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Growth response of *Spirulina platensis* in papaya skin extract and antimicrobial activities of *Spirulina* extracts in different culture media

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Abstract

Growth response of *Spirulina platensis* in papaya skin extract media and their antimicrobial activity were studied. Five different concentrations e.g. 10gm/L, 8gm/L, 6 gm/L, 4 gm/L and 2gm/L of Papaya (*Carica papaya*) skin extract media and BD_1 (control) medium were used in this study. After 8 days of cultivation, the optical density (0.33) was recorded in BD_1 medium and among the five different concentrations of papaya skin extract media the maximum was found (0.31) in 6gm/L. Antimicrobial activity of *Spirulina platensis* grown in three media namely Zarrouk , BD_1 media and media made from papaya skin extract was also studied. Only freeze dried *Spirulina platensis* powder extract showed inhibitory effect against bacteria and no antifungal activity was observed.

Key Words: Growth response, Antimicrobial activity, Papaya skin extract media, *Spirulina platensis*

Introduction

Spirulina platensis, a blue -green micro alga, has been used since ancient times as a source of food because of its high nutritional value (Dillon *et al.* 1995). It is rich in proteins, vitamins, minerals, carbohydrates and γ-linolenic acid. It is gaining more and more attention, not only for the foods aspects but also for the development of potential pharmaceuticals (Quoc and Pascaud, 1996). This alga is being widely studied, not only for nutritional reasons but also for its reported medicinal properties (Qureshi and Ali, 1996; Hayakawa *et al.,* 1997; Kim *et al.,* 1998; Miranda *et al.,* 1998; Mishima *et al.,* 1998; Hirahashi *et al.*, 2002; Subhashini *et al.*, 2004), antimicrobial activities (Demule *et al.,* 1996; Ozdemir *et al.,* 2004) as well as to inhibit the replication of several viruses, such as Herpes simplex and HIV-1 (Ayehunie *et al.,* 1998; Hernandez-Corona *et al.*, 2002). In Biological Research Division, BCSIR, Dhaka, *Spirulina* was cultured at pilot plant scale for over 19 years. Bangladesh medium (Jahan *et al*., 1994) was developed in this laboratory for commercial production of *Spirulina* in Bangladesh. Other medium BD_2, BD_3, BD_4, and BD_5 were developed in the same Laboratory for domestic scale culture of *Spirulina* in Bangladesh (Khatun *et al.,* 2006). Bangladesh is an agro-based country. In Bangladesh, huge number of vegetable is growing which have nutritional value. If these nutritional vegetable wastes used as nutrient source for *Spirulina* culture, it would be economically helpful. With a view to grow *Spirulina* at domestic level, research was aimed to develop a cheaper medium by utilizing locally available vegetable wastes such as skin of green papaya (*Carica papaya*) which contains necessary nutrients and mineral elements (Oloyede, 2005) which may be useful for growth of *Spirulina platensis.*

Materials and Methods

Media preparation

Fresh green papaya (*Carica papaya*) skin was peeled. Papaya skin was weighed and washed with tap water. 29gm papaya skin was blended in 200ml tap water. After blending, extract was sieved with 200µ mess cloth and stored at +4°C until use. For preparation of different concentration of papaya skin extract media, tap water was added separately with stored extract to make 10gm/L, 8gm/L, 6 gm/L, 4 gm/L and 2gm/L concentration and thoroughly mixed with 200mg/L urea, 5gm/L NaCl and 3gm/L $NaHCO_3$ to increase the concentration of nitrogen, salinity and pH respectively. BD_1 medium (Jahan *et al.,* 1994) was used as control for this experiment. Each experiment was done with 3 replicates.

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Inoculum preparation and maintenance

Culture was carried out in 1L conical flask. 500 ml of papaya skin extract medium was given in each flask. Stock culture (*Spirulina platensis*) was collected from Biological Research Division, BCSIR, Dhaka which was maintained in Zarrouk (1966) medium. Equal amount of inoculums (20ml/L) was added into each flask (initial OD 0.11). The flasks were shaken everyday in the morning and evening. These were kept in a room near the window, exposed to natural condition. The optical density (OD) of the culture was recorded by Spectrophotometer (Type-Helios Gamma, NC-9423 UVG 1702E) for the maintenance of the growth. PH, temperature, light intensity, salinity and dissolved oxygen level of the culture were recorded. The condition of *Spirulina* culture was observed under compound microscope once a week and recorded.

