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High-yield production and biochemical characterization of α-galactosidase produced from locally isolated *Penicillium sp.*

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Abstract

Background: α-Galactosidase is widely used in various biotechnological applications such as food processing, beet sugar, the pulp and paper industries, synthesis of oligosaccharides by trans-galactosylation, hydraulic fracturing of oil and gas wells, and medical applications.

Results: Screening and identification of fungi for α-galactosidase activity was performed. The isolate *Penicillium* sp. showed good α-galactosidase activity. α-Galactosidase production by the fungal strain *Penicillium* sp. cultivated in solid state fermentation (SSF) conditions using copra mannan extract as nutrient medium was investigated. The maximum α-galactosidase activity of 5.391 U/mL was obtained in defatted copra meal (dFCO) as carbon source, which is 2–3% greater as compared with commercial mannans and unprocessed copra meal. The highest product yield of α-galactosidase was obtained with media containing yeast extract (6.672 U/ml) as organic nitrogen and ammonium nitrate (6.325 U/ml) and as inorganic nitrogen source with media pH 5.5, and the time course of enzyme production was at the 5th day of fermentation, respectively. The optimum pH of α-galactosidase was obtained at pH 5 and optimum temperature at 60 °C. The enzyme was stable between pH 4 and 6 and retained more than 50% of residual activity for an 8-h incubation period. The Ca^{+2} ions enhanced the enzyme activity and Mn^{+2} ions have not altered the enzyme activity, whereas Hg^{+2} strongly inhibited the enzyme activity.

Conclusions: The findings of present investigations on α-galactosidase are of particular interest for its application in the food processing industry.

Keywords: α-Galactosidase, *Penicillium* sp., Copra meal, Food processing

Background

The enzymes responsible for hydrolyzing galactosidic linkages are termed as galactosidases. There are two types of galactosidases, viz., α-galactosidases and β-galactosidases depending on the configuration of the anomeric carbon atom of galactose in the substrate molecule on which they act. The α-galactosidases (α-D-galactosidegalactohydrolase, EC 3.2.1.22) are carbohydrases that catalyze the hydrolysis of α-galactosidic linkages in galacto-oligosaccharides such as raffinose, melibiose, stachyose, verbascose and ajugose, polysaccharide-like galactomannans, and glycoconjugates such as glycoproteins and glycolipids. For ceramide-trihexo-saccharides, their higher homologous and derivatives are also cleaved by the action of α-galactosidase (Katrolia et al. 2014; Dey and Pridham 1972.).

α-Galactosidases have widely used in both biotechnological and medical applications. For instance, α-galactosidases offer a promising solution for the degradation of raffinose family oliogo-saccharides (RFOs) present in soy beans and other leguminous food and feed that cause intestinal discomfort, flatulence, and low feed utilization in monogastritc animals (Naganagouda and Mulimani 2006; Naganagouda et al. 2007). In beet sugar industry, α-galactosidases are used to remove

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raffinose from beet molasses increasing the sucrose yield (Shibuya et al. 1997). α-Galactosidase is also used to improve the gelling properties of galactomannans to be used as food thickeners (Chen and Mustapha 2012). The enzymic conversion of RFOs in soymilk may be a rational alternative to improve the nutritional quality of this low-cost, high-quality protein supplement for humans and animals (Farzadi et al. 2011). In the pulp and paper industry, α-galactosidase could enhance the bleaching effect of β-mannanases on softwood pulp (Clarke et al. 2000). Furthermore, these enzymes are used for the treatment of Fabry's disease (Kang et al. 2019), in xenotransplantation (Liu et al. 2007) and in blood group transformation for safety transfusion (Balabanova et al. 2010).

α-Galactosidases are widely distributed in nature among plants, animals, and microorganisms. Due to high expression levels, extracellular secretion, ease of cultivation, and scope for improvement of yield by optimization of culture conditions, several microorganisms have been exploited for the production of α-galactosidase for use in various biotechnological and medical applications. α-Galactosidase from bacteria, especially probiotic bacteria like bifido and lactic acid bacteria are used as "live cultures" in fermented soymilk for removal of RFOs or in the production of α-galactosidases (Farzadi et al. 2011; Scalabrini et al. 1998).

The huge amounts of low-cost byproducts are generated by agro-industrial activities and these are susceptible to be transformed in culture media for the production of high-value-added bio-products such as ethanol and other biofuels, enzymes, and heterologous proteins (Álvarez-Cao et al. 2018; Álvarez-Cao et al. 2019).

