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Screening of commonly available solid process residues as substrate for L-asparaginase production by *Aspergillus terreus* MTCC *1782*

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ABSTRACT

This investigation examined the possibility of using a few agro process residues for their suitability to produce L- asparaginase in solid state fermentation (SSF). L-asparaginase secretion was found to be more pronounced in water moistened pomegranate (253 U/gds) followed by water moistened wheat bran (110 U/gds) and M9 medium moistened coconut oil cake (85 U/gds). Water appeared to be a better moistening agent than L-asparagine incorporated (proven inducer of L-asparaginase activity in submerged fermentation (SmF)) M9 medium for its ability to support better bio processing in SSF. While pomegranate showed consistent raise in L-asparaginase activity between third and fifth day of fermentation, wheat bran with water as moistening agent gave maximum enzyme activity on day three.

Keywords: Pomegranate skin powder, L- asparaginase, *Aspergillus terreus,* SSF

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INTRODUCTION

The enzyme L-asparaginase (L-asparagine amidohydrolase, E. C. 3.5.1.1) has attracted much attention as an amino acid degrading enzyme exhibiting antineoplastic activity (Bessoumy *et al.,*2004). Tumor cells are reported as taking in L-asparagine from blood circulation or body fluid as it cannot synthesize L–asparagines. The presence of L-asparaginase enzyme as chemotherapeutic agents may degrade the L-asparagine present in blood circulation and indirectly starve tumor cells and lead to cell death. Microbial enzymes are preferred over plant or animal sources due to their economic production, consistency, ease of process modification, optimization and purification. They are relatively more stable than corresponding enzymes derived from plants or animals[1].

L-asparaginase is a relatively wide spread enzyme and hydrolyze L-asparagine (essentialamino acid) to aspartic acid and ammonia.This enzyme was reported in microorganism such as *Aerobacter, Bacillus, Pseudomonas, Serratia,Xanthomonas, Photobacterium* [2], *Streptomyce*[3], *Proteus* [4], *Vibrio* [5] and *Aspergillus* [6].

L-Asparaginase is produced throughout the world by submerged fermentation (SmF). This technique has many disadvantages, such as the low yield levels, and consequent handling, reduction, and disposal of large volumes of water during the downstream processing. Solid-state fermentation (SSF) is a very effective technique as the yield of the product is many times higher when compared to that in SmF for a number of bio products[7]. This investigation deals with the production of L-asparaginase from *Aspergillus terreus* through solid state fermentation on six different agro process residues.

MATERIALS AND METHOD

Materials

All the chemicals used were of analytical grade and procured from Renkem, New Delhi, Himedia, Mumbai and Merck, Mumbai, India.The eightdifferent agro process residues were obtained from local market.

Microorganisms

The filamentous fungi *Aspergillus terreus* MTCC 1782 was obtained from Institute of Microbial Technology, Chandigarh, India. The sporesuspension in tween water was used as inoculum for periodic sub culturing in Potato Dextrose Agar slants.

Inoculum preparation

Inoculum culture was prepared in 250ml Erlenmeyer flask. 50 ml potato dextrose broth with a $_{P}$H of 5.4 was used for inoculum preparation.Spore suspension was added to the above at 1 % (v/v)and the flask was kept on rotary shaker at 120 rpm and30°C for 24hrs.

Fermentation studies

Eight different bioprocess residues were tried as substrates for L-asparaginase production. The substrates include pomegranate,coconut oil cake, groundnut shell,rice bran, wheat bran,tea residual dust, sugarcane bagasse and sugarcane pressmud.Thefermentation experiments were carried out by using 5 g each of substrateheld separately in petri-plates. They were all mixed with 7.5 mL each of sterile water and % moisture in the culture plates were maintained as 75% RH. A parallel set of 8 culture plates (one each for all the 8 substrates) were prepared as said above with the exception that M9 broth (7.5 mL per plate) was used instead of water. The plates (2X8) were all inoculated with 1 ml of fungal inoculum ($1x10^7$spores/ml). The content of the plates were then mixed thoroughlyusing a sterile spatula and kept for incubation at 30°C for 3-5 days. These studies can provide insights in to (1) type of substrate providing better enzyme activity in SSF, (2) maximum secreted levels of L-asparaginase for different substrate in SSF, (3) suggested period of harvest for maximum enzyme titers, (4) role of moistening agents (water and nutrient medium) on SSF of L-asparaginase activity and (5) influence ofL-asparagine(which is a component of M-9 medium) on L-asparaginase production during SSF.

