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Morphological and molecular identification of L-Asparaginase producing fungus from *Annona muricata*

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Abstract

The identification of L-Asparaginase producing fungal organisms has gained considerable attention because of its less allergic properties. The present investigation deals with the morphological and molecular identification of L-Asparaginase producing fungi from the leaves of the medicinal plant *A. muricata*. It was confirmed that the fungal isolate obtained from *A. muricata* has the potential to produce-Asparaginase and the fungal species was identified as *Aspergillus fumigatus*.

Keywords: L-Asparaginase, *Annona muricata*, *Aspergillus fumigatus*, Czapek Dox medium

Introduction

The tropical plant species *A. muricata* is very well known for its medicinal properties. The plant parts such as bark, leaves, seed and fruit have been used as a curative substance for a wide variety of health issues [1]. The anticancer enzyme L-Asparaginase has been derived from various microorganisms isolated from different parts of many medicinal plants [2, 3]. Surprisingly *A. muricata* is also a rich source of L-Asparaginase producing fungus [4]. The L-Asparaginase is from a fungal source is more suitable than other microbial sources because of its less allergic properties when it is used in the treatment of cancer [5]. Acute lymphoblastic leukemia is the condition where the blood cells become cancerous and in this case, L-Asparaginase is an amazing remedial measure for the treatment of acute lymphoblastic leukemia. The detection of L-Asparaginase as an anticancer drug and its further investigation began in 1953. Researchers found that the guinea pig serum has the capability to fight against lymphomas in rat and mice. Later they discovered that the L-Asparaginase enzyme present in guinea pig serum was responsible for the prevention of lymphomas [6, 7]. Microorganisms are widely used for the production of L-Asparaginase because of their effortless optimization and high yield by genetic modification. Nowadays fungal L-Asparaginase is preferred by many researchers because of their reduced side effects. The reviews showed that the Genus *Aspergillus* has the ability to produce L-Asparaginase [8].

The identification of L-Asparaginase producing fungi up to molecular level is very essential in anti-cancer research and it will be helpful for the enormous production of L-Asparaginase. The present investigation deals with the morphological and molecular identification of fungus isolated from the medicinal plant *A. muricata*.

Materials and Methods

Collection of plant sample

The leaf samples were collected from the medicinal plant *A. muricata* growing in the southern Western Ghat regions of Kerala.

Isolation and screening for L-Asparaginase

Mortar and pestle was used to ground the samples to very fine paste and serial dilution was used for the isolation of fungal organism [9]. The modified Czapek Dox agar medium with a pH 6.2 and phenol red as indicator were employed in the screening for L-Asparaginase. The formation of pink color around the colonies indicated the production of L-Asparaginase [10].

Identification of fungal species

The morphological and microscopical characteristics of isolated fungi were identified by following the method Lacto Phenol Cotton Blue Staining [11]. For the molecular identification,

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DNA extraction of the fungi was done [12]. Universal primers were designed to amplify D1-D2 region of fungal DNA. The PCR product was purified by using Thermo fisher scientific purification kit.

DNA Sequencing and BLAST

The PCR products were subjected to DNA sequencing for the determination of the exact order of nucleotides and the gene sequence [13]. BLAST was implemented to found region of local similarities [14].

Result and Discussion

Fungal isolates obtained from the medicinal plant *A. muricata* growing in the southern Western Ghats regions of Kerala were screened for L-Asparaginase production using modified Czapek Dox medium. In accordance with the progress of pink color around the colonies assessed that the isolated fungal organism from *A. muricata* has the potential to produce the anti-cancer enzyme L-Asparaginase. [15] (Figure 1)



Fig 1: Development of pink color around the colonies on Modified Czapek Dox agar pH 6.2 at room temperature

Morphological identification of fungal isolate

Lacto phenol cotton blue staining was preferred for the staining and observation of fungal isolate. The fungal organism was identified as *Aspergillus* species based on the morphological properties such as Hyphae and conidial heads. (Figure 2)

