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SHORT COMMUNICATION

Optimization of fermentation conditions for the biosynthesis of L-Asparaginase by *Penicillium* sp.

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Abstract

Extracellular L-asparaginase synthesis was carried out by *Penicillium* sp. and evaluated under different fermentation parameters employing submerged fermentation. L-asparaginase producers were detected by pink zone around the colony by plate assay method. *Penicillium* sp. 05 is the potential strain among the fungal isolates. L-asparaginase synthesis increased after the optimization of fermentation parameters. The optimum conditions were pH 6; temperature 35°C and inoculum size 1.0 mL recorded 167 IU/mL.

Keywords: L-asparaginase, *Penicillium,* submerged fermentation, pH, temperature, inoculum size.

Introduction

L-asparaginase (E.C 3.5.1.1) is a tetrameric protein belonging to oncolytic enzymes (David, 2005; Verma *et al.,* 2007). It catalyses the hydrolytic deamination of asparagine to yield aspartic acid and ammonium ion (Dominika and Jaskolski, 2001). L-asparaginase is a choice for acute lymphoblastic leukemia, lymphosarcoma and many other clinical experiments relating to tumour therapy in combination with chemotherapy (Peterson and Ciegler, 1969; Gulati *et al.,* 1997). This treatment brought a major breakthrough in modern oncology, as it induces complete remission over 90% of children within 4 weeks (Gulati *et al.,* 1997). With the development of its new functions, a great demand for L-asparaginase is expected in the coming years. With the increasing misuse of L-asparaginase, the serious problem of hypersensitivity is arising very fast with the bacterial L-asparaginase. Therefore, intensive search for new L-asparaginase without side effects is going worldwide (Sarquis *et al.,* 2004). Eukaryotic microorganisms like yeast and filamentous fungi such as *Aspergillus, Penicillium* and *Fusarium* have a potential for L-asparaginase production without side effects (Pinheiro *et al.,* 2001). This study was aimed to investigate the role of fermentation parameters on L-asparaginase production by *Penicillium* sp.

Materials and methods

Microorganism: Penicillium spp. was isolated from soil samples collected from Bangalore, India, according to the protocol of Seifert (1990). The isolated strains were tentatively identified in the laboratory as described by Rapper and Fennell (1965) and were maintained on potato dextrose agar (PDA).

Identification of fungal isolates: The isolated strains were tentatively identified in the laboratory as described by Rapper and Fennell (1965) and were maintained on potato dextrose agar (PDA). Further confirmation was done at Agharkar Research Institute, Pune.

Production of L-asparaginase: Production of L-asparaginase was carried out in the test fungi grown in modified Czapek-Dox's medium that contained glucose: 2 g; L-asparaginase: 10 g; potassium hydrogen phosphate: 1.52 g; potassium chloride: 0.52 g; magnesium sulphate.$7H_2O$; copper sulphate. $3H_2O$; zinc sulphate.$7H_2O$; iron sulphate. $7H_2O$ and distilled water; 1 L. The test fungus was grown for 4 d at room temperature (140 rpm).

Optimization of pH: Erlenmeyer flasks (250 mL) containing 100 mL of production medium was prepared by mixing with acid/alkali solution to obtain the required pH. The pH was adjusted in the range of 3-7 with increments of 1.0. Thus, prepared flasks were cotton plugged and autoclaved at 121°C for 15 min. The flasks were inoculated and incubated (Siddalingeshwar *et al.,* 2009).

Optimization of temperature: Production medium (100 mL) was separately taken in 250 mL Erlenmeyer flasks and prepared for submerged fermentation. Thus, prepared flasks were incubated and maintained at different temperatures ranging from 25-40°C (Siddalingeshwar *et al.,* 2009).

Optimization of inoculum size: The inoculum for L-asparaginase production was prepared separately by reviewing the 168 h old culture of the test fungi at different levels i.e., 0.25, 0.50, 0.75, 1.0 and 1.25 mL and fermentation studies were carried out according to Siddalingeshwar *et al.* (2009).

