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Research Article

Purification and Characterization of Amyloglucosidase Produced by *Aspergillus awamori* NA21 under Solid State Fermentation using Tapioca Powder

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ABSTRACT

Amyloglucosidase (α-1, 4 glucan glucohydrolase EC 3.2.1.3) hydrolyses amylaceous polysaccharides, removing successive glucose units in the β configuration from the non-reducing end of the chain. Amyloglucosidase is of considerable commercial importance to grain - alcohol fermentation, food, textile and pharmaceutical industry. The aim of this study is to purify and characterize amyloglucosidase Produced by *Aspergillus awamori* NA21 under Solid State Fermentation (SSF) using Tapioca Powder. The enzyme was purified by sequential ammonium sulfate fractionation, DEAE-ion exchange chromatography and Sephadex G-100 column chromatography. Thermostability is increased by addition of thermo stabilizers, PEG 6000 and Ethylene glycol at 5 mM. The enzyme apparent molecular weight is determined by sodium dodecylsulfate - polyacrylamide gel electrophoresis and gel filtration. The enzyme was purified approximately 68 fold with 54 percent recovery. The pH and temperature optima were 5.0 and 60°C, respectively. Enzyme was stable at temperature up to 40°C for 18 h and thermostability has increased to 24 h. The enzyme was stable at pH from 4.0 to 6.0. The enzyme was found to have apparent molecular weight of 61 kDa. The Michaelis Menten's constant Km for soluble starch was 3.12 mg/ ml. The V_{max} for soluble starch was 50.54 mg/ ml /min. The enzyme was activated by Ca^{2+} but inhibited by $Co^{2+,}$ $Hg^{2+,}$ EDTA and Pb^{2+}. The purified amyloglucosidase is stable in various organic solvents. This is the first report on purification of amyloglucosidase produced by *Aspergillus awamori* under SSF with tapioca powder as substrate.

INTRODUCTION

Fungal amylases in general and amyloglucosidase (glucoamylase) in particular have been a subject of extensive research throughout the world in the preceding couple of decades. Amyloglucosidase hydrolyses α -1, 4 and α-1, 6 amylaceous polysaccharides, removing successive glucose units in the β configuration as the sole product from the non-reducing end of the chain. The industrial use of amyloglucosidase is impaired by the limited glucose yields due to slow hydrolysis of α-(1, 6) - glucosidic bonds in starch. Moreover, amyloglucosidase production in the culture broth is

costly and generally associated with the production of various other proteins such as glucosyltransferase (Pestlin et al., 1997). The poor stability of enzyme under standard conditions of pH and temperature and end product inhibition of enzyme activity also affect glucose yields. The possibility of solving these problems encourages the search for production of a superior amyloglucosidase in purified form.

Many fungi and a few bacteria are reported for glucoamylase production (El-Safey et al., 2004 and Bhatti et al., 2007). A number of reports given by different workers showing the production of amyloglucosidase under submerged (SmF) or solid state fermentation (SSF) (El-Safey et al., 2004 and Mohamed et al., 2007). A large amount of amyloglucosidase production was achieved by Smf. But this technique is not only expensive but also energy intensive, hence solid state fermentation (SSF) is the alternative method, since obtained enzyme titers are higher in SSF than Smf .

SSF involves non-aseptic conditions with the use of cheap, simple and easily available agro industrial residues as substrates, low energy expenditure, less expensive downstream processing, less water usage, potential higher volumetric productivity, high reproducibility, easier control of contamination, less technology oriented, produces less pollution and generally simpler fermentation media (Krishna, 2005). As the moisture level is low, the volume of medium per unit weight of substrate is low. Therefore, the enzyme activity is usually very high. It was observed that the amyloglucosidase produced by SSF was more stable and total proteins were lower; moreover, undesirable proteolytic enzymes production was not observed during SSF. Filamentous fungi are ideally and the best adapted for SSF. The hyphal mode of fungal growth and their good tolerance to low water activity and high osmotic pressure conditions give fungi major advantages over unicellular microorganisms in the colonization of solid substrates and proper utilization of available nutrients.