Among filamentous fungi, in literature, the distribution of α-galactosidase in fungi, with generally regarded as safe (GRAS) status, such as *Aspergillus* and *Saccharomyces* sp. have been reported for the production of α-galactosidases because they can be cultivated on cheap agricultural residues and usually secrete high levels of enzyme into the culture media, which contribute a great deal in reducing the cost of enzyme production (Shankar and Mulimani 2007) and their application in commercial use in the food and feed industries.

The cost of the enzyme actually limits the profitability of most of the above cited applications. The use of cheap sub-products, such as molasses and whey, as substrates for production of *Saccharomyces cerevisiae* α-galactosidase (ScAGal) was previously reported and might favor the economy of the processes (Álvarez-Cao et al. 2018). The use of residues as culture media to produce the required enzymes can help with the economy of the process. Copra mannan is a sub-product of coconut industry after coconut oil extraction, rich in manno and galacto-oligosaccharieds.

The aim of the present study is to evaluate the production of α-galactosidase from the locally isolated fungal cultures. Further, there are very few reports using copra mannan as a source for the production of α-galactosidase. Therefore, the present investigation was carried out for the production of α-galactosidase from selected fungal strain under solid state fermentation. Further, the enzyme was studied with the biochemical characterization.

Methods

Chemicals

Locust bean gum (LBG) and guar gum were obtained from Sigma, USA. All other reagents used were of analytical grade. All solutions were prepared in double-distilled water obtained from a distilled water plant (Distillon 4S).

Preparation of buffers

Buffers were prepared according to Gomori (1946). All the preparations were carried out using double-distilled water and 0.5 M solution of potassium hydrogen phthalate was used to standardize pH meter.

Preliminary screening and identification of fungi

Different soil samples collected locally from garden and composts were used in the present study to isolate galactosidases producing fungi. The fungal strains were isolated from soil samples using dilution-plating technique. One gram of garden and compost samples was mixed in 9 ml distilled water. This suspension was serially diluted to 10^{-4}. One milliliter of the diluted samples from 10^{-3} to 10^{-4} dilutions was plated on sterile copra meal agar plate (Copra meal, 1 g; agar, 15 g; $NaNO_3$, 1 g; K_2HPO_4, 1 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; yeast extract, 1 g in 1000 ml distilled water and to eliminate the bacterial contamination, 0.080 g or 0.008% streptomycin was added to one of the medium) surface and incubated at 37 °C for 3–4 days. Microscopic observation was performed to determine the morphological characteristics of the fungal isolates.

Secondary screening

Those isolated organisms from the preliminary screening were cultured in liquid media containing 2% locust bean gum, 0.1% K_2HPO_4, and 0.05% $MgSO_4{\cdot}7H_2O$ at pH 5.5 in an Erlenmeyer flasks. After incubation on a rotary shaker (37 °C, 180 rpm) for 7 days, the culture broth was centrifuged (12085.2×*g* for 20 min) and the supernatant was collected for enzyme assay. Among 3 isolates tested, one isolate was found to be a potent galactosidase producer and were designated as *Penicillium* sp. The strains were maintained on potato dextrose agar and used for further study on α-galactosidase production.

Maintenance and propagation of fungal cultures

The fungal cultures were maintained and propagated on potato dextrose agar (PDA) medium.

Enzyme production

Inoculum preparation

The spore suspension was prepared by adding sterilized distilled water containing 0.01% Tween-80 and scrapping the spores of *Penicillium* sp. from 7-day grown slants.

Medium

The following medium was used. The composition of medium (g/liter) was as follows:

K_2HPO_4, 3 g
$MgSO_4{\cdot}7H_2O$, 0.5 g
Yeast extract, 5 g
Guar gum, 20 g
The pH of the medium was adjusted to 5.5.

Submerged fermentation

Different carbon sources like commercial mannans (LBG and guar gum), simple sugars (glucose, sucrose, lactose, galactose, mannose and xylose), copra meal, and defatted copra meal were used as potential inducers of the enzyme activities in *Penicillium* sp. For batch culture, 50 ml of basal medium was taken in a 250-ml Erlenmeyer flask and sterilized. After autoclaving, the flasks were inoculated with spores (2×10^6) of *Penicillium* sp. The flasks were incubated at 37 °C for 5 days on an orbital shaker at 120 rpm. The mycelium was removed from culture broth by filtration through muslin cloth followed by Whatman No. 1 filter paper and the clear supernatant phase was used as crude α-galactosidase.

