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Microbial amylases: A review

M. F. Martin[a], **E. A. Okpo**[b] and **I. E. Andy**[c]

Department of Microbiology, Faculty of Biological Sciences, University of Calabar, P.M.B. 1115, Calabar, Cross River State, Nigeria

[a-c]E-mail address: mafrimart2k8@yahoo.com , ekomarchims20@gmail.com , iniobongandy@gmail.com

ABSTRACT

Microbial amylases are enzymes produced by microorganisms to hydrolyze starch. There are three types of microbial amylases: alpha-amylase, beta-amylase and glucoamylase. Each of these amylases has a unique way of acting on starch to yield simple glucose monomers. Microorganisms, plants, and animals are sources of amylases, but much attention is given to microorganisms since the amylases produced by them have greater thermal stability and give rise to different sugar profiles, thus meeting industrial demands. Two major groups of microorganisms play pivotal role in amylase production: bacteria and fungi. Starch is the substrate used in amylase production. Between the two fermentation processes used in amylase production (i.e. submerged fermentation and solid state fermentation), the latter is more advantageous as it saves cost, generates little effluent, and has high volumetric productivity. Microbial amylases are greatly applied in pharmaceutical, food, chemical, paper and distilling industries.

Keywords**:** microbial amylases, substrate, fermentation, microbial

1. INTRODUCTION

Amylases are starch hydrolyzing enzymes (Reddy *et al*., 2003). Thus, Microbial amylases are enzymes produced by microorganisms to hydrolyze starch. They were first produced in 1894 by the Japanese scientist Jokichi Takamine at Peoria, Illinois (USA) from a fungal source and was used as a pharmaceutical aid for the treatment of digestive disorders (Rao *et al*., 2007). There are three types of amylases, namely: alpha amylase (endo-1,4-α-D glucohydrolase), beta amylase (β-1,4-glucan maltohydrolase), and glucoamylase (amyloglucosidase) (Rao *et al*.,

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2007). Each of the three types of amylases has a unique way of acting on starch as it serves as a substrate for their action.

Although amylases can be produced by plants, animals, and microorganisms, microbial amylases have been given much priority to because of their greater thermal stability, different sugar profiles, and a long history of safe use (Rao *et al*., 2007).

Different manufacturers have varying ways of producing amylases. However, there remain two main methods for amylase production, namely: Solid State Fermentation (SSF) and Submerged Fermentation (SmF). Nonetheless, SSF has numerous advantages over SmF (Pandey, 1994; Nigam and Singh, 1994). As would be discussed later, microbial amylases have wide range of applications due to new inventions in biotechnology (Zhang, 2017; Windish, 1965; Gey, 2016; Bhella 1985; Doyle, 1988; Chakravarthi, 2003; Gopaldas, 1986).

2. SOURCES OF MICROBIAL AMYLASES

Microbial amylases can be produced by bacteria and fungi (which include molds and yeast). The table below enlists some of the microbes falling under the above stated three categories (Pandey *et al*., 1999; Rao *et al*., 2007).

BACTERIA	**FUNGI**	
	MOLDS	**YEAST**
Bacillus subtilis	*Aspergillus oryzae*	*Saccharomycopsis capsularia*
Bacillus licheniformis	*Aspergillus kawachii*	*Amylomyces rouxii*
Bacillus megaterium	*Aspergillus niger*	
Clostridium thermosulfurogenes	*Aspergillus awamori*	
Aeromonas caviae	*Rhizopus oligosporus*	
Pseudomonas sp *(aerobic)*	*Rhizopus japonicas*	

Amongst the bacteria genera, the genus Bacillus (especially *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus amyloliquefaciens*) are greatly used for amylase production. However, amongst the fungi, *Aspergillus sp* and *Rhizopus sp* are the preferred strains for amylase production (Antai, 2005; Pandey *et al*., 1994).

3. MODE OF ACTION OF MICROBIAL AMYLASES ON ITS SUBSTRATE

As noted earlier, a substrate is a “reacting molecule” that is acted upon by its specific enzyme to yield a product or products. Thus, starch is the substrate for amylase production and the mechanism of action of the enzyme on its substrate is referred to as "lock-and-key model" (Prescott *et al*., 2005). Amylases are starch hydrolyzing enzymes (Reddy *et al*., 2003). There are three types of amylases, namely: alpha amylase (endo-1,4-α-D-glucohydrolase), beta amylase (β-1,4-glucan maltohydrolase), and glucoamylase (amyloglucosidase) (Rao *et al*., 2007).

Each of the three types of amylases has a unique way of acting on starch. For instance, alpha amylase (being an endoamylase) cleaves or breaks the α-1,4-glucosidic linkages in starch internally to give glucose, maltose, or dextrins (Rao *et al*., 2007). Beta amylase, as an exoamylase, cleaves the glycolytic bonds removing two glucose units at a time thus producing maltose (Abe *et al*., 1988; Gupta *et al*., 2003). Glucoamylase, on the other hand, cleaves both the α-1,4 and α-1,6-glucosidic linkages to yield glucose, maltose, and limit dextrins (Rao *et al*., 2007). The illustration below shows how amylases work.

