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Review paper

AMYLASES FROM YEASTS: AN UPDATE

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ABSTRACT
The use of microorganisms for starch degradation in various processes has not only eliminated the need to use various chemicals, but has also reduced the expenditure on these processes. In modern biotechnology, yeasts have gained sufficient importance due to several reasons; the most important being their harmless status for human consumption. The need to search for some good amylolytic yeast species, is therefore, the need of time, since such strains may not only be used for the bulk production of commercial products like bioethanol, but also to obtain amylases in larger amounts, which may then be used wherever required. The present review focuses on the various amylolytic yeast species and their potential industrial applications along with a brief discussion about amylases.

KEYWORDS: Amylases, amylolytic yeasts, starch, recombinant yeast.

INTRODUCTION

Yeasts, eukaryotic microorganisms classified under the kingdom Fungi, can be defined as those fungi that reproduce vegetatively by budding or fission, and their sexual states are not enclosed in a fruiting body (Kurtzman and Fell, 1998; Walker, 1998). Yeasts are placed in two main phyla, Ascomycota and Basidiomycota, depending upon their mode of sexual reproduction, i.e., budding or fission, respectively (Kurtzman and Fell, 1998). In general, the term yeast can be used to refer either true yeast, or the unicellular phase of dimorphic fungi (Pommerville, 2011; Waites *et al*., 2001).

Yeasts are found in natural environments, e.g., in soil, marine environment, fresh waters, or are associated with plants, animals and human beings (Rosa and Peter, 2006). However they are not as common as bacteria or fungi. Despite of this fact, yeasts remained a subject of research for many decades as they are associated with biotechnology since ancient times. For instance, beer was produced in Sumeria in 7000 B.C., wine production was prevalent in Assyria in 3500 B.C. and yeasts were used for bread making in Rome in 100 B.C. (Kurtzman and Fell, 1998). In modern biotechnology, though yeasts are not as widely used as bacteria are, they are being studied to discover their hidden potentials; some of the species are now being exploited commercially, e.g., *Saccharomyces cerevisiae*. They can either be used in their native forms, or may be improved later to be used on industrial scale.

Yeasts can also be used as biocontrol agents for post-harvest fungal diseases of stored fruits and grains. For this purpose, the stored goods are treated with antagonist saprophytic yeasts (Rosa and Peter, 2006; Satyanarayana and Kunze, 2009; Shimon *et al*., 2004). Another aspect of yeast biotechnology is the production of some industrially important molecules, such as enzymes, vitamins, lipids etc. Among all the compounds mentioned above, amylases are one of the widely used compounds used for biotechnological and industrial purposes these days. This review describes the various amylase producing yeasts and their biotechnological applications.

1.1 AMYLASES:

Amylases are group of enzymes that act on starch and other related compounds (e.g., glycogen and pullulan) to yield different products such as dextrins, maltose, small polymers of glucose and glucose units (Gupta *et al*., 2003; Harvathova, 2001; Jyoti *et al*., 2011; Reddy *et al*., 2003). They are widely distributed in nature and can be obtained from different sources, for example plants, animals and microorganisms. The different enzymes included in this group are widely used in various industries where starch hydrolysis is involved. For this purpose, amylases from microbial sources are significant owing to their productivity and thermostability (Fossi *et al*., 2004) and the amenability of microorganisms to genetic manipulation to obtain enzyme of desired characteristics (Souza and Magalhaes, 2010).

The substrate of amylases, Starch $(C_6H_5O_{10})_n$ is a form of carbohydrate occurring in most of the higher plants, it is present in pollen, leaves, stem, root, tubers, bulbs, rhizomes, fruits, woody tissues, flowers and the pericarp, cotyledons, embryo and endosperm of the seed (BeMiller and Whistler, 2009; Sohail *et al*., 2005; Tester *et al*., 2004).

Commercially, starch is mainly extracted from corn (maize), potato, wheat, cassava and rice; though there are some other plant sources also, such as sago, oats, arrow root, barley, pea, sorghum, oats and yam (Aiyer, 2005; Tester *et al*., 2004). Starch is synthesized by plants as semi-crystalline, granules of various shapes and sizes depending upon the plant source from which it is extracted (Aiyer, 2005; BeMiller and Whistler, 2009). Owing to its densely packed structure, with a density of 1.5 g/cm^3, starch is insoluble in water at room temperature, although reversible water sorption may occur (BeMiller and Whistler, 2009). It is a polymer of two different types of α-glucan molecules, amylose and amylopectin, which differ in the α-acetal linkages present within them.