Extraction of L-asparaginase

The samples were withdrawn at 3^{rd} and 5^{th} days under aseptic condition. 1gmeach of moldy substrate was taken into a beaker and distilled water (1:10) was added to it. Proper mixing was done byusing vortexing for 15 min in the presence of 20µL tween 60.The extract was filtered using Wattmanfilter No.1 followed by centrifugation at 4000rpm for 10 min. Supernatantsobtained were used as crude enzyme preparations and the same were used for the assay.

Assay of L-Asparaginase

Assay of enzyme was carried out as per [8]. In this assay, the rate of hydrolysis of L-asparagine was determined by measuring the ammonia released using Nessler's reaction. A mixture of 0.5 ml of 0.04 M asparagine was taken in a test tube, to which 0.5 ml of 0.5 M buffer(acetate buffer pH 5.4),0.5 ml of enzyme and 0.5 ml of distilled water was added to make up the volume up to 2.0 ml and incubate the reaction mixture for 30 min. After the incubation period, the reaction was stopped by adding 0.5 ml of 1.5 M TCA (Trichloroacetic acid). 0.1 ml was taken from the above reaction mixture and added to 3.7 ml distilled water and to that 0.2 ml Nessler's reagent was added and incubated for 15 to 20 min. The OD was measured at 450 nm. The blank was run by adding enzyme preparation after the addition of TCA.One U of L-asparaginase is the amount of enzyme which liberates 1 µ mole of ammonia perminute per ml [µmole/ml/min].Since the current experimentation was intended to screen the effectiveness of various solid substrates for maximum enzyme production during SSF, enzyme units were mentioned as U/gds where gds represents gram dry substrate.

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RESULTS AND DISCUSSION

Qualitative observation of fungal growth on the surface of substrates indicated (Figure 1) better mycelial growth and homogenous spreading across petriplates, maximum in tea residual dust and wheat bran. Growth was very less pronounced in sugarcane bagasse and sugarcane pressmud.

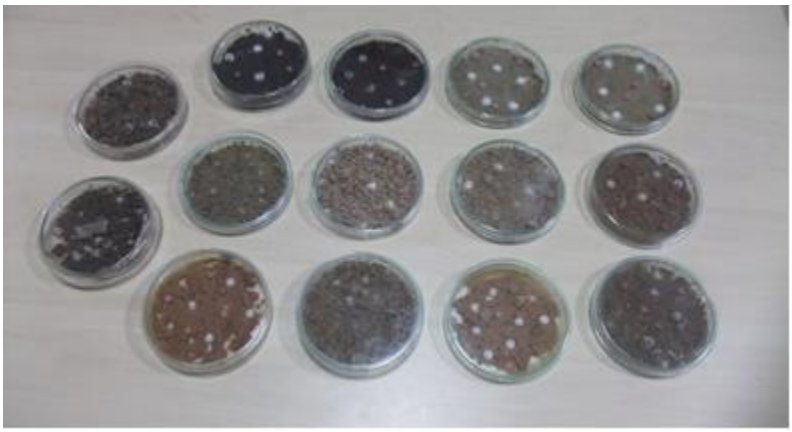

Figure 1 Growth of *A. terreus* on diverse solid substrates with water and M9 medium as the moistening agent.

Out of the eight substrates tested for their suitability to support SSF production of L-asparaginase, pomegranate appeared to be the best substrate supporting maximum enzyme activity of 253U/gds(water used as moistening system on day five).Wheat bran gave about 110U/gds (water used as moistening system on day three)activity while coconut cake supported 85U/gds (M9 medium as moistening system on day five) for of L-asparaginase activity (Figure 2).