Amyloglucosidase production through solid state fermentation using various starchy solid substrates such as rice bran, wheat bran, maize bran, cassava starch etc has been reported in the literature (Oriol et al., 1998). In this study, we are reporting the production of high yields of amyloglucosidase by a local fungal isolate *Aspergillus awamori* through SSF utilizing tapioca powder as solid substrate.

In the present study, we are describing the purification and characterization of the amyloglucosidase produced by *Aspergillus awamori* under SSF using nonconventional tapioca powder as starchy substrate.

MATERIALS AND METHODS

Microbial strain and its growth conditions:

An amyloglucosidase producing fungal strain was isolated from spoiled potato and identified as *Aspergillus awamori* NA21 by Mycology laboratory Department of Plant Pathology, CCS Haryana Agricultural University Hisar, Haryana, India. The fungus was grown in starch mineral medium (SMM) containing 3% soluble starch (Merck); $MgSO_4$ 7 H_20, 0.5%; yeast extract, 0.5%; KH_2PO_4, 0.1%; $FeSO_4$ $7H_20$, 0.005%; $CaCl_2$, 0.15%; pH 5.0 in shake flask at 30°C and was maintained on potato dextrose agar slants.

Chemicals and commercial enzymes:

Tapioca powder was from M/S Life Science Engineering, Madurai. Biotempase (α-Amylase) and Amylo 300 (amyloglucosidase); the commercial starch splitting enzymes in liquid formulations were from Biocon India Pvt. Ltd. India. All other chemicals used were of the analytical grade available commercially from Hi-media Pvt. Ltd. (Mumbai).

Analysis of solid substrates:

Tapioca powder used for solid state fermentation was analyzed for moisture and starch content. For determining moisture content, starchy material was dried in oven at 80°C till constant weight is obtained (Oriol et al., 1998). Starch was estimated in terms of total reducing sugar as determined by either anthrone method or dinitrosalicylic method (DNS) (McCreddy et al., 1950). Generation of reducing sugars from tapioca powder was achieved by acid or enzymatic hydrolysis (Aggarwal et al., 2001).

Anthrone method for sugar estimation:

The anthrone reagent (5 ml) was added to 1 ml of appropriately diluted acid hydrolysate and the mixture was held in boiling water bath for 15 min. The sample was analyzed for total sugar content colorimetrically using glucose as standard. Starch content in the sample was calculated by multiplying

the total reducing sugar by a factor of 0.9 (Clegg, 1956).

Amyloglucosidase production in SSF:

Amyloglucosidase production was studied under SSF using tapioca powder as substrates. For maximum enzyme titre during SSF, various parameters like incubation time, temperature, pH, mineral salt solution, moisture level and addition of various additives were optimized taking one at a time. Fermentation was carried out in 500ml Erlenmeyer flask containing solid substrate medium (25g) inoculated with 10 ml spore suspension (10^7-10^8 spores/ ml) mixed thoroughly and incubated under particular growth conditions under study. At the end of the fermentation period, the entire quantity of moldy substrates was homogenized with 100 ml of 0.1 M acetate buffer (pH 4.8), centrifuged and used as a crude source of amyloglucosidase. The supernatant obtained after centrifugation at 10,000 x g for 20 min was filtered through whatman paper No.1 to discard any fungal spore. The culture filtrate thus obtained was collected in test tubes and stored at 4°C for amyloglucosidase assay.

Amyloglucosidase assay:

Culture filtrate was suitably diluted and 0.5 ml was incubated with 0.5 ml of 1% (w/v) soluble starch in 0. 1 M acetate buffer pH 4.8 in blood sugar tubes at 30°C for 3 min. Glucose was estimated by DNS method (Miller, 1959). One unit of Amyloglucosidase activity was expressed as release of 1 µmol of glucose from starch per min at 30°C by 1 ml of enzyme solution. Standard curve was prepared with glucose (0.09 - 0.9 mg/ ml). Specific activity was expressed in terms of amyloglucosidase activity per mg of protein. Amyloglucosidase activity in case of SSF was expressed as units/g dry substrates (Ug-1 ds). All the experiments were carried out independently in triplicate and results presented are the mean of the three values.

Protein measurement:

Protein was measured by the absorbance at 280 nm following the method of Lowry *et al.* (1951) with bovine serum albumin (BSA) as a standard (20 - 200 µg /ml).

