Indian Journal of Experimental Biology
Vol. 54, January 2016, pp. 72-75

Note

Production of α-galactosidase from *Aspergillus foetidus* MTCC 6322 by solid state fermentation and its application in soymilk hydrolysis

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Received 13 January 2014; revised 27 October 2014

The production of α-galactosidase from the wild fungal strain *Aspergillus foetidus* MTCC 6322 using solid state fermentation (SSF), its characterization, and its efficacy in the hydrolysis of soymilk using response surface methodology were studied. The optimum conditions for production of α-galactosidase by SSF were: wheat bran (10 g), moisture content (64%), inoculum volume (1.0 mL; 6×10^7 spores/mL) with a yield of 4.1×10^3 units per gram dry substrate (U/gds) at 96 h. The enzyme showed optimum activity at pH 6.0, temperature 40°C, pH stability between 5.0-8.0, and temperature stability between 30-40°C. The enzyme was stable in the presence of trypsin, lipase, and collagenase and it showed susceptibility of the substrates such as raffinose, melibiose, guar gum and soymilk to hydrolysis in varying degrees. The optimized conditions for soymilk hydrolysis were: soymilk (10 mL) from defatted soybean meal (1.5%), α-galactosidase (0.15 UmL^{-1}) at 30°C, pH 6.0 and duration of 1 h.

Keywords: *Aspergillus foetidus* MTCC 6322, α-Galactosidase, Solid state fermentation (SSF), Soymilk hydrolysis, Response surface methodology (RSM)

α-Galactosidase catalyzes the hydrolysis of α-1,6-galactoside bonds, and release α-D-galactose[1]. Also, it hydrolyzes α–galactosidic linkages of raffinose and stachyose in the soymilk, and thereby improves its nutritive value[2]. Microorganisms are potential source of various enzymes[3-9]. There are few reports on enzyme production by solid state fermentation (SSF)[5,7,10] including α-galactosidase[11]. The SSF has been reported to have more advantages over submerged fermentation such as agricultural wastes as substrates, providing natural growth conditions, etc.[5,7]. However, there are no reports on the biotechnological applications of α-galactosidase from the wild strain *Aspergillus foetidus*. Production of α-galactosidase by SSF by a mutant strain of *Aspergillus foetidus* ZU-G1 using RSM[12] and the hydrolysis of soymilk is reported[13]. In this communication, we report efficient production of α-galactosidase from the fungal strain *Aspergillus foetidus* MTCC 6322, its characterization and application in optimization of soymilk hydrolysis.

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CSIR-CLRI Communication No.1022

Materials and Methods

Production, extraction and assay of α-galactosidase

The fungal culture *Aspergillus foetidus* MTCC 6322 reported to be a potent producer of tannase[14], was used in this study. About 10 g of substrate in 250 mL Erlenmeyer flask were moistened with 15 mL distilled water, initial pH 5.0 and sterilized. It was inoculated with 1 mL of *A. foetidus* MTCC 6322 spore suspension (6.0×10^7 spores/mL) and mixed thoroughly. The flasks were incubated at 30°C for 5 days. For extraction of the enzyme, 1 g of moldy bran was mixed with 10 mL of 0.2 M acetate buffer, pH 5.0. The mixture was filtered through muslin cloth and the filtrate obtained was centrifuged at 10000 rpm for 10 min at 4°C. The resulting supernatant was used as the enzyme source. All the experiments were carried out in triplicate. Enzyme activity was assayed by the method as described earlier[15]. One unit (U) of enzyme activity was defined as the amount of enzyme, which produced 1 μmol of *p*-nitrophenol/min at 405 nm under assay conditions. α-Galactosidase production under SSF was expressed as U/gds.

Characterization and application of α-galactosidase

α-Galactosidase produced by *Aspergillus foetidus* MTCC 6322 on a range of solid substrates was compared keeping other conditions constant. The physico-chemical characteristics of α-galactosidase were studied. α-Galactosidase activity at different pH (3.0-9.0), temperature (20-70°C), pH stability and temperature stability were also carried out. Stability in the presence of other enzymes (1:1) such as lipase (Sigma), trypsin (Hi-Media), amylase (SPIC), protease (SPIC) and collagenase (Sigma) was carried out and the relative activity was determined. The characteristic susceptibility of the substrates to hydrolysis by α-galactosidase was also studied.

