International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 7; Issue 6(E); June 2018; Page No. 13383-13385
DOI: http://dx.doi.org/10.24327/ijcar.2018.13385.2386

Research Article

SCREENING OF RHIZOSPHERE SOIL DERIVED FUNGAL ISOLATES FOR L- ASPARAGINASE

Sudha. N[1]* and Anandharaj B[2]

[1]Department of Microbiology & CLT, Dr. Umayal Ramanathan College for Women, Karaikudi, Sivaganga District, Tamilnadu, India
[2]Department of Microbiology, M.R. Govt.College, Mannargudi, Thiruvarur District, Tamilnadu, INDIA

A R T I C L E I N F O

Article History:

Received 8th March, 2018
Received in revised form 24th April, 2018 Accepted 16th May, 2018
Published online 28th June, 2018

Key words:

L- Asparaginase, L- Asparagine, czapekdox agar medium, *Aspergillus* species.

A B S T R A C T

L- asparaginase is an enzyme which is used in food industry and also as chemotherapeutic agent against the acute lymphoblastic leukemia and lymphosarcoma. L-asparaginase is a wide spread enzyme found in many plants, Animals, bacteria, fungi and algae. The present study was aimed at isolating fungal strains capable of producing L- asparaginase from soil samples of karaikudi, Sivaganga district. The fungal isolates were isolated from soil samples and screened for L- Asparaginase production using modified czapekdox agar medium which contain L- Asparagine as main source of carbon and phenol red indicator. On the basis of pink colour zone formed, positive fungal strains were selected. Quantitative enzyme assay was performed by measuring the ammonia liberated by Nesslerization. The highest producer was identified as *Aspergillus* species.

INTRODUCTION

L-Asparaginase (L- Asparagine aminohydrolase) is an enzyme which belongs to an amidase group that hydrolyses the amide bond in L-asparagine to L-aspartic acid and ammonia. They are naturally occurring wide spread enzymes with high molecular weight. The L- Asparaginase has attracted in the past decades because of its two broad applications in food industry and as an antineoplastic agent. In food Industry L-Asparaginase is used in the dough based products and starch rich products like potato chips and French fries to reduce acrylamide formation at very high temperatures of baking or frying. The other application of L- Asparaginase is as a chemotherapeutic agent in the treatment of acute lymphoblastic leukemia and lymphosarcoma. L-Asparagine is a nutritional requirement of both normal cell and cancer cells. The normal growing cells acquire L- Asparagine through diet or synthesized using L- Asparagine synthetase for protein synthesis. The tumour cells require high amount of external L-Asparagine supply for their rapid growth and survival as they are deficient in L- Asparagine synthetase activity, so they take L- Asparagine from blood circulation or any other body fluid. The L- Asparaginase as chemotherapeutic agents catalyzes the hydrolysis of L-asparagine into L-aspartate and ammonia and thereby prevents the rapid malignant growth of tumour cells (Lynette Lincoln and Sunil, 2014; Soni Yadav *et al.,* 2014).

L-asparaginase has been found widely in biological world. L-Asparaginase production using microorganisms has attracted attention, because of cost effective and eco-friendly nature (Kuldeep Kumar and Neelam Verma, 2012). A wide range of microorganisms such as filamentous fungi, yeasts and bacteria proved to be favourable sources of this enzyme. Several microbial strains like *E. Coli, Pyrococcus furiosus, Staphylococcus capitis, Aspergillus niger, Aspergillus terreus, Aspergillus tamari, Fusarium roseum, Saccharomyces Sp., Candida Sp., Vaucheria uncinata* have been reported for L-asparaginase production (Soniyamby Ambi Rani *et al.,* 2012; Dhanam Jayam and Kannan, 2014; Hassan Mustafa Arif and Zahid Hussain, 2014). With this outlook the present study was initiated for the isolation and screening of L- Asparaginase producing fungi from soil samples of Karaikudi, Sivaganga district.