Enzyme purification

Ammonium sulphate fractionation:

To the supernatant (1000 ml), 860 g of solid ammonium sulphate was added with constant stirring using magnetic stirrer to bring the saturation to 80% (Green and Hughes, 1955). After all the $(NH_4)_2SO_4$ was dissolved, stir for another 1 h to allow complete equilibration between the dissolved and aggregated proteins. The mixture was kept in the refrigerator for 1 h for complete precipitation. The mixture was then centrifuged at 10000 x g for 20 min. The precipitates obtained were dissolved in a small volume of 0. 1 M acetate buffer (pH 4.8).

Dialysis

The dissolved precipitates were dialyzed through dialysis membrane (M/s Sigma) against the acetate buffer (0.1 M pH 4.8) for 24 h (diluted to 1:10) with repeated changes till it became free of $(NH_4)_2SO_4$. Dialyzed samples were subjected to ion-exchange chromatography for further purification.

Ion-exchange chromatography:

Dialyzed protein sample was loaded on a DEAE-cellulose (anionic exchanger) (M/s Sigma) column (25 x 2.6 cm) pre-equilibrated with 0.1 M acetate buffer (pH 4.8) and was first eluted with the same buffer to wash out the unbound protein (Tsuboi et al.,1974). The bound protein was then eluted with a linear gradient of 0.1 to 0.5 M NaCl in the same buffer. The fractions of 5 ml each were collected at a constant flow rate of 35 ml/ h and analyzed for protein content (A_{280}) and amyloglucosidase activity. The fractions with high enzyme activity were pooled together and concentrated by osmosis against Polyethylene glycol (PEG 6000). Concentrated enzyme was further subjected to gel filtration chromatography using Sephadex G-100-column.

Gel permeation chromatography:

The amylolytic fractions obtained after ion-exchange chromatography were put on a gel permeation column (M/s LKB) of dimensions 75 x 1.6 cm which was pre-equilibrated with 0.1 M acetate buffer pH 4.8 (Ueda and Saha,1983). The column was eluted with same buffer (0.1 M, acetate buffer, pH 4.8) at a flow rate of 12 ml/ h. The fractions of 2 ml each were collected and monitored for protein content at A_{280} on DU-64 spectrophotometer and also analyzed for amyloglucosidase activity. The fractions with amyloglucosidase activity were pooled together, stored at 4°C and further used for enzyme characterization studies.

Enzyme characterization

Physio-chemical properties of the enzyme:

The temperature optimum of the purified enzyme was measured at pH 5.0 and at different temperature (20-100°C). The thermal stability was estimated by incubation of the enzyme at pH 5.0 for different intervals of time (0 - 24 h) at different temperatures 30°, 40°, 50°, 60°, 80°, 90° and 100°C. The residual activity was measured at 60°C. The activity of purified enzyme in 0.1 M acetate buffer with pH values 2.0 to 7.0 was measured for determination of the pH optimum. The pH stability was determined by incubation of purified enzyme solution at various pH values (2.0 - 7.0) at 60°C followed by measurement of the enzyme activity at optimum pH.

Kinetic constant:

Michaelis - Menten constant Km and Vmax were assayed for substrate (soluble starch) at 30°C. The substrate concentration was from 0.5 to 4.0 mg /ml dissolved in 0.1M acetate buffer, pH 4.8. Glucose was analyzed and the constants were calculated using the Line weaver- Burk plot.

Effect of stabilizer/salts on thermo stability:

In order to see the effect of stabilizers in increasing the thermo stability of enzyme, definite amount of various stabilizers like PEG-6000, glycerol, ethylene glycol, β-Mercaptoethanol (β-ME), SDS, sorbitol and NaCl at a concentration of 5 mM was mixed with 0.5 ml of enzyme solution and incubated at 40° for 24 h and residual enzyme activity was assayed under optimum conditions of assay i.e. pH 5.0 and temperature 60°C.

Effect of storage temperature:

In order to store the enzyme at a particular temperature different storage temperatures were tried (4°C, 30°C, 40°C).