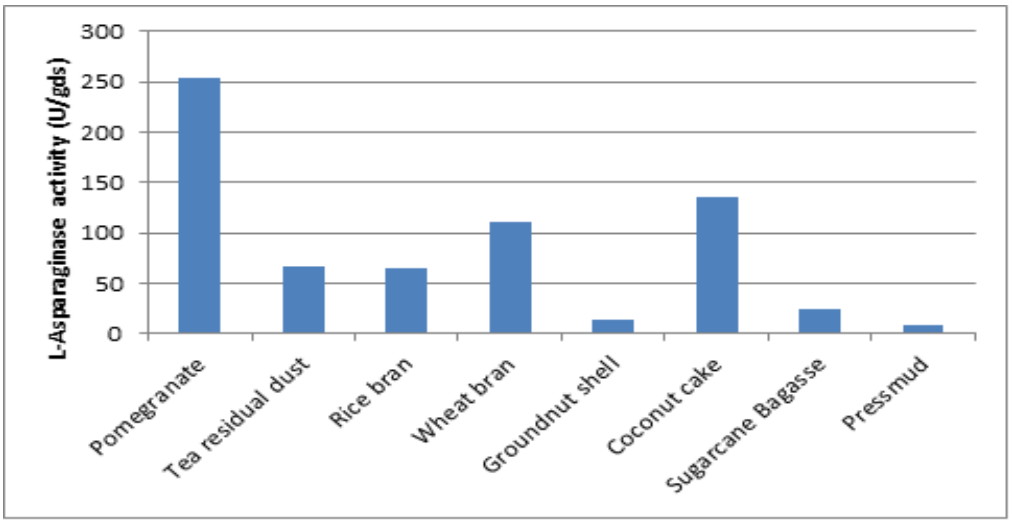

Figure 2: Maximum L-asparaginase titers supported by different solid substrates during SSF

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Role of water in SSF

While pomegranate showed consistent L-asparaginase secretion between third and fifth day of fermentation,with wheat bran maximum enzyme production occurred on day three itself 110U/gds (Figure 3).For coconut cake, enzyme secretion was prominent after third day only.Water was found to be favored moistening agent for SSF including pomegranate and coconut cake. Remarkable rise in enzyme titer was not noticed in M9 incorporated SSF plates for any substrates tested in comparison with that in water moistened SSF plate. This finding is quite contradictory to the earlier observation by the same group (data under publication) in SmF for L-asparaginase production which was clearly induced by the presence of specific substrate (L-asparagine) in M9 medium.

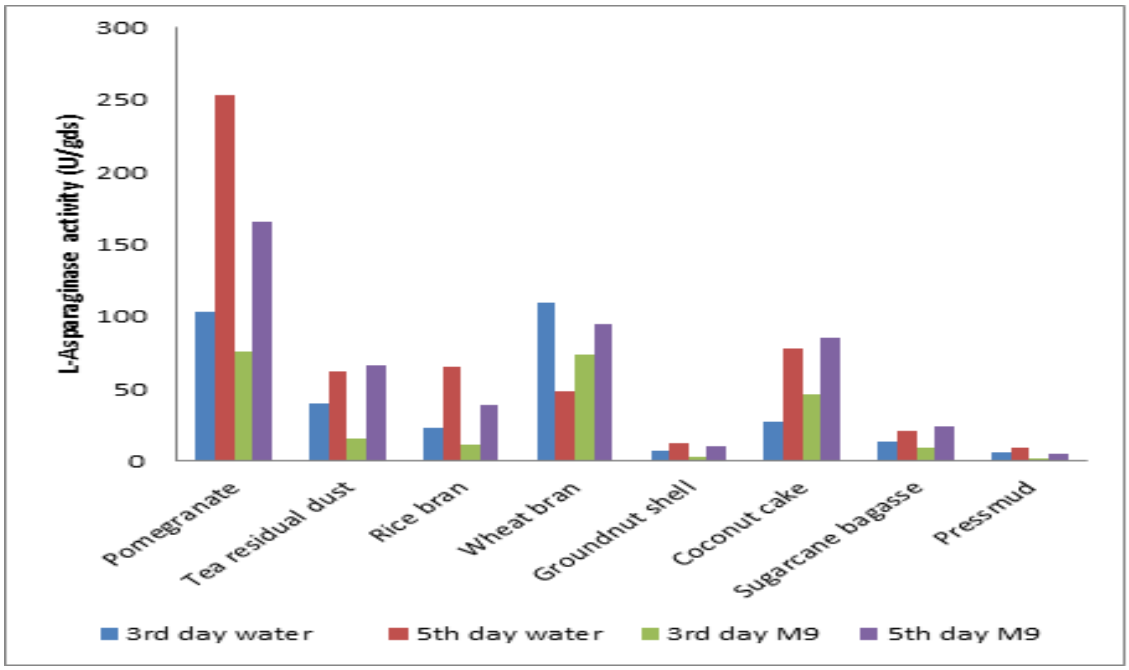

Figure 3: Effect of different solid substrate for the production of L-asparaginase in SSF.

The heterogeneous bound (non -assimilable) nutrients present in SSF make the organism to feel more at natural environment and hence is always under pressure to secrete enzymes which can convert the bound nutrients in to assimilable freely available monomers. While in SmF, when nitrogen sources other than L-asparagine is present (in freely assimilable form), the organism is not induced for producing L-asparaginase. Presence of L-asparagine in freely assimilable condition in SmF always induced better enzyme titers.

In our experimentation, water was proved as the best moistening agent for SSF in various solid substrates when compared with the specific substrate incorporated M9 medium. This could be because of the necessity of the solute free mass transfer assisting moistening agent for better bioprocessing in SSF. M9 medium though contains the specific substrate for L-

asparaginase;it's in ability to act as an ideal mass transfer medium could be the hampering factor why the water moistened plates yielded better enzyme titers.

