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Research Article

CHARACTERIZATION OF L- ASPARGINASE AND LIPASE PRODUCING ASPERGILLUS SP. DPB-365 ISOLATED FROM CHILLI RHIZOSPHERES

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Abstract

This paper describes production and characterization of L-Asparginase and Lipases from *Aspergillus* species isolated from chilli rhizosphere in the vicinity of Guntur, Andhra Pradesh. The isolated fungi were screened for L-asparaginase production using Czapek's agar media. On the basis of pink colour zone formed, ten fungal strains was selected and identified as *Aspergillus* species and names as *Apergillus* sp. DPB-365. This study investigates the optimization and production of L- Asparagine and Lipase. Days of incubation, temperature, pH, supplementary carbon and nitrogen sources on the production of L- Asparagine and Lipase was studied and accordingly optimum conditions were determined. The isolated soil fungi *Aspergillus species* is a strain that produces high levels of L- Asparagine, under optimized culture conditions, *viz.,* on the third day of incubation at an optimum pH of 7.5 and temperature of 37°C. The isolate *Aspergillus* sp. DPB-365 showed highest enzyme L- Asparginase production 71.2 IU/mg and Lipase production 65.5 IU/mg respectively. L-asparaginase has been used as anti-tumor agent for the effective treatment of acute lymphoblastic leukemia and as food processing aid to reduce the acrylamide formation during frying of starchy foods at high temperature.

Keywords: L- Asparginase, Lipase, Aspergillus.

INTRODUCTION

Enzymes are one of the essential products acquired for the needs of human through microorganisms. Enzymes like Amylases, Lipases, L- Asparginase, Glutaminase, Proteases and Cellulases has great industrial value in the present-day market. Among these Amylases plays a crucial role in enzyme market. Most of the prominent enzymes like protease, cellulases and amylase were used in many industries. L-asparaginase belongs to an amidase group that produces aspartic acid and ammonia by asparagine hydrolysis (Wriston and Yellin, 1973, Capizzi *et al.,* 1984). The search for other asparaginase sources, like eucaryotic microorganisms, can lead to an enzyme with less adverse effects. The importance of microorganisms as L-asparaginase sources has been focused since the time it was obtained from *Escherichiacoli* and its antineoplastic activity demonstrated in guinea pig serum (Broome, 1961; Mashburn and Wriston 1964; Roberts *et al.,* 1966; Schawartz *et al.,* 1966, Boyse *et al.,* 1967). This enzyme is widely distributed, being found in L-asparaginase is widely distributed, being found in animal, microbial and plant sources. Large number of microorganisms that include *Erwiniacarotovora, Pseudomonas stutzeri*, *Pseudomonasaeruginosa* and *E. coli*. It has been observed that eukaryotic microorganisms like yeast and fungi have a potential for asparaginase production. For example, the mitosporic fungi genera such as *Aspergillus, Penicillium*, and *Fusarium*, are commonly reported in scientific literature to produce asparaginase (Abdel Fatteh *et al*., 2002; Qin and Zhao, 2003; Wade *et al*., 1971). The literature reports suggested that the enzyme produced by different microbial strains differed in some physiological, biochemical, catalytic and immunological properties. So the continuous screening program is necessary for the isolation of novel microbial strains that could produce an effective enzyme.

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To our knowledge, reports on the production of L-Asparginase from *bacillus and Aspergillus species* is very limited. In the present investigation, the one-factor-at-a-time approach was used to select the best combination of incubation period, pH, temperature, carbon, nitrogen sources studied.

MATERIALS AND METHODS

Isolation of fungi: The rhizosphere soil samples from the chilli fields of 10 different areas of Guntur, district of Andhra Pradesh, India were collected for the study. Fungal strains were isolated on Potato Dextrose Agar (PDA) medium by soil dilution plate technique (Rapilly, 1968) using 10^{-3} to 10^{-5} dilutions. The plates were incubated at 28 ± 2°C for 5 days. Fungal colonies appeared in the plates were noted and sub cultured. After purified by single spore isolation method and they were maintained on potato dextrose agar (PDA) slants. Identification of Fungal isolates was based on culture characters as well as microscopic parameters (conidiophores branching, phial ides shape and position, spore size and shape) (Nagamani *et al.,* 2006). The pure cultures were stored in the refrigerator at 4°C for further studies.

L- Asparginase production: The production of L-asparaginase was carried out by using 20 g of carob pod as a substrate under solid state fermentation. The moisture content of the flask is 65% were maintained and inoculated 1 ml of inoculum (1x107spores/ml). The content of the flask were mixed thoroughly gently beating the flasks on the palm of hand and incubated in slanting position at 35 0C for 7 days. The pH 4.5 was maintained throughout the fermentation process.

