Romanian Biotechnological Letters *Vol. 16, No. 5, 2011*

ORIGINAL PAPER

Optimization of parameters for α-amylase production under solid state fermentation by *Trichothecium roseum*

Received for publication, February 4, 2011
Accepted, April 11, 2011

BALKAN B.[1*], BALKAN S.[2] and ERTAN F.[3]
[1]*Vocational College of Health Services, Kırklareli University, Kırklareli, Turkey*
[2] *Science and Art Faculty, Departmen of Biology, Kırklareli University, Kırklareli, Turkey*
[3] *Science Faculty, Departmen of Biology, Trakya University, Edirne, Turkey*
[*] ***Corresponding author**: Dr. Bilal Balkan*
Tel: +90 288 214 38 25, Fax: +90 288 214 30 63
E-mail: bilalbalkan@hotmail.com

Abstract

The production of extracellular α-amylase by Trichothecium roseum was studied in solid state fermentation (SSF). The effects of wheat bran (WB), rye straw (RS), corncob leaf (CL), sunflower oil meal (SOM) and rice husk (RH) were examined. WB exhibited the highest enzyme production. The appropriate moisture level, incubation period, moistening agent, incubation temperature, inoculum level and particle size were determined. Effect of supplementary carbon, nitrogen sources and metal ions were examined. Optimal conditions for the production of α-amylase by T. roseum on WB as substrate in 0.1 M phosphate buffer at pH 7.0 were determined as initial moisture content of 85 % (w/v), incubation period of 8 days, incubation temperature of 30 °C, particle size of 1,000 μm, lactose and urea (1 % w/w) as supplements. Addition of different metal ions (0.1M) to WB resulted in better α-amylase production with $CaCl_2$, addition of $CuSO_4$ to WB resulted in decreased enzyme activities. Under the optimized culture conditions, the maximum enzyme production was 1,048 U/g of WB. One and a half increase in α-amylase productions were achieved in optimal fermentation conditions as compared with the medium containing WB alone as the substrate.

Key words: α-amylase, solid state fermentation, *Trichothecium roseum,* optimization

Introduction

Alpha amylases (endo-1, 4-α-D-glucanohydrolases, E.C. 3.2.1.1) are extracellular endo enzyme that randomly cleave the 1,4-α-linkages between adjacent glucose units in the linear amylose chain and ultimately generate glucose, maltose and maltotriose units [1]. It is also extensively used in starch liquefaction, paper, food, pharmaceutical and sugar industries. Amylases accounts for about 30 % of the world's enzyme production [2]. To meet the demands of these industries, low cost medium is required for the fermentation of α-amylase [3].

Traditionally, amylase has been produced by submerged fermentation (SmF) and used in a one-way process in solution. In recent years, however, solid state fermentation (SSF) processes have been increasingly utilized for the production of this enzyme [4]. SSF involves simplicity of the fermentation media, with fewer requirements for complex machinery, equipment or control systems. It also offers a greater compactness of the fermentation vessel owing to a lower water volume, greater product yield, reduced energy demand, lower capital, and low recurring costs under industrial operation. Easy scale-up processes, less downstream processing and superior yield, absence of foam build-up, and easier control of contaminants due to the low moisture levels in the system are additional advantages of SSF [5].

It is known that commercial carbon sources such as glucose and starch are not advantageous for production of α-amylase since they are very expensive. Therefore, several investigators have described the utilization of cheap and easily available agricultural residues

such as wheat straw, wheat bran, coffee waste, banana waste, potato peel, and sugarcane bagasse as carbon source in SSF for the production of α-amylases [6, 7, 8, 9].

Turkey, being an agricultural country, has abundant agricultural waste produced from different crop residues. Thus, the use of agricultural waste in α-amylase production will decrease the impact of agricultural waste on the environment. SSF has been used for the production of fine chemicals of commercial value from microbial sources, such as enzymes, antibiotics, flavoring compounds and microbial biomass for use as animal feed [10].

Bacteria, yeasts and fungi can grow on solid substrates and find applications in SSF processes. Filamentous fungi are the best adapted for SSF. The hyphal mode of fungal growth and their good tolerance to low water activity and high osmotic pressure conditions make fungi efficient and competitive in natural micro flora for bioconversion of solid substrates [11]. Due to the ever increasing demand of this enzyme, people are still trying to increase the productivity of amylases by a variety of approaches optimization, usage of cheaper substrates, effective downstream processing, etc [12].