MATERIALS AND METHOD

Isolation of Fungi

Fungi were isolated from the soil samples collected from different areas of Karaikudi, Sivaganga district, Tamilnadu, India. The samples were serially diluted and cultured on PDA plate and incubated at 28°C-30°C for 72 hours temperature. The fungal isolates were identified by lacto phenol cotton blue

Corresponding author:* **Sudha. N
Department of Microbiology & CLT, Dr. Umayal Ramanathan College for Women, Karaikudi, Sivaganga District, Tamilnadu, India

staining and also on the basis of cultural and morphological features (Astrid, 1999).

Preliminary Screening for L-Asparaginase Production

The isolates were screened for L-Asparaginase activity using rapid-plate assay method. The Modified Czapek Dox medium supplemented with 1% L-asparagine and phenol red indicator was used for screening. The isolates were inoculated and incubated at 28 ± 2°C for 96 hours. The isolate that showed pink zone around the colonies which maximum zone diameter were selected and used for further studies (Gulati *et al.,* 1997; Niharika Yadav and Supriya Sarkar, 2014).

Enzyme Production by Submerged Fermentation

The positive fungal isolate was further subcultured on Czapek-Dox medium and stored at 4^0C. Production medium was prepared by using Czapek-Dox medium supplemented with 1% L-asparagine was used for the enzyme production. For inoculation, 25 ml of enzyme broth, 4 discs each of 5mm in diameter were obtained by using sterile cork borer from Czapek-Dox culture plate containing fungal culture. Inoculated flasks were incubated at 28 ± 2ºC for 10 days. The culture medium was filtered using Whatman no .1 filter paper, the filtrate was centrifuged at 5000 rpm for 10 min. The clear supernatant was used as the crude extra-cellular enzyme source (Kumar *et al.,* 2010; Niharika Yadav and Supriya Sarkar, 2014).

Mycelial Dry Weight

Mycelial biomass was collected on pre-weighed Whatman filter paper No1, dried to a constant weight at 50ºC and the difference in weight denoted the mycelial growth of fungus (Kumar *et al.,* 2010; Loureiro *et al.,* 2012).

Determination of L-Asparaginase Activity

Enzyme activity of the culture filtrates was determined at the end of cultivation time by quantifying ammonia formation using Nessler's Reagent. 500 µl 50mM Tris-HCl buffer (pH 8.0) with 20 mM L-asparagine (500µl) and 1.0 ml enzyme were combined and was incubated at 45^0C for 20 min and was terminated by adding 400 µl1.5 M trichloroacetic acid solution. After centrifugation, a 1 ml portion of the supernatant fluid is diluted to 3 ml with distilled water and treated with 1.0 ml of Nessler's reagent and 1.0 ml of 2.0 M NaOH. The color reaction was allowed to proceed for 20 min and the OD was measured at 425 nm (ELICO SL 159 UV – VIS spectrophotometer). The OD was then compared to a standard curve prepared from solutions of ammonium sulphate as the source. Blank was prepared without asparaginase enzyme sample (Produced in production medium). One unit (IU) of L-asparaginase is that amount of enzyme which librates 1 µmole of ammonia in 1 min at 45°C (Imada *et al.,* 1973; Niharika Yadav and Supriya Sarkar, 2014).

Estimation of Protein

Extracellular protein was estimated by Lowry method (Lowry *et al.,* 1951).The protein reacts with the Folin Ciocalteau reagent to give a coloured complex (Siddaligeshwara and Lingappa, 2011).

RESULT AND DISCUSSION

The soil samples collected from different areas of Karaikudi, Sivaganga district, Tamilnadu, India. The samples were serially diluted and plated on PDA medium and incubated at room temperature. All the isolates were screened for L-Asparaginase production by rapid plate assay method. The development of pink colour zone is positive for L-asparaginase production. The fungal isolate which showed pink colour zone around the colonies was selected as a L-asparaginase producer (Table: 1 & 2) (Fig: 1).

