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Review

Biotechnological production of α-amylases for industrial purposes: Do fungi have potential to produce α-amylases?

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Enzymes are substances produced by a living organism which acts as a catalyst to bring about a specific biochemical reaction. Amylases are a class of hydrolytic enzymes, widely spread in nature having varied application in different industrial processes and constitute a class of industrial enzymes. Fungal amylases have been widely used for the preparation of oriental foods. In spite of the wide distribution of amylases in nature, fungal amylases are used for industrial production due to advantages such as cost effectiveness, consistency, less time and space required for production and generally regarded as safe (GRAS). Due to the increasing demand for these enzymes in various industries, there is enormous interest in developing enzymes with better properties such as raw starch degrading amylases suitable for industrial applications. *Penicillium* and *Aspergillus* produces a large variety of extracellular enzymes, of which amylases and proteases are of significant industrial importance and serve in the production of a number of biotechnologically produced enzymes.

Key words: α-Amylase, *Penicillium, Aspergillus,* enzyme, hydrolytic.

INTRODUCTION

Microorganisms are the most important sources for enzyme production. Selection of the right organism plays a key role in high yield of desirable hydrolytic enzymes especially amylases. Recent discoveries on the use of microorganisms as sources of industrially relevant amylase enzymes have led to an increased interest in the application of microbial enzymes in various industrial processes. Amylases are the hydrolytic enzymes, widely spread in nature having varied application in different industrial processes and constitute a class of industrial enzymes and representing approximately 25-33% of the world enzyme market (Nguyen et al., 2002; Van der

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Table 1. Uses of amylases in various sectors of industry.

Sector	Use
Food industry	Production of glucose syrups, crystalline glucose
	Production of high fructose corn syrups
	Production of maltose syrups
	Reduction of viscosity of sugar syrups
	Reduction of haze formation in juices
	Solubilization and saccharification of starch for alcohol fermentation in brewing industries
	Retardation of staling in baking industry
Detergent industry	Used as an additive to remove starch based dirts
Paper industry	Reduction of viscosity of starch for appropriate coating of paper
Textile industry	Warp desizing of textile fibers
Pharmaceutical industry	Used as a digestive aid

Marrel et al., 2002); they can be obtained in bulk from different species of fungi. Due to the increasing demand for these enzymes in various industries, there is enormous interest in developing enzymes with better properties such as raw starch degrading amylases suitable for industrial applications and their cost effective production techniques (Burhan et al., 2003). Amylases from plant and microbial sources have been employed for centuries as food additives. Fungal amylases have been widely used for the preparation of oriental foods. In spite of the wide distribution of amylases, microbial sources, mainly fungal amylases, are used for the industrial production due to advantages such as cost effectiveness, consistency, less time and space required for production and ease of process modification and optimization (Burhan et al., 2003). The *Aspergillus* species produces a large variety of extracellular enzymes, of which amylases and proteases are of significant industrial importance (Pandey et al., 2000).

Some fungi of genus viz., *Penicillium* and *Aspergillus* serve in the production of a number of biotechnologically produced enzymes and other macromolecules, such as gluconic, citric, and tartaric acids, as well as several pectinases, lipase, amylases, cellulases and proteases (Akpan et al., 1999). Amylases are important enzymes employed in the starch processing industries for hydrolysis of starch into simple sugars (Alva et al., 2007). Amylases are widely distributed in plants, animals and microorganisms which show varying action patterns depending on the source (Pandey et al., 2000, Saboury, 2002; Morales et al., 2007).

However, amylases from fungal sources (especially *Aspergillus* spp.), have gained much attention because of the availability and high productivity of fungi, which are also amenable to genetic manipulation. The fungal amylases are preferred over other microbial sources because of their more acceptable generally regarded as safe (GRAS) status, the hyphal mode of growth, and good tolerance to low water activity and high osmotic pressure conditions make fungi most efficient for bioconversion of solid substrates (Raimbault, 1998) and thus attracting increasing attention as source of amylolytic enzymes suitable for industrial applications (Mishra and Maheshwari, 1996; Hernandez et al., 2006; Kathiresan and Manivannan, 2006). The few uses of amylases are depicted in Table 1.