Effect of metal ions and detergents on amyloglucosidase activity:

The effect of detergents and metal ions was studies on amyloglucosidase activity. For determining this effect, the purified enzyme was preincubated with 5mM concentration of detergents and metal ions for 24h at 40^{o}C. Then, the residual activity was determined using soluble starch as substrate under standard assay conditions (Miller, 1959).

Stability and activity in organic solvents:

The enzyme solution was mixed with different solvent solutions (20% v/v).The solvents used were Glycerol, Ethylene glycol, Methanol, Ethanol, Acetonitrile, Dimethylformamide Dioxane, Dimethylsulfoxide .An enzyme sample was exposed to solvent for 24 h at 40^{o}C after which its residual enzyme activity was measured using soluble starch as substrate under standard assay conditions (Miller,1959).

Molecular Mass determination:

The molecular weight of amyloglucosidase was estimated by gel permeation chromatography and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). For estimation of molecular mass on Sephadex G-100, the column (75 x 1.6 cm) was calibrated with standard marker proteins viz., phosphorylase b (97 kDa), BSA (68 kDa), carbonic anhydrase (29 kDa) and cytochrome c (12.4 kDa). A calibration curve of elution volume v/s log molecular weight was prepared.

For SDS-PAGE, denaturing discontinuous polyacrylamide gel electrophoretic method (Laemmli, 1970) was followed. The molecular weight for various proteins was calculated by calibration curve made by plotting log molecular weight v/s Rm values of standard molecular weight marker proteins. The reference proteins in molecular weight markers used were phosphorylase b (97 kDa), BSA (68 kDa) ovalbumin (43 kDa), Carbonic anhydrase (29 kDa), Lysozyme (14 kDa). Relative mobility (Rm) is defined as the distance migrated by protein divided by the distance migrated by the tracking dye.

RESULTS AND DISCUSSION

Amyloglucosidase production under SSF

Various isolates from spoiled potato were screened out for the best producer of amyloglucosidase. The isolate was purified, identified as *Aspergillus awamori* NA21 and then used for further study. Production of amyloglucosidase by *Aspergillus awamori* was optimized using locally available Tapioca powder. Tapioca powder exploited for amyloglucosidase production by *Aspergillus awamori* NA21 under SSF was found to contain 92% or 90% starch after acid or enzymatic hydrolysis respectively along with 2% moisture. Various parameters were optimized to obtain maximum amyloglucosidase production by *Aspergillus awamori* NA21. A number of fungi have been reported to produce amyloglucosidase through SSF using various agricultural and agro forest residue but low yield of amyloglucosidase was of concern in these cases. Our strain *Aspergillus awamori* NA21 produced very high yield of amyloglucosidase i.e. 347 U/g under standardized optimized conditions of SSF using tapioca powder as substrate.

