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Research Article

ISOLATION AND SCREENING OF L– ASPARAGINASE PRODUCING BACTERIA FROM VISAKHAPATNAM SOIL SAMPLES

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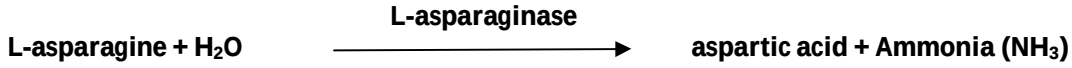
ABSTRACT

L-asparaginase (E.C No 3.5.1.1) is a potential anti-carcinogenic enzyme. Six different soil samples of Vishakhapatnam regions were screened for potential producers of L-asparaginase using modified czapek dox's agar containing L-asparagine and phenol red indicator with pH 6.8. The strain isolated from slaughter house soil sample (**BSH-3**) showed the maximum activity. The selected strain was characterized by biochemical tests as per the bergey's manual of determinative bacteriology and was found to be *Myroides* species. The enzyme production was carried out by submerged fermentation. The maximum enzyme activity was 85.7 IU/mg is showed by 24 hrs culture at 37^{o} C.

Keywords: L-asparaginase, enzyme activity, bacteria.

INTRODUCTION

L-asparagine is an essential amino acid used for nutritional requirement of both normal cells and cancer cells[1].L-asparaginase enzyme which cleaves L-asparagine into aspartic acid and ammonia[2]. Since several types of tumour cells require L-asparagine for protein synthesis they are deprived of an essential growth factor in the presence of L asparginase[3,4]. This enzyme is widely used in the treatment of acute lymphatic leukaemia mainly (ALL) in children's[5]. Lymphatic tumour cells require huge amount of L-asparagine for malignant growth.



L-asparaginase was produced by wide range of bacteria[6], fungi[7], actinomycetes[8], algae and plants[9] were reported. Microbes are better source for the production of L asparginase, because they can easily cultured, extraction and purification as well as the methods of this process from them is also convenient [10]. Commercially used L-asparaginase is obtained from *Erwinia Carotovora*[11] and *E.coli*[12].

The antineoplastic activity results from depletion of the circulating pools of L-asparagine by L-asparaginase[13].Since depletion of L-asparaginase from plasma by L-asparaginase results in inhibition of DNA and RNA synthesis with subsequent blastic cell apoptosis. Owing to the unique anti cancer mechanism of action L-asparaginase has been introduced to the multi drug chemotherapy in children and adults with acute lymphoblastic leukaemia, which has contributed to significant improvement of therapy outcomes and to achieve complete remission in approxly 90 % of patients. Despite its high therapeutic efficancy, L-asparaginase may lead to severe side effects such as fever, skin rashes, allergies and anaphylactic shocks[14].

The objective of this study was to isolate the potent bacterial strain producing large amount of L-asparaginase with maximum activity.

MATERIAL S AND METHODS

CHEMICALS

All chemical used in the present study were of analytical grade purchased from Himedia, Mumbai, India.

COLLECTION OF SAMPLE

Six soil samples were collected from different places at a depth of 30 to 40 cm in and around the Vishakhapatnam regions, Andhra Pradesh, India. The samples were collected into a sterile polythene bags and carried to department research lab for further microbial analysis.

ISOLATION OF MICROORGANISMS

Different soil sediment samples treated for bacterial isolation by serial dilution method using nutrient agar medium[15]. To avoid the fungal contamination nystatin (50µg/mL) added to the medium. The inoculated agar plates were incubated at 37° C for 24 hrs.

SCREENING OF L ASPARGINASE BY PLATE METHOD ASSAY

The isolated bacterial colonies were screened for L-asparaginase production on modified czapek dox agar containing glucose-2.0, L-asparagine-10.0, KH_2PO_4-1.52, KCL-0.52, $MgSO4.7H_2O$-0.52, $CuNO_3.3H_2O$-trace, $ZnSO_4.7H_2O$-trace, $FeSO_4.7H_2O$-trace (grams per litre of distilled water) at pH 6.2 and add 0.09 %(v/v) phenol red as a indicator to the media[16]. Control plates were of modified czapek dox's medium without L asparginase (add $NaNO_3$ as nitrogen source instead of asparagine) and phenol red dye. Strain with pink colour zones around the colonies were considered as L-asparaginase producing strains. Development of pink colour zones around the colonies was considered as positive result for L-asparaginase production.

