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REVIEW ARTICLE

BIOTECHNOLOGY

BIOTECHNOLOGICAL APPLICATIONS OF INDUSTRIALLY IMPORTANT AMYLASE ENZYME

Corresponding Author

SHIPRA DAS

[1]Hash Biotech labs Pvt. Ltd., Plot No. 354, phase IX, Industrial Area, Mohali- (Punjab), India

Co Authors

SURENDRA SINGH[1], VINNI SHARMA[2] AND MANOHAR LAL SONI2

[1]Hash Biotech labs Pvt. Ltd., Plot No. 354, phase IX, Industrial Area, Mohali- (Punjab), India
[2]Gyan Vihar School of Pharmacy, S.G.V.U., Jaipur, India

ABSTRACT

Amylases are digestive enzymes which hydrolyze glycosidic bonds in starch to glucose, maltose, maltotriose and dextrin. They have a lot of potential applications in both food and pharmaceutical industries. To improve the potential productivity of amylases, the organism genome must be modified and this is achieved in two ways: by (i) classical strain improvement by mutation and selection and (ii) the use of recombination Amylases are produced by microorganisms using submerged and solid state fermentation. However, solid state fermentation is attractive because of usage of cheap substrate, high yields and avoids substrate inhibition. This study reviews the microbial sources, strain improvement methods and fermentation characteristics of amylase production.

KEY WORDS

Amylase, Enzyme, Strain improvement, Solid State Fermentation

INTRODUCTION

The industrial enzyme producers sell enzymes for a wide variety of applications. The estimated value of world market is presently about US$ 2.7 billion and is estimated to increase by 4% annually through 2012. Detergents (37%), textiles (12%), starch (11%), baking (8%) and animal feed (6%) are the main industries, which use about 75% of industrially produced enzymes. Amylases constitute a class of industrial enzymes having approximately 25% of the enzyme market[1,2]. An extra-cellular amylase, specifically raw starch digesting amylase has found important application in bioconversion of starches and starch-based substrates[3]. The level of alpha amylase activity in various human body fluids is of clinical importance e.g. in diabetes, pancreatitis and cancer research[4,5,6], while plant and microbial alpha amylases are used as industrial enzymes[7]. Starch-degrading amylolytic enzymes are of great significance in biotechnological applications ranging from food, fermentation, textile to paper industries[8,9]. Although amylases can be derived from several sources, such as plants, animals and microorganisms, the enzymes from microbial sources generally meet industrial demands and had made significant contribution to the production of foods and beverages in the last three decades. The microbial amylases have almost completely replaced chemical hydrolysis of starch in starch processing industry[9].

History:

As diastase, amylase was the first enzyme to be discovered and isolated (by Anselme Payen in 1833)[10]. Interestingly, the first enzyme produced industrially was an amylase from a fungal source in 1894, which was used as a pharmaceutical aid for the treatment of digestive disorders[9,11]. Biodin and Effront were the first to use *B. subtilis* and *B. mesentericus* for the production of alpha amylase on commercial scale using large fermentors and LSF (Liquid State Fermentation). In fact, the employment of bacterial cultures for the production of commercial enzyme was pioneered by them[12]. It was also reported that the applicability of solid media such as soyabean cake with one or two parts of water was not favourable[13]. The culture vessel and the process were further improved[14], and LSF became accepted industrial practice throughout the world for the production of bacterial alpha amylase. Prior to the developments fungal amylase were extensively produced in the United States by the SSF techniques as pioneered by Takamine [15, 16]. Underkofler and coworkers studied the potential of SSF technique for the production of bacterial alpha amylase. The bacterial alpha amylase produced under the SSF technique was evaluated by Park and Rivera[17], in comparison to the enzymes obtained by SmF processes. The expertise in SSF processes at Central Food Technological Research Institute, CFTRI, Mysore, India and the economic advantages of the SSF over SmF compelled to revive interest in SSF process for the production of bacterial alpha amylase in late 1985[18, 19,20]

Types:

α-Amylase

(EC 3.2.1.1) The α-amylases are calcium metalloenzymes, completely unable to function in the absence of calcium., α-amylase breaks down long-chain carbohydrates by acting at random locations along the starch chain, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. α-amylase tends to

be faster-acting than β-amylase because it can act anywhere on the substrate. In human physiology, both the salivary and pancreatic amylases are α-Amylases. Also found in plants (adequately), fungi (ascomycetes and basidiomycetes) and bacteria (*Bacillus*)

β-Amylase

(EC 3.2.1.2) β-amylase is another form of amylase synthesized by bacteria, fungi, and plants. β-amylase catalyzes the hydrolysis of the second α-1,4 glycosidic bond, working from the non-reducing end, cleaving off two glucose units (maltose) at a time. During the ripening of fruit, β-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit. Both α-amylase and β-amylase are present in seeds; β-amylase is present in an inactive form prior to germination, whereas α-amylase and proteases appear once germination has begun. Animal tissues do not contain β-amylase.

γ-Amylase

(EC 3.2.1.3) γ-amylase cleaves α(1-6) glycosidic linkages, in addition to cleaving the last α(1-4)glycosidic linkages at the nonreducing end of amylose and amylopectin, yielding glucose. Unlike the other forms of amylase, γ-amylase is most efficient in acidic environments and has an optimum pH of 3.

Uses of amylase:

Amylases have potential application in a number of industrial processes such as in the food, textiles, paper industries[21], bread making[22], glucose and fructose syrups, detergents, fuel ethanol from starches[23], fruit juices[24], alcoholic beverages[25], sweeteners (Peppler and Periman, 1978), digestive aid and spot remover in dry cleaning[26]. Bacterial α-amylases are now also used in areas of clinical, medicinal, and analytical chemistry[27,28]. The most widely used thermostable enzymes are the amylases in the starch industry[29, 30]. Some of the applications are discussed in detail as follows:

1. Modern bread making techniques have included amylase enzymes (often in the form of malted barley) into bread improver thereby making the bread making process faster and more practical for commercial use[31].
2. In sugar processing industry, the first step includes gelatinization of the starch slurry which is achieved by heating starch with water at temperature around 100^{o}C, due to insolubility of starch at lower temperatures. This step involves dissolution of starch granules, thereby forming a viscous suspension. Because of this high viscosity it poses serious problem with mixing and pumping. To overcome this viscosity associated problems, geltinization is coupled with liquefaction which involves partial hydrolysis and loss in viscosity. This action is brought about by thermostable alpha amylase, which can act at temperatures around 70-100^{o}C depending upon the temperature profile of alpha amylase[32].
3. An inhibitor of alpha-amylase called phaseolamin has been tested as a potential diet aid[33].
4. A higher than normal concentration of amylases may reflect one of several medical conditions, including acute inflammation of the pancreas (concurrently with the more specific lipase), but also perforated peptic ulcer, torsion of an ovarian cyst, strangulation ileus, macroamylasemia and mumps. Amylase may be measured in other body fluids, including urine and peritoneal fluid.
5. In molecular biology, the presence of amylase can serve as an additional method of selecting for successful integration of a reporter construct in addition to antibiotic resistance. As reporter genes are flanked by homologous regions of the structural gene for amylase, successful integration will disrupt the amylase gene and prevent

starch degradation, which is easily detectible through iodine staining.

Organisms producing amylase:

Microbial amylases have successfully replaced chemical hydrolysis of starch in starch processing industries. Amylase has been derived from several fungi, yeasts, bacteria and actinomycetes, however, enzymes from fungal and bacterial sources have dominated applications in industrial sectors. Fungal sources are confined to terrestrial isolates, mostly to *Aspergillus* species and to only one species of *Penicillium*, *P. brunneum* [34,9]. The bacterial a-amylases were derived from *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *B. licheniformis* and *B. stearothermophilus* [35], *B. gavealeus*, *B. mesentericus*, *B. myocodes, B. polymyxa, B. vulgates, Bacillus aterrimus* [36], *Bacillus coagulance*, *Aspergillus* sp and *Bacillus* sp. mainly *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *B.licheniformis* are employed for commercial applications to produce alpha amylase[37,38,39,40, 41, 42]. Production levels were observed to be very low when *Streptomyces* strain were used under SSF[43]. Bacterial strains belonging to *Bacillus*, *Pseudomonas* (aerobic) and *Clostridium* (anaerobic) sp., actinomycetes strains belonging to *Streptomyces* sp. and fungal strains belonging to *Rhizopus* sp. have been reported to synthesize β amylase[44, 45, 46, 47]. Both fungal and bacterial systems are widely used for the production of alpha amylases but bacterial system are preferred because of several characteristic advantages it offers (Pandey et al., 2000a). The reasons are (i) *Bacillus* sp. are most widely used bacterial strain for alpha amylase production (ii) *Bacillus* sp. is the most studied in SSF because of its survival in low moisture content (iii) isolating thermostable microorganism is easier in bacteria[32].

Strain improvement for amylase production

Owing to their inherent control system, microorganisms usually produce commercially important metabolite in very low concentrations and although the yield may be increased by optimizing the cultural conditions, productivity is controlled ultimately by the organism's genome[48].

The exponential increase in the application of amylases in various fields in the last few decades have placed stress and demands extension in both qualitative improvement and quantitative enhancement through strain improvement, medium optimization and search for efficient fermentation process for higher enzymatic yield. Such improved strains can reduce the cost of the process and may also possess some specialized desirable characteristics[49]. Bacillus species such as *Bacillus subtilis* and *B. amyloliquefaciens* are the organisms of choice for amylase production[50,51]. Mutagenesis and genetic recombination techniques using protoplast fusion and transformation have been used widely by several workers as a tool of protein engineering to achieve strains with higher enzyme productivity or desired characters. Svensson and Sogaard (1993) reviewed the effects of mutation on the structure and function of GA and related enzymes. Following are the methods of mutagenesis for overproducing alpha amylase in microorganisms

Chemical mutagens

Different chemical agents such as nitrous acids or ethyl methane sulphonate (EMS) are used for the mutation of bacteria[52]. Mutagenesis induced by chemicals such as N-methyl-N′-nitro-N-nitrosoguanidine (NTG) or by UV radiation has been employed to obtain hyperproducing strains, in which α-amylase synthesis can often be doubled or even tripled[53,27]. Suntornsuk and Hang[54] reported strain improvement of *R. oryzae* for GA

production using NTG as well as UV radiation. One mutant, 3N4, produced more GA compared with the parent strain. The mutagenesis of a GA-producing strain of *A. awamori* resulted in a mutant that exhibited 80% increase in enzyme productivity [55].

Ribosomal engineering

Drug resistant mutation of microorganisms reflects structural and functional changes of the ribosome and RNA polymerase. These changes have significant effect on the secondary metabolism in the mutant strain. Thus ribosomal engineering is an effective approach to develop mutant strain that over produce secondary metabolites by screening various drug resistant mutants [56]. Alfa-amylase production by a strain of *Bacillus subtilis*, was enhanced by the introduction of mutations that produce streptomycin resistance. Increase in the activity of the ribosomal protein S12 is responsible for the increase in α-amylase production seen in the affected strain [57].

UV light

The major lethal and mutagenic effects of UV radiation to microrganism results from damage to DNA. Non-ionizing radiation, such as ultraviolet (UV) light, exerts its mutagenic effect by exciting electrons in molecules. The excitation of electrons in DNA molecules often results in the formation of extra bonds between adjacent pyrimidines (specifically thymine) in DNA. When two pyrimidines are bound together in this way, it is called a pyrimidine dimer. These dimers often change the shape of the DNA in the cell and can cause problems during replication. The cell often tries to repair pyrimidine dimers before replication, but the repair mechanism can also lead to mutations as well. UV-A generally causes indirect damage to DNA through the formation of chemical intermediates such as oxygen and hydroxyl radicals which interact with DNA to form strand breaks, alkali labile sites and DNA protein crosslinks[58]. Conversely, adsorption of energy from UV-B induces direct damage to DNA. The two major lesions induced by UV-B radiation are the cyclobutane pyrimidine dimer and the pyrimidine-pyrimidone photoproduct. These photoproducts may be detected and quantified using specific radioimmunoassays[59].

