Solid state fermentation for production of L – asparaginase in rice bran by Serratia marcescens SB08

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Citation

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Abstract

L – asparaginase, produced by various genera under solid-state and submerged cultivation, is an important chemotherapeutic agent used for the treatment of a variety of lymphoproliferative disorders and lymphomas, acute lymphoblastic leukemia in particular. In this study, L – asparaginase production by Serratia marcescens SB08 in rice bran supplemented with different nutrient factors in broth medium was investigated. With 50 % initial moisture content in rice bran and supplemented with yeast extract (0.5%, w/v) and at 36 h incubation time, a maximum L - asparagine production of 79.84U/g was achieved. When rice bran was combined with other starchy substances in the culture medium, quantum of L – asparaginase yield declined significantly. In rice bran, Serratia marcescens SB08 produced comparatively more L – asparaginase yield. Since rice bran is a very cheap, easily available source and more suited for L - asparaginase production in solid state cultivation, a detailed study for the large-scale fermentation processes using rice bran are suggested.

INTRODUCTION

L – asparaginase is an important chemotherapeutic agent used for the treatment of a variety of lymphoproliferative disorders and lymphomas, acute lymphoblastic leukemia in particular. In recent years, the use of L-asparaginase in the treatment of leukemia and other lymphoproliferative disorders has expanded immensely. For these reasons L – asparaginase has established itself to be an indispensable component in medicine (Umesh et al., 2007). Cancer cells differentiate themselves from normal cells in diminished expression of L – asparagine (Swain et al., 1993; Manna et al., 1995). Hence, they are not capable of producing L – asparagine and mainly depend on the L –asparagine from circulating plasma pools (Swain et al., 1993). Clinical trials indicate that this enzyme is also a promising agent in treating some forms of neoplastic cell disease in man (Oettgen et al., 1967). The principal source of L – asparaginases for clinical trials is the bacterium Escherichia coli (Adamson and Fabro, 1968). Although production and purification techniques of L – asparaginases have been developed, they generally provide a quantity of enzyme sufficient for limited trials only. To overcome this constraint, production of L – asparaginase by other bacterium and in other culture medium could be explored.

Several microorganisms including Serratia marcescens produce L-asparaginases (EC. 3.5.1.1), an antitumor enzyme (Wriston and Yellin, 1973). Although extensive studies have been carried out on the isolation and on the antileukemia properties of this enzyme (Heinemann and Howard, 1969; Heinemann et al., 1970), very little information is available on the production of this enzyme by S. marcescens (Khan et al., 1970; Netrval, 1977; Singh et al., 1977).

In recent years, the production of enzymes by solid state fermentation (SSF) has emerged. The SSF has numerous advantages over submerged fermentation, including superior productivity, simple technique, low capital investment, low energy requirement and less water output, better product recovery and reported to be the most appropriate process for developing countries (Carrizales and Jaffe, 1986). SSF holds tremendous potential for the production of secondary metabolites and has been increasingly applied in recent years (Sangeetha et al., 2004).

In this work, the different media ingredients along with rice bran were tested under solid state conditions for L – asparaginase synthesis by Serratia marcescens SB08. The results are encouraging for the production of L – asparaginase under solid state fermentation conditions. Perhaps, our attempt would be helpful for future researchers and industries to exploit this rice bran as a suitable substrate for large scale enzyme production.

MATERIAL AND METHODS

GROWTH OF ORGANISMS

S. marcescens SB08 was maintained in culture medium at 27° C containing (g/L): beef extract: 5, peptone: 5, glucose: 5, agar: 20 and at pH 7.0 on agar slants. Organisms were grown aerobically on a rotary shaker for 24 h in 500 mL Erlenmeyer flasks containing 100 mL of culture medium.

SOLID-STATE CULTIVATION IN FLASK

Rice bran was collected from a local market and grinded to obtain 0.5 mm size particle using a standard sieve and preserved at room temperature. 10 g (w/w) of this substrate was distributed in a 250 mL wide-mouth flask and then moisturized with 40 mL of L – asparagine broth medium. It had the following composition (g/L): Peptone: 0.5; Beef extract: 0.5; Yeast extract: 0.5; L – asparagine: 0.1. The initial pH of the medium was adjusted to 7.0 and sterilized at 121 oC for 15 min. The fermentation process was started by adding one mL (v/v) of inoculum (3 x 106 cells /mL) as prepared above, whole content mixed thoroughly and then incubated at 30 oC for 24 h in a stationary condition. A similar experiment was conducted for L - asparaginase production by replacing rice bran with 10 g (w/w) wheat bran. Every 6 hrs sample was withdrawn aseptically and used for determining the L - asparaginase activity. Moisture and biomass were measured by constant dry weight basis.