Amylose is a predominantly linear molecule constituting 20 – 30 % of starch (BeMiller and Whistler, 2009). It is composed of ~99% of α-1→4 and ~1% of α-1→6 linkages, with a molecular weight of ~1×10^5 – 1×10^6 g/mol (Tester *et al*., 2004). A few branches may be present in some high molecular weight amylose molecules which do not have any apparent effect on their properties. There may also be some linkages with phosphate groups. Each chain of amylose consists of ~1000 glucose units (Sohail *et al*., 2005). It can also form extended shapes, but due to the presence of hydrogen bonding between O2′ and O3′ oxygen atoms of contiguous residues, they tend to form helices. Amylopectin is a highly branched molecule comprising 70 – 80 % of starch (BeMiller and Whistler, 2009). It consists of α-1→4 glucose subunits (~95% of the molecule) linked by α-1→6 branch points (Aiyer, 2005). It is a larger molecule as compared to amylose, with a molecular weight of ~1×10^7 – 1×10^9 g/mol (Tester *et al*., 2004). The unit chains of amylopectin may contain 12 – 120 glucose subunits, out of which 12-20 are present in exterior chains, while interior chains may consist of 20 – 120 units (Tester *et al*., 2004). Amylopectin is mainly composed of three types of branch chains; A-chains, which are the greatest in number, are the outer unbranched chains, B-chains or the inner branched chains which are fewer, and the single C-chain which is the original chain containing the single reducing group within a molecule (Aiyer, 2005; BeMiller and Whistler, 2009).

After discussing the structure of starch, it can easily be understood that such a complex structure may not be hydrolyzed by a single enzyme, therefore, there are different types of starch hydrolyzing enzymes collectively referred as amylases. Amylases are considered to be of two types, liquefying and saccharifying. Saccharifying amylases yield a higher number of reducing sugars as compared to liquefying amylases (Abdullah, 2005) because the bond breakage is more extensive by saccharifying enzymes. The different types of amylolytic enzymes are classified on the basis of their mode of action on starch resulting in different products.

1.1.1 Types of Amylases:

The amylolytic enzymes are broadly classified into two groups; α-1→4 glucanases, which hydrolyze the α-1→4 linkages present within the substrate, and α-1→6 glucanases that act on the α-1→6 bonds (Polaina and MacCabe, 2007). Both these groups are further divided into two subgroups, endoglucanases and exoglucanases. Endoglucanases randomly hydrolyze the linkages in the interior of the starch molecule, resulting in linear and branched oligosaccharides of various chain lengths, while exoglucanases start hydrolyzing the molecule from the exterior non-reducing ends successively producing short chain end products. These exo- and endoglucanases comprise of different enzymes that catalyze the breakdown of starch forming different products depending upon their site of action within the molecule.

Some other starch converting enzymes are: transferases, that cleave α-1→4 glucosidic bonds and transfer part of the donor to a glucosidic acceptor resulting in the formation of new bond, i.e., intramolecular transglucosylation; and debranching enzymes that hydrolyze the α-1→6 linkages present within the substrate (Chang, 2012; Hii *et al*., 2012).

1.1.1.1 α-amylases

α-amylases (1,4-α-D-glucan-glucanohydrolases, E.C. 3.2.1.1) are type of endoglucanases that catalyze the hydrolysis of internal α-1→4 linkages present within the starch, forming low molecular weight compounds such as glucose, maltose and maltotriose units (Souza and Magalhaes, 2010). The endo-mechanism of these enzyme rapidly breaks down starch and results in a decrease in the viscosity of the solution, hence, they are said to be liquefying amylases (BeMiller and Whistler, 2009; Polaina and MacCabe, 2007). α-amylases cannot act on α-1→6 linkages, therefore, in the degaradation of amylopectin, α-limit dextrins are also produced along with glucose and other products. However, they are able to bypass these α-1→6 glucosidic bonds (Ray and Nanda, 1996). This enzyme has a characteristic substrate-binding cleft which can accommodate four to ten glucose units of the substrate, where each binding site has affinity for only one glucose unit. Thus the interaction of oligosaccharides with several binding sites creates a multipoint linkage resulting in

the correct arrangement of the substrate molecule towards the catalytic site. However, mere substrate binding is not sufficient; the glucose units of the substrate oligosaccharide must interact with the catalytic site as well. Differences in the number of substrate binding sites and the location of catalytic region are responsible for the substrate specificity and the final product formed after hydrolysis. Most of the α-amylases are metalloenzymes and require calcium to maintain their stability and structural integrity (Vihinen and Mantsala, 1989). However excessive calcium ions may have an inhibitory effect (Polaina and MacCabe, 2007). All the α-amylases yield product by the retaining mechanism, i.e., the products formed have the same α-configuration as that of the substrate bond hydrolyzed.