Table 1 Isolation of fungal Isolates from various soil samples

S. No.	Samples	No. Of Isolates
1	Garden Soil	4
2	Chemical factory campus soil	3
3	Paddy field soil from Konapet	3
4	Vegetale field soil from Koththari	3
5	Sugarcane field soil from Karuviappatti	4

Table 2 Plate Assay Method

S. No	Positive Isolates code	Fungi	Zone diameter (cm)
1	GAR3	*Aspergillus sp.*	12
2	PFK1	*Penicillium sp.*	6
3	PFK2	*Aspergillus sp.*	4
4	VFK3	*Mucor sp.*	8

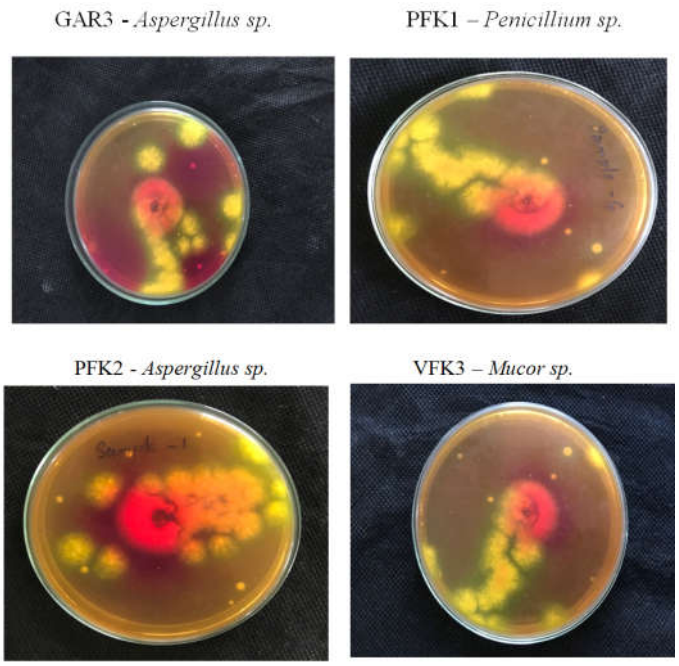

Fig 1 L- Asparaginase activity detected by Plate assay method , showing pink colour zone around the colony

The positive fungal isolate was identified as *Aspergillus* sp.*(*GAR3), *Penicillium* sp.*(* PFK1), *Aspergillus* sp.*(* PFK2), *Mucor* sp.*(* VFK3) by lacto phenol cotton blue stain based on cultural and morphological features (fig: 2).

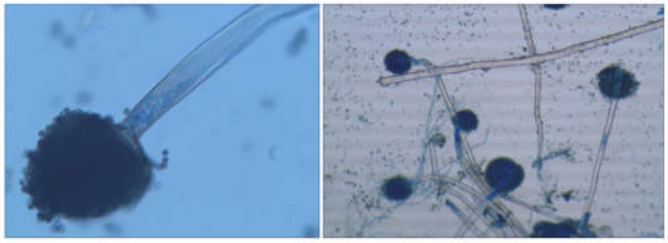

Fig 2 Lactophenol cotton blue mount of GAR3 - *Aspergillus sp.*

In this experiment, the L-asparaginase production by *Aspergillus* sp. (GAR3) was studied for 10 days of incubation period when cultured on Czapek-Dox medium supplemented with 1% L-asparagine (Fig: 3).

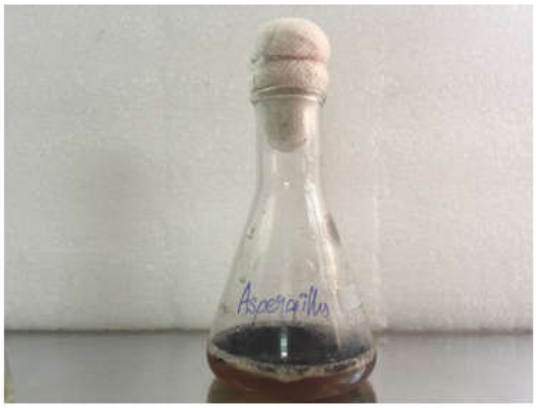

Fig 3 GAR3 - *Aspergillus* sp. in Czapek-Dox liquid medium

The activity was determined after 2, 4, 6, 8, 10 days of incubation. The L- Asparaginase enzyme activity was calculated by plotting a standard graph using Ammonium sulphate, from the standard graph it was calculated that *Aspergillus* species showed highest activity 80 IU / ml on eighth day. The protein estimation was performed by Lowery method. The protein content was found to be 29 ng /g and the specific activity was found to be 1IU / ml (Table: 2).The mycelial dry weight was also identified as 0.868mg/g (Table: 2). The organism was maintained for various physico-chemical characters.