Enzyme assay

α-Galactosidase activity was carried out according to method of Dey and Pridham (1972). One milliliter of reaction mixture contains 100 μl of suitably diluted enzyme with 50 μl of 10 mM chromogenic substrate (p-nitrophenyl α-D-galactopyranoside, PNPG) and 850 μl of 0.2 mM phosphate buffer pH (7.5) at 50 °C for 10 min. The reaction was terminated by adding 2 ml of 0.2 M sodium carbonate solution. The amount of p-nitrophenol released was estimated from absorbance at 405 nm. Enzyme activity was expressed as the amount of enzyme required to liberate 1 μmol of product per minute under the assay conditions.

Defatting methods of copra

In order to remove the oil content from the copra, the treatment of copra was carried out according to the methods of Lin and Chen (2004). The copra was finely ground with a grinder for 5 min and sieved (30 mesh); the powder was designated as CO. CO was boiled for 2 h with two volumes of distilled water. The cooled CO suspension was then placed at 4 °C overnight to allow the oil to solidify and finally be removed. The dried and sieved residues were designated as FCO. FCO was then defatted by the solvent extraction using n-hexane for 24 h. One liter of solvent n-hexane was mixed with 100 g of ground copra in a beaker and left overnight. The copra suspension was then filtered through Whatman No. 1 filter paper. The product was designated as dFCO, after the residues were oven-dried and sieved. All samples were kept in a desiccator until used.

Biochemical characterization of α-galactosidase

Optimum pH

In order to determine optimum pH, 100 μl of suitably diluted (in 0.2 M acetate buffer of pH 5) enzyme was incubated with 900 μl LBG substrate prepared in different buffers ranging from pH 3.0 to 9.0 for 20 min at 50 °C. The following range of buffers was used: pH 3.0–5.6 (acetate), pH 5.8–7.2 (citrate-phosphate), and pH 7.2–9.0 (tris-HCl buffer). The initial pH of the fermentation medium was set to 3.5, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 before inoculation. The pH was monitored but not controlled during the course of fermentation.

pH stability

To determine the pH stability, the enzyme was incubated with substrate at the desired pH for 16 h at 4 °C. Residual activity was calculated in each sample by α-galactosidase against enzyme control sample at optimum pH.

Optimum temperature

Optimum temperature was determined by incubating 100 μl of suitably diluted enzyme and 900 μl of 0.5% LBG (prepared in 0.2 M acetate buffer pH 5.0) substrate for 20 min at different temperatures ranging from 30 to 80 °C.

Thermostability

For determination of thermal stability, the enzyme was pre-incubated with the substrate without any stabilizers at 60 °C. Residual activity in each sample was calculated by doing assay against enzyme control sample at pH 5.0 and 60 °C.

Effect of nitrogen source

Effect of various inorganic, organic (0.5% in the medium), and combined nitrogen sources (0.25% each in the medium) for the production of alpha-galactosidase (using 2% defatted copra meal, dFCO) was carried out.

Effect of metal ions

The metal ions to be tested were added to the assay system (5 mM) and the enzyme assay was carried out. The results are expressed as a percentage of control.

Results

Screening and identification of fungi for α-galactosidase activity

Totally three α-galactosidase-producing fungal strains were identified in preliminary screening. Active α-galactosidase-producing strains that formed colonies on the copra meal agar plate were selected for secondary screening. In the secondary screening, the liquid medium with locust bean gum (LBG) as sole carbon source was used for the isolation of potent α-galactosidase-producing strains. In preliminary screening, a total of three isolates were obtained from soil sample. In secondary screening, out of three isolates, one of the isolate (*Penicillium* sp.) showed good α-galactosidase activity and these strains were selected for detailed taxonomical and optimization studies.

Optimization of culture parameters for the production of α-galactosidase from *Penicillium*

Optimization of composition of culture media and optimization of culture parameters like pH of media and incubation period are important for obtaining high enzyme activity from *Penicillium* sp.

Effect of carbon sources and enzyme induction studies

Results of these α-galactosidase activities are given in Table 1 and Fig. 1. It is evident that copra meal defatted (dFCO) was the best inducer of α-galactosidase synthesis in *Penicillium* sp. The use of commercial mannan-like locust bean gum and guar gum as substrate is uneconomical for large-scale production of alpha-galactosidase. In order to use locally available copra waste into value-added products like enzymes, in this study, defatted copra meal was used as potent inducer of α-galactosidase production. Among commercial mannans used for induction of α-galactosidase, 2% LBG induced maximum production of α-galactosidase (4.784 U/ml), then 2% guar gum (3.126 U/ml). Copra meal without defatting will not support the fungal growth because of high oil content, which inhibited the mannanase production. Coconut oil separated the culture broth from contacting with air and amount of dissolved oxygen in the cultural broth decreased, which in turn affected the growth of the fungi. The simple sugars like glucose, sucrose, galactose and xylose will not induced the enzyme production. The monosugars could cause catabolite repression, as reported for *Penicillium* sp. Hence, induction of enzyme activity was not observed. The enhanced production of enzyme was observed in *Penicillium* sp. using 2% defatted copra meal as carbon source compared to LBG.