Enzyme Assay: Assay of enzyme was carried out as per Imada*et al*. 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. The enzyme activity was expressed in International unit.

Lipase production: One ml of olive oil along with 100 ml basal salt solution (Peptone: 0.5 g; MgSO4.7H2O: 0.05 g; KCl: 0.05 g; KH2PO4: 0.2 g; NaNO3: 0.05 g) in 250 ml of Erlenmeyer flask were autoclaved at 15 psi for 15 minutes. These flasks were inoculated with *Aspergillus* sp. DPB-365and incubated at28±1 °C in shaking incubator (80 rpm) for 5 days.

Enzyme assay: Crude enzyme extract was prepared from the culture supernatant which was centrifuged at 10,000 rpm for 15 min at 4°C. Lipase activity was measured spectrophotometrically using pnitrophenyl acetate (pNPA) as a substrate at 45°C in 100 mM phosphate buffer of pH 7.0 (Licia *et al*., 2006). The substrate for this reaction was composed of solution A and B. Solution A contained 40 mg of p-nitro phenyl actetate dissolved in 12 ml of isopropanol, solution B contained 0.1 g of gum arabic and 0.4 ml of triton X-100 dissolved in 90 ml of distilled water. The substrate solution was prepared by adding 1 ml of solution A and 19 ml of solution B. The assay mixture contained 1 ml of the substrate, 0.5 ml of buffer, 0.1 ml of enzyme and final volume was made up to 3 ml with distilled water. The enzyme activity was stopped by adding 0.2 ml isopropanol and liberation of *p*-nitrophenol at 28 °C was detected in spectrophotometer at 400 nm. One enzyme unit was defined as 1 µmol of p-nitrophenol enzymatically released from the substrate per minute Syed *et al*., (2010).

Optimization of medium and cultural conditions: Optimization of different cultural conditions such as incubation period, pH, temperature, carbon, and nitrogen sources on the production of L-Asparginase was determined.

Effect of incubation period on L- Asparginaseand lipase production: To study the effect of incubation period on L-Aspargine production, the enzyme production by *Aspergillus* sp. DPB-365 was studied for every 24 hours up to 96 hours. For this study, the RN-7 culture was inoculated into 500 ml conical flask, containing sterile M9 broth and was incubated from 24 hours up to 96 hours at 37°C and triplicates were maintained. The optimum time, showing maximum production was taken for further experiments.

Effect of pH on L- Asparginaseand lipase Production: To study the effect of different pH on L- Aspargine production, the isolate *Aspergillus* sp. DPB-365 was grown in sterile M9 broth with various pH levels ranging from 5.0 to 9.0. and was maintained by using phosphate buffer. 0.5 ml of the 24-hour old inoculum was transferred into the sterile M9 broth at different pH and incubated at 30°C for 48 hrs and assayed for enzyme activity. The most favourable pH achieved at this step was used for further study.

Effect of temperature on L- Asparginase and lipase production: To study the effect of temperature on L-Aspargine production, the isolate *Aspergillus* sp. DPB-365 was grown in sterile M9 broth at different temperatures, ranging from 30 to 55^{O}C for 48 hrs of incubation and temperature was maintained by using respective incubators. 0.5 ml of the 24-hour old inoculum was transferred into a sterile M9 broth and was incubated and further assayed for enzyme activity.

Optimization of L- Asparginaseand lipase Production by *Aspergillus* sp. DPB-365

Effect of Different Carbon Sources on L- Asparginaseand lipaseproduction: To study the effect of carbon sources on L-Aspargine production by growing the bacterial strain *Aspergillus* sp. DPB-365 on M9 broth, the broth was supplemented with different carbon sources such as Galactose, Maltose, Fructose, Starch, Sucrose, and Cellulose, each at a concentration of 1% (w/v). 0.5 ml of the inoculum was added to a sterile M9 broth with different carbon sources, incubated at 30°C for 72 hrs and assayed.

Effect of Different Nitrogen sources on L- Asparginaseand lipase production: Different nitrogen sources like Yeast extract, Tryptone, Ammonium sulphate, Urea, Peptone and Malt extract were added to the M9 broth separately at a rate of 1% (w/v), and 0.5 ml of the inoculum from the bacterial isolate *Aspergillus* sp. DPB-365 was added to sterile M9 broth, incubated at 30°C for 72 hrs and was assayed for enzymatic activity.

