



ISSN (E): 2277- 7695  
ISSN (P): 2349-8242  
NAAS Rating: 5.03  
TPI 2019; 8(3): 220-223  
© 2019 TPI  
www.thepharmajournal.com  
Received: 17-01-2019  
Accepted: 20-02-2019

**Dani Benchamin**  
Zoology Research Centre,  
St. Stephen's College,  
Pathanapuram University of  
Kerala, India

**Sreejai R**  
Zoology Research Centre,  
St. Stephen's College,  
Pathanapuram University of  
Kerala, India

**Athira L**  
Zoology Research Centre,  
St. Stephen's College,  
Pathanapuram University of  
Kerala, India

**Jensy Roshan F**  
Zoology Research Centre,  
St. Stephen's College,  
Pathanapuram University of  
Kerala, India

**Sujitha S**  
Zoology Research Centre,  
St. Stephen's College,  
Pathanapuram University of  
Kerala, India

**Beena S Kurup**  
Zoology Research Centre,  
St. Stephen's College,  
Pathanapuram University of  
Kerala, India

**Correspondence**  
**Dani Benchamin**  
Zoology Research Centre,  
St. Stephen's College,  
Pathanapuram University of  
Kerala, India

## Production and characterization of L-Asparaginase isolated from *Aspergillus fumigatus*

**Dani Benchamin, Sreejai R, Athira L, Jensy Roshan F, Sujitha S and Beena S Kurup**

### Abstract

The present research describes the production and characterization of L-Asparaginase from *Aspergillus fumigatus* isolated from the medicinal plant *Annona muricata*. This fungal isolate have shown potential to produce L-Asparaginase on modified Czapek Dox medium under optimal conditions. Characterization of enzyme was revealed the finest conditions for highest production.it was observed that the maximum production of L-Asparaginase at a pH of 8, 40 °C of temperature, 20 minutes of incubation period, Presence of Fe<sup>2+</sup> ions and. 20mM substrate concentration.

**Keywords:** *Aspergillus fumigatus*, *Annona muricata*, production, characterization

### Introduction

L-Asparaginase is an anti-cancer enzyme widely distributed in nature. Chemically it is known as mono methoxy polyethylene glycol succinimidyl L- Asparaginase <sup>[1]</sup>. L-Asparaginase is also commercially accessible under the brand formulations such as Preventase, Medac, Ciderolase and Oncaspar. These are broadly used as the potent antileukemic agent. As a nutritional requirement, L-Asparagine is accessed by both normal and cancer cells for the production of protein. Major fungal sources reported to produce asparaginase were *Aspergillus tamari*, *Aspergillus terreus*, *Aspergillus nidulans*, *Penicillium sp*, *Fusarium sp*, *Helminthosporium sp*.<sup>[2]</sup> and *Aspergillus fumigatus* <sup>[3]</sup>. Comparative analysis of L-asparaginase from different microorganisms revealed that, the beneficial properties differ with their source <sup>[1]</sup>. Because of its anti-cancer properties, L-asparaginase is also in great demand in food processing industries. The rationale behind the importance of L-Asparaginase in food industry depends upon the fact that it can reduce the amount of acrylamide formation during frying or baking food process <sup>[4]</sup>

The aim of the present study is the production and characterization of fungal L-Asparaginase isolated from the therapeutic plant *A. muricata*. The L-Asparaginase producing fungal organisms from *A. muricata* were previously described by Abhini *et al.*, in 2013 <sup>[5]</sup> and Benchamin *et al.*, in 2019 <sup>[3]</sup>

### Materials and methods

#### Isolation of fungal organism

The fungal organism used in this study was isolated from infected leaves of the plants *A. muricata* collected from southern Western Ghats regions of Kerala. Modified Czapek Dox medium with pH 6.2 were used for the maintaining the isolated organisms <sup>[6]</sup>.

#### Screening of L-Asparaginase

The fungi obtained were screened by plate assay method for their abilities to produce asparaginase during their progress on modified Czapek's Dox media accompanied with pH 6.2 and indicator phenol red. The fungal isolates that exhibited pink color around the colonies were regarded as capable to produce L-Asparaginase enzyme. Gulati *et al.*, (1997) <sup>[7]</sup>.

