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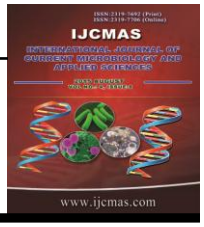

Original Research Article

Antifungal Activity of Cell Extract of *Spirulina platensis* against Aflatoxin Producing *Aspergillus* Species

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Keywords

Spirulina platensis, *Aspergillus flavus*, Aflatoxin B_1

A B S T R A C T

Spoilage of the food items results in the production of Mycotoxins by few fungi. The types of toxins include Aflatoxin, Fumonisins, Tricothecene, Ochratoxins, Zearalenones and Cyclopiazonic Acid. Aflatoxins are highly toxic secondary metabolites secreted by *Aspergillus flavus* and *Aspergillus parasiticus*. 18 different types of toxins had been identified. Among them, the major types include B1, B2, G1 and G2, named after their fluorescing property under ultra violet light, as blue and green, respectively. Aflatoxin B_1 is the most potent, naturally occurring chemical liver carcinogen. The present study focuses on the study of Anti-fungal activity of the cell extract of *Spirulina platensis* against aflatoxin producing *Aspergillus sp* (Vinay Kumar et al., 2013). The aflatoxin was extracted from *Aspergillus flavus* MTCC 2798 strain after incubation of the strain at 28ºC for 7 days and it was confirmed by Mass-spectrometry. This proved that the test strain definitely produced aflatoxin. 0.1ml of the cell extract of *Spirulina platensis* was prepared and used for anti-fungal activity studies by agar well diffusion method on Czapek Doz agar medium. Methanol was used as control. Zone of 14mm and 19mm were observed in plates inoculated with *Aspergillus flavus* and *Aspergillus niger*. It was concluded that the cell extract of *Spirulina platensis* has the potency to cease the growth of the toxic fungi which in turn can be used as a bio-

Introduction

Fungi are known to cause a variety of spoilage in different kinds of food commodities owing to the increase in moisture, temperature, humidity and pH. Aflatoxins are highly toxic secondary metabolites secreted by *Aspergillus flavus* and *Aspergillus parasiticus*. There are 18 different types have been identified. Among them, the major types include B1, B2, G1, G2, named after their fluorescing property under ultra violet light, as blue and green, respectively. Because aflatoxin contamination is unavoidable, numerous strategies for their detoxification have been proposed. These include physical methods of separation, thermal inactivation,

irradiation, solvent extraction, adsorption from solution, microbial inactivation, and fermentation (Arijit Das et al., 2012).

Algae can be a very interesting natural source of new compounds with biological activity that could be used as functional ingredients. The cyanobacterium *Spirulina* is used as food by humans because of its chemical composition, which has high quality and quantity of proteins, essential amino acids, minerals, polyunsaturated fatty acids and vitamins. It has been reported to prevent oxidative damage, and hence can indirectly reduce cancer formation in human body.

Materials and Methods

Culture media was obtained from Himedia Laboratories Pvt Ltd., Mumbai, India. Analytical grade chemicals were obtained from Nice Chemicals Pvt Ltd, Cochin, India. Dry *Spirulina platensis* powder was obtained from of ERR, Egmore, Chennai.

Source of fungal strain

Aflatoxin producing standard strain of *Aspergillus flavus* MTCC 2798 was obtained from Microbial Type Culture Collection, Chandigarh, India. The spore suspension was aseptically transferred onto Czapek Dox Broth and spot inoculation was done on Czapek Dox Agar. The colony morphology and microscopic features were also noted. Pure culture was maintained at 4°C until use (Manjusha Chakranarayan and Anita Pati, 2013).

Extraction and partition of aflatoxin

The mycelial broth was taken into a blender jar and 100ml of methanol was added followed by 25ml of 0.1N HCl. Contents were blended for 3 minutes at high speed and filtered through Whatmann filter paper No.1. 50ml of the filtrate was collected and transferred to a separating funnel. 50ml of 10% sodium chloride solution was added and swirled for 30 seconds. To this, 50ml of hexane was added and gently shaken for 30 seconds.

The phases were allowed to separate and the lower aqueous phase was taken into another separating funnel. To this aqueous phase, 25ml of dichloromethane was added and moderately shaken for 30 seconds. When the phases separated, the lower dichloromethane layer was transferred to another separating funnel and partitioning was repeated twice with dichloromethane. The resulting fraction was then evaporated to 2–3ml at 40°C in a hot air oven (Denault and Underkolfer, 1967).