Table 1 Effect of different carbon sources on production of α-galactosidase

Carbon source (2% w/v)	Maximum α-galactosidase activity (U/ml)
	Penicillium sp.
Commercial mannans	
Locust bean gum	4.784 ± 0.29
Guar gum	3.126 ± 0.21
Copra mannans	
Copra meal (CO) (unprocessed)	1.471 ± 0.09
Copra meal (dFCO) sxcv (defatted)	5.391 ± 1.30
Simple sugars	
Glucose	0.001 ± 0.00
Sucrose	0.004 ± 0.00
Lactose	0.021 ± 0.00
Galactose	0.001 ± 0.00
Mannose	0.001 ± 0.00
Xylose	0.001 ± 0.05

Results are the representative of average of three experiments in duplicate SE at 5% level

Effect of nitrogen source

The effect of various inorganic and organic nitrogen sources on alpha-galactosidase synthesis was also studied and the results are given in Table 2. From the above findings it is clear that the *Penicillium* sp. showed significant enzyme activity even in the absence of any organic and inorganic nitrogen sources. The highest product yield of α-galactosidase was found to be 6.736 U/ml.

Effect of initial pH of medium on enzyme production

Penicillium sp. grows well at acidic pH and produces α-galactosidase having good activity between pH 5 and 6 with enzyme showing high optimum temperature for its activity.

The aim of the present investigation was to study the effect of different ranges of pH on the production of α-galactosidase by *Penicillium* sp.

Time course of α-galactosidase production

The time course of α-galactosidase production was studied. The results presented in Fig. 2 showed that α-galactosidase was maximally produced at the 5th day of fermentation, in optimized and non-optimized medium. When the fungal cultures were cultivated on optimized media with defatted copra meal as carbon source, the peak in α-galactosidase production on optimized media was on day 5 for the *Penicillium* sp. The measured

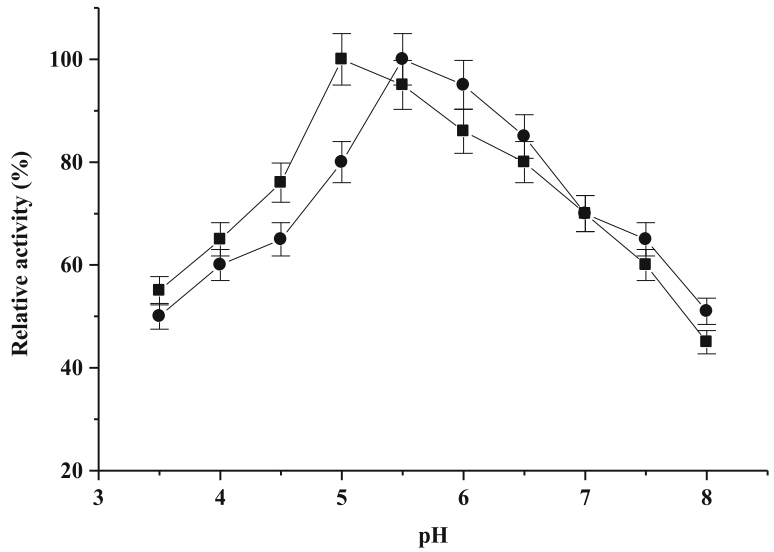

Fig. 1 Alpha-galactosidase production by *Penicillium* sp. (black square) *at different initial pH*

enzyme activities were 40.01 U/ml and 33.126 U/ml, respectively. As pointed, α-galactosidase activity was stable up to the 5th day and after which the enzyme activity fell possibly due to shift of pH and catabolic activity of the organism.