#### Identification of the fungal Isolate

Lacto phenol cotton blue staining technique <sup>[8]</sup> was used for the determination of morphological and microscopical features. The process of identification expanded to further methods such as DNA sequencing <sup>[9]</sup> and BLAST <sup>[10]</sup>

**Enzyme assay**

For the determination of L-asparaginase activity, the standard method of Imada *et al.*, (1973) was used [11].

**Characterization of enzyme [11]**

**Effect of pH**

The effect of pH on L-asparaginase activity was evaluated at a pH ranging from 6 to 10 with incubation at 37 °C for 30 minutes.

**Effect of Temperature**

L-Asparaginase activity was checked at different temperatures ranges from 25 °C to 45 °C.

**Effect of Substrate concentration**

To study the effect of substrate concentration, different asparagine concentrations (1mM to 60mM) were used.

**Effect of Metal ion concentration**

To investigate the effect of various metal ion concentrations, enzyme assay was progressed with Mg<sup>2+</sup>, Fe<sup>2+</sup>, Na<sup>+</sup> and K<sup>+</sup> and the activity of the enzyme was characterized.

**Effect of Incubation period**

Different incubation period ranges from 10 to 60 minutes at 37 °C. were employed to study the effect on L-Asparaginase production.

**Result and discussion**

**Isolation and screening**

Production of L-Asparaginase was displayed by detecting pink zone around the colonies, and was chosen for determination of enzyme activity [12] (Figure1).



Fig 1: Pink color around the colonies on Modified Czapek Dox agar media.

**Identification of fungus**

By using Lacto phenol cotton blue staining, the isolated fungal strain was identified as *Aspergillus* species and DNA Sequencing and BLAST identified the fungal isolate as *Aspergillus fumigatus*

**Characterization of enzyme**

**Effect of pH**

The maximum enzyme production was recorded at pH 8 which was 23.83 U/ml. (Figure 2) Therefore, pH 8 was determined as the optimum pH for this enzyme.

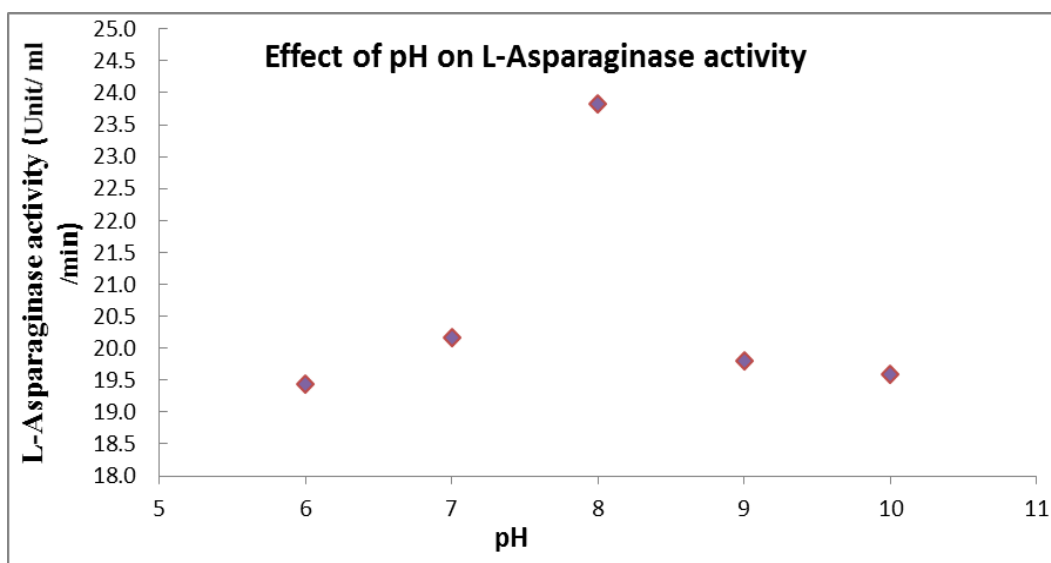


Fig 2: Effect of pH.