Statistical Analysis: All measurements were carried out in triplicate. Statistical analyses were performed using one-way analysis of variance (ANOVA), and the significance of the difference between means was determined by Duncan's multiple range tests. Differences at $P< 0.05$ were considered statistically significant.

RESULTS

A total of 10 isolates were obtained from chilli rhizosphere in the vicinity of Guntur, Andhra Pradesh. The preliminary characterization like cultural and biochemical characteristics was done by Bergey's manual of systemic bacteriology. All the isolates belong to *Aspergillus* species according to their preliminary and biochemical studies. Different incubation (7, 8, 9, 10, 11, 12, 13 and 14 days) periods were studied for the L- Asparginase and Lipase production. Maximum enzyme production was observed by 12 days of incubation period (Table-1). In this study enzyme production of each strain is based on the specific growth rate of the strain. Growth rate and enzyme synthesis of the culture are the two main characteristics which are mainly influenced by incubation time (Ellaiah *et al*., 2002).

Table 1. Effect of incubation period on L- Aspargine and Lipase production by *Aspergillus* sp. DPB365

Sl.no	Incubation period	L- Aspargine production (IU/mg) by Aspergillus sp. DPB365	Lipase production (IU/mg) by Aspergillus sp. DPB365
1	7	18	11
2	8	27	17
3	9	36	28
4	10	42	33
5	11	59	45
6	12	71.2	65.5
7	13	60	44.7
8	14	55	21.3

*The overall model is significant with p<0.05

Different pH levels were maintained for the L- Asperginase and Lipase production by Aspergillus sp. DPB365. Maximum enzyme production was obtained from neutral pH. Acidic

phase to a neutral phase enzyme activity increased up to a pH of 4.0 and upon further increase in pH, enzymatic activity decreased (Table-2).

Table 2. Effect of pH on L- Aspargine and Lipase production by *Aspergillus* sp. DPB365

Sl.no	pH	L- Aspargine production (U/L) sp. DPB365	Lipase production (IU/mg) by Aspergillus sp. DPB365
1	4	2	18
2	5	16	24
3	6	57	32
4	7	71.2	65.5
5	8	61	41
6	9	44	32
7	10	25	21

*The overall model is significant with p<0.05

Various temperate levels were maintained for the L-Asperginase and Lipase production by Aspergillus sp. DPB365. Maximum enzyme production was obtained from 35°C nearly room temperature. Further increase in temperature enzymatic production was decreased (Table-3).

Table -3. Effect of temperature on L- Aspargine and Lipase production by *Aspergillus* sp. DPB365

Sl.no	Temperature (□C)	L- Aspargine production(U/L) by *Aspergillus*sp. DPB365	Lipase production (IU/mg) by *Aspergillus*sp. DPB365
1	4	5	2
2	15	22	14
3	20	35	21
4	25	51	30
5	30	65	42
6	35	71.2	65.5
7	40	60	41
8	45	48	22

*The overall model is significant with p<0.05

Different carbon sources were tested for L- Asparginase and Lipase production by *Aspergillus* sp. DPB365. Maximum enzyme production was obtained from Glucose was used as carbon source followed by rhamnose (Table-4). Majority of the carbon sources greatly influenced the enzyme production by *Aspergillus* sp. DPB365.

Table 4. Effect of carbon sources on L- Asparginaseand Lipase production by *Aspergillus* sp. DPB365

Sl.no	Carbon sources	L- Asparginase production (IU/mg)Aspergillussp. DPB365	Lipase production (IU/mg) by Aspergillus sp. DPB365
1	Glucose	71.2	36.4
2	Maltose	65	42.4
3	Rhamnose	70	55.1
4	Fructose	68	52.6
5	Xylose	55	58.7
6	Arabinose	52	62.7
7	Starch	63	48.4

*The overall model is significant with p<0.05

Different nitrogen sources were tested for L- Asparginase and Lipase production by *Aspergillus*sp. DPB365. Maximum enzyme production was obtained from glutamine followed by proline (Table-5).

Table -5. Effect of nitrogen sources on L- Aspargineand Lipase production by *Aspergillus* sp. DPB365

Sl.no	Nitrogen sources	L- Aspargine production (IU/mg)sp. DPB365	Lipase production (IU/mg) by Aspergillus sp. DPB365
1	Proline	73.5	42.5
2	Urea	70	51.6
3	Glutamine	88	43.8
4	Sodium nitrate	65	31.4
5	Ammonium sulphate	60	28.2
6	Peptone	68	60.5
7	Beef extract	62	51.2

*The overall model is significant with p<0.05

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