ENZYME PRODUCTION BY SUBMERGED FERMENTATION METHOD

L-asparaginase production was carried out by submerged fermentation. A 250mL Erlenmeyer flask containing 50mL of sterilized medium was used for production. A loop full of log phase culture was inoculated into the medium. The flask was placed in an incubating orbital shaker at 120 rpm at 37° C for 24 hrs. Un-inoculated medium served as control. The bacterial cell mass was separated by centrifuged at 10000 rpm for 10 min at 4° C. The liquid supernatant was used as crude enzyme source to determine the enzyme activity and protein assay.

ESTIMATION OF L-ASPARAGINASE ENZYME ACTIVITY

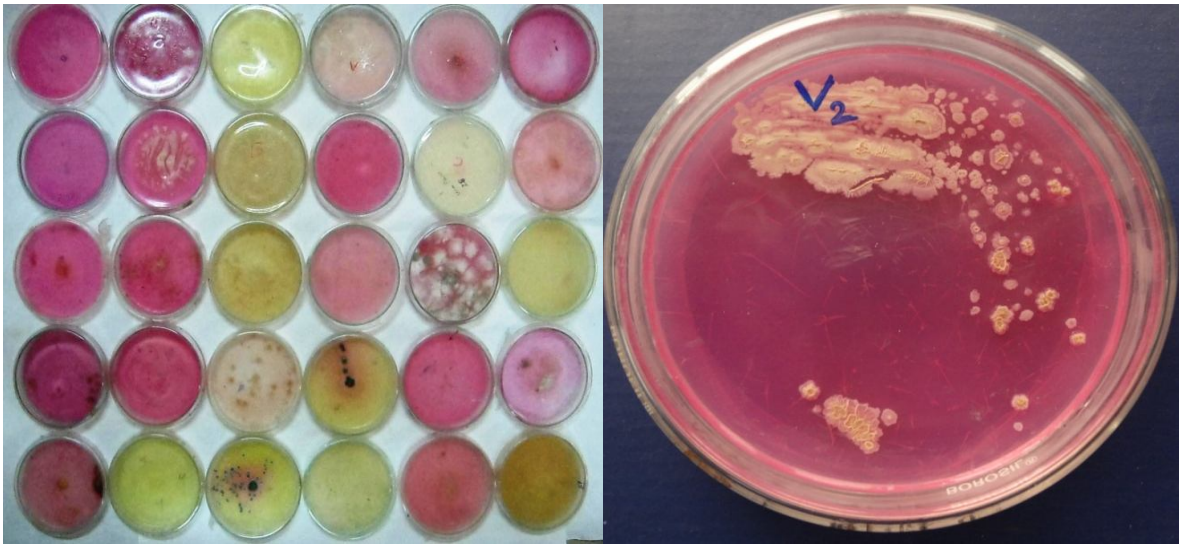
By using mashburn and wriston[17] determination of L-asparaginase activity was carried out. In this assay, the rate of hydrolysis of L-asparagine was determined by measuring the released ammonia using nessler's reagent. A mixture of 100µL enzyme extract, 200µL 0.05M Tris-Hcl buffer (pH 8.6) and 1.7mL of 0.01M L-asparagine was incubated for 10min at 37° C. The reaction was stopped by addition of 500µL of 1.5M TCA. After centrifugation at 1000rpm at 4° C, 0.5mL of the supernatant was diluted to 7mL with distilled water and adds 1mL of nessler's reagent. The color reaction was allowed to develop for 10min and the OD was checked at 450nm with spectrophotometer. The ammonia liberated was entrapped from a curve derived with ammonia sulphate as standard curve. One unit (IU) of L-asparaginase was defined as the amount of enzyme which liberates 1µm of ammonia per minute under the assay condition pH 8.6 at 37°C[18].

ESTIMATION OF PROTEIN

Estimation of protein was determined by using lowery et al method[19]. A stock solution of standard protein, BSA at a concentration of 1000 µg/mL was made. From these solution aliquots of 0.2 to 1mL of working standard at concentration of 100 µg/mL was taken in test tubes. All the test tubes were makeup to 1mL with distilled water. 1 mL of FC reagents was added to each test tube. After 30min of incubation, the absorbance was measured at 660nm using UV-VIS spec.