UV light has been shown to be lethal and mutagenic in a variety of organisms. The correlation between the quantity of energy absorbed by DNA and the observed biological effects (survival and mutation frequency) are illustrated in the wavelength region between 254 and 320 nm. Mutational experiments were performed to produce morphological mutants from *Aspergillus wentii* Wehmer (IMI 17295) by UV and X-ray irradiation. Among the morphological mutants, five representative types were recognized. Marked variation existed in amylase activity between the morphological mutants and the wild type [60]. UV irradiation is found to be best for the improvement of strains like *Aspergillus niger* for maximum production of various enzymes[61]. Suntornsuk and Hang[62] have reported that the strain improvement in *Rhizopus oryzae* by UV, resulted in the production of more glucoamylase by a mutant than the parent strain.

Genetic Recombination using Protoplast fusion:

Hopwood[63] defined recombination as any process which helps to generate new combinations of genes that were originally present in different individuals. Protoplast fusion is one of the technique of genetic recombination.

Protoplasts are the cells devoid of their cell walls may be prepared by subjecting cells to the action of wall degrading enzyme in isotonic solutions. Cell fusion followed by nuclear fusion may occur between the protoplast of the strains, which would not otherwise fuse and the resulting fused protoplast may regenerate a cell wall and grow

as a normal cell. Protoplast fusion has been achieved using filamentus fungi, yeast, streptomycetes and bacteria. Amylase hyper-producing, catabolite-repression-resistant, recombinant strains are produced by intraspecific protoplast fusion of thermophilic fungus *Thermomyces lanuginosus* strains, using well-characterized, morphological, and 2-deoxy-D-glucose resistant markers (Rubinder et al., 2000). Protoplasts of a catabolite-repression-resistant strain of *Malbranchea sulfurea* and a mutant of it over-producing amylase were isolated and fused using electrofusion. The yield of hybrids was 5×10^{-5}. One stable hybrid, DGCS 1, was insensitive to glucose repression and produced approx. twice the -amylase activity produced by either of its parents[64]. To produce a new hybrid yeast strain that is able to produce ethanol directly from starch without the need for a separate saccharification process, protoplast fusion was performed, between the amylolytic yeast *Saccharomycopsis fibuligera* K-33 and *Saccharomyces cerevisiae* D-71. *S. fibuligera* K-33 produces glucoamylase and α-amylase. The intergeneric protoplast fusion frequency was 1.95×10^{-4}. Amylase activity of the fusant was 2.5× higher than that of the parental strain K-33 at 2 days [65].

Genetic recombination through transformation:

Transformation is a mode of genetic recombination in which involves the uptake of naked DNA molecules from one bacterium (the donor cell) by another bacterium (the recipient cell). Palva, 1982 isolated, gene coding for α-amylase from *Bacillus amyloliquefaciens* by direct shotgun cloning using *B. subtilis* as a host. The genome of *B. amyloliquefaciens* was partially digested with the restriction endonuclease. MboI and 2- to 5-kb fragments were isolated and joined to plasmid pUB110. Competent *B. subtilis* amylase-negative cells were transformed with the hybrid plasmids and kanamycin-resistant transformants were screened for the production of α-amylase. The amount of α-amylase activity produced by this transformed *B. subtilis* was about 2500-fold higher than that for the wild-type *B. subtilis* Marburg strain, and about 5 times higher than the activity produced by the donor *B. amyloliquefaciens* strain [66].