This review covers the progress made in research on fungal α-amylase, a highly demanded industrial enzyme in various sectors as depicted in Table 1. The article reviews the fungal sources of α-amylases, production aspects, industrial applications and some recent research developments in the field of microbiology and biotechnology.

FUNGAL SOURCES OF α-AMYLASES

Production of α-amylases from *Aspergillus* species

The mycelial growth and amylase production by a mycotoxigenic strain *Aspergillus flavus* was evaluated in culture medium containing starch, glycerol, wheat bran or corn by Figueira and Hirooka (2000) and reported that the medium composed of milky stage corn supernatant promoted the best mycelial growth and amylase production whereas the isolation, screening, selection and mutation of *Aspergillus oryzae* for α-amylase production showed that mutant strains demonstrated 2.6 fold increased activity over the parental strain in terms of enzyme production (Abdullah, 2005).

Xu et al. (2008), while working on optimisation of nutrient levels for the production of *α*-amylase by *A. oryzae* in solid state fermentation (SSF) with spent brewing grains (SBG), using response surface methodology (RSM) based on Plackett-Burman design (PBD) and Box-Behnken design (BBD) found that corn steep liquor (1.8%), $CaCl_2$ (0.22%) and $MgSO_4.7H_2O$ (0.2%) are the most compatible supplements to the substrate SBG to influence α-amylase activity positively. Some fungal strains of each of the two filamentous fungi viz*., A. niger*

and *A. flavus* were analysed for their α-amylase activity (Shafique et al., 2009) and reported that all the test strains exhibited their maximum α-amylase activity after 48 h of incubation. The wastes from foods and drinks industries were studied by Sidkey et al. (2010) focusing on the possibility of using different fermented enviro-agro-industrial wastes as very cheap and available substrates for obtaining microbial α-amylases that are of great industrial importance and isolated seventy three fungi and bacteria from twenty different wastes, e.g. food industrial wastes, daily home wastes, expired food stuff wastes and some agricultural wastes from Al-Madinah, Al-Munawwarah, K.S.A.

However, Khokhar et al. (2011) reported that filamentous fungi are important due to their high enzyme production potential. Fifteen fungal isolates of three genera, *Aspergilus*, *Penicillium* and *Trichoderma* were examined for their ability to produce amylases. It was found that all isolates exhibited enzymatic potential and reported that *Penicillium*, *Aspergillus raperi* and *Aspergillus speluneus* were hyper active in starch medium and showed the increased growth in starch medium as compared to control. During his study on the fungal strain of *A. oryzae* used for the production of α-amylase by solid state fermentation from agro-industrial wastes, Ahmed (2011) reported that enzyme production was growth associated and maximum activity (8.23 U/ml) was obtained after 120 h when incubated at 30°C on wheat bran with initial moisture content of 60% and initial medium of pH 5. It was also found that enzyme activity increased when the solid medium was supplemented with additional nitrogen source.

The *Aspergillus* strains obtained by Kumar and Duhan (2011), were screened for their ability to produce amylase on starch agar plates, among the five strains, *A. niger*, showed highest clearing zone on starch agar plates as well as amylase activity in solid state fermentation. Different substrates like wheat bran, rice bran, soybean meal and black gram bran were screened for enzyme production and rice bran was found to be the best substrate for the enzyme production.