Table 2 Effect of various nitrogen sources on production of alpha-galactosidase (using 2% defatted copra meal, dFCO)

Nitrogen source (0.5% in the medium)	Maximum mannanase activity (U/ml)
	Penicillium sp.
Inorganic nitrogen source	
Ammonium sulfate	5.729 ± 1.39
Ammonium nitrate	6.325 ± 1.55
Sodium nitrate	5.446 ± 1.25
Potassium nitrate	5.623 ± 1.30
Ammonium acetate	5.429 ± 1.14
Urea	5.463 ± 1.30
Organic nitrogen source	
Yeast extract	6.736 ± 1.62
Beef extract	5.821 ± 1.30
Peptone	5.916 ± 1.47
Casein	5.431 ± 1.30
Soybean meal (defatted)	5.402 ± 0.99
Combined form*	
Yeast extract + ammonium nitrate	6.672 ± 2.00
Peptone + ammonium nitrate	5.838 ± 1.80
Casein + ammonium nitrate	5.841 ± 1.43
Soybean meal + ammonium nitrate	5.624 ± 1.30

Results are the representative of average of three experiments in duplicate
SE at 5% level

Characterization of α-galactosidase from isolated *Penicillium* sp.

Optimum pH

It is well established that the hydrogen ion concentration of enzymatic reaction mixture influences the rate of reaction with the substrate. The pH optimum was determined by using different buffers of pH ranging from 3.0 to 8.0. The pH activity profiles for the strain *Penicillium* sp. are shown in Fig. 3. From the figure, it is observed that the enzyme was considerably active from pH 3.5 to 6.0 and maximum activity of α-galactosidase was obtained at pH 5.0. The enzyme activity was gradually increased from 3.5 to 5.0. There was gradual decrease in enzyme activity after pH 5.0.

pH stability

The effect of pH on the stability of the enzyme is depicted in Fig. 4. The enzyme from *Penicillium* sp. was incubated in buffers of varying pH for 16 h at 4 °C and the residual activity was assayed. The enzyme was stable between pH 4.0 and 7.0 and retained about 90% of its activity (Fig. 4).

Effect of temperature

Temperature is a critical variable in any enzymatic process, producing opposite effects by simultaneously increasing enzyme reactivity and inactivation rate. As a consequence, an optimum temperature will exist for any enzyme catalyzed process where those effects are optimally balanced. The temperature dependence of α-galactosidase was determined by incubating the enzyme in 0.2 M acetate buffer (pH 5.0). The effect of temperature on enzyme activity profile was shown in Fig. 5. It is clear that, the enzyme showed optimum

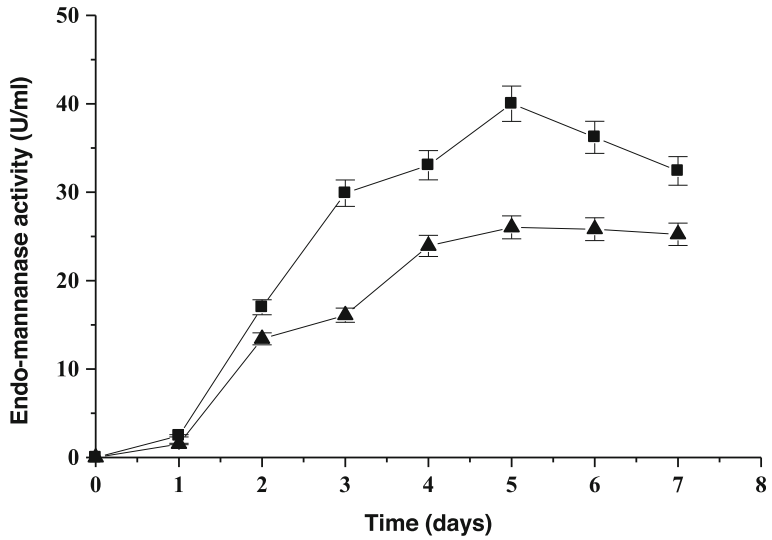

Fig. 2 Time course of endo-β-1, 4 mannanase production by *Penicillium* sp. in SmF on defatted copra meal before and after optimization (black square indicates optimized and black triangle, non-optimized)

activity at 60 °C, whereas α-galactosidase from the source *Penicillium* sp. exhibited its optimal activity at 65 °C.

α-Galactosidase from *Penicillium* sp. exhibited thermo stability up to 60 °C, which appears to be a characteristic shared by other bacterial α-galactosidase.

Thermal stability

The effect of thermostability on α-galactosidase produced from *Penicillium* sp. is displayed in Fig. 6. The enzyme was pre-incubated in 0.2 M acetate buffer (pH 5.5) at 60 °C for different incubation time, i.e., 0 h to 12 h without any stabilizers. From the Fig. 6 it is noted that α-galactosidase produced from the *Penicillium* sp. showed good thermal stability. The enzyme retained more than 50% of residual activity for 8 h incubation period.

