

L- asparaginases from fungi of Bhitarkanika mangrove ecosystem

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Abstract. 208 fungi isolated from mangrove ecosystem of Bhitarkanika were screened for L asparaginase activity. Among them 85 fungi exhibited enzyme activity in biomass and 20 fungal strains showed L asparaginase activity in culture filtrate. *Penicillium* sp. strain PF 52 and RF 22 were found to be good source of L asparaginase as they exhibited highest activity in biomass and culture filtrate respectively. During screening programme, *Scopulariopsis* strain FMG 133 and single unidentified fungal strain FMG 181 showed good enzyme activity i.e. 10.28 & 7.85 i. u. /ml/g activity in biomass and 6.25 & 11.44 i.u./ml/g in the well as culture filtrate of 10 day old culture. Over all *Aspergillus*, *Penicillium*, *Fusarium*, *Helminthosporium*, *Scophulariopsis*, *Paecilomyces* and *Pestalotiopsis* were found to be good source of L asparaginase enzymes in this study.

Keywords: Enzymes, Asparaginase, mangrove, fungi, *Penicillium*, *Aspergillus*

INTRODUCTION

L asparaginase enzymes are known as antitumor agent and studied well worldwide Swain *et al.*, 1993, Boyd and Philips, 1971). They are characterized by their ability to degrade L asparagine into L aspartic acid and ammonia in a hydrolytic reaction. This causes a rapid depletion in serum asparagine, an exogenous supply of which is required for growth of certain tumors Ferrare *et al.*, 2004; Borkotaky and Bezbarbah (2002); Abdefattah and Olama (2002). Asparaginases are found in diverse sources in nature, including bacteria, yeasts, molds, plants and vertebrates. The enzymes derived from microorganisms are the major sources of the enzymes for practical clinical use Northrup *et al.*, 2002. However, very rare reports are available on the asparaginases producing microbes from mangrove origin Das *et al.*, 2006, Bacterial asparaginase from mangrove of Andaman islands and Pichavaram was reported by some workers Kathiresan (2000), Shome and Shome (2001). No available reports are found on the asparaginases from Bhitarkanika mangrove ecosystem. Hence, a wide scale screening was made of the fungal isolates obtained from this mangrove area with the objective to obtain some good microbial strains.

MATERIALS AND METHOD

Study sites. The present study was carried out on fungi collected from Bhitarkanika mangrove forests of Orissa coast. It is located on the East Coast of India (20° 4' 20' 8' N latitude and 86° 45' 87' 5' E Longitude) and extends upto the northeastern part of the Mahanadi delta in the Kendrapara district of Orissa. Tides are semi-diurnal in nature with an altitude of 2-3 m in upstream areas and 3.5-6.0 m near the river mouth. Due to regular inundations through tidal action the soil is highly slushy. The surface soil is composed of silt and clayey loam and is about 3-4 m in depth. The soil though well-aerated, is saline. In the elevated areas away from the creeks and channels, the soil is more sandy and comparatively less moist and saline. The soil pH varies from 6.3 - 7.1

Source materials. The fungi were isolated from phyllosphere and rhizosphere of mangrove plants, and air samples collected from the different locations including outer estuaries,

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inner estuaries, creeks at lower elevation and higher elevation and transitional zones. The area included were Khola, Brahmani river, Brahmamari creek, Bhitarkanika, confluence point of Bhitarkanika and Khola, Mahishmari Creek, Dangmal area.

Collection of samples. Plant and air samples were collected from mangrove area by visiting the different locations with the help of country boat. Air samples were collected by opening presterilised specific medium for 10 seconds in open environment of mangrove region and were closed tightly with parafilm so that entrapment of more air did not occur. A total 26 plant and 22 air samples were collected and brought to the laboratory in ice containers to preserve their natural condition.