Obtaining cell extract of *Spirulina platensis*:

2g of dry powder of *Spirulina* was mixed with 10ml methanol. The mixture was then agitated at 25ºC for 60 minutes. The agitation was interrupted every 15 minutes by the addition of 10ml methanol each time and the procedure was continued. The content was filtered using Whatmann filter paper No.1 and the filtrate was discarded. To the filtered powder, 20ml of hexane was added and washing procedure was thus repeated twice. The powder was then evaporated in hot air oven at 40°C and dissolved in 25ml of distilled water and used for the study (Medina-Jaritz et al., 2011).

Preparation of spore suspension

Spore suspension of *Aspergillus niger* and Aflatoxigenic *Aspergillus flavus* were prepared by propagation in Czapek Dox Agar. This was followed by extraction from the medium with 0.2 solution of Tween 80.It

was then filtered and used for study.

Agar well diffusion method

0.1ml of the spore suspension of the two fungal strains was spread onto Czapek Dox Agar using a sterile L-Rod for uniform distribution.

Using the sterile well puncture, 6mm wide well was made on the plate. Then 0.1ml of the *Spirulina* cell extract was poured into the wells. Controls were placed without the cell extract. The plates were incubated at 28ºC for 3 days (Michele Moraes de Souza, 2011).

Results and Discussion

Growth on Czapek Dox agar

Aspergillus flavus colonies were granular, flat, with radial grooves, yellow at first, but turned dark yellow-green with entire margin on prolonged incubation period with moderate to rapid growth. Conidial heads were typically radiate. The reverse side was colorless to yellow.

Growth on Czapek Dox broth

For the detection of aflatoxin production, the mold was grown on Czapek Dox broth at 25ºC for 8 days beyond which the medium developed a characteristic yellow hue, which was probably due to the production of secondary metabolites.

Antifungal activity

Antimicrobial activity (Arijit Das et al., 2012; Sakthivel and Kandasamy Kathiresan, 2012) of cell extract of *Spirulina platensis* against the two fungal strains, *Aspergillus niger, Aspergillus flavus* was studied.

Among the two strains, *Aspergillus niger* was the most sensitive, which showed inhibition zone of 19mm, while *Aspergillus flavus* showed inhibition zone of 15mm.

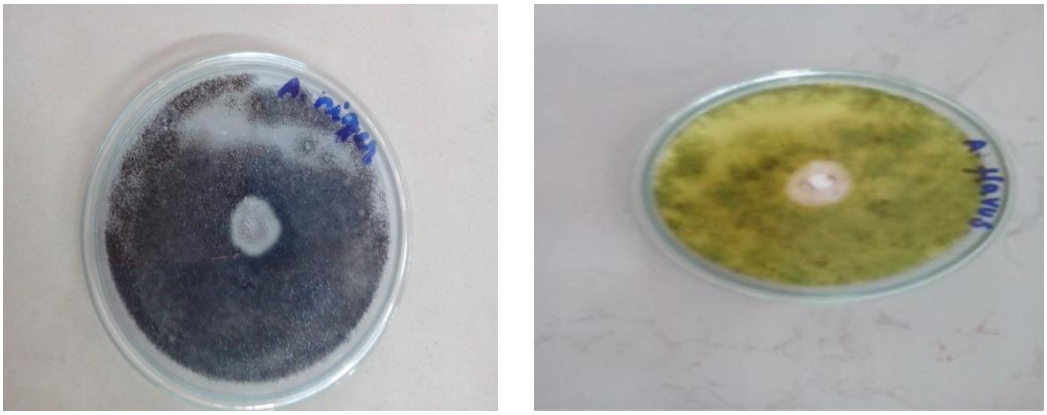
The present study resulted in determining the antifungal activity of methanolic cell extract of *Spirulina platensis* against the aflatoxigenic *A. flavus* and *A. niger*. The study paves way for the use of the microalgae as a biopharmaceutical. This has a potential application to be used as an alternative to suppress the multiplication of the toxic fungi which is a potential human carcinogen.

Table.1 Anti-fungal activity of the cell extract of *Spirulina platensis* against *A. flavus, A. niger*

ORGANISM	ZONE OF INHIBITION-TEST	ZONE OF INHIBITION-CONTROL
A.flavus	14mm	No zone
A.niger	19mm	No zone

Figure.1 Growth characteristics on Czapek Dox Agar

Figure.2 Anti-fungal activity of the cell extract of *Spirulina platensis* against the two fungal strains



Being no specific antidote to aflatoxicosis, the microalgae with its outstanding benefits, including the source of a variety of vitamins and proteins, can be used with adequate carbohydrate content. The free radical scavenging ability and the potential benefits of the microalgae include anti-inflammatory, anti-histamine, anti-aging, antidepression, anti-radiation properties, makes it an effective agent in controlling a variety of ailments in human.

The availability of the microalgae in the form of powder or capsules make it easily digestible. Typically, many older people have difficulty digesting complex proteins and are on restricted diets. They find Spirulina's protein an ideal way of ensuring they receive the nourishment needed.

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