Effect of metal ions

Table 3 shows the effect of metal ions on α-galactosidase activity. It is clear that α-galactosidase from the source was strongly inhibited by Hg^{+2}. Inhibition by mercuric ions suggested that the enzyme contains an essential sulfhydryl group. Significant inhibitory effect was also observed in the presence of Ag^{+}, Zn^{+2}. Ca^{+2} enhanced the α-galactosidase activity in the strain.

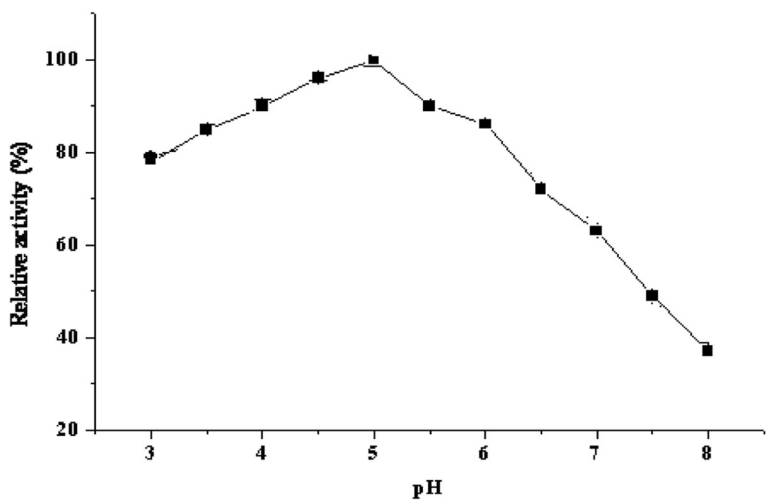

Fig. 3 Effect of pH on alpha-galactosidase activity in *Penicillium* sp.

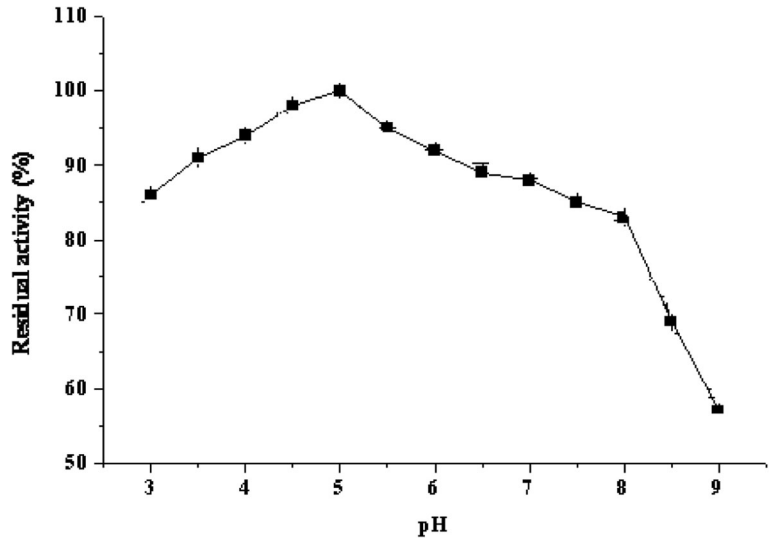

Fig. 4 pH stability of alpha-galactosidase in *Penicillium* sp.

Discussion

A screening method for detection of α-galactosidase-producing microorganism had been studied. Similar to ours, findings by Youssef et al. (2006), Hossain et al. (1996), and Lin and Chen (2004) have isolated the *Aspergillus* fungal cultures for β-mannanase activity using copra meal as a carbon source for cultivation. The biotechnological applications of α-galactosidase includes the removal of raffinose family oligosaccharides (RFOs) in soymilk, beet sugar industry, flatulence, blood group conversion, etc. (Raja et al. 2020; Bhatia et al. 2019).

Carbon occupies a unique position among the essential elements required by microorganisms. When it is consumed, it undergoes three metabolic changes, viz., conversion into cell substances, carbon dioxide and accumulation in several metabolic products. Based on the type and nature of carbon source in the medium, the organism tries to grow and thus produces number of enzymes.

