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Research Article

SCREENING OF L-GLUTAMINASE FROM SEAWEED ENDOPHYTIC FUNGI

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Abstract: This work studied glutaminase enzyme production by seaweed endophytic fungi. Totally 50 fungal endophytes isolated from seaweed samples, only 10 showed glutaminase production as evident by change of the colour of the medium from yellow to pink due to change in the pH of the medium. The strain 7 of *Penicillium* sp., showed maximum enzyme production (31.62 U/ml) and strain S1 the minimum enzyme production (10.24 U/ml).
Keywords: Seaweeds, endophytic fungi, screening, L-glutaminase.

1. Introduction

Seaweeds are marine macroalgae forming an integral part of marine coastal ecosystems. Fungi both parasitic and saprobic have been reported from marine algae (living on the tissue, as epiphytes). Only few studies have investigated the endophytes of marine algae [1]. An endophyte is described as an organism that lives inside the tissue of a host plant without causing any symptom of disease [1, 2]. The interactions between the endophytic fungi and their hosts are complex including mutualism, commensalism, latent and virulent pathogenicity[3]. Certain endophytic fungi might promote growth and improve the ecological adaptability of their hosts by enhancing plant tolerance to environmental stresses and resistance to phytopathogens and/or herbivores[4]. The marine-derived endophytic fungi of many species of algae (red, green and brown) have yielded bioactive metabolites of novel chemical structures, with an array of bioactive properties, such as, antimicrobial, antifungal and insecticidal, as well as cytotoxic effects on tumor cells [5, 6].They have not been studied to any extent for their enzyme potential[7].

L-glutaminase (L-glutamine amidohydrolase E.C 3.5.1.2) is a hydrolytic enzyme, that deaminates L- glutamine to L-glutamic acid and ammonia. The action of L-glutaminase plays an important role in the nitrogen metabolism of both prokaryotes and eukaryotes. L-glutaminase has attracted much attention in both pharmaceutical and food industrial applications. It is used as a flavor enhancer by increasing the glutamic acid content in food by the hydrolysis of L-glutamine to L-glutamic acid and ammonia. It is also used in enzyme therapy for cancer especially for acute lymphocytic leukemia [8, 9, 10]. Its importance as biosensors for monitoring the glutamine levels in mammalian and hybridoma cells is well known. In *Pseudomonas* sp., recombinant glutaminase was patented for its activity against cancer and HIV virus therapy [11].

L-glutaminase can be derived from animal and plant tissues. But microbial enzymes are significantly meeting the industrial demands. For example bacteria, fungi and yeast are the potential sources for the production of L-glutaminase enzyme. Even there are a few reports on the production of extra-cellular L-glutaminase under SSF (solid state fermentation) using microbial strains [12, 13, 14].

Terrestrial microorganisms such as *Escherichia coli*, *Pseudomonas species*, *Acinetobacter* sp., *Bacillus* sp., *Hansenula*, *Cryptococcus*, *Candida*, *Aspergillus oryzae* and *Beuveria bassiana* were earlier reported for L-glutaminase synthesis [15]. Not only this even L-glutaminase activity was reported from marine microorganisms such as *Micrococcus luteus Pseudomonas fluorescens*, *Vibrio cholerae* and *Beuveria bassiana* [16] and also from marine actinomycetes [17]. Based on the available literature no reports are available for L-glutaminase production from seaweed associated microbes. The main objective of this study is to isolate industrially important glutaminase enzyme from endophytic fungi of seaweed.

2. Experimental methods

2.1 Source of endophytic fungi

The endophytic fungi were isolated from healthy seaweed samples collected from Mandapam coastal region such as *Codium* sp., *Halimeda gracilis*, *Halimeda macroloba*, *Caulerpa racemosa*, *Gelidiella acerosa*, *Gracilaria corticata*, *Gracilaria edulis and Gracilaria crassa* and from Pondicherry coast such as *Ulva fasciata* and *Chaetomorpha antennina*.

2.2 Isolation of endophytes from seaweeds

Surface sterilization method was followed to ensure that all isolated fungi are endophytic [18, 19]. The chemical sterilization procedure was followed by placing the algae in the beaker containing disinfectant ethanol (80%) by specified timing and then immersed into the water. Time duration required for each individual algal species was performed and standardized before isolating endophytes from algal samples.