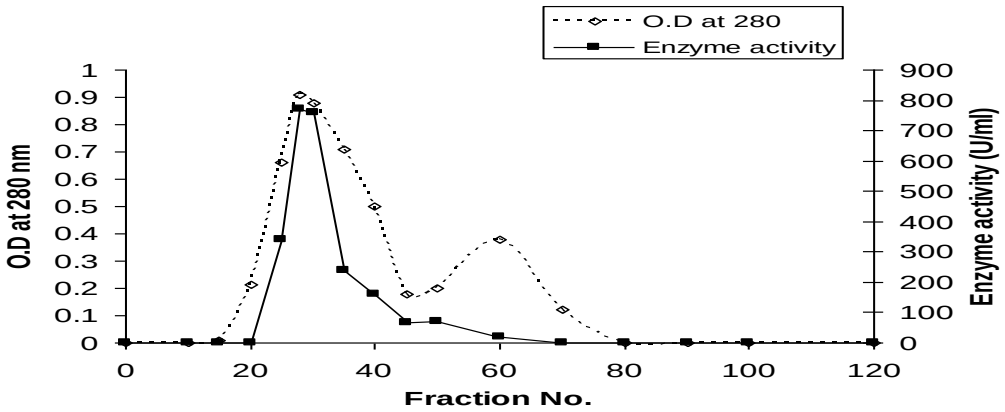

Fig. 1 Elution profile of protein and enzyme on Sephadex G-100

Table 1 Optimum conditions for maximum amyloglucosidase production under SSF

Parameters	SSF range	Optimum
Incubation period (h)	24-168	144
pH of medium	5.0-8.0	5.0
Temperature (^oC)	30-50	30
Selection of Moistening agent (g/l) S1) Na_2HPO_4; 11, NaH_2PO_4; 6.0, KCl; 3.0, $MgSO_4.7H_2O$; 0.1, pH 5.0 S2) NH_4NO_3; 2.0, K_2HPO_4; 5.0, $MgSO_4.7H_2O$; 0.5, $CaCl_2$; 0.1, $FeSO_4$; 0.1, $MnSO_4$; 0.1, pH 5.0 S3) K_2HPO_4; 0.1, $(NH4)H_2PO_4$;1.0, $MgSO_4.7H_2O$; 0.5, $CaCl_2$; 0.1, $FeSO_4$; 0.1, $MnSO_4$; 0.1, pH 5.0 S4) Tap water		S1
Substrate: Distilled water (Moistening agent) ratio	1:1 - 1:2.5	1:1.5
Supplementation: 1% Glucose 1% Ammonium sulphate 1% $CaCO_3$	1%	$CaCO_3$
Amyloglucosidase activity(Ug^{-1} ds)		347±31.60[a]
Specific activity (U mg^{-1}protein gds^{-1})		112±28.23

[a] Values are mean ± SD.

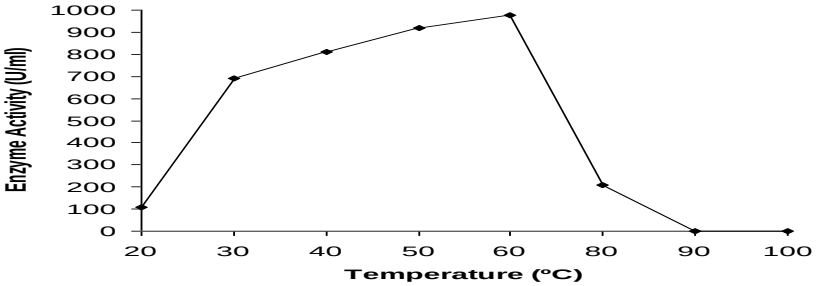

Fig. 2 Effect of temperature on activity of amyloglucosidase purified from *Aspergillus awamori* NA21

Purification of amyloglucosidase:

The results of purification studies are summarized in Table 1. The crude extract obtained after $(NH_4)_2SO_4$ fractionation (80%) yielded 8 fold purification with 80% enzyme recovery. More than 90% unwanted proteins were removed during this step of purification. The enzyme was eluted as a single peak with maximum enzyme activity in fraction number 27 when dialyzed protein sample was subjected to DEAE-cellulose ion-exchange chromatography. By gel-permeation chromatography on Sephadex G-100 column two peaks of protein and a single major peak of enzyme activity were obtained (*Figure 1*). By this procedure, amyloglucosidase was purified approximately 68 fold with 54% recovery. Soni et al., (1995) have found that about 96.82 % unwanted proteins present in 2-day old culture broth of *A. awamori* NRRL-3112 were separated by precipitation with ammonium sulphate.

Optimum temperature and thermo stability

The purified enzyme exhibited an optimum temperature of 60°C, as it had maximum activity of 975 U/ ml. Enzyme had significant activity of 810 and 900 U/ ml at 40° and 50°C, respectively. Incubation at temperatures below 40°C or above 60°C resulted in sharp decline in the enzyme activity. Enzyme retained only 21% of its maximum activity at 80°C and was completely inactive at 90°C (*Figure 2*). The enzyme was found to be quite stable up to 50°C for 6 h with only subtle losses in the enzyme activity. Enzyme was completely inactive at 50°C after 24 h of incubation, while it had retained 65 and 60% of its maximum activity at 30° and 40°C, after 24h respectively.

When thermo stability of enzyme was checked at higher temperature i.e. 80°C, it retained only 28% of its activity after 12 h and lost the activity sharply thereafter. The effect of different stabilizers showed that PEG-6000, glycerol, ethylene glycol and sorbitol each at 5 Mm concentration not only maintained the original activity, but also resulted in the increase in activity of the enzyme by 20, 15, 30 and 5%, respectively (Table 2). It was observed that enzyme was stable up to one month at 4°C without significant loss in activity.Amyloglucosidase activity was measured after 24 hours of incubation at 40^{o}C and resultant enzyme activity was assayed under standard conditions.