The purpose of the present study was to investigate the production of α-amylase under SSF conditions. In this paper, we report a number of factors that influence α-amylase production by *Trichothecium roseum* through SSF. To the best over knowledge this is first report in α-amylase production from *T. roseum*.

Materials and Methods

Screening of α-amylase producing strains

39 fungi were isolated from air and morphological, physiological and biochemical characterizations were performed by Aydoğdu H. and Asan A. [13]. The screening procedure for α-amylase was based on a plate culture method which uses soluble starch (2%) as the sole carbon source. The screening plate medium contained 1.0 g $NaNO_3$, 1.0 g K_2HPO_4, 0.5 g $MgSO_4$.$7H_2O$, 0.01 g $FeSO_4$, 20 g soluble starch, and 25 g agar per liter. The initial pH was adjusted to 5.0. The medium was sterilized by autoclaving at 121 ^oC for 15 minutes. Fungi were plated on the agar medium and incubated at 30 ^oC for 7 days. Starch hydrolyzing activities were detected as clear zones after exposure to iodine.

Preparation of inoculum

The spores of *T. roseum* were dislodged from a fully sporulated (7 days old) PDA slant culture using 7 mL of sterile distilled water. The spores were dislodged using the inoculation needle under aseptic conditions and the suspension, with appropriate dilution, was used as inoculum.

Enzyme production in SSF

The SSF process was carried out in 250 mL Erlenmeyer flasks containing 5 g of WB. Distilled water was added in such a way that final substrate moisture content was 65 % (w/v). After sterilization by autoclaving, flasks were cooled and inoculated with a 10 % (v/w) inoculum level. The contents of the flasks were mixed thoroughly to ensure uniform distribution of the inocula and incubated at 30 ^oC for 7 days.

Enzyme extraction

From the fermented matter, crude enzyme was extracted by mixing a known quantity of fermented matter with distilled water on rotary shaker (220 rpm) for one hour. The slurry was squeezed through muslin cloth. The extract was filtered through Whatman filter paper No. 1. The suspension was then centrifuged at 7,000×g a 4 ^oC for 10 min and the supernatant was used for enzyme assay.

α-Amylase assay

Soluble starch (1.0 %) was dissolved in boiling 0.1 M acetate buffer (pH 5.0) and then cooled to 30°C. Fresh iodine reagent was prepared by diluting 1.0 mL of stock solution (0.5 % I_2 in 5.0 % KI) into 500 mL of distilled water containing 5 mL of 5 N HCL. For the assay, the reaction mixture consisting of 0.1 mL of enzyme and 0.2 mL of soluble starch were incubated at 40 °C for 10 min. The reaction was stopped by adding 5.0 mL of iodine reagent. The absorbance was measure at 620 nm against a blank [14]. One unit of the *α*-amylase activity was defined as the amount of enzyme that hydrolyses 0.1 mg of starch in a minute when 2.0 mg of starch is present, under the assay conditions while the enzyme productivity has been expressed as U/g of dry substrate. All the experiments were conducted in triplicate and the mean of the tree with standard deviation (SD) was represented.

Optimization of fermentation process under SSF

Various process parameters influencing enzyme production during SSF were optimized. The strategy followed was to optimize each parameter, independent of the others and, subsequently, optimal conditions were employed in all experiments.

In a sequential order, the various process parameters were optimized for maximal enzyme production as follows: various substrates [wheat bran (WB), rye straw (RS), corncob leaf (CL), sunflower oil meal (SOM) and rice husk (RH)], initial moisture content [45, 55, 65, 75, 85 and 95 % (w/v)], incubation period (3, 4, 5, 6, 7, 8, 9 and 10 days), moistening agents [acetate buffer (pH 4.0, 5.0, 6.0; 0,1M), phosphate buffer (7.0, 8.0; 0,1M) and distilled water (pH 6.5)], incubation temperature (20, 25, 30, 35 and 40°C), inoculum level [5, 10, 15, 20 and 25 % (v/w)], particle size (212, 600, 850, 1,000, >1,000 and natural mixed particle size), supplementary carbon nitrogen and metal ions [carbon sources; soluble potato starch, maltose, sucrose, glucose, lactose (%1 w/w); nitrogen sources; yeast extract, peptone, ammonium sulphate, ammonium chloride, urea (%1 w/w); metal ions; $CuSO_4.5H_2O$, $MgSO_4.7H_2O$, $ZnSO_4.7H_2O$, $FeSO_4.7H_2O$, $CaCl_2$ (0.1 M)].

Dry weight determination

Dry weight of the samples was determined by drying them in a hot air oven at 80°C for 24h.