Amylase production

Although the details of the specific fermentation processes adopted by different manufacturers vary, there are two main methods for amylase production, submerged fermentation and solid–state fermentation. At industrial scale, most of the enzymes are manufactured by submerged fermentation (SmF) techniques. However, in the last decades, there has been an increasing trend towards the utilization of the solid state fermentation (SSF) technique to produce several enzymes from thermophilic microorganisms[67,68,69, 70,71,72,73.] Solid-state fermentation has gained renewed interest from researchers for the production of amylases in view of its several economic and engineering advantages and has been often employed to produce amylases[74,75,9,27,76,77]. Few important advantages of solid state fermentation (SSF) over the traditionally employed submerged fermentation (SmF) are higher yields in a shorter time period, better oxygen circulation, resemblance to natural habitats for filamentous fungi, high volumetric productivity, relatively higher concentration of products, less effluent generation, requirement for simple fermentation equipment etc[79,9,27], less effects in downstream processing, and low energy consumption. Production of alpha amylase via SmF system is known to cause potential problems[18]such as: presence of product in low concentration, handling and reduction and disposal of large volumes of water during downstream processing. These unit operations are cost intensive and poorly understood[80]. Use of SSF system effectively overcomes

these problems as the yield is several times higher and is cost effective. Moreover the product is recovered from the fermented products in lower volume of solvent, thereby achieving a much simpler and cheaper downstream. Bioreactor design aspects especially at the industrial level have not been given enough attention by the researchers and it is not possible to currently indicate an ideal type of bioreactor for these SSF systems (Regulapati, 2007). The production of microbial alpha amylase by bacteria is dependent on the type of strain, composition of medium, methods of cultivation, cell growth, nutrient requirement, metal ions, pH, temperature, time of incubation, thermostability and the control of contamination during fermentation[81]. High aeration rates were found to be essential for better yield of enzyme, however foaming problems resulted[82].

REFERENCES

1. Sidhu GS, Sharma P, Chakrabarti T and Gupta JK, Strain improvement for the production of a thermostable alpha amylase by *Bacillus* species. Enz. Microbial Technol, 21: 525-530, (1997).
2. Rao MB, Tanksale AM, Gathe MS, Deshpande VV, Molecular and Biotechnological aspects of microbial proteases. Microbial. Microbial. Rev, 62: 597-635, (1998).
3. M.W. Fogarty. Microbial Amylases. In: W.M. Fogarty (eds.), *Microbial Enzymes and Biotechnology*, Applied Science Publishers Ltd, London, UK, 1983, pp. 1–92.
4. Song SY. et al., Med. J, 44: 74-75, (2004).
5. Shigemura M. et al., Clin Chem Lab Med, 42(6): 677-80, (2004).
6. Abou-Sheif Ma. et al., Clin Chem Acta*.,* 346(2):161-70, (2004).
7. Lin LL, Biotechnol. Appl. Biochem, 28:61-68, 1998
8. Lin LL, Hsu WH, Chu WS, A gene encoding for α-amylase from thermophilic *Bacillus* sp., strain TS-23 and its expression in *Escherichia coli.,* J. Appl. Microbiol, 82: 325-334, (1997).
9. Pandey A, Soccol CR and Mitchell D, New developments in solid state fermentation. *Process* Biochem, 35: 1153-1169, (2000a)
10. Hill R and Needham J, Ed. The Chemistry of Life: Eight Lectures on the History of Biochemistry, Cambridge University Press, London, England, 17, (1970).
11. Crueger W and Crueger A, Ed. Industrial Microbiology, Sinauer Associates, Sunderland, MA, 189–218, (1989).
12. J. C. Hoogerheide Microbial enzymes other than fungal amylases. In: L. A. Underkofler and R. J. Hickey (eds.), *Industrial fermentations* Chemical Publishing Co., New York. 1954, Vol. II.
13. Boidin A and Effront J. Bacterial enzymes, U.S. Pat, 1,227,374 and 1,227,525, 1917.
14. Wallerstein I, Enzyme preparations from microorganisms. Commercial production and industrial application. Ind. Eng. Chem, 31: 1218-1224, (1939).
15. J. Takamine, Process of making diastatic enzyme, U.S. Patent 525,820 and 525,823, 1894.
16. Takamine J, Enzymes of *Aspergillus oryzae* and the application of its amyloclastic enzyme to the fermentation industry. Ind. Eng. Chem, 6: 824-828, (1914).
17. Park YK, Rivera BC, Alcohol production from various enzyme converted starches with or without cooking. Biotechnol. Bioeng, 24: 495-500, (1982).
18. Ramesh MV and Lonsane BK, Solid state fermentation for the production of alpha amylase by *Bacillus megaterium* 16M. Biotechnol Lett, 9: 323-328, (1987a).