Temperature is a critical factor for maximum enzyme activity and it is a prerequisite for industrial enzymes to be active and stable at wider range of temperatures. The enzyme exhibited high activity (80-100%) over a broad range of temperature (40-60°C). This property makes it suitable for industrial applications because it is quite difficult to maintain specific temperature during large scale processing. The thermal stability of the enzyme may be due to the presence of protective factors within the cell and the higher average degree of hydrophobicity of the proteins (Imanaka et al., 1986). Nagasaka *et al.* (1998) reported that amyloglucosidase from *Corticium rolfsii* have optimum temperature of 65^{o}C on raw starch. Moreover, the enzyme could safely be stored in the refrigerator if it is to be used for longer periods.

Table 2. Purification of amyloglucosidase from *Aspergillus awamori* NA21

Purification step	Total activity (U)	Total protein(mg)	Specific activity(U/mg)	Fold purification	Percent recovery[b]
Crude Enzyme	108312	676.11	160.2	1	100
$(NH_4)SO_4$ Fractionation (80%)	86650	67.61	1281.6	8	80
DEAE cellulose	80150	14.71	5446.8	34	74
Sephadex G 100	58488	5.36	10893	68	54

Optimum pH and pH stability:

Amyloglucosidase purified from fungal strain showed a pH optima of 5.0. The enzyme was found to be stable over a pH range of 4.0 to 6.0 as it retained 80-94 percent of its maximum activity (*Figure 3*). Selvakumar *et al.* (1996) reported that the enzyme was stable over the pH range 3.4-4.8 with optimal activity at pH 4.0 when produced by *Aspergillus niger* during SSF. Nagasaka *et al.* (1998) reported that five different amyloglucosidase purified from *Corticium rolfsii* were stable over a pH range of 3.0-8.0.

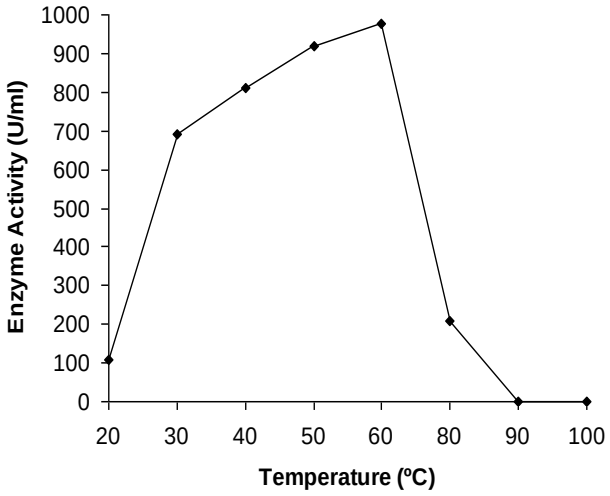

Fig. 3 Effect of temperature on activity of amyloglucosidase purified from *Aspergillus awamori* NA21

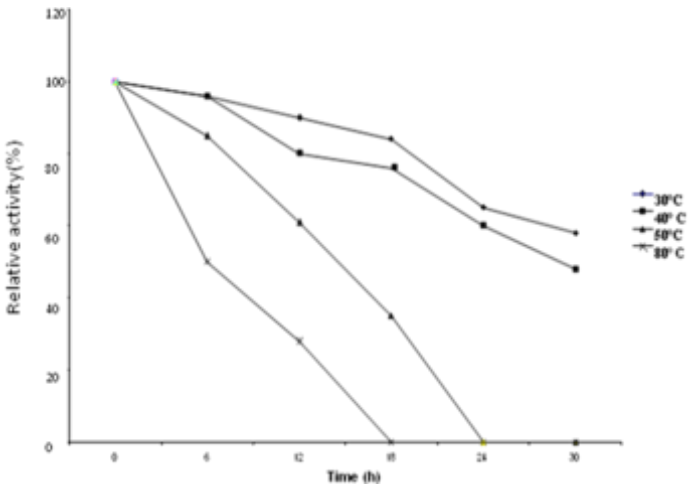

Fig. 4 Effect of temperature on stability of amyloglucosidase purified from *Aspergillus awamori* NA21