Different substrates such as raffinose, melibiose, guar gum powder, soybean meal in 0.2 M acetate buffer (pH 6.0) at the concentration of 1% (10 mL) were hydrolyzed by 0.15 U/mL of α-galactosidase at 37°C for 1 h and the reducing galactose was estimated by the method of Miller[16].

Soymilk was prepared according to the modified method[17]. About 1% defatted soybean meal (Hi-Media) was suspended in 100 mL of 0.2 M acetate buffer (pH 5.0) and heated to boil for 20 min followed by centrifugation for 10 min at 8000 rpm. The supernatant containing soymilk was used for further experimentation of hydrolysis. For hydrolysis reaction, 10 mL of soymilk from defatted soybean meal (1%) and 0.15 U/mL of α-galactosidase were incubated for 1 h at 37°C and the reaction was terminated by placing the mixture in boiling water bath for 20 min. The galactose liberated was estimated as reducing sugar by the method of Miller[16]. RSM was performed using the 'Design Expert' software package (Version 6.0.11, Stat-Ease Inc. Minneapolis, USA).

Results and Discussion

The production of α-galactosidase by SSF using various substrates are reported (Fig. 1). Among different substrates used, wheat bran supported maximum production of α-galactosidase with yield of 3.3×10^3 U/gds. Substrates, such as wheat bran and soybean meal[12], soyflour[11], red gram plant waste[18], soybean vinasse[1] have been reported for α-galactosidase by SSF. In this study, wheat bran was chosen as the best substrate in contrast to an earlier report wherein wheat bran has been shown to be a 'not good' substrate[18]. The effect of moisture has profound influence on α-galactosidase production by SSF. A moisture content of 64% yielded higher α-galactosidase activity of 3.4×10^3 U/gds (Fig. 2). In the present study, the α-galactosidase activity is shown to be 4.1×10^3 U/gds using the strain *Aspergillus foetidus* MTCC 6322 as compared to the reported activity of 2.2×10^3 U/gds from *Aspergillus foetidus* ZU-G1[12].

α-Galactosidase activity is shown to be optimum at pH 6.0 and 40°C (Fig. 2). However, α-galactosidase from *Streptomyces* sp. S27 exhibited optimum activity at 35°C and pH 7.4[19] and the same authors have reported optimum activity at 50°C and pH 4.8 for α-galactosidase produced by *Rhizopus sp*[20]. The relative stability at different pH and temperature were in the range of 5.0 to 8.0 and 30-60°C, respectively. The present study is similar to an earlier report on pH stability studies where the enzyme retains more than 80% of its activity between pH 5.0 and 10.0[20].

The α-galactosidase activity has been found stable in the presence of trypsin, collagenase and lipase whereas it is low in the presence of protease and amylase. The latter ones are commercial enzymes used in leather process. In an earlier report, α-galactosidase has been shown to be stable in the presence of enzymes such as subtilisin A, proteinase K, collagenase, trypsin and α-chymotrypsin[20] whereas the same authors observed significant activity loss after incubation with trypsin and proteinase K and remained stable in the presence of α-chymotrypsin, subtilisin A and collagenase[19].

Experiment with different substrates such as raffinose, melibiose, guargum powder and soymilk in the presence and absence of α-galactosidase revealed soymilk to be the best for hydrolysis in