The optimum conditions for the activity of α-amylases vary greatly, depending upon the producing organism and the environment from which it is obtained. Most microbial α-amylases have a pH range of 2.0 – 10.5 for their activity, while the optimum temperature for this enzyme may be as low as 25 – 30 °C for *Fusarium oxysporum* to as high as 100 °C in case of *Bacillus licheniformis*. The molecular weight is also quite variable, with a range of 50 kda to 60 kda for most microbial α-amylases (Vihinen and Mantsala, 1989); however 10 kda for *Bacillus subtilis* and 210 kda of *Chloroflexus aurantiacus* amylases have also been reported (Chang, 2012).

1.1.1.2 β-amylases

β-amylases (α-1,4-D-glucan maltohydrolase, E.C. 3.2.1.2) also hydrolyze the α-1→4 linkages, but they differ from α-amylases in their mechanism, i.e., they begin the hydrolysis from the non-reducing ends, until a reducing end or a branch point is encountered, therefore they are included in α-1→4 exoglucanases. The products formed are β-maltose and β-limit dextrins when the substrates are amylose and amylopectin respectively. These enzymes can neither hydrolyze nor can bypass the α-1→6 glucosidic bonds present in the substrate, therefore, the degradation of starch is incomplete; about 50% degradation takes place when the substrate is amylopectin, while it is 75 – 90% in case of amylose as it is less branched (Polaina and MacCabe, 2007; Ray and Nanda 1996). β-amylases are considered to be saccharifying amylases as more sugars are produced in the reaction (Kavanagh, 2011; Polaina and MacCabe, 2007; Ray and Nanda, 1996). Though β-amylases are similar to α-amylases in that these enzymes also have a number of glucosyl unit-binding subsites, they differ in their structure where the starch chains bind to active sites found into a pocket rather than into a cleft. Their mechanism of hydrolysis results in a conversion of configuration at the anomeric center (BeMiller and Whister, 2009).

The molecular weight of β-amylases varies greatly; 31.6 kda – 160 kda have been reported (Vihinen and Mantsala, 1989). The optimum pH of β-amylases is slightly higher than α-amylases. Most microbial β-amylases have an optimum pH of 6 – 7 for their activity, however, slightly acidic (4.5 – 5.5) and slightly alkaline (7.5 – 8.5) pH optima have also been reported. As far as the temperature is concerned, β-amylases are thermostable; while the optimum temperature their activity may vary from <40 °c up to 70 °C depending upon the producing organism (Ray and Nanda 1996).

1.1.1.3 Glucoamylases

Glucoamylases (1,4-α-D-glucan glucanohydrolases, E.C. 3.2.1.3), also known as amyloglucosidases, are a type of exoglucanases that can cleave both α-1→4 and α-1→6 glucosidic bonds present in starch (Kavanagh, 2011; Polaina *et al*., 2004), although their action on α-1→6 linkages is slower (Polaina and MacCabe, 2007). Because of their action on both types of acetal linkages, these enzymes are capable of hydrolyzing the starch (amylose or amylopectin) completely. This hydrolysis takes place with an inversion of the anomeric configuration (Sauer, 2000).

Structurally, glucoamylases usually have five to seven glucosyl unit-binding subsites, where the catalytic regions are located between first and second subsites from the non-reducing ends (BeMiller and Whistler, 2009). Glucoamylases are very important because they cannot only degrade starch, but are also able to hydrolyze glycogen, pullulan and many more substrates (Pandey, 1995). The molecular weight of glucoamylases falls in a range of 20 kda to 306 kda (Vihinen and Mantsala, 1989). Their optimum pH lies in the acidic range, i.e., 4.5 – 5.0, and they remain stable at acidic pH for prolonged periods. The optimum temperature for the activity of glucoamylases ranges between 40 °C and 60 °C (James & Lee, 1997; Pandey, 1995; Vihinen and Mantsala, 1989).