Similarly, Lin and Chen (2004) have made observation that fourfold enhanced production of mannanase by growing *A. niger* in medium containing 2% defatted copra meal. Therefore, based upon the same amount of carbon sources, the defatted copra carried the higher mannan content, which would induce more enzyme production by the microorganisms. Copra waste was apparently a better substrate because of its high reducing sugar content. Defatted copra also contained more protein and minor elements, which were more beneficial to fungal growth than other carbon sources. The medium must be designed to provide the essential elements

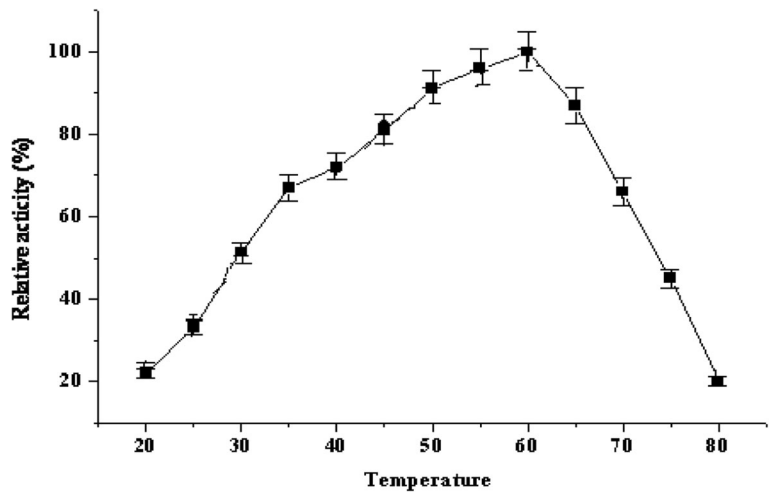

Fig. 5 Effect of temperature on the production of alpha-galactosidase in *Penicillium* sp.

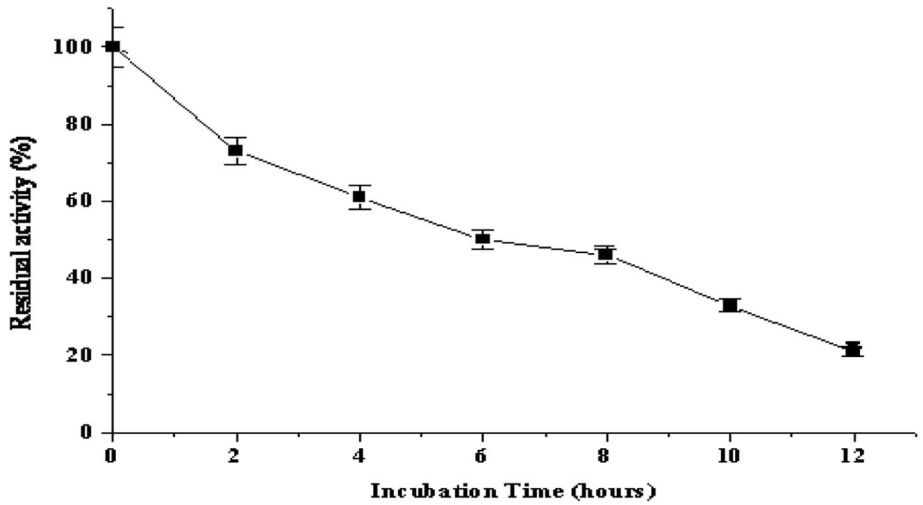

Fig. 6 Thermo stability of alpha-galactosidase from *Penicillium* sp.

carbon, nitrogen, sulfur, oxygen, phosphorous, magnesium, calcium, and numerous other trace elements such as iron, copper, cobalt, zinc, manganese, and molybdenum, which may be required to support active culture function. However, it is highly important in formulating an effective medium for use in a competitive enzyme production process to produce one which is both cheap and where possible, reproducible over a long production period. These relate to the phenomenon of induction, catabolite repression, and end product inhibition and in case of extracellular enzymes and protein release mechanisms.

Similar to our findings, Anisha (2017) have reported highest α-galactosidase obtained in submerged fermentation on various carbon sources.

The mechanisms that govern the formation of extra cellular enzymes are influenced by the availability of precursors for protein synthesis. Furthermore, the nitrogen source can significantly affect the pH of the medium during the course of fermentation (Lin and Chen 2004).

Table 3 Effect of metal ions on α-galactosidase activity

Metal ion (1 mM)	% Relative activity *Penicillium* sp.
Ag^{+2}	13 ± 1.30
Hg^{+2}	11 ± 1.10
Zn^{+2}	15 ± 1.50
Mn^{+2}	100 ± 10.00
Co^{+2}	55 ± 5.50
Ca^{+2}	120 ± 12.00
Pb^{+2}	43 ± 4.30
Mg^{+2}	31 ± 3.10

Results are the representative of average of three experiments in duplicate SE at 5% level

Nitrogen is the principal element governing cell formation and synthesis of various constituents. It influences the synthesis of various enzymes especially in microorganisms, which are either accumulated inside the cell or excreted into the culture medium. The type of nitrogen and also its concentration influences markedly the yield and the rate of enzyme production.