19. Ramesh MV and Lonsane BK, A novel bacterial thermostable alpha amylase from bacterial bran produced under solid state fermentation. Biotechnol. Lett, 9: 505-508, (1987b)
20. Ramesh MV and Lonsane BK. Chem. Mibobwl. Technol Lebensm, 11:155-159, (1988)
21. W. M. Fogarty and Kelly, C. J. Developments in microbial extracellular enzymes. In: A. Wiseman (eds.), *Topics in Enzyme and Fermentation Biotechnology*, Ellis Horwood Ltd. Publishers, England, 1979, Vol. 3: pp. 289.
22. Cheetham PSJ, Topics in enzyme and fermentation technology. Willey, New York, Chapter 6, Vol. 4, (1980).
23. H. UpaDek and B. Kottwitz. Application of amylases in detergents In: J.H. Van Ee, O Misset and E.J. Baas (eds.), *Enzymes in detergency*. Marcel Dekker Inc, New York, 1997.
24. Wiseman A, Ed. Topics in enzyme and fermentation technology Willey, New York, Vol. 4, (1980).
25. A.M. Macleod. In: J. R. A. Pollock, (eds.), *Brewing Science*, Academic Press, London, Vol. 1, 1979, 46-232.
26. Peppler HJ, Periman D, Ed. Microbiological Technology, 2nd Edn, Academic Press, New York, 7-16, (1978).
27. Pandey A, Nigam P, Soccol VT, Singh D and Mohan R, Advances in microbial amylases. Biotechnol. Appl. Biochem, 31, 135-152, (2000b)
28. Arican B, Highly thermostable, thermophilic, alkaline, SDS and chelator resistant amylase from a thermophilic *Bacillus* sp. isolate A3-15. Bioresource Technology, 99: 3071–3076, (2008).
29. Emmanuel L, Stephan J, Bernard H and Abdel B, Thermophilic archeal amylolytic enzymes. Enzyme Microb. Technol, 26: 3-14, (2000).
30. Sarikaya E, Higassa T, Adachi M and Mikami B, Comparison of degradation abilities of α and β amylases on raw starch granules. Process. Biochem, 35: 711-715, (2000).
31. Anthea M, Hopkins J, McLaughlin CW, Johnson S, Warner MQ, LaHart D, Wright JD, Human Biology and Health, Englewood Cliffs, New Jersey, USA, 76-1, (1993).
32. Regulapati R, Malav PN and Gummadi SN, Production of thermostable alpha amylase by solid state fermentation- A review. American J. Food Technol, 2(1):1-11, (2007).
33. Udani J, Hardy M and Madsen DC, Blocking carbohydrate absorption and weight loss: a clinical trial using Phase 2 brand proprietary fractionated white bean extract. Altern Med Rev, 9 (1): 63–9, (2004).
34. Haska R and Ohta Y, Starch/Starke, 46: 480-485 (1994).
35. Bessler J, Schmitt K, Maurer DR, Schmid, Directed evolution of a bacterial amylase: Toward enhanced pH-performance and higher specific activity, Protein Sci, 12: 2141–2149, (2003).
36. Beckford LD, Kneen E and Lewis KH, Bacterial amylase production on wheat bran. Ind. Eng. Chem, 37: 692-697, (1945).
37. Abe J, Nakajima K, Nagano H, Hizukuri S and Obata K, Carbohydr. Res, 175: 85-92, (1998).
38. Bhella RS and Tossar I, Can. J. Microbiol, 31: 149-153, (1985).
39. Siedenberg D, Gerlach SR, Czwalinna A and Schugerl K J. Biotechnol, 56: 205-216, (1997).
40. Kikuchi S, Shibayama J, Yasui M, Takeuchi T, Yamamoto I and Sato M, Biosci. Biotechnol. Biochem, 1993: 57, 845-846