ENZYME PRODUCTION ON DIFFERENT MEDIA SUPPLEMENTS

The effect of different media supplements including carbon, nitrogen and other starchy materials along with rice bran was studied in this work by using L – asparagine broth medium. 1% (w/v) carbon source was separately tested in 40 mL of the above medium containing 10 g (w/w) rice bran for enzyme activity. For checking the effect of nitrogen sources on L - asparaginase production, 0.5% (w/v) organic nitrogen source and 0.3% (w/v) inorganic nitrogen source were separately added in 40 mL of the above medium. The influence of different media ingredients on L - asparaginase production was examined by replacing appropriate sources in the same medium with rice bran as a main substrate. 10 g of starchy material was used to replace the rice bran in the same minimal medium containing sucrose (1%) and yeast extract (0.5%). One mL of cells (3 x 106 cells/mL) was used as an inoculum to initiate fermentation and then all of the flasks were incubated at 30oC for 24 h in a stationary condition.

SOLID-STATE CULTIVATION IN ALUMINUM TRAY

One mL (v/v) of inoculum (3 x 106 cells/mL) was transferred to a 500 mL wide-mouth flask containing 25 g (w/w) rice bran moisturized with 100 mL L – asparagine broth medium and then sterilized at 121o C for 15 min. After 24 h incubation in stationary condition, substrate was thoroughly mixed with inoculum to obtain dough. In tray culture method, one Kg (w/w) pulverized (0.5 mm particle size) rice bran was distributed and uniformly layered on an aluminum tray (30 x 15 cm). Then, the same minimal medium was sprinkled on the surface of the rice bran until it acquired proper moisture content. 25 g (w/w) dough was spread on the surface of rice bran and dispersed thoroughly. This tray was closed invertically with another one tray by keeping a space between two trays with wooden strips and then incubated at 28oC for 48 h.

EXTRACTION OF ENZYME

After 48 h incubation, 50 mM citrate buffer (pH-6.8) (1:10 ratio) was added to fermented dough and homogenized with a constant stirring. This suspension was filtered through Whatman filter paper number 1 and the filtrate was again centrifuged at 6000 rpm for 15 min. This solid free supernatant was used as enzyme source for assaying L – asparaginase activity.

L – ASPARAGINASE ASSAY

L-asparaginase activity was determined by measuring the amount of ammonia formed by nesslerization (Wriston and Yellin, 1973). A 0.5 mL sample of crude enzyme, 1.0 mL of 0.1 M sodium borate buffer (pH 8.5) and 0.5 mL of 0.04 M L-asparagine solution were mixed and incubated for 10 min at 37oC. The reaction was then stopped by the addition of 0.5 mL of 15% trichloroacetic acid. The precipitated protein was removed by centrifugation and the liberated ammonia was determined by direct nesslerization. Suitable blanks of substrate and enzyme containing samples were included in all assays. The yellow color was read in a spectrophotometer (Hitachi – 3210 UV - Vis) at 500 nm. One unit (U) of L-asparaginase was the amount of enzyme which liberates 1 µmole of ammonia in 1 min at 37oC.

RESULTS AND DISCUSSION

The cell free culture supernatant analyzed by paper chromatography using n-butanol: pyridine: distilled water as mobile phase (50:28:32 ratio) indicated that Serratia marcescens SB08 was capable of producing optimum quantum of L - asparaginase in the fermentation medium.

Therefore, this strain was further studied in rice and wheat bran in solid state fermentation conditions for enhanced L - asparaginase production and results were presented in Figure 1.

Figure 1

Figure 1: showing the effect of incubation time on L - asparaginase production when SB08 growing in rice bran and wheat bran under solid state condition.