**Effect of Temperature**

The influence of temperature on enzyme production was observed at 40 °C which was 21.26 U/ml. (Figure 3).

Temperature at 40 °C was considered as the optimum for this enzyme.

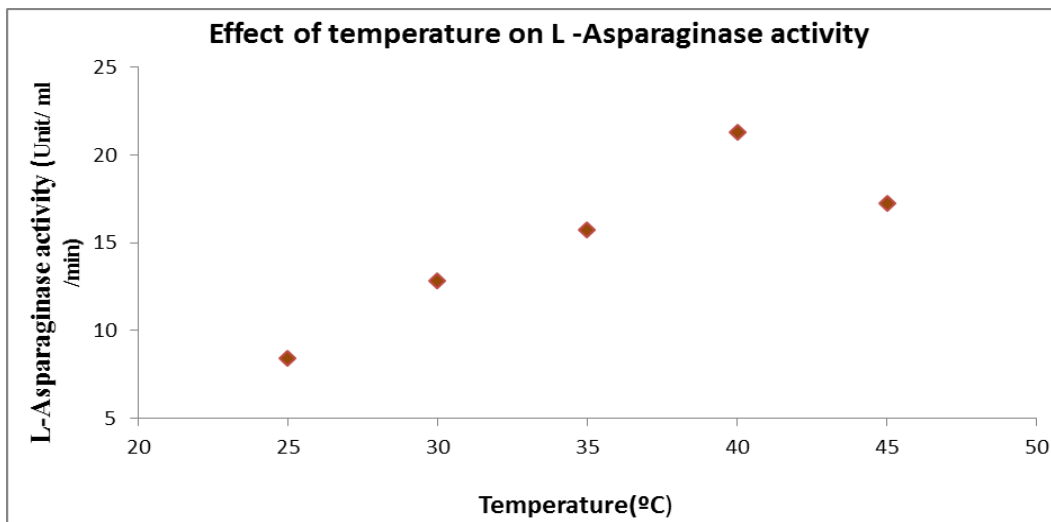


Fig 3: Effect of temperature.

**Effect of Substrate concentration**

At 20 mM substrate concentration, the enzyme retains maximum production which was 17.6 U/ml (Figure 4) and 20

mM substrate concentration was regarded as the optimum for this enzyme.

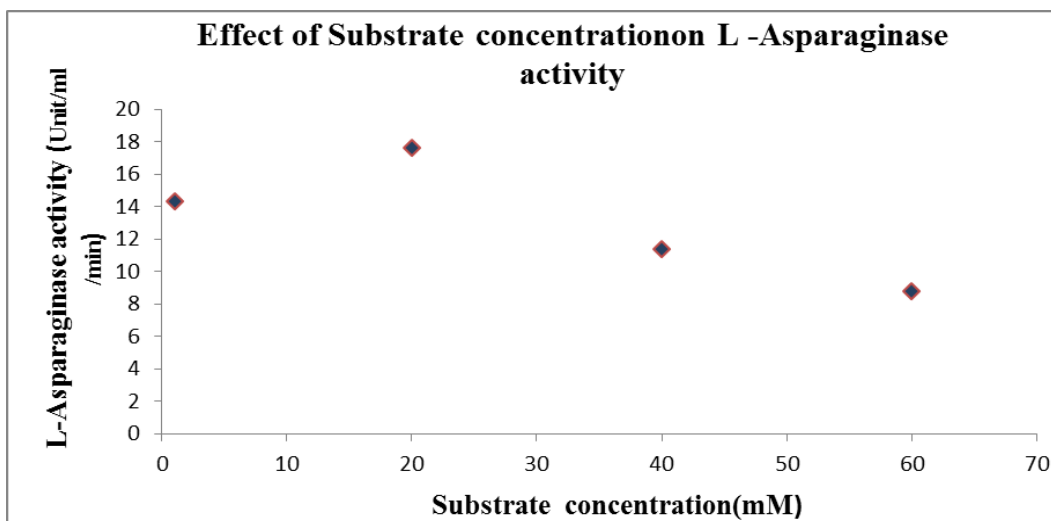


Fig 4: Effect of Substrate concentration.