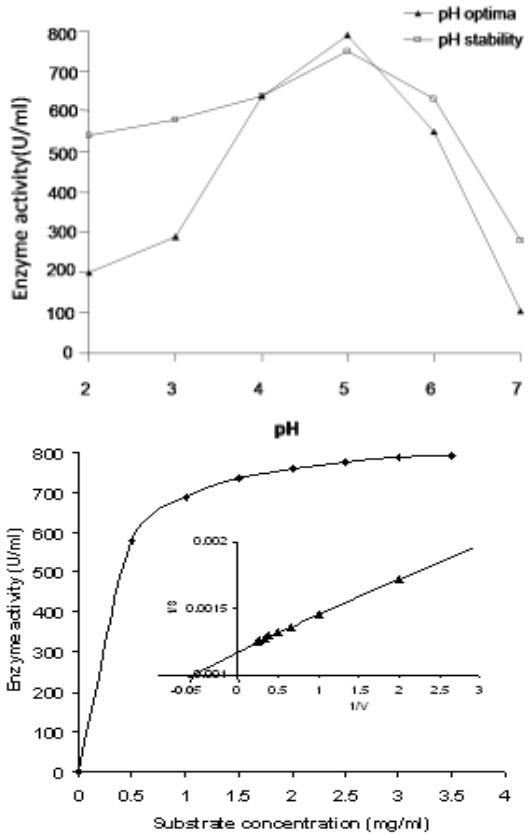

Fig. 5 Effect of pH and substrate concentration on activity and stability of amyloglucosidase purified from *Aspergillus awamori* NA21.

Kinetic constants:

A plot of enzyme velocity v/s. substrate concentration showed a typical hyperbolic curve which revealed that the enzyme followed Michaelis-Menten equation (*Figure 4*). From a double reciprocal plot (*Figure 4*) an apparent Km of the enzyme for soluble starch was found to be 3. 12 mg /m1 and its V_{max} was 50.54 mg/ ml /min. Amyloglucosidase from *Rhizopus oryzae* NRRL395 has Km and V_{max} of 3.34 mg/ ml and 136 µmoles /mg/ min respectively (Yu and Hang ,1991). The decreased value of Km for starch may be due to an increased number of interactions between the active site of the enzyme and the substrate molecule, resulting in an increased affinity of the enzyme with starch.

Table 3. Effect of stabilizers on thermo stability of purified amyloglucosidase.

Reagent	Concentration (mM)	relative activity a	percent increase in activity
Control	-	100	-
Glycine	5	100	0
PEG- 6000	5	120	20
Glycerol	5	115	15
Ethylene glycol	5	130	30
Sorbitol	5	105	5
β ME	5	100	-
SDS	5	100	-
NaCl	5	100	-

Table 4. Effect of metal ions and ion chelator on activity of purified amyloglucosidase

Metal ions/ Chelator	Concentration (mM)	Relative Activity
Control		100
Hg^{2+}	5	15
Zn^{2+}	5	80
Mg^{2+}	5	80
Co^{2+}	5	20
Cu^{2+}	5	14
Ca^{2+}	5	160
Mn^{2+}	5	50
EDTA	5	10

Table 5. Activity of soluble amyloglucosidase in presence of organic solvent

Solvent (20%)	Activity (%)
None	100
Glycerol	102
Ethylene glycol	104
Methanol	102
Ethanol	107
Acetonitrile	106
Dimethylformamide	80
Dioxane	82
Dimethylsulfoxide	84

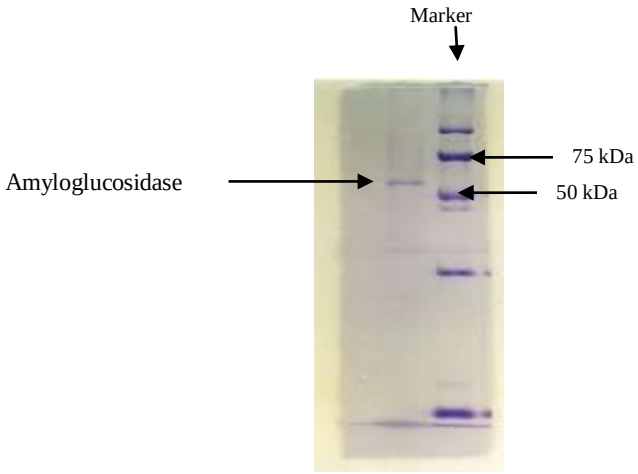

Fig. 7 Electrophoretic pattern of standard markers and purified amyloglucosidase (61kDa) on SDS PAGE.