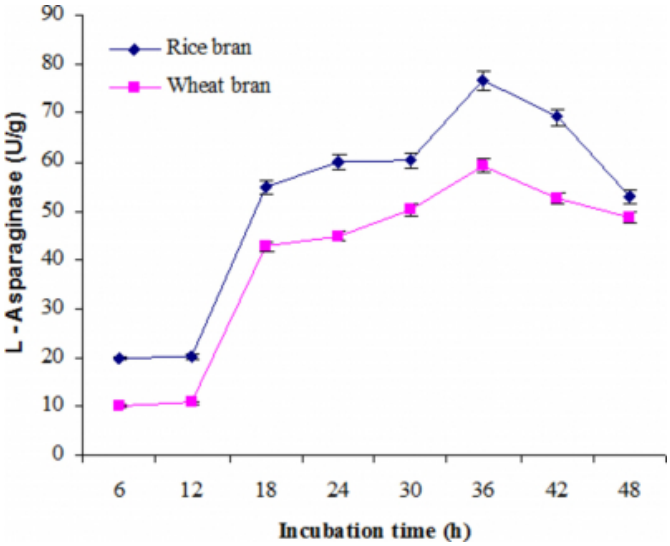
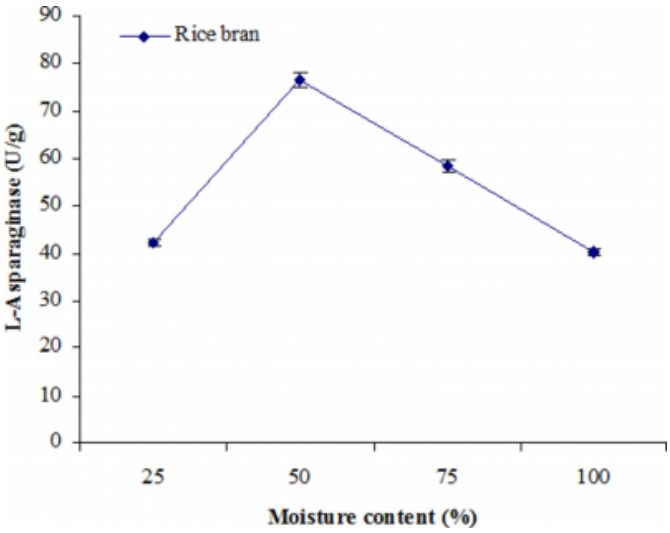


Figure1 shows the effect of incubation time on L – asparaginase production by Serratia marcescens SB08 grown in rice bran and wheat bran under the solid state cultivation in flask. It revealed that L - asparaginase activity gradually increased with the increase of incubation time until 36 hrs and maximum productivity sharply declined at further increase of incubation time. This indicated that when the incubation time exceeded 36 hrs, the cells of Serratia marcescens SB08 attained death phase resulting in declining of enzyme production. The result thus demonstrated the optimum incubation time for L - asparaginase production as 36 hrs and Serratia marcescens SB08 showed better enzyme yield on rice bran than wheat bran at this optimum incubation time.

The moisture content in rice bran tested for maximum L – asparaginase production indicated enhanced enzyme production at 50 % moisture content. The results are furnished in Figure 2.

Figure 2

Figure 2: showing the effect of moisture content on L - asparaginase production in rice bran by SB08



Moisture content below and above 50% was significantly lowering the formation of L - asparaginase and the biomass yield of Serratia marcescens SB08. As reported by Forgarty and Kelley (1980) and Alazard and Raimbault (1981), water acts as a vehicle for substrate transport and as a reactant so it may be expected that activity of water affects enzymatic conversion during fermentation.

In rice bran supplemented with yeast extract (0.5%), Serratia marcescens SB08 produced maximum L - asparaginase of 79.84 U/g at 36 hrs incubation time. The same strain produced 78.14 U/g of L – asparaginase in rice bran supplemented with starch (0.5 %) at 36 hrs incubation time. These results are comparatively better than L – asparaginase yield of 54.99 U/g produced by Serratia marcescens SB08 in wheat bran under the same culture conditions. Though L – asparagine broth medium also supported enzyme production in rice bran, an addition of yeast extract and starch found to enhance the growth of Serratia marcescens SB08 as well as enzyme yield in flask culture (Table 1).