Extraction of L-asparaginase: The culture filtrate in the production medium was withdrawn periodically at 24 h in aseptic condition and then it was filtered through Whatman filter No.1 and the clear extract was centrifuged at 2000-3000 rpm for 15 min and the supernatant was used as enzyme source and assayed

Assay of L-asparaginase: Assay of enzyme was carried out as per Imad *et al.* (1973). Aspargine (0.04 M; 0.5 mL) was added to 0.5 mL of 0.5 M acetate buffer (pH 5.4). To this 0.5 mL of enzyme and 0.5 mL of distilled water was added to make up the volume to 2.0 mL and reaction mixture was incubated for 30 min. After the incubation period, the reaction was stopped by adding 0.5 mL of 1.5 M TCA (Trichloroacetic acid). The reaction mixture (0.1 mL) was 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 and blank was run by adding enzyme preparation after the addition of TCA. The enzyme activity was expressed in International units/mL. One IU of L-asparaginase is the amount of enzyme which liberates 1 μmol of ammonia per min per mL (μ mole/mL/min).

Results and discussion

All 20 strains of *Penicillium* spp. isolated from soil samples produced clear zones on asparagine plate and they were selected for further process. Among the 20 strains, *Penicillium* sp. 05 was found to be the best and high L-asparaginase producing strain. It showed 0.45 cm of pink cleared zone around the colony (data not shown). The data obtained in the present study on the effect of pH and temperature on submerged fermentation is shown in Table 1. The findings revealed that the production of L-asparaginase increased with the increase in pH of the medium up to 6 and thereafter, the decrease in L-asparaginase production was observed. The maximum production of L-asparaginase (147 IU) was obtained at pH 6 and the minimum production of L-asparaginase (56 IU) was observed at pH 4.

The findings in the study are in close agreement with the findings of Ali *et al.* (1994) who reported pH 7 and 4.5 optimum for the maximum production of L-asparaginase under submerged fermentation (SmF). Gulati *et al*. (1997) reported pH 6.2 was optimum for L-asparaginase production by *Aspergillus terreus*. Similarly, Sarquis *et al.* (2005) reported L-asparaginase production of 58 U/L when *A. terreus* strains were cultivated in the medium having pH of 6.2.

The production of L-asparaginase increased significantly with the increase in fermentation temperature from 25-35°C and decreased above 35°C. The maximum L-asparaginase production was recorded at 35°C (157 IU) and the least production was observed at 25°C (45 IU) at 72 h of fermentation. Any temperature beyond the optimum range is found to have some adverse effect on the metabolic activities of the microorganisms and it is also reported by various researchers that the metabolic activities of the microbes become slow at low or high temperature (Tunga *et al.,* 1999). Sarquis *et al*. (2004) reported 30°C as the suitable temperature for L-asparaginase production through SmF by *A. terreus* and *A. tamarii*.

Table1. Effect of pH, temperature and inoculum size on L-asparaginase production.

	Enzyme activity (IU/mL)
pH	
3	45
4	56
5	96
6	147
7	117
Temperature (°C)	
25	45
30	86
35	157
40	125
Inoculum size (mL)	
0.25	77
0.5	96
0.75	135
1.0	167
1.25	115

The impact of L-asparaginase production by *Penicillium* sp. 05 increased as the inoculum size increased up to 1.0 mL after 72 h of fermentation. Further increase in the inoculum size did not show any significant increase in the activity of L-asparaginase. The inoculum size 1.0 mL showed maximum activity of L-asparaginase around 167 IU. The lowest amount of L-asparaginase activity of 77 IU was observed at inoculum size of 0.25 mL (Table 1). This indicates that the inoculum density does not have effect on fermentation processes. It has some optimum value depending upon the microbial species and fermentation processes (Tunga *et al.,* 1998).

Conclusion

L-asparaginase synthesis by *Penicillium* sp. was evaluated under different fermentation parameters employing submerged fermentation. *Penicillium* sp. 05 is the potential strain among the fungal isolates and L-asparaginase synthesis increased after the optimization of fermentation parameters. The optimum conditions were pH 6; temperature 35°C and inoculum size 1.0 mL recorded 167 IU/mL. To conclude, this fermentation conditions may be used to increase the enzyme yield for biotechnological applications.

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