ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 12, pp. 14834-14836, December, 2016

International Journal of Recent Scientific Research

Research Article

ISOLATION AND SCREENING OF L-ASPARAGINASE ENZYME FROM MICROFUNGI

Rathish R[1]., Madanraj P[1] and Ambikapathy V[2]

[1]Department of Microbiology, Marudupandiyar College, Vallam, Thanjavur
[2]Department of Botany and Microbiology, A.V.V.M Sri Pushpam College (Auto), Poondi, Thanjavur

ARTICLE INFO

Article History:

Received 05th September, 2016
Received in revised form 08th October, 2016
Accepted 10th November, 2016
Published online 28st December, 2016

Key Words:

Soil sample, Microfungi, L-asparaginase,

ABSTRACT

Marine micro fungi are a great ability in producing enzymes in a minimal medium containing L-asparaginase enzyme and phenol red as indicators. Isolation and identification of microfungi from marine soil samples. Some of the potential microfungi were isolated and characterized by using standard manual. The screening of microfungi on the basis of enzyme L-asparaginase activity by standardized to hydrolysis from the various sources. Different culture condition was examined such as pH, temperature, incubation period and nutrient sources were optimizing for enzyme production. The results were discussed in detail

INTRODUCTION

Microbial L-asparaginase is one of the most important industrial enzymes of interest on accounting for about 40 % of the total worldwide enzyme sales (Elshafei *et al*., 2012). The enzyme L-asparagine amido hydrolase E.C.3.5.1.1 belongs to an amidase group that catalyzes the hydrolysis of the amino acid L-asparagine to L-aspartic acid and ammonia. A remarkable achievement in the field of medicine was the development of the L-asparaginase enzyme much significance in medical field for the treatment of leukemia especially acute lymphoblastic leukemia (ALL) and an effective antitumor agent (Verma *et al*., 2007). L-asparaginase is also widely used in baking and food industries to reduce the formation of carcinogenic acrylamides in biscuits and in deep fried potato (Pedreschi *et al*., 2008 and Mohan Kumar and Manonmani, 2012).

L-asparaginase is widely distributed in plants, animals and microorganisms. L.asparaginase is a tetramer protein that deaminates Asn and Gln. L-asparaginase inhibits protein synthesis in T-cells by catalyzing the conversion of L-asparagine to L-aspartate and ammonia, and this catalytic reaction is essentially irreversible under physiological conditions. L-asparagine is a major requirement by the cells for the production of protein. It can be produced within the cell by an enzyme called asparagines synthetase or can be absorbed from outside. Tumor cells, more specifically lymphatic tumor cells, require high amount of asparagines for their rapid malignant growth. Therefore, L-asparagine is an essential amino acid for the growth of tumor cells, whereas the growth of normal cells is not dependent on its requirement as it can be synthesized in amounts sufficient for their metabolic needs with their own enzyme L-asparagine synthetase. The presence of L-asparaginase deprives of an important growth factor and them failure to survive. Thus the development of enzyme as a potent anti-tumor or anti leukemic drug (Neelam and Kuldeep, 2007). L-asparaginase has its application in food industry. L-asparaginase can be used as a food processing to reduce the formation of acrylamide, a suspected carcinogen, in starchy food products. Acrylamide is a chemical compound, formed in starchy foods when they are baked or fried. During heating the amino acid asparagine, naturally present in starchy foods, is converted into acrylamide in a process called the Maillard reaction. The reaction is responsible for giving baked or fried foods their brown color, crust and toasted flavour. By adding asparaginase before baking or frying the food, asparagine is converted into another common amino acid, aspartic acid, and ammonium.

L-asparaginase from microbial sources has gained much attention because of its high productivity. It is extracellular and therefore secreted in to the fermentation medium. Among microbes, this enzyme is produced by bacteria, fungi and actinomycetes. Microbial strains like *Escherichia coli* (Younes *et al*., 2008), *Erwinia caratovora* (Vaibhav *et al*., 2010), *Pseudomonas aeruginosa* (Manikandan *et al*., 2010), *Streptomyces gulbargensis* (Amena *et al*., 2010), *Aspergillus*

*Corresponding author: **Rathish R***
Department of Microbiology, Marudupandiyar College, Vallam, Thanjavur

terreus (Balasubramanian *et al*., 2012), *Aspergillus niger* (Laan *et al*., 2008), *Penicillium brevicompactum* (Elshafei *et al*., 2012), *Cladosporium* sp (Kumar and Manonmani, 2013) are the main source of L-asparaginase. Bacterial L-asparaginase has been reported to cause hypersensitivity leading to allergic reactions and anaphylaxis (Moola *et al*., 1994). Hence, L-asparaginase from eukaryotic microorganisms is gaining much importance as it is known to have less adverse effects (Sarquis *et al*., 2004).