Isolation of fungi. Phyllosphere and rhizosphere fungi were isolated by keeping pre-sterilised plant material of 1cm in size on potato dextrose agar (pH 5) and incubated at 30°C for 6 days. The plates containing trapped air samples were also incubated similarly. After 6 days, the fungi grown on different plates of various samples were isolated and purified. All pure cultures of fungi were identified according to morphological characterization by following slide culture method Raper *et al.*, 1984; Bennett and Hunter, 1972.

Screening of fungi for L- asparaginase activity. Fungi were screened for the L- asparaginase activity in Biomass and extracellular condition by culturing them in glucose asparagines medium (25ml in 150ml Ehrlenmeyer flasks) 4.5 pH, at 30°C for 10 days in static incubation. The cultures were harvested after the due incubation period and the mycelial mats were washed with ice cold distilled water. One gram of fresh fungal biomass was thoroughly homogenized in 10 ml of cold normal saline with sterilized sand and mycelial extract obtained was centrifuged at 3000 rpm for 20 min and supernatant was directly used as the source of enzyme. To evaluate the extracellular enzyme activity, culture filtrate was centrifuged at 3000 rpm and supernatant was assayed for enzyme activity. The enzyme L asparaginase was assayed by estimating the amount of ammonia released in the reaction Soni, 1989. The amount of Ammonia released by the test sample was calculated with reference to the standard graph. The enzyme activity was expressed in terms of enzyme units (i. u./ml).

RESULTS

A total of 208 fungi were isolated from phyllosphere, rhizosphere and air samples. These fungi were identified as *Penicillium*, *Aspergillus*, *Fusarium*, *Helminthosporium*, *Paecilomyces*, *Alternaria*, and *Cladobotrytis*. Among 208 fungi studied for L asparaginases, 85 fungi produced good amount of enzyme in biomass indicating the production of intracellular and cell bound enzymes. However maximum enzyme was

produced by *Penicillium* PF 52 i.e. 16.71 i.u./ml/g followed by *Penicillium* sp.RF2 (5.41 i. u./ml/g) and *Helminthosporium* RF3 (4.88 i. u. /ml/g). Other fungi showed poor enzyme activity.

Only 20 fungi appeared positive for the extracellular production of L asparaginase into culture medium after 10 days of growth. The highest enzyme activity recorded was 9.94 and 7.29 i.u./ml/g produced by *Paecilomyces* RF21 and *Penicillium* RF 22 followed by *Aspergillus* AF 7 (6.26 i.u. /ml/g).

It is very interesting to note that all fungi which are producer of biomass - bound enzyme in this present study, were not necessarily found to be producer of extracellular L -asparaginase. The phyllosphere fungi endowed with both intracellular and extracellular enzyme activity exhibited higher quantity of intracellular enzymes as compared to extracellular. Among them *Scophulariopsis* sp. strain FMG 133 produced 10.28 and 6.25 i. u. /ml/g enzymes in biomass and culture filtrate, respectively (Figure 1). In contrast, almost all rhizosphere fungi produced asparaginase extracellularly in more than intracellular ones. Fungal strains producing lower intracellular and higher extracellular L asparaginases were *Penicillium* sp. strain RF22 and single unidentified fungal strain FMG 181 that showed 9.9 and 11.44 i.u. /ml/g enzymes in 10 days old culture filtrate (Figure 2).

