 moisture analyzer was used to dry out the fermented bran to around 12% moisture content and milling was done to 425 µm particle size using a micro-milling machine (Glen Creston, Stanmore, UK). Amylase preparation was done using the milled fermented bran [17].

Study on the effect of Temperature and pH on Amylase production

For studying the effect of temperature on amylase production the submerged fermentation process was carried out at different temperatures like 25º C, 30º C, 35º C, 40º C [23,25,29,33,34] respectively. And for studying the effect of pH fermentation medium was henceforth prepared at by varying pH values 4, 5, 6, 7, 8, 9 and 10 for the amylase production [32,37].

Amylase assay

According to Akpan et al (1999) the amylase activity per gram weight of the milled fermented bran was assayed at around 55º C using 0.1M acetate buffer pH 4.5 for 1 hour with 4%(w/v) soluble starch. By using the 3,5-dinitrosalicylic acid method, the determination of the reducing sugars was done [19,33,35].

Stabilization of amylase preparations

To the milled amylase preparations Soya bean flour (SBF) and partially hydrolyzed starch (PHS) were added as stabilizers approximately 1,5,10,20 % (w/v) [21]. By using the method, Flor and Hayashida (1983) the determination of the thermal stability of amylase preparations was done [24,25].

Storage stability of the amylase preparation was strictly monitored at 4 to 30º C for 12 weeks by determining the residual amylase preparation in each duplicate plate [27,28]. The duplicate portions were incubated for 15 minutes at various temperatures between 50-100º C and amylase activity was measured under standard assay conditions [29,31].

CONCLUSION

The bacterial isolates which were recorded to have the largest clearance zone and utilize glucose in the media were identified and characterized as Bacillus megaterium, Staphylococcus aureus [20,21]. There was rise in the activity of amylase enzyme when the incubation period was 24 hours followed by increase in incubation period to 48 hours. But decline in amylase activity was observed when incubation period was further increased to 72 hours and 96 hours [22]. The amylase activity was increasing with rise in the temperature [22]. The amylase activity, yield from Bacillus megaterium, was high at 30º C, which further increased at 40º C but the activity sharply decreased at 50º C[21,23]. Interestingly the enzyme activity still increased when the temperature was increased to 60º C[23]. But further rise in the temperature caused decline in the activity of amylase enzyme from Bacillus megaterium [20]. pH when increased from 4 to 5, there was a slight decline in the activity of amylase activity but it was observed to increase when pH rose to 6. Maximum activity was observed at pH 7 but gradually declined with rise in pH beyond 7. The maximum production of amylase enzyme from Bacillus spp. was observed at 35º C [6]. But at 40º C the production of amylase enzyme was observed to stop. Thus there was decline in the amylase production from Bacillusspp as the incubation temperature increased [21,30]. This was because as the temperature kept rising, the growth of bacteria too declined as a result the amylase enzyme produced by the bacterial strain decreased. The optimum activity of the enzyme was observed to be at 6.8 pH. The pH affects the bacterial growth thus indirectly affects the production of the enzyme amylase from Bacillusspp [30]. In presence of stabilizers the thermal deactivation of the enzyme was not observed. This may be due to the carbohydrate or the glycoprotein components present in the stabilizers, there was an influence on the thermal stability of the enzyme [27]. The stable complexes formed from the carbohydrate components of these substrates [20,21,22]. Proteolysis of the enzyme doesn't occur which in turn increased the stability of the enzyme amylase [21,22]. There is 95% correlation between the proteolysis and slow inactivation of amylase enzyme during storage [20,21].

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Corresponding Author: Aratrika Chatterjee
Department of Life Sciences, CHRIST (Deemed to be) University, Bangalore, Karnataka-560029, India.
E-mail: aratrika.chatterjee@science.christuniversity.in

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