MATERIALS AND METHODS

Isolation of fungi from soil samples (Palaniswamy et al., 2008)

The fungi in this study, isolated from soil samples were collected from *Thengapattinam, coastal* areas of Kanyakumari District. The dilution plate method was employed for the isolation of fungal strains. The isolated fungi were maintained on modified Czapek Dox medium. In 10^{-3} dilution is better for the identification of fungal isolates.

Screening of L-asparaginase producing fungi (Gulati et al., 1997)

The fungi obtained from the above steps were subjected for rapid screening of L-asparaginase production by plate assay. Modified Czapek Dox's (mCD) medium11, pH 6.2, used for fungi contained 0.2% (w/v) glucose, 1% (w/v) L-asparagine, 0.152% (w/v) K_2PO_4, 0.052% (w/v) KCl, 0.052% (w/v) MgSO4.$7H_2O$, 0.003% (w/v) $CuNO_3$. 3H2O, 0.005% (w/v) $ZnSO_4.7H_2O$, 0.003% (w/v) $FeSO_4.7H_2O$, 1.8% (w/v) agar, initial pH 6.2 was supplemented with 0.009% (v/v) phenol red as indicator. Control plates were MCD medium containing $NaNO_3$ as nitrogen source instead of L-asparagine. The plates were inoculated with the 38 selected fungal isolates and incubated at 30°C for 48 hours. The isolates that showed pink zone around the colonies indicated L-asparaginase production and zone was measured.

RESULT AND DISCUSSION

In this present study thirteen fungal species were isolated from the soil sample. According to physicological and morphological identification of fungi were identified to the genera *Aspergillus* sp., *Curvularia* sp., *Penicillium* sp. and *Trichoderma* sp (Table 1).

Table 1 Isolation of microfungi from marine soil sample

S.No	Name of the fungi	*Thengapattinam*
1	*Aspergillus flavus*	5
2	*A.fumigatus*	11
3	*A.niger*	6
4	*A.candidus*	5
5	*A.sydowi*	2
6	*A.terreus*	8
7	*A.sulhureus*	4
8	*Curvularia lunata*	5
9	*Penicillium chrysogenum*	9
10	*P.citrinum*	11
11	*Penicillium sp*	3
12	*Trichoderma harzianum*	11
13	*T.kongii*	12

Haqeeqat and Sahera, (2016) reported that the soil samples were analyzed with respect to different types of fungi. The most common fungi, *Aspergillus niger*, *Penicillium stoloniferum*, *Penicillum* sp. and *Rhizopus* sp. was recorded and also the highest zone index was exhibited by an *Aspergillus* isolate (Vasini Roy, 2016).

The isolated fungi were screened L-asparaginase activity. Among this isolates showed pink zone around the colonies on modified Czapek dox agar. The maximum zone of diameter were measured in *A.terreus*, *A.sulphureus*, *Trichoderma kongii*, *Penicillium chrysogenum*, *P.citrinum* and *Trichoderma harzianum* was 25, 22, 23, 21 and 20 mm zone were measured (Table 2). The preliminary screening of filamentous fungi is based on the semi qualitative methods described by Gulati *et al*. (1997). Similar reports were recorded in the maximum L-asparaginase production was observed at pH 7.0 and temperature 30°C at 178 hours of fermentation period. Further, these isolates were subjected for secondary screening for enzyme activity by Imada *et al*. (1973).

Many soil fungal species have been reported that production of L-asparaginase. Eg., *Emericella nidulans* from different soils of Tumkur University Campus, Karnataka, India (Jayaramu *et al*., 2010), *Aspergillus flavus* (KUFS20) from the garden soil of Coimbatore, Tamil Nadu, India (Rani *et al*., 2011) and Pencilllin species from soil samples of Bangalore, Karnataka, India (Mushtaq *et al*., 2012). Hosamani *et al*. (2011) reported the screening of *Fusarium equiseti* from rhizosphere soil of various plants around Karnataka university campus, Dharwad, Karnataka and suggested that the presence of the fungus might be due to the presence of a natural source of amino acids in the root exudates of the plants in the rhizosphere soil.

Table 1 Isolation of microfungi from marine soil sample

S.No	Name of the fungi	*Thengapattinam*
1	*Aspergillus flavus*	5
2	*A.fumigatus*	11
3	*A.niger*	6
4	*A.candidus*	5
5	*A.sydowi*	2
6	*A.terreus*	8
7	*A.sulhureus*	4
8	*Curvularia lunata*	5
9	*Penicillium chrysogenum*	9
10	*P.citrinum*	11
11	*Penicillium sp*	3
12	*Trichoderma harzianum*	11
13	*T.kongii*	12

Reference

1. Amena, S., Vishalakshi, N., Prahhakar, M., Dayanand, A. and Lingappa, K., (2010) Production, purification and characterization of L- asparaginase from Streptomyces gulbargensis. *Braz J Microbiol;* 41(1): 173-178.
2. Balasubramanian, K., Ambikapathy, V. and Panneerselvam, A., (2012) Production, isolation, and purification of L-asparaginase from Aspergillus terreus using submerged fermentation. *Int J Adv Pharma Res*; 3(2): 778-783.
3. Elshafei, A.M., Hassan, M.M., AbouZeid, M.A., Mahmoud, D.A. and Elghonemy, D.H., (2012) Purification, characterization and antitumor activity of L-asparaginase from *Penicillium brevicompactum* NRC 829. *Br Microbiol Res*; 2(3): 158-174.
4. Gulati, R., Saxena, R.K. and Gupta, R., (1997). A rapid plate assay for screening L-asparaginase producing micro-organisms. *Lett. Appl. Microbiol*; 24:23-26.