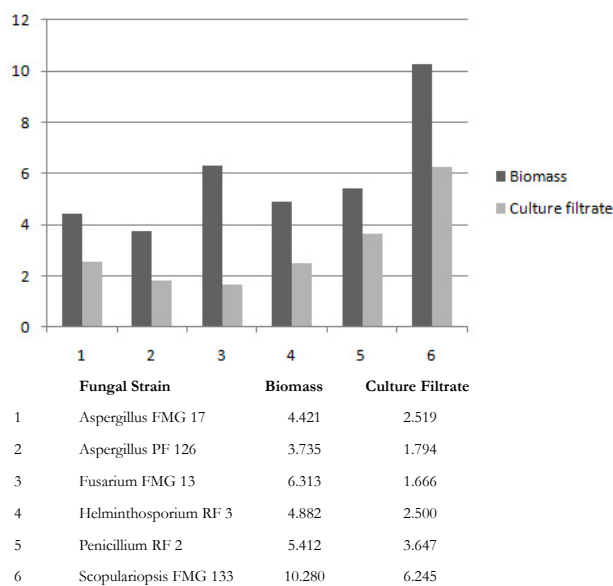


Figure1. Fungal strains producing higher intracellular (biomass) and lower extracellular (culture filtrate) L asparaginase i.u./ml/gm

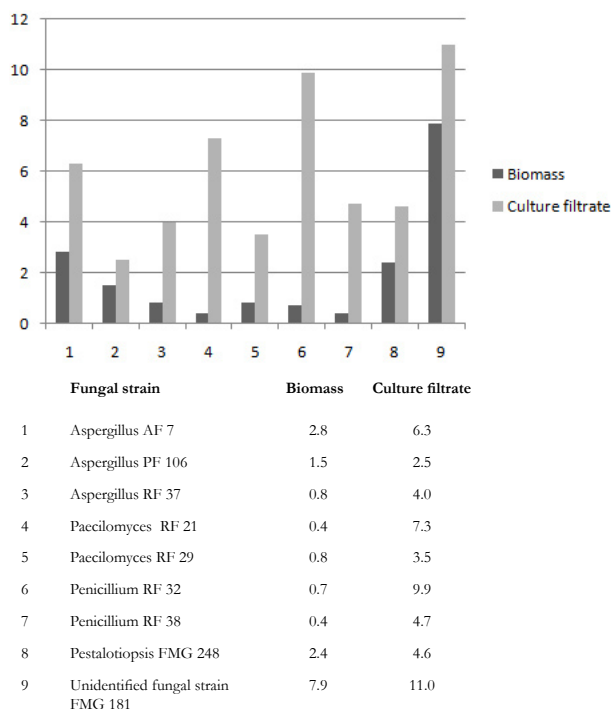


Figure 2. Fungal strains producing lower intracellular (biomass) and higher extracellular (culture filtrate) L asparaginase i.u./ml/gm

DISCUSSION

The findings of achieving extracellular L asparaginases from mangrove origin itself important as studies on amino acid degrading enzymes for both essential and non essential amino acids from marine microbes represent an important approach in chemotherapy for cancer (Geckil and Gerner, 2004; Dhevagi and Poorani, 2006). Cell bound asparaginases may be the general phenomenon in fungi as most of fungal isolates were found as producer of asparaginase in biomass (Saxena and Sinha, 1981). In present study, more number of fungi from rhizosphere have shown extracellular asparaginase as compare to phyllosphere fungi. It may be due to the more availability of extracellular metabolite especially free amino acids around the rhizosphere. Because of that surrounding microbes might have adapted the uptake and utilization systems for available nutrients. This has also confirmed with our observations regarding more production of extracellular enzymes from rhizosphere fungi. In general, most of the microbial L –asparaginases are intracellular in nature except few, which are secreted outside the cell (Savitri *et al.*, 2007, Narayana *et al.*, 2008). In our study also some fungi could be observed with biomass asparaginase only. It may indicate the cell bound activity of asparaginase enzyme that either released outside cell and become inactive or not produced out at all. The two fungal strains *Scopulariopsis* sp. strain FMG 133 and single unidentified strain FMG 181 were found to be most potent system to be exploited as both strains showed L asparaginase activity in intra and extracellular conditions. Besides this, three other potent fungal

strains namely, *Penicillium* sp. strain PF52 (intracellular), and *Paecilomyces* sp. strain RF21 and *Penicillium* strain RF22 (extracellular) exhibited the potential for being explored in strain improvement programme for the further utilization in mass scale and clinical use.

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