Sample preparation for screening of antimicrobial activity

S. platensis was cultivated under photoautotrophic growth conditions. Zarrouk (1966), BD_1 (Jahan, *et al.,* 1994) and newly made Papaya Skin Extract media were used for cultivation. The initial pH was adjusted to 9.5. The culture temperature was maintained at 30° ± 0.1 °C. *S. platensis* was collected after 8 days of culture and dried in 3 different ways - freeze dry, sun dry and shade dry.

Preparation of various extracts of *S. platensis*

Freeze-dried, sun dried and shade dried *S. platensis* samples were mixed well with different solvents separately at the ratio of 0.5:10 w/v. Three different solvents (ethanol, methanol and chloroform) were used for the preparation of extracts of *S. platensis.* Twenty gram dried *Spirulina* powder was steeped separately in methanol and chloroform, 20gm dried powder was steeped in ethanol, then all the samples were kept for 72 hours at room temperature. Soaked mixtures were filtered (using What man filter paper-I).Then the filtrate was subjected to rotary vacuum evaporator (STUART, RE3022C) at 50º C and concentrated to gummy materials under reduced pressure. The gummy materials were then collected in small vials and then dried in room temperature. Thus crude extracts were obtained. The extracts were kept at +4 °C until use. The gummy extracts were dissolved in dimethyl sulfo-oxide (DMSO) prior to use in antimicrobial activity test.

Determination of antibacterial and antifungal activities

In vitro antibacterial studies were carried out against four bacterial pathogens viz *Salmonella typhi* CRL.(ICDDR.B), *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 *Bacillus cereus* BTCC 19 and two fungal pathogens viz *Aspergillus fumigatus* DSM 819 and *Candida albicans* ATCC 10239 which were obtained from the Microbiology Laboratory of IFST, BCSIR, Dhaka. Bacterial inoculums were prepared by Clinical and Laboratory Standards Institute (CLSI) guideline. Bacterial cultures were emulsified in normal saline and turbidity was matched with 0.5 McFarland turbidity standard. The agar cup method (Barry, 1980) was followed to investigate the antibacterial activity of the extracts. Wells of 6 mm diameter was punched over the agar plates using a sterile cork borer. The bottoms of the wells were sealed by pouring 50 - 100 µl of molten MHA into the scooped out wells. Using a micropipette, extracts of different solvents were added to different wells in the inoculated plate. These plates were then kept at 4ºC for 2-4 hours and then incubated at 37 ºC for 24 hours. Inhibition zones around the wells confirm the antibacterial activity of the respective extracts.

The poisoned food technique (Grover and Moore, 1962) was used to screen antifungal activity. 0.1 ml extract of *Spirulina platensi*s in respective solvent was taken by sterilized pipette in a sterile petriplate and then 20 ml of Sabouraud dextrose agar medium was poured into the petriplate, mixed well and allowed to solidify. Inoculation was done at the centre of each plate with 5 mm mycelium block for fungus. The mycelium block was prepared with the help of cork borer from the growing area of a 5 days old culture of the test fungi on sabouraud dextrose agar. The inoculated plates were incubated at 25ºC ±2 for 3 to 5 days. After 5 days of incubation the diameter of fungal radial mycelia growth was measured. The average of three measurements was taken as redial mycelial growth diameter of the fungus in mm. The percentage inhibition of mycelia growth of the test fungus was calculated by following method:

I=(C-T)/C x 100. Here, I=Percentage of inhibition, C=Diameter of the fungal colony in control, T= diameter of the fungal colony in treatment.