Table 3 Enzyme activity of the strain GAR3 - *Aspergillus sp.*

Day	Dry weight	Enzyme activity (IU/ml)	Specific activity (IU/ml)
2	0.051	5	0.23
4	0.685	20	0.25
6	1.016	28	0.35
8	0.868	80	1.00
10	0.056	10	0.12

CONCLUSION

From this study, it is determined that soils can provide a good source of L- asparaginase producing fungi. The positive L-asparaginase producing isolates were screened and was found to belong to genera *Aspergillus.* The enzyme production can be optimized by studying various parameters. Further studies on purification can contribute to therapeutic value of the enzyme.

References

1. Lynette Lincoln and Sunil S. More. Isolation and production of clinical and food grade L- Asparaginase enzyme from fungi. *Journal of Pharmacognosy and Phytochemistry* 2014, 3(3): 177-183.
2. Soni Yadav, Sitansu Kumar Verma, Jitendra Singh, Ajay Kumar. Industrial Production and Clinical Application of L- Asparaginase: A Chemotherapeutic Agent. *International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering,* vol. 8, No.1, 2014.
3. Kuldeep Kumar and Neelam Verma. The various sources and Application of L-Asparaginase. *Asian Journal of Biochemical and Pharmaceutical Research* 2012, 3(3): 197 - 205.
4. Dhanam Jayam, G and Kannan, S. The various sources of L- Asparaginase. *International journal for Recent Scientific Research* vol. 5, issue,2, pp.342-346, 2014.
5. Hassan Mustafa Arif, Zahid Hussain. Important Sources and Medical Applications of L- Asparaginase. *International Journal of Pharma Research and Review,* 2014, 3(6):35-45.
6. Soniyamby Ambi Rani, Lalitha Sundaram, Praveesh Bahuleyan Vasantha, Isolation and Screening of L-asparaginase producing fungi from soil samples. *International Journal of Pharmacy and Pharmaceutical Sciences* 2012 4:279-282.
7. Astrid. L. Preparation of Lactophenol Cotton Blue Slide Mounts. *Community Eye Health* 1999; 12(30):24.
8. Gulati R, Saxena RK, Gupta R. A rapid plate assay for screening L-asparaginase producing micro-organisms. *Lett Appl Microbiol* 1997; 24:23-6.
9. Niharika Yadav. C and Supriya Sarkar. Production of L-asparaginase by *Fusarium oxysporum* using submerged fermentation. *International Journal of Pharmaceutical Science Invention* 2014, volume 3 issue 61 PP; 32-40.
10. Kumar, S., Pakshirajan, K., VenkataDasu, V., 2010. Localization and production of novel L-asparaginase from *Pectobacteriumcarotovorum* MTCC 1428. Process Biochem. 45, 223–229.
11. Loureiro CB, Borges KS, Andrade AF, Tone LG, Said S. Purification and Biochemical Characterization of native and pegylated form of L-asparaginase from *Aspergillus terreus* and evaluation of its antiproliferative activity. *Adv. Microbiol* 2012; 2: 138-45.
12. A. Imada, S. Igarasi, K.Nakahama, and M. Isono, "Asparaginase and glutaminase activities of micro organisms," *Journal of General Microbiology*, vol. 76, no. 1, pp. 85–99, 1973.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193:265-275.
14. Siddaligeshwara KG, Lingappa K. Production and characterization of L-asparaginase A tumour inhibitor. *Int J PharmTech Res* 2011;3:314-319.

How to cite this article:

Sudha. N and Anandharaj B (2018) 'Screening of Rhizosphere Soil Derived Fungal Isolates for L- Asparaginase', *International Journal of Current Advanced Research,* 07(6), pp. 13383-13385.
DOI: http://dx.doi.org/10.24327/ijcar.2018.13385.2386

****