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Microbes: Potent Source of Amylolytic Enzymes

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Amylases are amylolytic enzymes (Reddy et al., 2003). Thus, amylase produced by microbes plays important role in starch hydrolysis. Jokichi Takamine at Peoria, Illinois (USA) first observed the production in 1894 from a fungal source and was used as a medicinal aid for the treatment of digestive malfunctions (Rao et al., 2007). Three major 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). All these amylase shows different mode of actions on substrate which is supplied for hydrolysis. Amylases are generally produced by various organisms such as plants, animals, and microorganisms. Microbial amylases are highly diverse in nature as in terms of abiotic factors as temperature and sugar variability and safe use (Rao et al., 2007). Solid State Fermentation and Submerged Fermentation are the two main methods of amylase production. However, Solid State Fermentation has several advantages over Submerged Fermentation (Pandey, 1994). In the last two decades as the emergence of industrial biotechnology microbial amylase have their diverse range of applications (Zhang, 2017; Dey, 2016; Chakravarthi, 2003).

Microbial sources of amylolytic enzymes
Bacterial sources

Bacterial production of amylases is cheaper and faster than other producing microorganisms. It is very easy to modify the genetic constituents of bacteria to get the desirable recombinant enzymes (Gupta et al 2003, De Souza and Magalhaes 2010, Mojsav 2012, Hussain et al 2013, Sundarram and Muethy 2014). Many reports reveal that wide range of bacterial species is used for the production of amylase. Most of these are Bacillus species (B. subtilis, B. stearothermophilus, B. amyloliquefaciens, B. licheniformis, B. coagulans, B. polymyxa, B. mesentericus, B. vulgaris, B. megaterium, B. cereus, B. halodurans, and Bacillus sp. Ferdowsicous). Some halophytic strains are also reported for the production of amylases Haloarcula hispanica, Halobacillus sp., Chromohalobacter sp., Bacillus dipsosauri, and Halomonas meridiana (Kathiresan and Manivannan 2006).

Fungal sources

Aspergillus and Penicillium are the main genus of fungus to produce the amylase enzyme. Since fungus has penetrate on hard substrate and facilitate the hydrolysis process. Fungal amylase has advantage of being secreted extracellularly. Thus, fungal species are highly suitable for solid-based fermentation. Efficient amylase producing species are Aspergillus (A. oryzae, A. niger, A. awamori, A. fumigatus, A. kawachii, and A. flavus), as well as Penicillium species (P. brunneum, P. fellutanum, P. expansum, P. chrysogenum, P. roqueforti, P. janthinellum, P. camemberti, and P. olsonii), Streptomyces rimosus, Thermomyces lanuginosus, Pycnoporus sanguineus, Cryptococcus flavus, Thermomonospora curvata, and Mucor sp. (De Souza and Magalhaes 2010, Mojsav 2012, Hussain et al 2013, Sundarram and Muethy 2014 (De Souza and Magalhaes 2010, Mojsav 2012, Hussain et al 2013, Sundarram and Muethy 2014).

Genetically Modified Organisms (GMO) as source of Amylase

Recombinant DNA technology is the molecular techniques used to enhance enzyme production (Nielsen and Borchert 2000, Corbin et al 2016, Jung et al 2016, Son et al 2016). It involves the selection of suitable elite gene, gene insertion into an appropriate vector, transformation in bacterial system to produce high quantity of recombinant protein and purification of the protein for downstream process.

Zhang et al. (2016) deleted amyR gene (encoding a transcription factor) from A. niger CICC2462, which results to the production of enzyme/protein specifically with lower background protein secretion. Wang et al. (2016) generated a new strategy to express the α-amylase from Pyrococcus furiosus in B. amyloliquefaciens. This extracellular thermostable enzyme is produced in low amount in P. furiosus, but its expression in B. amyloliquefaciens was significantly increased and had good stability at higher temperature (optimum 100°C) and lower pH (optimum pH 5). By mimicking the P. furiosus system, they obtained a novel amylase with yields approximately 3000- and 14-fold higher amylase units/ml than that produced in B. subtilis and Escherichia coli, respectively.

Industrial Applications of Microbial Amylase

Amylase industry makes up approximately 25% of the total world enzyme industries (Mojsav 2012). It is widely used in foods, detergents, pharmaceuticals, and paper and textile industries (Hussain et al 2013 , De Souza and Magalhaes 2010). Its applications in the food industry involves in the production of various syrups (corn, maltose, glucose), juices and alcohol by fermentation and baking (Mojsav 2012). It is also used as a food additive and in detergents preparations. Amylase plays an important role in beer and liquor productions brewing from sugars (starch based). Fermentation process includes ingestion of sugars by yeast, and production of alcohol. It is suitable and efficient method for production of microbial amylase enzyme under moisture and optimum growth conditions. In traditional beer production malted barley is mashed and hydrolyzed starch into sugars by amylase at an optimum temperature.

The potentiality and industrial applications of enzymes are determined by the ability easy, low cast, with wide range of activity. As stated above, different methods have been established for enzyme production. Since the crude enzyme extract works efficiently in general but for specific application such as pharmaceuticals, molecular applications, purification of the enzyme is required. This is achieved by various downstream processes like precipitation, dialysis, chromatographic techniques immunoprecipitation, polyethylene glycol/Sepharose gel separation, and aqueous two-phase and gradient systems (Gopinath et al 2013), With these developments, microbial amylase production has successfully replaced its production by chemical processes, especially in industries (Sanaraj and Stella 2013). For further improvement in the industrial process, the above-mentioned DOE and encapsulation methods can be implemented.

Future Perspectives

Amylase possesses efficient potential for use in different industrial and medicinal applications. The integration with modern technologies, like white, pink and green biotechnology, will enhance its industrial production on a large scale. This will be further facilitated by implementation of established fermentation technologies with appropriate microbial species (bacteria or fungi) and interventation of other biotechnological aspects. Enhancing the amylase production for industrial and medicinal applications can be achieved by the technologies of high-throughput screening and processing with efficient microbial species, along with the ultimate coupling of genetic engineering of amylase-producing strains.

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