Results and Discussion

Screening for α-amylase production from fungi which is isolated from the air of Edirne City, was performed by means of the plate culture method. The ability of starch hydrolyzing activities of fungi was estimated in terms of diameter of clear zone/diameter of fungus colony ratios. The highest diameter of clear zone/diameter of fungus colony ratio was found with *Trichothecium roseum.*

In SSF, the selection of a suitable solid substrate for a fermentation process is a critical factor and thus involves the screening of a number of agro-industrial materials for microbial growth and product formation [4]. In the present studies five substrate, viz. WB, RS, CL, SOM and RH were used for growth and α-amylase production by the *T. roseum.* The results were shown in Fig. 1. All the substrates supported growth and enzyme formation by the culture. A high titer of α-amylase activity (690 U/g) was obtained in a medium containing WB alone as the substrate. It was reported that wheat bran was found to be the best substrate and suitable for necessary manipulation [2, 4, 15, 16]. Widespread suitability of WB may be due to the presence of sufficient nutrients and its ability to remain loose even in moist conditions thus providing a large surface area [17]. The order of substrate suitability was

WB>RH>SOM>RS>CL. In subsequent experiments, WB was used as the substrate for the production of α-amylase.

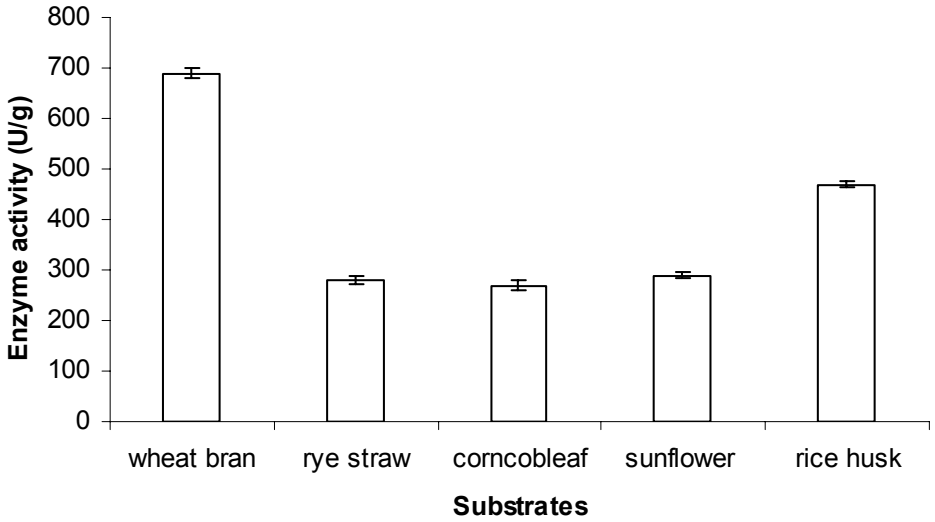

Figure 1. Effect of various substrates on α-amylase production in SSF. Incubation period 7 days, inoculum level 10 % (v/w), initial moisture level 65 % (w/v), incubation temperature 30°C, moistening agent distilled water.

Initial moisture content plays an important role in enzyme production during SSF. In SSF, most of the microbial growth and product formation takes place at or near the surface of the solid substrate. Thus, it is very crucial to provide optimized water level that controls the water activity of the fermenting substrate for achieving maximum product [2]. High titer (780 U/g) was attained when the initial moisture level was 85 % (w/v) in comparison with that at low moisture levels and high moisture level. The influence of the substrate humidity is presented in Fig. 2. High moisture level decreases porosity, changes WB particle structure, reduces gas volume and decreases diffusion [18]. Low moisture content reduces the solubility of nutrients provided to the organism by solid substrate; a lower degree of swelling and higher water tension [19].

The optimum moisture content for growth and substrate utilization depends upon the organism and water holding capacity of substrate used for cultivation [11]. In SSF initial moisture levels are between 55 % and 75 % which was prepared by using different agricultural by-products (corncob leaf, rye straw, wheat straw and wheat bran) to produce α-amylase from *Penicillium chrysogenum* [20]. It was reported that for *Aspergillus sp.* [15], *Aspergillus oryzae* [21], *Penicillium expansum* MT-1 [9], *Bacillus subtilis* [22] and *Bacillus sp.* [23] initial moisture levels was 80, 70, 70, 30 and 30 %, respectively. Optimal moisture level has been found to be 90 % for amylase production by *Thermomyces lanuginosus* [4].

