J. Sci. Trans. Environ. Technov. 2020, 13(3) : 131-133

Isolation and Screening of Microfungi by L-Asparaginase enzyme assay

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Article History
Received: 12.11.2019
Revised and Accepted : 11.02.2020
Published: 16.03.2020

https://doi.org/10.56343/STET.116.013.003.007
http://stetjournals.com

Abstract

Marine micro fungi are having great ability in producing enzymes in a minimal medium containing L- asparaginase enzyme. Thirteen fungal species were isolated from the soil samples of Thengapattinam coastal area of Tamilnadu, southern India. The screening of microfungi was on the basis of enzyme L-asparaginase production under different culture conditions varying in pH, temperature, incubation period and nutrient sources for optimizing the enzyme production. *Aspergillus fumigattus, Penicillium citrinum, and Trichoderma harzianum* had higher enzyme activity.

Keywords : *Aspergillus*, micro fungi, L-asparaginase, *Pencillium*, soil sample, *Trichoderma*

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 for the treatment of leukemia especially acute lymphoblastic leukemia (ALL) and as an effective antitumor agent. 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.

L-asparaginase is widely distributed in plants, animals and microorganisms. L. asparaginase is a tetramer protein that deaminates Aspargine and Glycine. 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 the tumor cells fail to survive. As such this enzyme is a potent anti-tumor or anti leukemic drug. L-asparaginase has its application in food industry as well. L- asparaginase can be used in 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 terreus* (Balasubramanian *et al*., 2012), *Aspergillus niger* (Laan *et al*., 2008), *Penicillium brevicompactum* (Elshafei *et al*., 2012), *Cladosporium* sp (Kumar and Manonmani, 2013)

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P - ISSN 0973 - 9157
E - ISSN 2393 - 9249

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 for better identification of fungal isolates.

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

The preliminary screening of filamentous fungi is based on the semi qualitative methods described by Gulati *et al*. (1997). . Further, these isolates were subjected for secondary screening for enzyme activity as described by Imada *et al*. (1973). 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) K2PO4, 0.052% (w/v) KCl, 0.052% (w/v) MgSO4.7H2O, 0.003% (w/v) CuNO3. 3H2O, 0.005% (w/v) ZnSO4.7H2O, 0.003% (w/v) FeSO4.7H2O, 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 NaNO3 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 diameter was measured.

RESULT AND DISCUSSION

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

Haqeeqat and Sahera, (2016) reported fungi in the soil samples of Aurangabad, India, of which,the most common fungi were *Aspergillus niger*, *Penicillium stoloniferum*, *Penicillum* sp. and *Rhizopus* sp.

The isolated fungi were screened L-asparaginase activity. The maximum zone of diameter were measured in *A.fumigaus, P.citrinum* and *Trichoderma harzianum.*Vasini Roy (2016) also found the highest zone of inhibition was exhibited by an *Aspergillus* isolate .

Table 1. Isolation of microfungi from marine soil sample collected from Thengapattinam, southern India

S.No.	Name of the fungi	Zone Diameter (mm)
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

Many soil fungal species have been reported to produce L-asparaginase. For 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 L-Asparginase 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.

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