1.1.1.4 α-Glucosidases

α-glucosidases or maltases (α-D-glucoside glucohydrolase, E.C. 3.2.1.20) are able to cleave the α-1→4 and α-1→6 glucosidic bonds within the substrate. They act on non-reducing ends of short chain oligosaccharides produced from

large polymers by the action of other amylolytic enzymes, and liberate glucose with α-configuration (Chang, 2012; Vihinen and Mantsala, 1989). The molecular weight of most α-glucosidases varies between 12 kda and 160 kda. Their optimum pH lies in the acidic range, or near neutrality; while most of these enzymes are stable at low temperatures (Vihinen and Mantsala, 1989).

1.1.1.5 Pullulanases

Pullulanases (1,6-α-D-glucanohydrolase) are debranching enzymes that hydrolyze starch and a related carbohydrate polymer called Pullulan. This group consists of five different enzymes classified on the basis of their target glucosidic bond and the products obtained: Pullulanase type I and type II, and Pullulan hydrolase type I, II and III.

Pullulanases type I (pullulan-6-glucanohydrolase, E.C. 3.2.1.41) are able to cleave the α-1→6 linkages present in the substrate, while type II pullulanase, also called amylopullulanase (E.C.3.2.1.41) can hydrolyze both α-1→4 and α-1→6 glucosidic bonds. Both these enzymes can act on starch and glycogen as well, releasing maltotriose as final product; however, amylopullulanase may also produce maltose and glucose alongwith maltotriose. Pullulan hydrolase type I, also called Neopullulanase (pullulan-4-D-glucanohydrolase, E.C. 3.2.1.135), and Pullulan hydrolase type II, known as Isopullulanase (pullulan-4-glucanohydrolase, E.C. 3.2.1.57) hydrolyze the α-1→4 linkages, producing panose and isopanose respectively as the final product; isopullulanase typically cleaves the first α-1→4 bond after the α-1→6 bond (Vihinen and Mantsala, 1989). Apart from pullulan, both these enzymes can work efficiently on cyclodextrins as well; however they have practically no action on starch. Pullulan hydrolase type III is able to hydrolyze both α-1→4 and α-1→6 bonds producing maltose and maltotriose as final products. This enzyme can degrade starch, pullulan and glycogen (Hii *et al*., 2012).

The molecular weights of pullulanases (particularly type I and type II) vary from 80 kda – 145 kda. The optimum pH for their activity usually falls in the acidic range. In case of isopullulanase, they are stable over a pH range of 4 – 7, the optimum being 3.5 – 4.0; the optimum temperature for their activity ranges between 30 °C and 40 °C (Vihinen and Mantsala, 1989).

1.1.1.6 Isoamylase

Isoamylase (glycogen α-1,6-glucanohydrolase, E.C. 3.2.1.68) is a debranching endoenzymes that acts on internal linkages producing linear maltodextrins with α-configuration (BeMiller and Whistler, 2009). Apart from starch, this enzyme can hydrolyze glycogen as well. To be specific, it is the only known enzyme that can debranch glycogen completely; however, it is unable to act on pullulan (Hii *et al*., 2012). It also has low affinity for short chain oligosaccharides (Vihinen and Mantsala, 1989). The molecular weight of most microbial isoamylases is higher than α- or β-amylases, ranging from 65 kda – 121 kda. They are relatively thermolabile (Vihinen and Mantsala, 1989) and have maximum temperature stability at 45 – 55 °C. The pH optima for their activity and stability lie in the acidic range, i.e., pH 3 – 4 (Hii *et al*., 2012; Vihinen and Mantsala, 1989).

1.1.1.7 Transferases

As mentioned above, transferases breakdown starch by the formation of new glucosidic bonds. This category mainly includes CGTase, amylomaltases and branching enzymes.

Cyclomaltodextrin Glucanosyltransferases (CGTase E. C. 2.4.1.19) and Amylomaltases (E.C. 2.4.1.25) attack the α-1→4 glucosidic bonds and form new α-1→4 bonds. The action of CGTase results in the production of cyclic, non-reducing, α-1→4 linked oligosaccharides, known as cyclomaltodextrins (or simply cyclodextrins). In this reaction, a mixture of cyclic products are produced having six, seven or eight glucose units, known as α-, β-, and γ-cyclodextrins respectively (BeMiller and Whistler, 2009; Hii *et al*., 2012), and high molecular weight CGTase limit dextrins. Amylomaltases are similar to CGTase with respect to enzyme reaction, however they produce linear products. Branching enzymes (E.C. 2.4.1.18) cleave the α-1→4 bonds and result in the formation of α-1→6 linkages (Chang, 2012; Hii *et al*., 2012). The optimum temperature range for the activity of CGTase is 45 – 60 °C, while the optimum pH ranges between 4.5 and 7. The molecular weight may fall between 67 kda to 145 kda depending upon the source (Vihinen and Mantsala, 1989).