Whereas, the purification and characterization of α-amylase from *A. flavus*, showed that the activity of the purified α-amylase increased with increasing enzyme concentration and incubation time and the enzyme exhibited maximum activity at 30°C and pH 6.4 with the optimum starch concentration of 15 mg/ml (Sidkey et al., 2011). The amylase production by *A. niger* under solid state fermentation using agro industrial wastes was studied by Suganthi et al. (20110 who reported that *A. niger* showed the highest production of amylase. They also reported that sucrose and nitrogen improved the yield in the same medium. *A. flavus* was investigated for the production of amylase (Ileasanmi et al., 2012), implicated in the bio-deterioration of starch-based fermented foods and showed that 30°C incubation temperature was optimum for amylase production by this isolate. It was further revealed that an incubation period of six days was optimum for amylase production by this isolate. However, when *A. niger* was grown in a medium with rice as carbon and growth source and in a defined synthetic medium with varying carbon and nitrogen sources at 25°C producing amylase (Adejuwon, 2012), it was reported that optimum amylase activity in rice was expressed on the eighth day of incubation as 0.58 units and in the synthetic growth medium with starch as carbon source and tryptone as nitrogen source, optimum amylase activity was expressed on the seventh day as 0.47 units. Similarly, *A. niger* was investigated utilizing *Ipomoea batatas,* it has been reported that submerged fermentation holds tremendous potentiality in high biomass yield of alpha-amylase (Sundar et al., 2012). The effect of varying pH, temperature and nitrogen sources of the medium on the productivity of α-amylase was also studied and it was reported that the maximum activity of α-amylase was recorded as 450 U/mg after 7 days of submerged fermentation at pH 7.0 and room temperature of 28°C.

A. flavus was studied by Bhardwaj et al. (2012) who reported that the highest yield of amylase production was obtained by the addition of magnesium sulphate (0.1%) and calcium chloride (0.02%), respectively. It was further reported that supplementation of the enzyme production medium with non-ionic surfactants in general and Tween 80 in particular resulted in an enhanced secretion of the starch hydrolyzing proteins in the medium. The extracted amylase enzyme was purified by diethyl amino ethyl (DEAE) cellulose and Sephadex G-50 column chromatography and the enzyme activity was measured by using synthetic substrate starch.

The partially purified enzyme exhibits maximum activity at the optimum pH (7.0), temperature (60 to 70°C) and substrate concentration (1.5 to 2.0%) under standard assay conditions. Among the four different *Aspergillus* species examined, *Aspergillus flavipes* showed maximum production of amylase (Doss and Anand, 2012). The amylolytic enzymes produced by *A. flavus* isolated from mouldy bread with the aim of establishing some factors that affect its activity shows that *A. flavus* grows in synthetic medium containing starch as the sole carbon source and synthesize enzymes which exhibited amylolytic activities.

The production of the enzyme increases with increase in days of incubation with optimum activity occurring on the tenth day of incubation (Ayansina and Owoseni, 2010). Very recently, Alhussaini (2013), worked on the mycobiota of wheat flour to isolate and identify the fungal species, which contaminated the stored flour. The study revealed that the *Aspergillus* genus was the most active producer of α-amylase. Adejuwon and Ladokun (2013) worked on the effect of carbon source of growth medium on α-amylase production by *Aspergillus rubrum* isolated from yam (*Dioscorea alata*) using potato dextrose agar, rice (*Oryza sativa*) supported fungal growth and α-amylase

production. It was also found that least activity was expressed by *A. rubrum* when galactose was carbon source.

Production of α-amylases from *Penicillium* species

The filamentous fungi have been widely used for the production of amylases under solid state fermentation, wherein certain cultural parameters may provide good growth of microorganisms and thereby better enzyme production. Amylase enzyme extracted from fungi finds potential application in a number of industrial processes such as bread making, brewing, starch processing, pharmacy, textile and paper industries. Amylases have almost completely replaced chemical hydrolysis of starch in starch processing industry (Pandey et al., 2000) and constitute a class of industrial enzymes representing approximately 25-33% of the world enzyme bank (Nguyen et al., 2002; Van der Marrel et al., 2002). While as, Balkan and Ertan (2005) studied the fungi and screened their ability to produce α-amylase, *Pencillium chrysogenum* showed high enzymatic activity and α-amylase production by *P. chrysogenum* cultivated in liquid media containing maltose (2%) reached its maximum in 6-8 days at 30°C.

However, Kathiresan and Manivannan (2006) studied the effects of pH, temperature, incubation time, salinity, sources of carbon and nitrogen on submerged fermentation process in production of α-amylase by *Pencillium fellutanum* isolated from coastal mangrove soil and reported that the production medium without addition of sea water and with provision of maltose as carbon source, peptone as nitrogen source, incubated for 96 h maintained with pH of 6.5 at 30°C, was optimal for production of α-amylase. Another study was carried out on *Pencillium rugulosum* isolated from a soil sample (Tiwari et al., 2007), for production of α-amylase which showed that the maximum production of amylase by *P. rugulosum* was observed at 3rd day of incubation with an improvement in its production in the presence of galactose as sole carbon source.