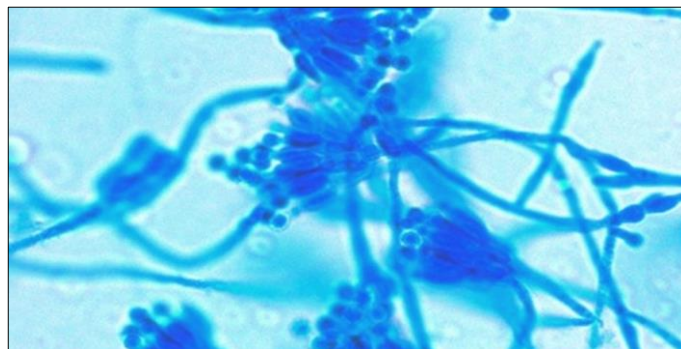


Fig 2: Microscopic view of *Aspergillus fumigatus*

Molecular identification of fungal isolate

The process of identification was broadened to molecular level with DNA sequencing and BLAST. It was recognized as *Aspergillus fumigatus* by obtaining the DNA sequence (Figure 3) and percentage of similarity (Figure 4).

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TCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGT
GAGGGCCCTCTGGGTCCAACCTCCCACCCGTGTCTAT
CGTACCTTGTGCTTCGGCGGGCCCGCGTTTCGACG
GCCGCCGGGAGGCCTTGCGCCCCCGGGCCCGCGCC
CGCCGAAGACCCCAACATGAACGCTGTTCGAAAGT
ATGCAGTCTGAGTTGATTATCGTAATCAGTTAAACT
TTCAACAACGGATCTCTTGGTTCGGCATCGATGAA
GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
GAATTCAGTGAATCATCGAGTCTTTGAACGCACATT
GCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGA
GCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGG
GCCCCGTCCCCCTCTCCCGGGGACGGGCCCCGAAA
GGCAGCGGCGGCACCGCGTCCGGTCTCGAGCGTAT
GGGGCTTTGTACCTGCTCTGTAGGCCCGCCGGCG
CCAGCCGACACCCAACCTTATTTTCTAAGGTTGACC
TCGGATCAGGTAGGGATAACCCGCTGAACTAAGCAT
ATCAATAAGCGGGAGGAAAAGAAACCAACAGGGAT
TGCCTCAGTAACGGCGAGTGAAGCGGCAAGAGCTCA
AATTTGAAAGCTGGCCCCCTTCGGGGTCCGCGTTGTA
ATTTGCAGAGGATGCTTCCGGGTGCAGCCCCCGTCTA
AGTGCCCTGGAACGGGCGGTCATAGAGGGTGAGAAT
CCCGTCTGGGACGGGGTGT
```

Fig 3: DNA Sequence of isolated fungal organism

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Aspergillus fumigatus strain KARVS04 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1439	1439	100%	0.0	100%	KC119200.1
<input type="checkbox"/> Aspergillus fumigatus strain KARVS03 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1439	1439	100%	0.0	100%	KC119199.1
<input type="checkbox"/> Uncultured Aspergillus genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene	1432	1432	100%	0.0	99%	HG936917.1
<input type="checkbox"/> Uncultured Aspergillus genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene	1432	1432	100%	0.0	99%	HG936915.1
<input type="checkbox"/> Aspergillus fumigatus strain F7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1432	1432	100%	0.0	99%	KR023997.1
<input type="checkbox"/> Aspergillus sp. 4 BRO-2013 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S rRNA gene	1432	1432	100%	0.0	99%	KF367537.1
<input type="checkbox"/> Aspergillus sp. 2 BRO-2013 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S rRNA gene	1432	1432	100%	0.0	99%	KF367498.1
<input type="checkbox"/> Aspergillus fumigatus strain EMBT12 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1426	1426	100%	0.0	99%	MG552677.1
<input type="checkbox"/> Uncultured Aspergillus genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene	1426	1426	100%	0.0	99%	HG936916.1
<input type="checkbox"/> Aspergillus fumigatus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S rRNA gene	1426	1426	100%	0.0	99%	KM491894.1
<input type="checkbox"/> Aspergillus sp. 5 BRO-2013 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S rRNA gene	1426	1426	99%	0.0	99%	KF367538.1
<input type="checkbox"/> Aspergillus fumigatus strain LCF20 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1426	1426	100%	0.0	99%	FJ867935.1

Fig 4: BLAST result showing 100% similarity with *Aspergillus fumigatus*.

Conclusion

This investigation revealed that the leaf sample of the medicinal plant *A. muricata* is a rich source of L-Asparaginase producing fungi. The isolated fungal species *Aspergillus fumigatus* suggested for the large scale production of L-Asparaginase for industrial and Pharmaceutical purposes.

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