T. roseum produced high titer (790 U/g) of enzyme at 8 days (Fig. 3). After 8th days the decrease has been observed in the enzyme production. The reason for this might have been due to the denaturation of the enzyme caused by the interaction with other components in medium [24]. It could have been also be due to the fact that the microorganism was on its exponential phase during the 8 days of fermentation and resulted in the maximum production of enzyme. At the later stage, when nutrients were depleted, it reached its stationary phase and could have started producing secondary metabolites, resulting in a lower yield of enzyme [25].

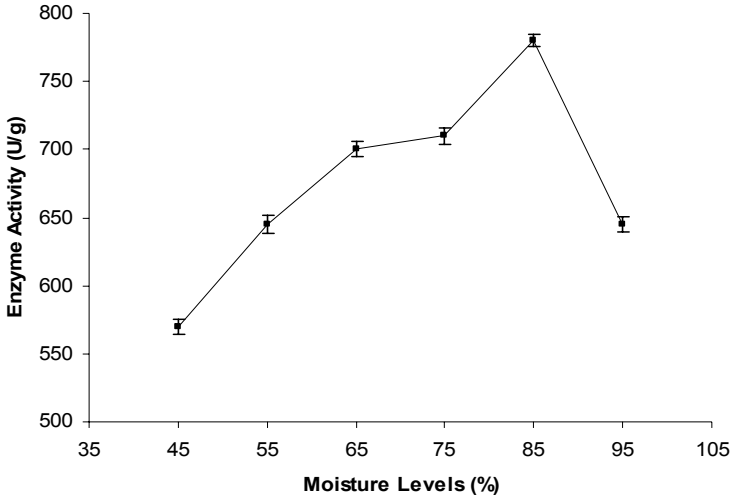

Figure 2. Effect of initial moisture content (% w/v) on α-amylase production in SSF system for WB. Incubation period 7 days, inoculum level 10 % (v/w), incubation temperature 30°C, moistening agent distilled water, natural mixed particle size.

It is difficult to monitor and control pH in the SSF system, since pH electrodes able to measure the pH of the moist solids, in the absence of free water, are not available [26]. Each microorganism possesses a pH range for its growth and activity with an optimum value in between the range. An attempt to overcome the problem of pH variability during the SSF process is obtained by the substrate formulation considering the buffering capacity of the different components employed or by the use of buffer formulation with components that have no deleterious influence on the biological activity [27]. In the present study, all media with different moistening agents supported enzyme production but maximum α-amylase production (810 U/g) was recorded when phosphate buffer (0.1 M; pH 7.0) was used as the moistening agent (Table 1). Our results were also accordance with the observations made by earlier worker [2], stating that pH 7.0 was optima for *Aspergillus flavus*. The best moistening agents were tap water (pH 6.5) and distilled water (pH 6.8) for wheat bran was reported [27].

Table 1. Effect of the nature of moistening agent on α-amylase production in SSF system for WB. Incubation period 8 days, moisture level 85 % (w/v), incubation temperature 30°C, inoculum level 10 % (v/w), natural mixed particle size.

Moistening agent	Enzyme activity (U/g)
Acetate buffer (pH 4.0)	340 ± 5.2
Acetate buffer (pH 5.0)	735 ± 7.8
Acetate buffer (pH 6.0)	780 ± 12.8
Phosphate buffer (pH 7.0)	810 ± 5.6
Phosphate buffer (pH 8.0)	790 ± 10.2
Distilled water (pH 6.5)	780 ± 9.6

The significance of temperature in the development of a biological process is such that it could determine the effects of protein denaturation, enzymatic inhibition, promotion or suppression of the production of a particular metabolite, cell viability and death [21].

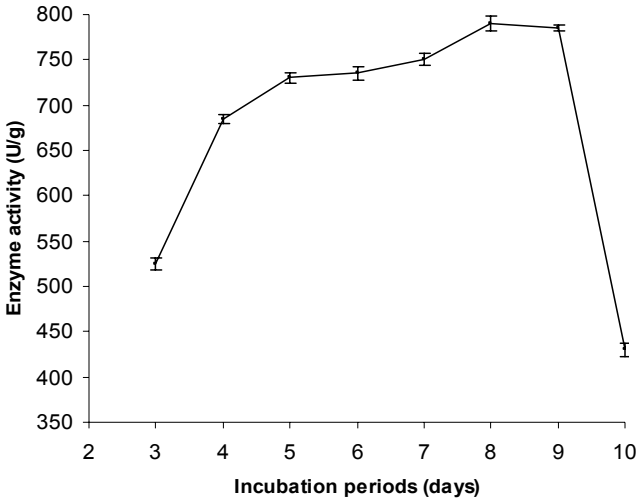

Figure 3. Effect of incubation period on α-amylase production is SSF system for WB. Inoculum level 10 % (v/w), incubation temperature 30°C, moistening agent distilled water, natural mixed particle size, moisture level 85 % (w/v).