Figure 3

Table 1: Showing the influence of different medium supplements in minimal medium for L – asparaginase production when SB08 under solid state fermentation

Production Medium		Biomass (mg/g)	L- asparaginase activity (U/g)
P1	Rice bran + MM	1.00	75.40
P2	P1 + yeast extract	1.11	79.84
P3	P1 + Salicin	0.83	56.02
P4	P2 + P3	0.97	67.20
P5	P4 + L - asparagine	0.91	60.04
P6	P1 + Starch	1.03	78.14
P7	P6 + Sucrose	0.63	46.12
P8	P6 + Sucrose + Yeast	0.61	43.98
P9	P1 + Rice flour	0.98	69.21
P10	P1 + Wheat bran	0.78	54.99
P11	Rice bran + distilled water	0.89	60.12
P12	Rice bran + Wheat bran (2 g each)	0.83	23.98

The concentration of yeast extract, salicin, starch, sucrose and L – asparagine were 0.025 %, 0.5 % and 1.0 % (w/v) respectively. 7.5 g of rice bran plus 2.5 g of other starchy materials were used in P9 – P12.

Rice bran alone, without addition of minimal medium, served as a potential carbon source for the solid state cultivation of Serrratia marcescens SB08 for L – asparagine production (60.12 U/g). When it was combined with different starchy raw materials, as shown in Table 1, the combination of rice bran (7.5 g) with wheat bran (2.5 g) gave 23.98 U/g. Although a multi-combination of starchy materials with rice bran did not favor enzyme synthesis, they supported the growth of Serratia marcescens SB08 on solid surface.

Additional carbon supplements in rice bran, exclusively galactose, lactose and sucrose in L – asparagine broth medium, were found to induce maximum L – asparaginase activity whereas supplementation of xylose and fructose lowered L – asparaginase production drastically (Table 2). Anto et al. (2006) has reported that media supplementation with sucrose in rice bran increased enzyme production (46.12 U/g). The results indicated that the selective additions of carbon supplements favored L - asparaginase enhancement under solid state fermentation in rice bran.

Figure 4

Table 2: Showing the influence of carbon sources in minimal medium incorporated with carbon sources for L – asparaginase production when SB08 growing in rice bran under solid state fermentation

Carbon source	L – asparaginase activity (U/g)
Galactose	49.93
Lactose	48.00
Sucrose	46.12
Fructose	20.13
Xylose	19.86

Addition of organic and inorganic nitrogen sources in L – asparagine broth medium, was found to produce varied L – asparaginase activity by Serratia marcescens SB08. The effect of additional nitrogen sources on L - asparaginase production studied was reported in Table 3.

Figure 5

Table 3: Showing the influence of nitrogen sources in minimal media incorporated with nitrogen sources for L – asparaginase production when SB08 growing in rice bran under solid state fermentation

Nitrogen source	L – asparaginase activity (U/g)
Peptone	59.14
Yeast extract	79.84
Beef extract	65.02
Ammonium chloride	43.56
Di-ammonium hydrogen phosphate	29.80
Sodium nitrate	24.38

The concentration of organic and inorganic nitrogen sources were 0.5 % (w/v) and 0.025 % (w/v) respectively.

Additional nitrogen supplements, exclusively organic nitrogen in L – asparagine broth medium, were found to induce maximum L – asparaginase activity whereas supplementation of inorganic nitrogen sources lowered L – asparaginase production drastically (Table 3). The results indicated that the selective additions of nitrogen supplements favored L - asparaginase enhancement under solid state fermentation in rice bran.

Tray culture method of enzyme production attempted to compare with the results of solid state cultivation of Serratia marcescens SB08 for enzyme production in flask conditions resulted in the production of 71.15 U/g at 48 hrs incubation time and at pH 7 and bed thickness in the inferior limits at

2.0 cm. The bed thickness in tray was the most significant variable for having as response enzyme production and productivity. L - asparaginase produced by Serratia marcescens SB08 in flask culture method (79.84 U/g) is somewhat better than the enzyme yield obtained in tray culture method. This variation in the enzyme yield suggests that further optimization study will be required to improve the productivity of this enzyme in tray culture method.

CONCLUSIONS

Serratia marcescens SB08 is a promising agent for industrial application since it gave a significant L –asparaginase (79.84 U/g) activity in rice bran under solid state conditions, which is comparatively better than wheat bran (54.99 U/g). As rice bran is a low cost substrate, easily available and showing suitability for solid state cultivation of microbes, it is suggested as a potential substrate for L –asparaginase production. No additional nutrient supplements are required for improving L –asparaginase productivity of Serratia marcescens SB08, which indicates the noteworthy use of this substrate in enzyme industry. Overall, we conclude that rice bran served as a more suitable substrate, compared to other available starchy materials, for solid state cultivation of Serratia marcescens SB08 for L –asparaginase production and suggest the use of this strain to large scale fermentation processes and in pharmaceutical industry.

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