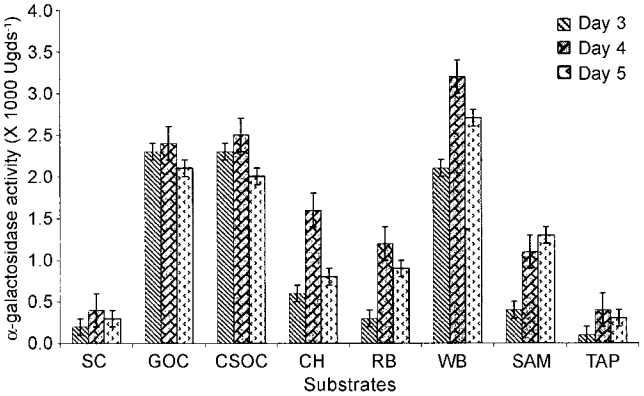

Fig. 1— Effect of different substrates on α-galactosidase production

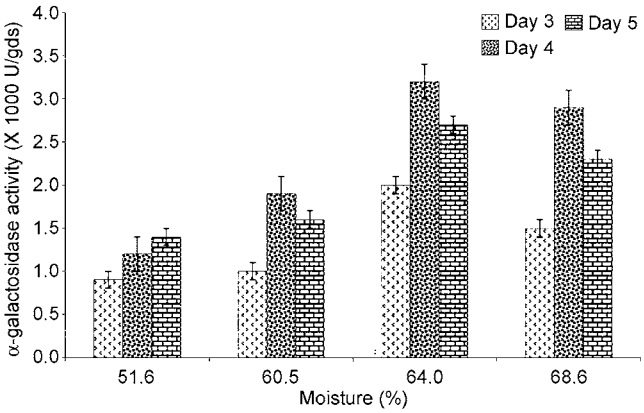

Fig. 2— Effect of moisture content on α-galactosidase production

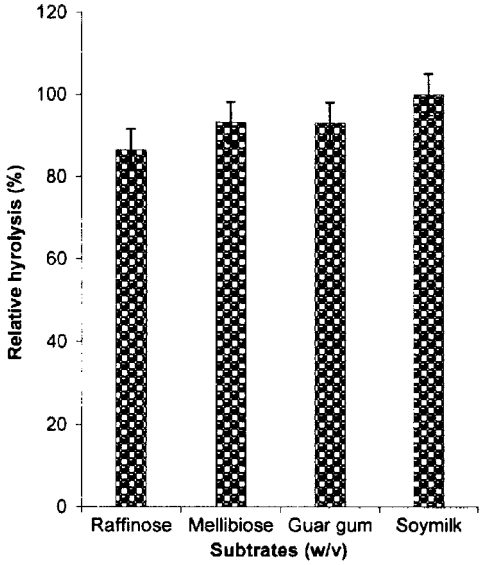

Fig. 3— Effect of α-galactosidase on different substrates

terms of galactose yield (Fig. 3). Hence, it was chosen for further study. Cao *et al*.[19] observed high hydrolysis ability of α-galactosidase from *Steptomyces* sp. S27 with melibiose, raffinose and stachyose whereas guar gum and locust bean could not be hydrolyzed.

The determination coefficient (R^2) of the RSM model was 0.8769 indicating that 87.69% of variability in the response could be accounted by the model. The parameters optimized for soymilk hydrolysis by RSM were soymilk 1.5 %; enzyme 1.5 U; temperature 30°C, pH 6.0; incubation period 1 h with reducing galactose of 0.374 mg/mL which is comparable with that of reducing sugar reported for α-gal I of α-galactosidase from *Aspergillus foetidus* ZU-G1[13].

Earlier, maximum hydrolysis of soymilk was reported at 50°C with the production of raffinose and stachyose at 2 h using α-galactosidse from thermophilic fungus *Humicola* sp.[15]. Raffinose family sugars present in soymilk were hydrolyzed to the extent of 79 and 66% by free and immobilized α-galactosidase from *Gibberella fujikuroi* within 3 h[21] whereas, 85% reduction was reported within 2 h of using soluble enzyme from *Aspergillus oryzae*[22]. Another study reports hydrolysis of soymilk to reducing sugars at 65°C within 2 h. Extensive hydrolysis to reducing sugars after treatment suggests its potential application in soymilk and related food industry[23], Hydrolysis treatment reported in the present study within 1 h at 30°C could be effectively used in the food industry.

Conclusion

The present study has demonstrated efficient production of α-galactosidase from the fungal strain *Aspergillus foetidus* MTCC 6322 using solid state fermentation (SSF) as well as its stability. The statistical optimization of hydrolysis of soymilk as shown in the above study has revealed its potential use in soymilk and related food industry.

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