These results are in concordance with the data of many investigators, who have reported that fungi produce more enzymes on addition of complex organic nitrogen sources (Anisha 2017; Naganagouda et al. 2009). Concerning the inorganic nitrogen sources used as shown in Table 2, a maximum activity was observed with ammonium nitrate in the *Penicillium* sp. (6.325 U/ml). In case of organic nitrogen sources, a maximum activity was observed with yeast extract rather than peptone, beef extract, casein, and soybean meal (Table 2), respectively. In addition yeast extract showed a maximum production comparable with different inorganic nitrogen sources.

Among several cultural parameters influencing the product formation by microorganisms, the hydrogen concentration of the culture medium is considered extremely important. The higher concentration of hydrogen or hydroxyl ions exhibits the growth of microbial culture and thereby influence the yield of enzyme or metabolite production. There are distinct three types of microorganisms according to their tolerance of hydrogen and hydroxyl ions. These are acidophilic, alkalophilic, and mesophillic strains which grow at varying ranges of pH in the cultivation medium.

The influence of age of the culture is very important in influencing the growth as well as product formation either extracellular or intracellular under shake or stationary conditions of fermentation.

Denaturation of enzyme possibly due to prolonged incubation could be the reason. Lin and Chen (2004) have compared time course of β-mannanase production by *Aspergillus niger* in shake cultures. They further reported that in shake culture 2nd day maximum activity was obtained.

α-Galactosidase produced by the genus *Penicillium* sp. and indeed most fungi have pH optima within the range of acidic pH ranging from 2.5 to 6.0. Slightly acidic pH optima of the enzymes of the present investigation match the values characteristic for the fungal glycoside hydrolases (Christov et al. 1999).

α-Galactosidases from various fugal sources exhibit acidic pH optima and several fungal α-galactosidases are stable over a wide range of pH (pH 3–12), which is a favorable property for biotechnological applications (Cao et al. 2009; Janika et al. 2010; Katrolia et al. 2012).

α-Galactosidase produced from mesophilic and thermophilic fungi adapted to wide range of temperatures ranging from 50 to 65 °C (Kotiguda et al. 2007; Simerska et al. 2007; Rezessy-Szabo et al. 2007; Viana et al. 2009).

Similar to our findings several researchers reported that thermostable α-galactosidases are useful in food processing and other biotechnological applications, where operational conditions can denature thermo-labile enzymes (Brouns et al. 2006; Pessela et al. 2007).

Generally, metal ions have been shown to be relevant in enzyme activity. It has been reported that some metal ions such as Ag^{+}, Cr^{+3}, Cu^{+2}, Fe^{+3}, Hg^{+2}, Mn^{+2}, Pb^{+2}, and Zn^{+2} inhibited α-galactosidase activity (Gote et al. 2006; Álvarez-Cao et al. 2018).

Conclusions

Based on our findings from the present study, we conclude that the locally available copra waste can be economically employed as substrate in SSF for the production of α-galactosidase by *Penicillium* sp. Cheap and easily available media, which is very economical, was formulated. The fermentation medium containing 2% defatted copra meal was found to be the best medium along with yeast extract and ammonium nitrate for obtaining high enzyme titers by *Penicillium* sp. when compared to commercial mannans, viz., LBG, guar gum, etc. Optimization of culture parameters for obtaining maximal enzyme titers will lead to scale up of process for obtaining α-galactosidase in large quantities for industrial use. The fungus *Penicillium* sp. does not produce toxins; their metabolic products enjoy generally recognized as safe (GRAS) status, and this can be used in the food industry. Further work for its complete application of α-galactosidase and its application in food industry would be conducted with the aid of other biochemical techniques.

Abbreviations

sp.: Species; SSF: Solid state fermentation; CO: Finley grinded copra meal; FCO: Dried and sieved residues of copra meal; dFCO: Defatted copra meal; RFOs: Raffinose family oliogo-saccharides; GRAS: Generally regarded as safe; LBG: Locust bean gum; PDA: Potato dextrose agar; PNPG: p-Nitrophenyl α-D-galactopyranoside

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Authors' contributions

NVK designed this research and performed the experiments. ACM, VT, and AGG guided and supervised the research work. NVK, ACM, and VT wrote the first draft and analyzed the result. AGG read the manuscript for correction. All authors read and approved the final manuscript.

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The authors declare that they have no competing interests.

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