Result and Discussion

Generally *Spirulina platensis* are spiral shaped. Straight filaments have better survival capacity, and under less favor-

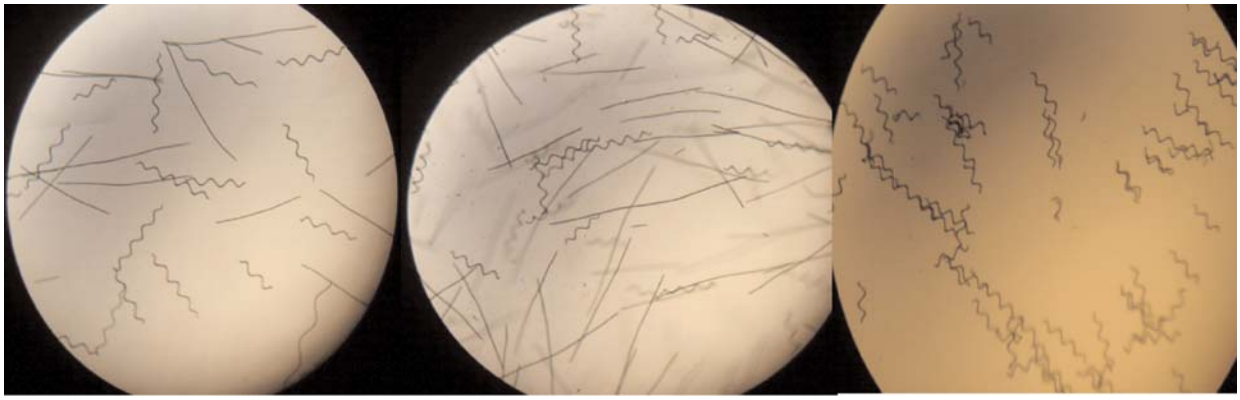
able conditions coiled filaments turn straight (Noor *et al.,* 2008). During initial growth period the maximum filaments condition were found to be straight, but after 14 days most of the filaments were turned to spiral shape in 6gm/L papaya skin extract media. The effect of papaya skin extract media on morphological condition of *Spirulina platensis* are presented in the Table I and Fig 1.

Table I: Effect of papaya skin extract media on morphology of *Spirulina platensis* (6gm/L)

Time period	Conditions (Average of 18 observations)
Day 0	Filaments are very good and healthy, some are in spiral shaped and some are in straight shaped and light blue- green in color.
After 8 days	Some filaments are in spiral shaped, some are in broken condition and numbers of filaments were more than previous observation and light blue green in color.
After 14 days	Number of filaments were less than previous observation and maximum filaments are in spiral shaped and light blue-green in color.

different *Spirulina* strain is between 30-35°C with 40°C definitely injurious.

Venkataraman (1983) stated that even a short exposure of *Spirulina* cultures to direct intense sunlight will result in bleaching of algal cells. In this respect, experimental flasks were kept at room temperature near the window and light intensity was 120 Lux to 2.5 Klux. The range of dissolved oxygen level was 1-4mg/L. Salinity of the culture medium was 10 ppt. Salinity plays a direct role on the purity of the culture Bonin (1992). Fig. 2 shows the growth of *Spirulina platensis* in the experimental culture media. Despite having started with a similar initial inoculum, the growth of *Spirulina platensis* was started to change from the second day of cultivation. After 8 days of cultivation, the highest OD 0.33 was observed in control media BD_1, whereas OD 0.31, 0.28, 0.20, 0.19 and 0.18 was observed in concentration 6, 4, 2, 8 and 10 of papaya extract media, respectively. It was observed that after 8 days of cultivation, optical density was decreased in experimental medium except concentration 2 of papaya extract medium. Papaya skin extract culture media of concentration 6, yielded 500mg/l of *Spirulina platensis* per 8 days whereas control medium, BD_1 yielded 750mg/L. In BD_3 and BD_4 media, yield was 664 mg/l and 665 mg/l, respectively (Begum *et al.*, 1998).