The sterilized algae were cut into small pieces and placed on the Petri dish containing 2% of malt extract agar medium (five segments of algae per Petri dish). Then the plates were incubated at room temperature. After incubation period the algal pieces were monitored for fungal hyphae growing from the cut end of the each sample. Control plates were used for the verification of the surface sterilization technique. The growth of fungal hyphae were monitored for

two weeks. The fungal hyphae were removed from the edge of the algal portion and transferred into the 2% malt extract agar medium and allowed for growth. After incubation the fungal samples were sub-cultured and stored for further studies.

2.3 Screening of L- Glutaminase producing endophytes

2.3.1 Qualitative assay for L-glutaminase production

The modified Czapek Dox medium (glucose 2 g, L-glutamine 10 g, KH_2PO_4 1.52 g, KCl 0.52 g, $MgSO_4.7H_2O$ 0.52 g, $FeSO_4.7H_2O$ 0.01 g, agar 20 g, distilled water 1000 mL) was used for plate assay. A 2.5% stock solution of phenol red was prepared in ethanol (pH 6.2) and 3 mL of this was added to 1000 mL of Czapek Dox medium. A mycelial plug (5 mm dia.) cut from the growing margin of the colony of an endophyte was placed in a Petri dish containing 20 mL of this medium. After 72 h of incubation at 26±1°C, the appearance of a pink zone around the fungal colony showed L-glutaminase activity [20].

2.3.2 Quantitative assay of L-glutaminase enzyme

L-glutaminase activity was measured by the modified method [21]. The fungus was grown for 5 days at 26±1°C in liquid modified Czapek Dox (CD) medium. A reaction mixture containing 0.5 mL of 0.5 M tris HCl buffer (pH 8.2), 0.1 mL of 40 mM L-glutamine, 1.0 mL of suitably diluted enzyme source (culture filtrate of an endophyte) and 0.4 mL of distilled water (total volume of 2.0 mL) was incubated at 37°C for 30 min. The reaction was terminated by adding 0.5 mL of 1.5 M tricholoroacetic acid (TCA). Blank tubes were prepared by adding the enzyme source after the addition of TCA. After termination of the reaction, 3.7 mL volume of distilled water and 0.2 mL of Nessler's reagent were added to 0.1 mL of the above reaction mixture and incubated for 20 min. The amount of ammonia released during the reaction was determined by measuring the absorbance at 450 nm. One international unit (IU) of L-glutaminase is the amount of enzyme needed to liberate 1 µmol of ammonia in 1 min at 27°C.

3. Results and Discussion

Many microorganisms, such as bacteria, yeasts, moulds and filamentous fungi, have been reported to produce L-glutaminase of which the most potent producers are fungi. Microbial Glutaminases of microbial origin are supposed to be more stable than that from animal and plant sources. It also acts as proteolytic endopeptidase, which hydrolyses the peptide bonds present in the interior of the protein molecules.

In the present study, 50 different endophytes were isolated from green and red seaweeds. The isolated strains were screened using Czapek Dox medium supplemented with phenol red for L- glutaminase production. The active glutaminase producing fungal colony changed the colour of the medium from yellow to pink. This colour change was due to change in the pH of the medium, as L-glutaminase causes the breakdown of amide bond in L-glutamine and librated ammonia (Fig. 1).

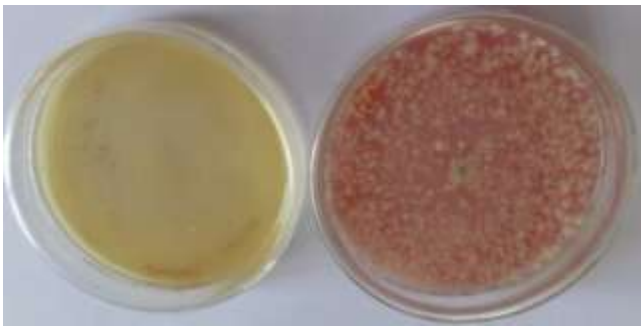

Fig 1: Plate showing S7 strain for glutaminase production

From the 50 isolates, 10 showed positive results. Further the amount of L- glutaminase production was screened using spectrophotometric assay. Among the 10 strains, the S7 strain showed maximum enzyme production (31.62 U/ml) and the strain S1 exhibited minimum enzyme production (10.24 U/ml) (Fig. 2). Further, the maximum glutaminase producing S7 strain was morphologically identified as *Penicillium* sp.