SSF was carried out at different incubation temperatures ranging from 20-40°C. *T. roseum* showed the highest productivity (810 U/g) at a temperature of 30°C (Fig. 4) which may be advantageous as it can reduce the rate of evaporation during incubation. There was an increase in the enzyme production with an increase in temperature up to 30°C. A further increase in the temperature above 30°C not only inhibited fungal growth but also the production of α-amylase. Production of α-amylase is closely related to the growth of fungus as the optimum temperature for α-amylase production is similar to optimum temperature for the growth of fungus. Similar results have been previously reported for α-amylase production [2, 9].

Inoculum level was also an important factor for the production of α-amylase. Higher inoculum concentration increased the moisture content to a significant extent. The free excess liquid present in an unabsorbed form will therefore give rise to an additional diffusion barrier together with that imposed by the solid nature of the substrate and lead to a decrease in growth and enzyme production [28].

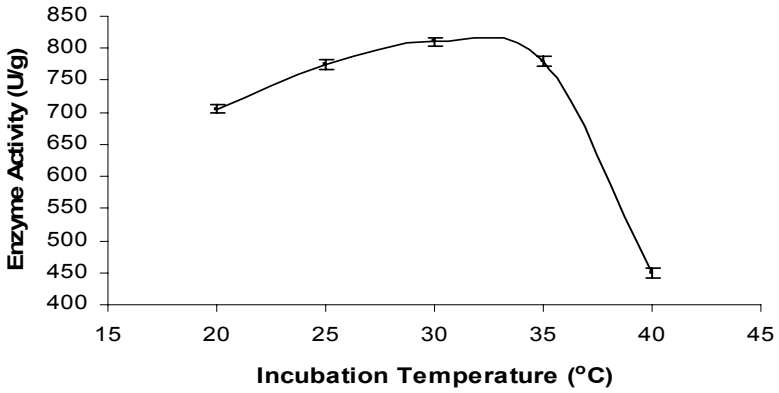

Figure 4. Effect of incubation temperature on α-amylase production in SSF system for WB. Inoculum level 10 % (v/w), incubation period 8 days, moistening agent phosphate buffer (pH 7.0, 0.1 M), natural mixed particle size, moisture level 85 % (w/v).

Low inoculum size required longer time for the cells to multiply to sufficient number to utilize the substrate and produce the desired product [25]. Various inoculum levels [5, 10, 15, 20 and 25 % (v/w)] were tried to study their effect on enzyme production. The higher enzyme production (815 U/g) was obtained at 10 % (v/w) inoculum level (Fig. 5). A similar result has been previously reported for α-amylase production by *Thermomyces lanuginosus* [4].

The particle size is a critical factor in SSF. Various particle size (212, 600, 850, 1,000, >1,000 μm and natural mixed) were tried to study their effect on enzyme production. The higher enzyme production (825 U/g) was obtained at 1,000μm (Table 2). Similar trends were reported for α-amylase with wheat bran and loquat kernel flour of 1,000 μm particle size [22, 9]. It has been reported that banana fruit stalk particles of 400 μm favoured for maximal α-amylase production by *Bacillus subtilis* CBTK 106 compared to larger particles [6]. Hashemi et al. [29] also reported that a particle size of 900 μm was the most effective for the production of α-amylase by *Bacillus sp.* KR-8104. The lower enzyme productions were obtained at small and big particle size. With smaller particle, the surface area for growth is greater, but the interparticle porosity is less. With larger sizes, porosity is greater, but the saturated surface area is less. These two opposing factors, a decrease in surface area, an increase in porosity and probably interact to determine the values corresponding to optimum growth and enzyme production [30].

Table 2. Effect of substrate particle size (μm) on α-amylase production in SSF. Substrate WB, incubation period 8 days, moisture level 85 % (w/v), incubation temperature 30 ^{o}C, moistening agent phosphate buffer (pH 7.0, 0.1 M), inoculum level 10 % (v/w).

Particle size (μm)	Enzyme activity (U/g)
212	760 ± 10.5
600	780 ± 7.1
850	805 ± 11.1
1,000	825 ± 12.1
>1,000	765 ± 5.5
Natural mixed	815 ± 10.1

The solid substrates may not provide all the nutrients needed by the organism for maximum enzyme production during SSF or some of the vital nutrients necessary for optimum growth and product formation may be present at suboptimal levels. Hence the exogenous addition of various nutrients to the solid medium improves the growth of organism and thus the product yield [24, 31]. Carbon source represents the energetic sources that are available for the growth of the microorganism.