41. Outtrup H and Norman BE, Strach/Stake, 36: 247-251, (1984).
42. Mamo G and Gessesse A, *Biotechnol. Tech*. 11: 447-450, (1997)
43. Dey S and Agrawal SO, Characterization of thermostable alpha amylase from thermophilic *Streptomyces megasporus* strain SD 12. Ind. J. Biochem. Biophys, 36: 150-157, (1999).
44. W.M. Fogarty and C.T. Kelly. In: W.M. Forgarty and C.T. Kelly (eds.), *Microbial Enzymes and Biotechnology*, 2nd edn, Elsevier Science Publishers, London, 1990, pp. 71-132.
45. Hyun HH, Zeikus JG, Appl. Environ. Microbiol, 49: 1162-1167, (1985).
46. Reddy PRM, Swamy MV and Seenayya, G., World J. Microbiol Biotechnol, 14: 89-94, (1998).
47. Swamy MV, Sai RM and Seenayya G, Lett. Appl. Microbial, 18: 301-304, (1994).
48. Stanbury A, Whitaker and Hall SJ, Fermentation Technology' Principles of Fermentation Technology', Pergamon Press, Oxford, 1-24, (1995).
49. Prabakaran M, Thennarasu V, Mangala RA, Bharathidasan R, Chandrakala N and Mohan N, Comparative studies on the enzyme activities of wild and mutant fungal strains isolated from sugarcane field. Ind. J. Sci. Technol, 2 (11), (2009).
50. Qirang J and Zhao W, Selection and breeding of a high productivity strain of alpha amylase from multi resistant mutant of *Bacillus*. Wuxi Qinggongyo Xueguan Xuebae, 13: 21-26, (1994)
51. Haq I, Ashraf H, Qadeer MA and Iqbal J, Pearl millet, a source of alpha amylase production by *Bacillus licheniformis*. Bioresource Technology, 96: 1201-1204, (2005).
52. Bin G, Lais X, Youfang D. and Yanquan L, Screening of alpha amylase high-producing strains from *Bacillus subtilis*. J. Zhejiang, 23: 88-92, (1999).
53. Yoneda Y and Maruo B, Mutation of *Bacillus subtilis* causing hyperproduction of α-amylase and protease, and its synergistic effect. J. Bacteriol, 124: 48-54, (1975).
54. Suntornsuk W and Hang YD, Strain improvement of *Rhizopus oryzae* for production of L(+)-lactic acid and glucoamylase. Lett. Applied Microbiol, 19: 249-252, (1994)
55. Witkowska-Gwiazdowska A, Ilnickaolejniczak O, Kostka G and Czajkowska D, An *Aspergillus awamori* mutant with enhanced glucoamylase activity. Acta Biotechnol, 1: 235-239, 1991
56. Wei S. and Wu XB, Advance in microbial ribosome engineering. 49(8): 981-986, (2009).
57. Kurosawa K, Hosaka T, Tamehiro N, Inaoka T and Ochi K, Improvement of α-Amylase Production by Modulation of Ribosomal Component Protein S12 in *Bacillus subtilis* 168. *Appl Environ Microbiol*. 72(1): 71–77, (2006).
58. Peak MJ, and Peak JG, Photochem. Photobiol, 50: 379-383, (1989).
59. Mitchell DL, Haipek CA and Clarkson JM, Mutat. Res, 143: 109-112, (1985)
60. Basu S and Chakrabarty SL, Variability in amylase production among morphological mutants of *Aspergillus wentii*. Folia Microbiologica, 19: 199-202, (1974).
61. Kang SW, EH Ko, Lee JS and Kim SW, Overproduction of beta-glucosidase by *Aspergillus* higher mutant from lignocelluloses biomass. Biotechnol. Lett, 21: 647-650, (1999).
62. Suntornsuk W and Hang YD, Strain improvement of *Rhizopus oryzae* for production of l(+)-lactic acid and glucoamylase. Lett. Appl. Microbiol, 19: 249–252, (2008).