Initial condition **After 8 days** **After 14days**

Fig. 1: Microscopic view of *Spirulina platensis* cultured in 6gm/L Papaya skin extract media

Spirulina requires relatively high pH values between 9.5-9.8 Bonnin (1992). In the experimental papaya skin extract media pH was varied from 9.4 to 9.8 (Table II). During experimental period, the range of temperature of the culture medium was found between 27.4°C and 30.4°C. Richmond (1992), reported that the optimal temperature for growth of

Antimicrobial activity of *Spirulina platensis*

In this experiment, different solvent extracts of *Spirulina platensis* were screened for their antimicrobial activity. Only freeze dried powder extracts showed antibacterial activity against four pathogenic bacteria viz *Salmonella typhi,*

Table II: pH of the experimental media (Average of triplicates)

Different concentrations ns (gm/L)	Days							
	Initial pH	After 2 days	After 4 days	After 6 days	After 8 days	After 10 days	After 12 days	After 14 days
Conc. 2	9.5	9.6	9.6	9.7	9.8	9.8	9.8	9.7
Conc. 4	9.4	9.6	9.6	9.6	9.7	9.8	9.7	9.7
Conc. 6	9.7	9.6	9.5	9.6	9.6	9.7	9.7	9.6
Conc. 8	9.7	9.5	9.5	9.5	9.5	9.6	9.7	9.7
Conc. 10	9.5	9.3	9.3	9.5	9.5	9.5	9.7	9.6
Control medium, BD_1	9.4	9.6	9.6	9.6	9.7	9.8	9.9	9.8

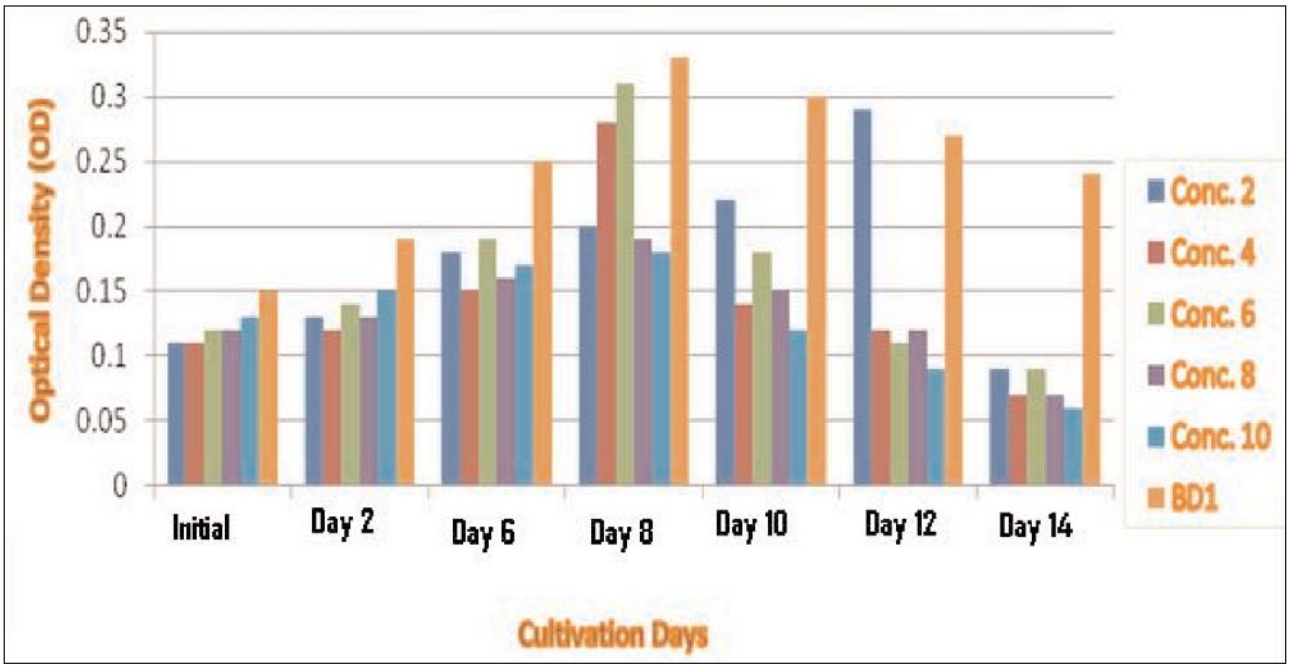


Fig. 2: Bar graph showing the growth of *Spirulina platensis* in experimental culture media (papya skin extract and BD_1 media)