5. Haqeeqat, A.A. and Sahera, N., (2016). Isolation and identification of fungi from soil samples of different sites in Aurangabad City, India. *International J. Scientific Research;*5 (3): 419
6. Hosamani, R. and Kaliwal, B.B., (2011). Isolation, molecular identification and optimization of fermentation parameters for the production of L-asparaginase, an anticancer agent by *Fusarium equiseti*. *Int J Microbiol Res;* 3:108-19.
7. Imada, A., Igarasi, S., Nakahama, K. and Isono, M., (1973). Asparaginase and glutaminase activities of micro-organisms. *J Gen Microbiol*; 76:85-99.
8. Jayaramu, M., Hemalatha, N.B., Rajeshwari, C.P., Siddalingeshwara, K.G. and Mohsin, S.M., (2010). A novel approach for detection, confirmation, and optimization of L-asparaginase from *Emericella nidulans*. *Curr Pharm Res*; 1:20-4.
9. Kumar, N.S.M. and Manonmani, H.K., (2013) Purification, characterization and kinetic properties of extracellular L-asparaginase produced by *Cladosporium* sp. *World J Microbiol Biotechnol;* 29(4): 577-587.
10. Laan, V.D., Stor, M.C., Lange, D.I. and Mohrmann, L., (2008). *Aspergillus niger* asparaginase variants and their commercial uses. US Patent No. WO2008128974.
11. Manikandan, R., Pratheeba, C.N., Pankaj, S. and Sah, S., (2010) Optimization of L-asparaginase production by Pseudomonas aeruginosa using experimental methods. *Nat Sci*; 8(2): 1-6.
12. Moola, Z.B., Scawen, M.D., Atkinson, T, Nicholls D (1994). *Erwinia chrysanihemi* L-asparaginase: epitope mapping and production of antigenically modified enzymes. *Biochem J;* 302: 921-927.
13. Mushtaq, M.S., Siddalingeshwara, K.G., Karthic, J., Sunil, D.P., Naveen, M. and Prathiba, K.S., (2012). Rapid screening and confirmation of L-asparaginase from *Penicillium* sp. *Int J Res Pharmacol Pharm*; 1:147-50.
14. Palaniswamy, M., Pradeep, B.V., Sathya, R. and Angayarkanni, J., (2008). Isolation, identification, and screening of potential xylanolytic enzyme from litter degrading fungi. *Afri J Biotech*; 7:1978-1982.
15. Rani, S.A., Lalitha, S. and Praveesh, B.V., (2011). *Invitro* antioxidant and anticancer activity of L-asparaginase from *Aspergillus flavus* (KUFS20). *Asian J Pharm Clin Res*; 4:174-7.
16. Sarquis, M.I.D.M., Oliveira, E.M.M., Santos, A.S. and Da-Costa, G.L., (2004). Production of L-asparaginase by filamentous fungi. *Mem Inst Oswaldo Cruz*; 99(5): 489-492.
17. Saxena, R.K. and Sinha, U., (1981) L-asparaginase and glutaminase activities in the culture filtrates of *Aspergillus nidulans*. *Curr Sci*; 50(5): 218- 219.
18. Vaibhav, D.D., Mangesh, D.V. and Lambert, R., (2010) Production of intracellular L-asparaginase from Erwina carotovora and its statistical optimization using response surface method. *Int J Chem Sci Appl;* 1(1): 25-36.
19. Verma, N.K., Kumar, G. and Kaur-Anand, S., (2007) L-asparaginase: A promising chemotherapeutic agent. *Crit Rev Biotechnol*; 27(1): 45- 62.
20. Vasini Roy K.V., (2016) Isolation and screening for L. Asparginase producing fungi IJBPAS; 5(9): 2196-2202.
21. Younes, G., Alireza, E., Sara, R.A. and Gholamreza, Z., (2008) An optimized medium for screening of L-asparaginase production by *Escherichia coli*. *Am J Biochem Biotechnol;* 4(4): 422-424.

****

How to cite this article:

Rathish R, *et al.*2016, Isolation and Screening of L-Asparaginase Enzyme From Microfungi. *Int J Recent Sci Res.* 7(12), pp. 14834-14836.