4. PRODUCTION, EXTRACTION / PURIFICATION, AND APPLICATION OF MICROBIAL AMYLASE

4. 1. Production of Microbial Amylase

The substrate used for the production of microbial amylase is starch. A substrate is a 'reacting molecule' on which a specific enzyme acts on to yield a product even products (Prescott *et al*., 2005). There are two main methods of amylase production that utilizes the substrate (starch), namely: Solid State Fermentation (SSF) and Submerged Fermentation (SmF) (Rao *et al*., 2007).

SSF is a technique for growing microorganisms such as fungi and bacteria on a moist solid substrate. Due to modern invention, the processes of SSF can be easily done using a bioreactor (Suryanarayan, 2001).

The processes include:

- Filling the cleaned modules of the bioreactor with any fermentation medium (like corn, soybeans etc.) alongside with minerals like vermiculites or any supporting materials capable of absorbing aqueous solutions.
- Assembling the modules to form a "stack" operated in tandem thus isolating the interior from the exterior of the bioreactor.
- Introduction of sterilization fluid (steam or ethylene oxide) into the stack through the communicating channels to sterilize the matrix at 121^{o}C for steam or at 50^{o}C for ethylene oxide.
- Cooling of the sterilized matrix using cooling water and sterile cool air.
- Introduction of appropriate amount of inoculum (either bacteria or fungi) into the stack and adjustment of moisture content.
- Stirring of the modules using a mixer to ensure uniform distribution of the inoculum. Stirring stops prior to fermentation.
- Monitoring of the internal environment of the bioreactor using a probe during the growth period.
- Regulating the heat generated during fermentation either by evaporating cooling or by conduction. Since conduction does not result in dehydration of the fermenting medium, then it is the best method of heat regulation.
- Extraction and purification of the amylase produced.

In SmF process, microbial growth occurs inside a liquid or broth medium unlike SSF where the growth occurs at or near the surface of the solid substrate (Pandey, 1994). SmF processes include:

- Inoculation of a pure culture (from petri dish, culture tube, or slant tube) into a small flask of nutrient broth.
- Agitation of the flask's content using a temperature controlled flask shaker.
- Pipetting of a small amount of the culture in the flask (during the exponential growth phase or log phase) into the next flask.
- Acclimatization of the culture through repeated sub-culturing before studying the fermentation kinetics.

Recently, SmF has been relegated to the background by scientist whilst SSF has the upper hand due to its advantages, namely:

i. High volumetric productivity is obtained.
ii. Relatively higher concentration of the enzyme is produced.
iii. Less effluent is generated.
iv. Low cost of production is required.
v. It is easy to use.
vi. Raw starch is efficiently digested (Hata *et al*., 1997).

4. 2. Extraction and Purification of Microbial Amylases

After amylase is produced, it is extracted and purified using the following four major processes as highlighted by Rao *et al*, (2007):

- Centrifugation.
- Selective concentration of the supernatant.
- Selective precipitation of the enzyme.
- Chromatographing of the crude enzyme.

Centrifugation is the process of using centrifugal force field to selectively separate and settle particles suspended in a liquid medium (Ebong *et al*., 2005).

Selective concentration of the supernatant is best achieved using ultra filtration which is a process of separating a mixture of particles into respective particle components using membrane filters (Ebong, 2003).

Selective precipitation of the enzyme involves the precipitation of enzyme on the basis of its solubility (or extent in which it can dissolve in a solvent).

Chromatographing of the crude enzyme involves separating the enzyme in a solution due to its migration rate along a stationary phase (Ebong *et al*., 2005).

4. 3. Application of Microbial Amylases

Microbial amylases (especially thermostable alpha amylase) have various industrial applications because of their greater thermal stability, different sugar profiles, and a long history of safe use (*Rao et al*., 2007). They can be exploited for high fructose corn preparation, for the production of alcohol and brewing, for paper coating, for the preparation of detergents amongst all others (Abe *et al*., 1988; Bailey and Ollis, 1986). Industries requiring microbial amylases include: clinical, medicinal, analytical chemistry, textile, food, and distilling industries (Rao *et al*., 2007; Malleshi, 1988; Mensah, 1990).

5. CONCLUSION

Amylases have played a pivotal role in both biotechnological and pharmaceutical industries. Thermophilic bacteria and fungi are efficient producers of thermostable amylases. For an efficient large scale production of amylases, the structural and functional relationships of these enzymes have to be known in detail. Moreover, understanding these relationships will lead to improving the stability of the existing enzymes as well as the discovering of many new ones.

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