However, solid state fermentation using banana peel as a substrate (Vijayaraghavan et al., 2011) for the production of amylase by *Penicillium* sp. and partially purified enzyme by the combination of ammonium sulphate precipitation, Sephadex G-75 gel filtration chromatography and dialysis, showed that the enzyme had optimum activity at a pH of 7.0 and incubation temperature 50°C. The *Penicillium* species isolated from decaying apple fruit (Adejuwon, 2011) grown in a synthetic medium containing starch as sole carbon source showed that culture filtrates exhibited amylase activity, and that the presence of cations Mg^{++}, Ca^{++}, K^{+} and Na^{+} stimulated the activity of the enzyme. It was further observed that the enzyme activity was inhibited in the presence of EDTA and was enhanced in the presence of metal ion Mn^{2+} and Fe^{2+}. The extracellular amylase production was studied by Metin et al. (2010) on *Penicillium citrinum,* and reported that amylase exhibited broad substrate specificity because it acted on all the substrates tested and showed that enzyme was activated by Mn^{2+}, Ca^{2+}, Co^{2+}, Fe^{3+}, Ba^{2+}, NH_4^{+} and Al^{3+}. The other ions and EDTA had no effect on its activity. It was further observed that enzyme activity was inhibited in the presence of phenyl methane sulfonyl fluoride (PMSF), N-bromo succinimide (NBS) and 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide methyl *p* toluene sulphonate (CMC), suggesting that serine, tryptophan residues and carboxyl groups play an important role in the catalytic process.

The loquat kernel flour (LKF) could serve as a sole source of nitrogen and carbon for the fungus to grow and synthesize α-amylase (Erdal and Taskin, 2010) as the feasibility of waste loquat kernels as substrate in solid state fermentation for α-amylase production by *Penicillium expansum* has been evaluated. The *Penicillium* strains from the Howzsoltan lake were studied by Abbas et al. (2011), to produce α-amylase and it was reported that some filamentous fungi can survive and grow in high concentration of salt; they analyzed 100 water samples and isolated 65 samples as 9 species of *Penicillium* and showed that solid state fermentation (SSF) medium could increase the α-amylase activity to tenfold, in comparison with subaro broth as submerged fermentation (SmF).

However, purification and characterization of α-amylase from *Penicillium janthinellum* and its application in detergent industry was studied (Sindhu et al., 2011) and it was concluded that after 96 h of incubation using wheat bran as substrate for SSF, amylase was purified. The culture and nutrient requirements of *Penicillum crysogenum* for production of α-amylase in production media containing different pH, temperature, incubation period, inoculum size, carbon sources, nitrogen sources and metal ions were analyzed under submerged fermentation (Vidya et al., 2012).

It was found that the optimum pH, temperature, inoculum size and incubation period for enzyme production were 6, 50°C, 4% and 6th day of incubation. It was also found that minimal medium can be used under submerged fermentation for the maximum production of amylase under controlled conditions. Adejuwon and Ladokun (2013), worked on the effect of carbon source of growth medium on α-amylase production by strains of *Penicillium solitum* isolated from yam (*D. alata*) using potato dextrose agar, rice (*O. sativa*) supported fungal growth and α-amylase production.

CONCLUSION

Amylases extracted from fungi have potential applications in a number of industrial processes and constitute a class of industrial enzymes representing approximately 25-33%

of the world enzyme bank. Demand and selection of the right organism plays a key role in high yield of desirable amylase enzyme. A large number of amylase enzymes are available commercially which are very costly, but microbial amylases have successfully replaced chemical hydrolysis of starch in starch processing industries which ultimately will save our billions of dollars and will meet the rising industrial demands. Although amylases can be obtained from several sources such as plants and animals, the enzymes extracted from fungal sources are GRAS.

Conflict of Interests

The author(s) declare there is no conflict of interests.

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