63. D. A. Hopwood. Genetics of Industrial Micro-organisms. In: 0. K. Sebec and A.J. Laskin (eds.), *American Society of Microbiology*, Washington, DC, 1979, pp 1.
64. Gupta AK and Gautam SP, Improved production of extracellular α-amylase, by the thermophilic fungus *Malbranchea sulfurea*, following protoplast fusion. World. J. Microbiol. and Biotechnol, 11(2): 193-195, (1995).
65. Choi SH, C Sung, Oh MJ and CJ Kim, Intergeneric protoplast fusion in *Saccharomycopsis fibuligera* and *Saccharomyces cerevisiae*. J. Fermentation and Bioeng, 84 (2): 158-161, (1997).
66. Palvaa I, Molecular cloning of α-amylase gene from *Bacillus amyloliquefaciens* and its expression in *B. subtilis* Gene Volume, 19 (1): 81-87, (1982).
67. Busch JE and Stutzenberger FJ, Amyloytic activity of Thermomonospora fusca. World J. Microbiol. Biotechnol, 13(6): 637-642, (1997).
68. Haseltine C, Rolfsmeier M and Blum P, The glucose effect and regulation of α-amylase synthesis in the hyperthermophilic archaeon *Sulfataricus solfataricus*. J. Bacteriol, 178, 945-950, (1996).
69. Fadel-M, Production of Thermostable amylolytic enzyme by *Aspergillus niger* F-909 under solid state fermentation. Egyptian J. Microbial, 35: 487-505, (2000).
70. Lonsane BK and Ramesh MV, Production of bacterial thermostable α-amylase by solid state fermentation: a potential tool for achieving economy in enzyme production and starch hydrolysis. Adv. Appl. Microbial, 35: 1-56, (1990).
71. Sodhi HK, Sharma K, Gupta JK and Soni SK, Production of a thermostable α-amylase from *Bacillus* sp. PS-7 by solid-state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production. Process Biochem, 40(2): 525-534, (2005).
72. Sadhukhan RK, Manns S, Roy SK and Chakraborty SL, Thermostable amyloytic amylase enzyme from a celluolytic fungus *Myceliophthora thermophilla* D14 ATCC 48104. Appl. Microbioal. Biotechnol., 1990, 33: 692-696.
73. Robinson T, Mchnlan G, Marchant R and Nigam P, Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Biosensor. Technol, 77: 247-255, (2001).
74. Carrizales V and Jaffe W, Solid State fermentation an appropriate biotechnology for developing countries. Interscience, 11: 9-15, (1986).
75. A. Pandey. In: V. S. Malik and P. Sridhar (eds.), *Industrial Biotechnology,* IBH and Oxford Publishing Co., New Delhi, 1992, 525-537.
76. Pandey A, Selvakumar P, Soccol CR and Nigam P, Curr. Sci, 77: 149-162, (1999)
77. Pandey A, Soccol CR, Nigam P, Brand D, Mohan R and Roussoss S. Biotechnology potential of agro-industrial residues, Part II. Cassava bagasse. *Biores. Technol*., 74: 81-87, (2000c).
78. Pandey A, Soccol CR, Rodriguez-Leon J and Nigam P, Ed. Solid state fermentation in Biotechnology, Asia Tech Publishers, Delhi, 1-221, (2001).
79. Nigam P and Singh D, Enzymes and microbial system involved in starch processing enzyme. Enz. Microbial Technol, 17: 770-778. (1995)
80. Datar R, Economics of primary separation steps in relation to fermentation and genetic engineering. Process Biochem, 21: 19-26, (1986).
81. Prescott S and Dunn's A, Ed. Industrial Microbiology, 4th Ed, CBS Publishers and

Distributors, New Delhi, India, 550-565, (1987).

82. Philip JB, Ronald EH and Martin WT, Purification and properties of a raw commercial thermostable *Bacillus stearothermosphilus* alpha-amylase. Food Biotechnol, 2: 67-80, (1988).