RESULTS AND DISCUSSION

A total number of 64 bacterial isolates were screened for L-asparaginase production from the six different soil samples using nutrient agar medium at 37° C for 24hours. Most of the bacterial isolates have found to be positive for L-asparaginase production during screening process[20]. Out of 64, 21 isolates showed L-asparaginase activity on plate method assay by pink colour zones. Further these isolates were subjected to the secondary screening for enzyme activity by mashburn & wriston method [17]. Among them, seven isolates showed higher activity range between 75.6 IU/mg & 85.7 IU/mg protein. The bacterial isolate BSH-3 showed maximum activity of 85.7 IU/mg of protein. Other bacteria showed low enzyme activity.



Screening Plates **Positive Plate**

Fig: 1: Isolation & Screening of L-asparaginase producing bacteria using modified czapek dox's containing L-asparagine and phenol red indicator agar

Fig: 2: Positive plate showing L-asparaginase activity by BSH-3 strain

Table 1: Isolation and screening of L-asparaginase producing bacteria from different habitats

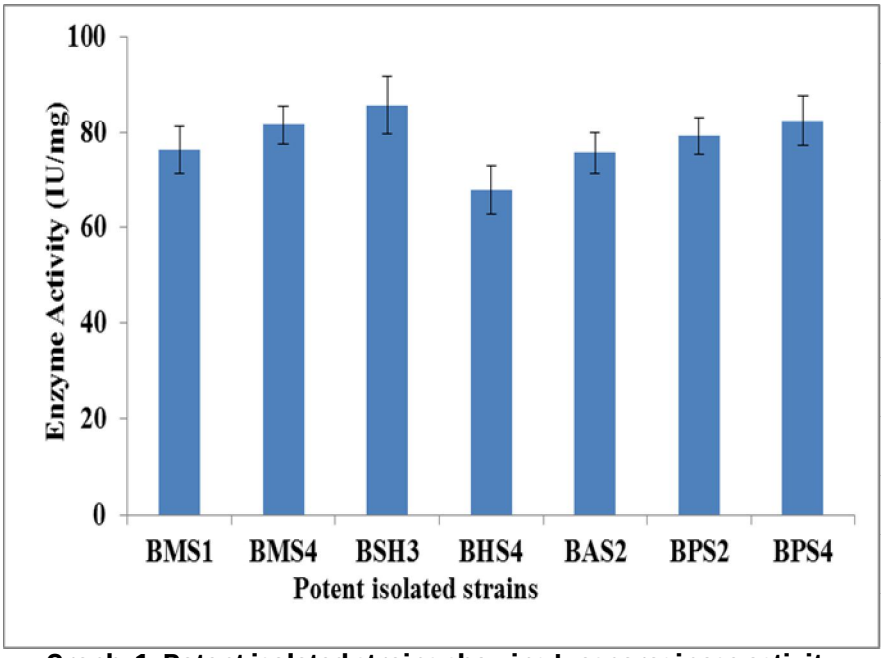
Source of soil samples	Sample code	Total no of isolates	No of potent isolates showing L asparaginase
Marine sample	BMS	18	05
Slaughter house	BSH	10	03
Construction sample	BCS	06	02
Hilly area sample	BHS	08	04
Agriculture field sample	BAS	12	03
Port area sample	BPS	10	04

Table 2: Morphology and cultural characteristics of L-asparaginase producing bacterial isolates

Bacterial Isolate Designation	Cultural Characteristics	Gram Reaction and Microscopic morphology
BMS-1	Large, yellowish, rough irregular colonies	Gram –ve bacilli
BMS-4	Minute, Yellowish, irregular colonies	Gram –ve cocci in pairs
BSH-3	**Yellowish, circular, slimy colonies**	**Gram –ve bacilli in clusters**
BHS-4	Minute, white, irregular, oval shaped	Gram +ve bacilli with bulged edges
BAS-2	Minute lemon yellow colonies	Gram +ve cocci in clusters
BPS-2	Orange yellow, minute mucoid colonies	Gram –ve cocci in chains

Table 3: Bacterial isolates showing excellent L-asparaginase activity

Sample code		L-asparaginase activity (IU/mg)
BMS	1	76.3
	4	81.5
BSH	**3**	**85.7**
BHS	4	67.8
BAS	2	75.6
BPS	2	79.3



Graph. 1: Potent isolated strains showing L-asparaginase activity

CONCLUSION

From this work, it was clearly shows that slaughter house and marine soil samples can provide a rich source of L-asparaginase producing bacteria when compared to other soil sediments and construction area soil sediments show very low L-asparaginase producing bacteria. In contrast, almost all potent isolates showed nearest enzyme activity (Refer Graph). However, in future the BSH-3 bacteria will be taken to categorize its genus, species and to optimize enzyme production as well as purification.

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