The supplementation of WB with the different carbon sources; glucose, soluble potato starch, sucrose, lactose, maltose at 1 % (w/w) concentration showed marginal increased production of enzyme with lactose (950 U/g) in comparison to 825 U/g in control (Table 3). The result is similar to those reported for other fungal α-amylases [29, 32, 33].

Among the nitrogen sources, urea increased α-amylase production to 870 U/g (Table 3). Presence of organic nitrogen sources, urea and peptone, has been reported to enhance α-amylase enzyme production by *Aspergillus niger* in wheat bran containing solid substrate medium [16]. Addition of yeast extract to WB resulted in decreased enzyme activity.

Addition of different metal ions to WB resulted in better α-amylase production (865 U/g) with $CaCl_2$, addition of $CuSO_4$ to WB resulted in decreased enzyme activities (Table 3).

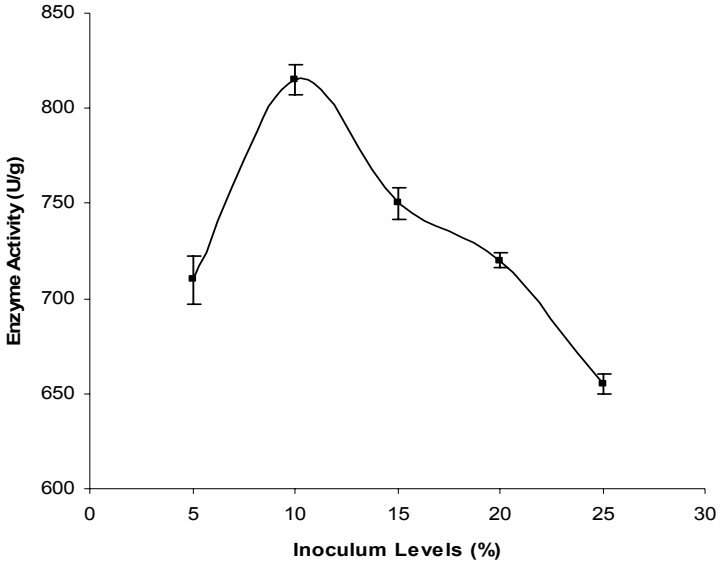

Figure 5. Effect of inoculum level (% v/w) on α-amylase production in SSF system for WB. Incubation period 8 days, moisture level 85 % (w/v), incubation temperature 30°C, moistening agent phosphate buffer (pH 7.0, 0.1 M), natural mixed particle size.

Table 3. Effect of different supplements on α-amylase production in SSF system for WB. Incubation period 8 days, moisture level 85 % (w/v), incubation temperature 30°C, moistening agent phosphate buffer (pH 7.0, 0.1 M), particle size 1,000 μm, inoculum level 10 % (v/w).

Supplement	Enzyme Activity (U/g)
Carbon sources (%1 w/w)	
Soluble potato starch	830 ± 5.5
Maltose	845 ± 6.5
Sucrose	855 ± 7.1
Glucose	880 ± 7.5
Lactose	950 ± 8.6
Nitrogen sources (%1 w/w)	
Yeast extract	810 ± 9.2
Peptone	860 ± 7.6
Ammonium sulphate	860 ± 4.5
Ammonium chloride	865 ± 9.2
Urea	870 ± 8.8
Metal ions (0.1 M)	
$CuSO_4.5H_2O$	555 ± 7.5
$MgSO_4.7H_2O$	810 ± 9.5
$ZnSO_4.7H_2O$	810 ± 10.4
$FeSO_4.7H_2O$	860 ± 8.6
$CaCl_2$	865 ± 7.2
None (control)	825 ± 5.8

The reduction in iodine-staining capacity of starch versus release of reducing power showed a pattern typical of endo-attacking enzymes. This fact confirmed the endo-action of the *T. roseum* enzyme on starch substrates and was identified it as a α-amylase.

In our study, the activity of amylase produced by *T. roseum* with WB in SSF was determined to be 690 U/g. In SSF, the activities of amylase produced by *Aspergillus sp*., *Thermomyces lanuginosus* and *Penicillium expansum* MT-1 with wheat bran and loquat kernel flour as substrate were reported to be 128, 261 and 406 U/g, respectively [2, 4, 9].