1.1.2 Industrial importance of Amylases

The present era is marked with the use of natural products for different purposes to minimize the environmental pollution caused by chemicals. Today, the focus of different industries is switched to microorganisms and their products, such as enzymes, to carry out their work in an eco-friendly manner. Amylases are also being extensively used in different industrial and biotechnological processes; they constitute about 25% of the enzyme industry (Souza and Magalhaes, 2010). The use of amylases began in U.S. in 1894, and was meant for the treatment of digestive disorders (Aiyer, 2005; Stewart, 1987). But today, a large number of amylolytic enzymes are not only commercially available, but they also have almost completely replaced the use of chemicals (Pandey *et al*., 2000). They have extended their applications to different areas; they are used either in industries which require starch hydrolysis such as food and bakery products, or are used on their own for different purpose, e.g., textile, detergent, paper industry etc. Table 1 shows the use of different types of amylases in various fields.

Table 1. Amylases and their commercial use

TYPE OFAMYLASES	INDUSTRIAL USE(S)	REFERENCE(S)
α-amylase	Baking; brewing; laundry detergents; automatic dishwashing detergents; liquefaction in grain wet milling; animal feed; textile desizing	Aehle, 2007; Gupta *et al*., 2003; Polaina and MacCabe, 2007
β-amylase	Baking; brewing; saccharification	Aehle, 2007; Ray and Nanda, 1996
Glucoamylase	Fruit juice clarification; baking; brewing; saccharification in grain wet-milling; toothpaste; alcohol production	Aehle, 2007; Kumar *et al*., 2012; Polaina and MacCabe, 2007
Isoamylase	Automatic dishwashing detergents; production of glucose syrups	Aehle, 2007; Harada, 1984
Pullulanases	Brewing; automatic dishwashing detergents; saccharification in grain wet-milling; production of glucose syrup	Aehle, 2007; Harada, 1984; Hii *et al*., 2012; Polaina and MacCabe, 2007
CGTase	Automatic dishwashing detergents; production of cyclodextrins	Aehle, 2007; Hii *et al*., 2012

1.2 YEAST AS SOURCE OF AMYLASES:

Amylases are widely present in nature and can be obtained from plants, animals, and microorganisms such as bacteria, fungi and yeasts. Microbial amylases are preferred for commercial use because of their stability. A large number of bacterial and fungal species have been found to be amylolytic after extensive studies, and are being used commercially. Yeasts are also a good source of amylases but, unfortunately, less research has been done on yeasts in this regard. However, scientists have now focused on these organisms to find out their amylolytic potential. It is reported that the GRAS status of fungal amylases favour their application for biotechnological processes (Souza and Magalhaes, 2010). It is also well known that yeasts are safer for human consumption as compared to molds. Therefore, the use of yeast amylases confers several advantages for commercial use: the shorter generation time of yeasts than molds ensures more productivity, because of their GRAS status their products are safe for human consumption, and usually yeasts get an edge over bacteria due to their extracellular products.

After several studies, a few yeast species have been reported to be amylase producers. These species may not only be used for starch hydrolysis and amylase production, but may also be used for other purposes. For example, in the production of ethanol, if starch is used as raw material, it first needs to be saccharified, and then the simple sugars obtained are used by *Saccharomyces cerevisiae* to produce ethanol. In such cases, if some amylolytic yeast specie is used, then the additional step of saccharification may be eliminated, making the process easier. Some of the amylase producing yeast species is briefly discussed here.

1.2.1 *Saccharomycopsis fibuligera*

Saccharomycopsis fibuligera, previously reported as *Endomycopsis fibuligera*, is one of the best producers of amylases among yeasts. It is food-borne, dimorphic, ascomycetous yeast, commonly found in starchy substrates (Hostinova, 2002; Kurtzman and Fell, 1998). Amylase production from this specie was first reported by Wickerham in 1944 (Kurtzman and Fell, 1998). This strain has been extensively studied due to its high amylolytic activity in submerged cultures (Gonzalez *et al*., 2008). The potential of this organism to degrade starch is due to the production of two different types of amylases; α-amylases and glucoamylases, in most cases. However, some strains have also been found to produce α-glucosidases as well. *S. fibuligera* IFO 0111 is also capable of degrading raw starch (Hostinova, 2002) and there are some reports of direct conversion of starch to ethanol by this organism, however the yield is very low for commercial use (Reddy and Basappa, 1993).