Soniyamby*etal*. [9]had reported the highest L-asparaginase activity when orange peel is used as the substrate (70.67±1.14 U/g) and minimum for groundnut shell 10.27±0.40 U/g). Production of L-asparaginase by *Aspergillus terreus* was reported for other substrates too. For coconut cake, it was 40 U/g, fortea residual waste, it was 25U/g, for wheat bran,it was 30U/g, rice bran 25U/g and sugarcane bagasse30U/g[9]. Suresh and Raju [10]had reported production of L- asparaginase using *Aspergillus terreus* by using different agro residues. The maximum L-asparaginase activity was reported in sesame oil cake as a substrate (68.49 U/gds). While we reported L-asparaginase titers for coconut oil cake (136 U/gds), for rice bran 65 U/gds, for wheat bran (110 U/gds), Suresh and Raju [10]observed coconut oil cake with enzyme activity of 58 U/gds, 40 U/gds, 28 U/gds for above said substrates respectively. Optimization of solid culturing parameters could be the decisive factor that favored better productivity in our experimentation as was reported in many earlier studies.Mitchell and Lonsane [11],reported that the production enzyme is often simple& trivial, when agro-industrial by-products like wheat bran, rice bran or wheat straw are used as substrate. Because the moisture level is optimum (only bound water), the volume of medium per unit weight of substrate is low. Hence, enzyme activity could be usually very high.

We observed the production of maximum L-asparaginase at 96hrs (5^{th} day) by using *Aspergillus terreus* in SSF. Similar findings were reported earlier by Balasubrahmaniam *et al.*[12], Holker *et al*.[13], Lee *et al.*[14] and Akilandeswari *et al.*[15]. Earlier report for maximum L-asparaginase production (6.05 IU) at 72 hour using *Aspergillus terreus* by SSF was reported by Siddhalingheshwara *et al*.[16]. Khamna *et al.*[17] observed maximum productionof L-asparaginase by *Aspergillus terreus* at 178.hrs of fermentation period.

CONCLUSION

Of all the solid substrate tested pomegranate produced maximum enzyme titers in SSF. Water was the best moistening agent irrespective of the presence of L-asparagine in the M-9 medium used as the alternative moistening agent for comparison.Enzyme titers were high for fifth day of cultivation for most of the substrates rather than on third day.

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REFERENCES

[1] Savitri NA, andAzmi W. Ind J Biotechnol 2003;2:184-194.

[2] Peterson RE, Ciegler A. App Microbiol 1969; 17(6):929-930.
[3] Dejong. Appl Microbiol 1972; 23:1163-1164.
[4] Tosa T, Sano R, Yamamoto K, Nakamura M, Ando K, and Chibata I. App Environ Microbiol 1971; 22(3):387-392.
[5] Kafkewitz D, and Goodman D. App Environ Microbiol 1974; 27(1): 206-209.
[6] Sarquis MIdeMoura, Oliveira EMM, Santos AS andDa Costa GL. Memórias do Instituto Oswaldo Cruz 2004;99(5): 489-492.
[7] Lonsane BK, Ghildyal NP, Budiatman S and Ramakrishnan S. Enzyme Microb Technol, 1985; 7: 228-256.
[8] Imada A, Igarasi S, Nakahama K and Isono M. J General Microbiol 1973; 76:85-99.
[9] Soniyamby Ambi Rani ,Lalita S and Praveesh Bahuleyan Vasantha. International Journal Of Pharmacy And Pharmaceutical Sciences2012;279-282.
[10] Suresh JV and Jayaraju. Journal of Chemical, Biological and Physical Sciences 2013;314-325.
[11] Mitchell DA and Lonsane BK. Elsevier Sci Publ Ltd; London and New York1992;1-13.
[12] Balasubramanian K, Ambikapathy V and PanneerselvamA. International Journal of Advances in Pharmaceutical Research2012;3:778 – 783
[13] Holker U, Hofer M and Lenz. J Appl Microbiol Biotechno2004;64:174-186.
[14] Lee SY, Nakajima I, Ihara F, Kinoshita H and Nihirs T. Mycopathol 2005; 160:321-325.
[15] Akilandeswari K, Kavitha K and Vijayalakshmi M. Int J Pharmacy and Pharmaceutical Sciences 2012; 4:363-366
[16] Siddalingeshwara KG, and Lingappa K. International Journal of Pharm Tech Research 2011;3(1): 314-319.
[17] Khamna S, Yokota A andLumyong S. International Journal of Integrative Biology 2009; 6: 22–26.