The maximum productivity of α-amylase (1,048 U/g) was achieved by utilizing WB as the solid substrate for 8 days at 30 ^{o}C, at an initial moisture content of 85 %, a pH of 7.0, an inoculum level of 10 % (v/w), with lactose (1 % w/w), urea (1 % w/w) and $CaCl_2$ (0.1 M in moistening agent) as supplements. From the above mentioned results, we conclude that one and a half increase in α-amylase productions were achieved in fermentation conditions as compared with the medium containing WB alone as the substrate.

The maximum amylase activities under optimum conditions were reported to be 164, 534 and 1,012 U/g for amylase produced by *Aspergillus sp*., *Thermomyces lanuginosus* and *Penicillium expansum* MT-1, respectively [2, 4, 9].

Conclusions

The present study describes the suitability of *T. roseum* for the large scale production of high titres of α-amylase using simple, cheap and economically feasible substrate, WB, with the supplementation of simple nutrients, like lactose, urea and $CaCl_2$. The fungus performances could be improved by further investigation in larger scale operation and by mutagenic methods.

References

1. R.C. RAY, S. KAR, S. NAYAK, M.R. SWAIN, Extracellular α-amylase production by *Bacillus brevis* MTCC 7521. *Food Biotechnology***,** 22, 234-246 (2008).
2. M.K. CHIMATA, P. SASIDHAR, S. CHALLA, Production of extracellular amylase from agricultural residues by a newly isolated *Aspergillus species* in solid state fermentation. *African Journal of Biotechnology*, 9, 5162-5169 (2010).
3. I. HAQ, H. ASHRAF, M.A. QADEER, J. IQBAL, Production of alpha-amylase by *Bacillus licheniformis* using an economical medium. *Bioresour. Technol.,* 87, 57-61 (2003).
4. A. KUNAMNENI, K. PERMAUL, S. SINGH, Amylase production in solid state fermentation by the thermophilic fungus *Thermomyces lanuginosus*. *Journal of Bioscience and Bioengineering*, 2, 168-171 (2005).
5. A. PANDEY, Recent process developments in solid state fermentation. *Process Biochemistry*, 27, 12-17 (1992).
6. C. KRISHNA, M. CHANDRASEKARAN, Banana waste as substrate for α-amylase production by *Bacillus subtilis* (CBTK 106) under solid state fermentation. *Appl. Microbiol. Biotechnol.*, 46, 106-111 (1996).
7. H.XU, L. SUN,D. ZHAO, B.ZHANG, Y. SHI, Y. WU, Production of α-amylase by *Aspergillus oryzae* As 3951 in solid state fermentation using spent brewing grains as substrate. *J. Sci. Food. Agric*., 88, 529-535 (2008).
8. P.S. MURTY, M.M. NAIDU, P. SRINIVAS, Production of α-amylase under solid state fermentation utilizing coffee waste. *J. Chem. Technol. Biotechnol*., 84, 1246-1249 (2009).
9. S. ERDAL, M. TASKIN, Production of α-amylase by *Penicillium expansum* MT-1 in solid-state fermentation using waste Loquat (*Eriobotrya japonica* Lindley) kernels as substrate. *Romanian Biotechnological Letters*, 15, 5342-5350 (2010).
10. G.V. SANGHVI, R.D. KOYANI, K.S. RAJPUT. Thermostable xylanase production and partial purification by solid state fermentation using agricultural waste wheat straw. *Mycology,* 1, 106-112 (2010).
11. M. RAIMBAULT, General and microbiological aspects of solid substrate fermentation. *Electron. J. Biotechnol.*, 3, 1-15 (1998).