1.2.2 *Debaryomyces occidentalis*

Initially classified as *Schwanniomyces occidentalis*, later named as *S. castellii* and then *S. alluvius* (Strasser *et al*., 1989), this organism was first identified by Kloecker in 1909, and was placed in the genus *Debaryomyces* by Kurtzman and Robnett (1991), on the basis of its sequence similarity in partial ribosomal RNA sequences with many several *Debaryomyces* species (Kurtzman and Fell, 1998). However, the two names, i.e., *S. occidentalis* and *S. alluvius* are still used by scientists. This strain is important because of its ability to hydrolyze starch completely. Two types of amylases are known to be produced by this strain; α-amylase, and glucoamylase. Because of its unique enzyme system, this organism is capable of degrading pullulan and glycogen too (Spencer *et al*., 2002). It also possesses fermentative ability to produce ethanol (Wilson and Ingledew, 1982).

1.2.3 *Pichia anomala*

Pichia anomala is reported to produce β-amylase (Ray and Nanda, 1996), however further studies are required to properly evaluate its amylolytic potential and its industrial use in this regard. It is ascomycetous yeast specie frequently found in natural habitats (Walker, 2011) and in food products (Passoth *et al*., 2006). It has several biotechnological applications, e.g., in food fermentation, as flavouring agent, biofuel production etc. (Passoth *et al*., 2006; Walker, 2011). Apart from those discussed above, several other yeast genera have been reported to possess the starch degrading potential (Table 2).

Table 2. Different amylolytic yeast strains

AMYLOLYTIC YEAST SPECIE(S)	REFERENCE
Lipomyces kononenkoae	Estrela *et al*., 1982; Ramchandran *et al*., 2005; Spencer-Martins, 1982
Aureobasidium pullulans	Linardi and Machado, 1990
***Candida* spp.**	Linardi and Machado, 1990
Debaryomyces vanrejii	Linardi and Machado, 1990
***Rhodotorula* spp.**	Linardi and Machado, 1990
***Trichosporon* spp.**	Buttner *et al*., 1987; Linardi and Machado, 1990
***Cryptococcus* spp.**	Linardi and Machado, 1990; Wanderley *et al*., 2004
Filobasidium capsuligenum	De Mot and Verachtert, 1985
Saccharomyces cerevisiae	Olasupo *et al*., 1996

1.2.4 Recombinant Yeast Strains:

Besides those species which are naturally amylolytic, different recombinant amylase producing yeast strains have been developed and are being used for research and commercial purposes,for example, *Saccharomyces cerevisiae*. Despite of its ethanologenic abilities, it cannot degrade starchy materials and hence cannot be used for the first generation bioethanol. Therefore, recombinant strains of *S. cerevisiae*have been developed so as to eliminate the additional steps of liquefaction and saccharification, thereby reducing the time and cost of the process. Yamakawa *et al*. (2012) developed a recombinant strain of *S. cerevisiae* displaying both α-amylase and glucoamylase for the production of

bioethanol from raw starch substances. This strain was found to be efficient ethanol producers for 23-cycle repeated fermentation from raw starch, without any loss in amylase productivity.
Similarly, Li *et al*. (2011) described the expression of α-amylase from *Rhizopus oryzae* in *Pichia pastoris*. They obtained a higher yield of enzyme from this recombinant strain and concluded that this method can be useful for large-scale production of the enzyme.

CONCLUSION

For the purpose of reducing environmental pollution and bioremediation, natural substances are now being used in industries in place of chemicals. Starch is one of the naturally occurring carbohydrate sources that has widespread industrial and biotechnological applications. Those processes that involve starch hydrolysis initially used chemicals, but now starch degrading microorganisms and their enzymes are being used. On the other hand, yeasts are exploited in food and fermentation industries and for biotechnological aspects since ancient times. Therefore, if some good amylase producing yeast species are obtained, they may greatly reduce the cost of different processes. For instance, in the production of bioethanol, if some starch degrading yeast specie is used, then the additional step of starch saccharification may be eliminated. The GRAS status of yeast ensures the safety of product for human consumption. Further studies are required to fully understand the amylolytic system of these strains in order to exploit them on a large scale.

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