Effect of metal ions and detergents on amyloglucosidase activity

Among the various metal ions tested, only Ca^{+2} was found to be an activator of the enzyme and it caused 60% increase in enzyme activity. On the other hand, Co^{+2}, Cu^{+2} and Hg^{+2} were found to be the potent inhibitors of the enzyme and caused 80 to 85% inhibition. EDTA resulted in 90 percent loss of enzyme activity (Table 3). The inhibitory effect of Hg^{+2}, Cu^{+2}, Ag^{+} and EDTA and activating effect of Mn^{+2}, Fe^{+2} on amyloglucosidase activity are also reported in the literature (Selvakumar et al.,1996). In contrary enzyme was reported to be unaffected by Na^{+}, K^{+}, Ca^{+2}, Mg^{+2} and Glycine (Selvakumar et al., 1996). In the present study, amyloglucosidase seems to be the calcium metalloenzyme as Ca^{+2} exerted strong activating effect on amyloglucosidase activity.

Effect of organic solvents on amyloglucosidase activity

Stability and activity of enzymes in organic solvents depend not only on the properties and concentration of the organic solvents, but also on the nature of the enzymes. Enzyme, being proteins, lose their activity after addition of organic co-solvents concentrations higher than 10-20% (Gupta et al., 1997). Therefore, the effect of various organic solvents at concentration of 20% (v/v) on the enzyme activity was examined. The results revealed that *Aspergillus awamori* NA21 amyloglucosidase was stable for 24 h in all organic solvents tested with the exception of Dimethylformamide Dioxane, Dimethylsulfoxide. A significant degree of stabilization in the presence of organic solvents has been reported for this amyloglucosidase than amyloglucosidase obtained from *Aspergillus niger* (Ulbrich-Hofmann and Selisko, 1993). Its marked stability and activity in organic solvents suggests that this amyloglucosidase is highly suitable as a biotechnological tool with a variety of applications including organo synthetic reactions and preparation of enantiomerically pure pharmaceuticals.

Physical properties

The molecular weight of the purified enzyme was determined by SDS-PAGE and gel filtration. The purified enzyme appeared as a single band corresponding to molecular weight of 61 kDa (*Figure 5*). This value was similar to that revealed by gel filtration. Interestingly, our fungal strain produces single form of amyloglucosidase as indicated by single band corresponding to 6l kDa in SDS PAGE. A lot of variations in the molecular weight of glucoamylases ranging from 49 to 112 kDa in different organisms have been reported (Selvakumar et al., 1996).

Present study was undertaken with the aim of purification of amyloglucosidase produced by solid state fermentation using tapioca powder as substrate for use in bioethanol fermentation in distilleries as a substitute of molasses. By employing solid state fermentation, amyloglucosidase can be produced at low cost along with utilization of unused tapioca powder. Amyloglucosidase from fungal isolate was purified to 68 fold with standard biochemical techniques. The temperature and pH profile of purified enzyme make it suitable enzyme for carrying out simultaneous saccharification and fermentation at one go in the industry where temperature maintenance during fermentation is a big challenge to ask. Moreover, storage of enzyme in refrigerator even for a month without significant loss in enzyme activity gives additional advantage as far as economy of process is concerned in industry.

CONCLUSION

This is the first report on purification of amyloglucosidase produced by *Aspergillus awamori* under SSF with tapioca powder as substrate. Our strain produced very high yield of amyloglucosidase i.e. 347 U/g under standardized optimized conditions of SSF. The present work would be continued for optimization of starch based

bioethanol production in alcohol industry using this enzyme preparation.

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