12. S. LALIT, K. BHARTI, L.A. IYER, purification of a bifunctional amylase/protease inhibitor from ragi (*Eleusine corocana*) by chromatography and its use as an affinity ligand. *Journal of Chromatography,* 878, 1549-1554 (2010).
13. H. AYDOGDU, A. ASAN, Airborne fungi in child day care centers in Edirne city, Turkey. *Environ. Monit. Assess*, **147**, 423-444 (2008).
14. A.M. ABOU-ZEID, Production, purification and characterization of an extra cellular amylase enzyme isolated from *Aspergillus flavus. Microbios.*, 89, 55-56 (1997).
15. P. ELLAIAH, K. ADINARAYANA, Y. BHAVANI, P. PADMAJA, B. SRINIVASULU, Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated *Aspergillus* species. *Process Biochem.*, 38, 615-620 (2002).
16. H. ANTO, U. TRIVEDI, K. PATEL, Alpha amylase production by *Bacillus cereus* MTCC 1305 using solid state fermentation. *Food Technol. Biotechnol.*, 2, 241-245 (2006).
17. S. SIVARAMAKRISHNAN, D. GANGADHARAN, K.M. NAMPOOTHIRI, C.R. SOCCOL, A. PANDEY, Alpha amylase production by *Aspergillus oryzae* employing solid-state fermentation. *J. Sci. Ind. Res*., 66, 621-626 (2007).
18. B.K. LONSANE, N.P. GHILDYAL, S. BUDIATMAN, S.V. RAMAKRISHNA, Engineering aspects of solid state fermentation. *Enzyme Microb. Technol.*, 7, 258-265 (1985).
19. F. ZADRAZIL, H. BRUNNET, Investigation on physical parameters important for the SSF of straw by white rot fungi. *Eur. J. Appl. Microbiol. Biotechnol.*, 11, 183-188 (1981).
20. B. BALKAN, F. ERTAN, Production of α-amylase from *Penicillium chrysogenum* under solid state fermentation by using some agricultural by-products. *Food Technol. Biotechnol.*, 45, 439-442 (2007).
21. F. FRANCIS, A. SABU, K.M. NAMPOOTHIRI, G. SZAKACZ, A. PANDEY, Synthesis of α-amylase by *Aspergillus oryzae* in solid state fermentation. *Journal of Basic Microbiology*, 42, 320-326 (2002).
22. Z. BAYSAL, F. UYAR, C. AYTEKIN, Solid state fermentation for production of α-amylase by a thermotolerant *Bacillus subtilis* from hot-spring water. *Process Biochem.,* 38, 1665-1668 (2003).
23. Z. BAYSAL, F. UYAR, M. DOGRU, H. ALKAN, Production of extracellular alkaline α-amylase by solid state fermentation with a newly isolated *Bacillus sp*. *Preparative Biochemistry and Biotechnology*, 38, 191-197 (2008).
24. M.V. RAMESH, B.K. LONSANE, Solid state fermentation for production of alpha amylase by *Bacillus megaterium* 16 M. *Biotechnology Letters*, 9, 323-328 (1987).
25. S. RAMACHANDRAN, A.K. PATEL, K.M. NAMPOOTHRI, S. CHANDRAN, G. SZAKACS, C.R. SOCCOL, A. PANDEY, Alpha amylase from a fungal culture grown on oil cakes and its properties. *Brazilian Archives of Biology Tecnology*, 47, 309-317 (2004).
26. B.K. LONSANE, G.S. CASTANEDA, M. RAIMBAULT, S. ROUSSOS, G. VINIEGRA, N.P. GHILDYAL, M. RAMAKRISHNA, M.M. KRISHNAIAH, Scale up strategies for solid state fermentation systems. *Process Biochemistry*, 27, 259-273 (1992).
27. H.K. SODHI, K. SHARMA, J.K. GUPTA, S.K. SONI, Production of a thermostable α-amylase from *Bacillus sp*. PS-7 by solid state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production. *Process Biochemistry*, 40, 525-534 (2005).
28. P.K.A. MUNISWARAN, P. SELVAKUMAR, N.C.L.N. CHARYULU, Production of cellulase from coconut coir pith in solid state fermentation. *J. Chem. Tech. Biotechnol.*, 60, 147-151 (1994).
29. M. HASHEMİ, S.H. RAZAVİ, S. A. SHOJAOSADATI, S. M. MOUSAVI, K. KHAJEH, M. SAFARI. Development of a solid state fermentation process for production of an alpha amylase with potentially interesting properties. *Journal of Bioscience and Bioengineering*, 110, 333-337 (2010).
30. P.K.A. MUNISWARAN, N.C.L.N. CHARYULU, Solid substrate fermentation of coconut coir pith for cellulase production. *Enzyme Microb. Technol.*, 16, 436-440 (1994).
31. M.V. RAMESH, B.K. LONSANE, Ability of solid state fermentation technique to significantly minimise catabolite repression of α-amylase production by *Bacillus licheniformis* M 27. *Appl. Microbiol. Biotechnol.*, 35, 591-593 (1991).
32. V.H. MULIMANI, G.N. PATIL, RAMALINGAM, α-amylase production by solid state fermentation: a new practical approach to biotechnology courses. *Biochemical Education*, 28, 161-163 (2000).
33. S. RAMACHANDRAN, A.K. PATEL, K.M. NAMPOOTHIRI, F. FRANCIS, V. NAGY, G. SZAKACS, A. PANDEY, Coconut oil cake a potential raw material for the production of α-amylase. *Bioresource Technology*, 93, 169-174 (2004).