**Effect of Metal ion concentration**

To explore the effect of various metal ions in enzyme production, Fe<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> and Na<sup>+</sup> were used and revealed

that maximum activity was noticed in the presence of Fe<sup>2+</sup> which was 32.26 U/ml and the lowest activity was noticed in presence of K<sup>+</sup> which was 2.93 U/ml. (Figure 5)

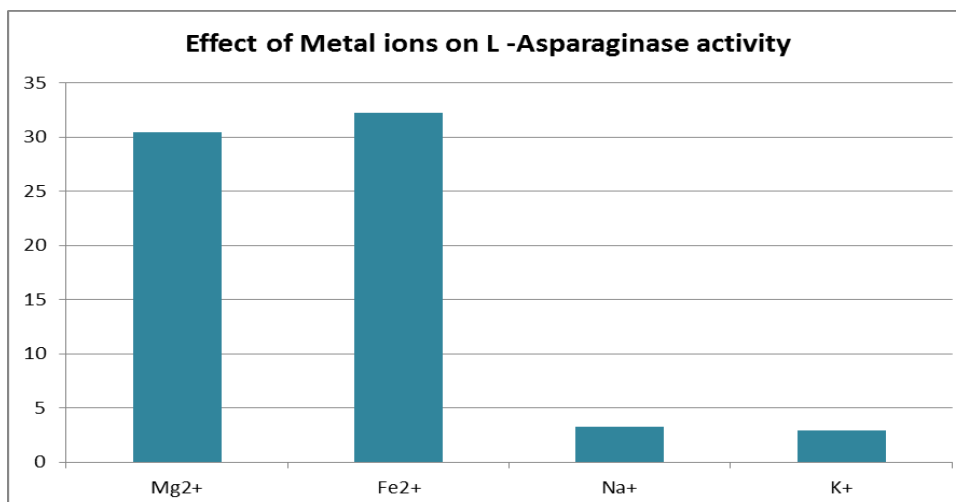


Fig 5: Effect of Metal ions concentration.

### Effect of Incubation period

Significant difference was observed on enzyme production when analyzed under different reaction periods and confirmed

highest enzyme production at 20 minutes which was 19.9 U/ml. (Figure 6)

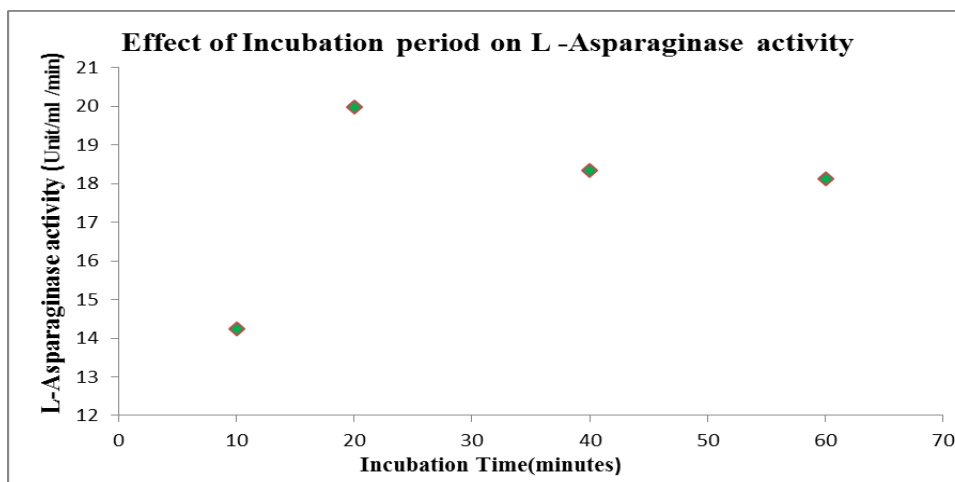


Fig 6: Effect of Incubation period.

### Conclusion

The present investigation revealed that the fungal isolate *Aspergillus fumigatus* from the medicinal plant *Annona muricata* has the capability to produce L-Asparaginase under optimal conditions. Hence, L-Asparaginase from *A. fumigatus* suggested for the industrial and therapeutic applications

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