Escherichia coli, Staphylococcus aureus and *Bacillus cereus*. Antifungal activity was not observed by any type of powder extracts. Concerning the antibacterial effects, methanol extracts of *Spirulina platensis* (cultured in IFP media) gave the highest zone of inhibition (20mm) against *Bacillus cereus* (Table III). *Staphylococcus aureus* was the second most inhibited bacteria with most of the extracts of *Spirulina platensis* (cultured in BD_1 media). The growth of *Salmonella typhi* was moderately inhibited and *Escherichia coli* was partially inhibited by all extracts of *Spirulina platensis* (cultured in IFP media). It is clear from the Table III that the diameter of the inhibition zone depends mainly on types of the powder, type of solvents used, amount of extracts used and the tested bacterial organisms. In different studies, freeze dried extracts of *S. platensis* have shown antimicrobial activity against pathogenic bacteria. They have also reported that the extracts extracted in different solvents were effective against both Gram-positive and Gram negative organisms (Ozdemir *et al.,* 2004; Kaushik and Chauhan, 2004; Bhowmik *et al.,* 2009). This is in agreement with our findings, since the *S. platensis* extracts had effects on both types of bacteria used in this study.

Table III: Inhibition zone activities of freeze dried *Spirulina platensis* cultured from different media on four species of bacterial growth

Name of the organism	Zone of Inhibition in mm								
	Chloroform			Ethanol			Methanol		
	BD_1	IFP	PSEM	BD_1	IFP	PSEM	BD_1	IFP	PSEM
Salmonella typhi	7	12	8	7	12	8	8	13	8
Staphylococcus aureus	14	8	7	14	8	7	15	9	8
Bacillus cereus	9	17	8	10	19	9	12	20	10
Escherichia coli	-	9	7	-	8	7	8	8	7

(-) indicates no zone of inhibition

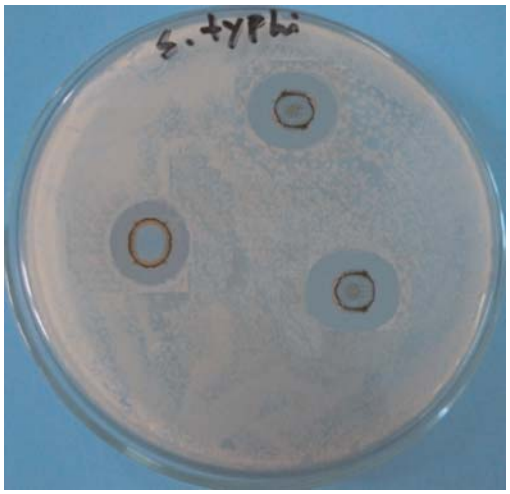


Fig. 3: Antimicrobial activity of *Spirulina platensis* cultured in IFP media against *Salmonella typhi*

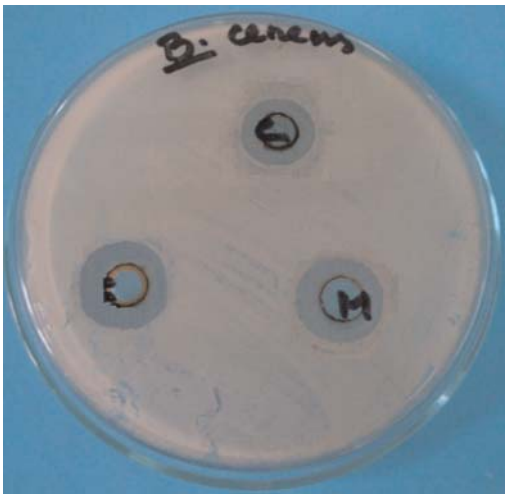


Fig. 4 : Antimicrobial activity of *Spirulina platensis* cultured in IFP media against *Bacillus cereus*

Conclusion

The present study suggests that vegetable wastes can be used as a good source of nutrititive media for *Spirulina* culture at domestic level. Active ingredients present in *S. platensis* have diverse biological activity. Identification of these active ingredients present in *S. platensis* may be interesting topic to study antimicrobial activity of *S. platensis.*

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