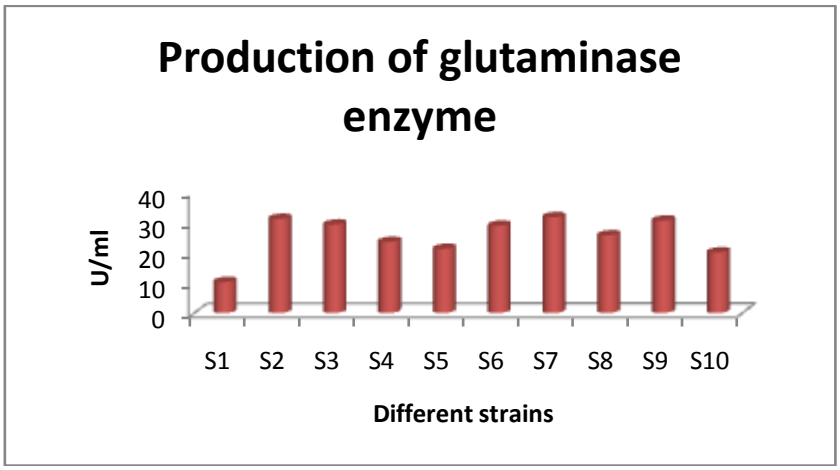

Fig 2: Production of glutaminase from endophytic fungus (U/ml)

In previous study, forty fungal isolates from food and other sources. It was further screened using minimal glutamine agar containing L-glutamine and phenol red as indicator for glutaminase production [22]. All the strains were positive for glutaminase activity in a rapid plate assay, as evidenced by pink halos. Among 400 marine isolates screened from various marine niches, one isolate showed maximum productivity of glutaminase (71.23 U/l) [23]. Of 25 actinomycetes isolated from marine sediments of Capecomarin. SBU1 exhibited promising activity of L-glutaminase [24].

Twenty one isolates of *Aspergillus wentii* isolated from different soil samples and named serially from KGSD1 to KGSD21. They also showed the enzyme production based on semi qualitative method [25].

Mangrove sediments from the east coast of India exhibited higher L-glutaminase activity than sediments of other biotypes were reported[26]. L-glutaminase-producing bacteria both from seawater and marine sediments from Cochin coastal regions and observed that their populations were on the order of 10^5cells/per ml or g [27]. L-glutaminase producing actinomycetes were reported from marine sediment sample collected from rhizosphere region of the mangrove *Rhizophora apiculata.* Among the 20 actinomycete strains isolated, the potential strain was identified as *Streptomyces olivochromogenes* [17]. In another report, the marine bacterium *B. diminuta* as a source for L-glutaminase (48.4 U/ml), an enzyme of industrial and pharmaceutical significance recently was isolated from the sea water collected from the coastal area of Arabian sea [28].

The marine actinomycetes from sediment samples collected from Pitchavaram mangrove ecosystem situated along the southeast coast of India. In this study, about 38% of the isolates produced L-asparaginase [29]. Thirty marine bacterial strains from sediment and water samples collected from different places along the beach of Bay of Bengal in Visakhapatnam by dye based procedure. Among the thirty isolates LG24 gave highest activity of 22.68U/ml with in 120h where L-glutamine supplemented as sole carbon and nitrogen source in the media [30].

A total of 20 actinomycetes strains were isolated from various body parts of the fish, *Chanos chanos* (skin, gills and gut contents). Among the 20 strains tested, the strain LG-10 was identified as *Streptomyces rimosus* which showed L-glutaminase activity (17.51 IU/ml) and was indicated as an ideal organism for industrial production of extracellular L-glutaminase [31].

The endophytic fungi isolated from seaweeds occurring along the coast of Tamil Nadu for production of L-asparaginase [32]. But as such there is no work on L-glutaminase production from endophtic fungi of seaweeds.

So in the present study among the 50 isolates, one single isolate obtained from *Gracilaria edulis* showed maximum enzyme production (31.62 U/ml). It was further identified as *Penicillium* sp.

4. Conclusion

In the present investigation, the endophytic fungi of marine origin were isolated from seaweed samples. The isolates were screened for glutaminase production. The strain S7 identified as *Penicillium* sp. showed maximum production and S1 the minimum production. The yield obtained in the present investigation would further be increased its industrial importance in large-scale production of microbial metabolites of biotechnological and medical importance.

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