Available online at www.banglajol.info

Bangladesh J. Sci. Ind. Res. **49(4),** 205-210, 2014

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

E-mail: bjsir07@gmail.com

Growth response of *Spirulina platensis* (nordst) geitler, in Cabbage extract and antibacterial activities in different culture media

N. Akhtar[1*], M. M. Ahmed[1], A. Hossain[2], M. A. A. Jahan[1] and H. Akhter[1]

[1]*Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh*
[2]*Department of Microbiology, Stamford University, Dhaka, Bangladesh*

Abstract

Growth response of *Spirulina platensis* (Nordst) Geitler in cabbage skin extract media and their antibacterial activities were studied. Five different concentrations e.g. 10 , 8 ,6 , 4 and 2 gm/L of cabbage skin extract media and one BD1 (control) medium were used in this experiment. Highest optical density was observed in 10 gm/L cabbage skin extract medium (0.35) followed by BD1 medium (0.30) after fifteen days of culture. Antibacterial activity of *S. platensis* was studied. Only freeze dried *S. platensis* powder extracts showed inhibitory effect against tested bacterial pathogen.

Key Words: Growth response; Antibacterial activity; Cabbage skin extract media; *Spirullina platensis*

Introduction

Spirulina platensis (Nordst) Geitler is a blue-green photosynthetic microscopic unbranched, filamentous alga. Sometimes found to grow as an unialgal culture in nature in some alkaline lakes with a very high pH. *Spirulina* has a long history of use as food for over 1000 years (Hayashi *et al*.1994) But very recently, the interest in *Spirulina* was increased mainly in its nutritive value. It is rich in protein, vitamins, especially vit-B_{12} and pro-vitamin A (beta-carotene), iron, essential amino acids, minerals and essential fatty acids like gamma linolenic acid (GLA) (Switzer, 1982, Venkataraman, 1983). New clinical research reveals health benefits of *Spirulina*, which contains 15-20% carbohydrates. The primary forms of carbohydrates are rhamnose and and glycogen, these two polysaccharides are easily absorbed by the body, with minimum insulin inversion (Henrikson, 1989). Cell extract of *Spirulina* has shown antibacterial activity against *Bacillus subtillis* and *Saccharomyces cerevisiae*. The presence of high quantities of acrylic acid in *Spirulina* was substantiated at the end of the seventies. This substance shows anti-microbial activity, in a 2 mg/L of biomass concentration (Balloni *et al.* 1980).

In Biological Research Division, BCSIR, Dhaka, *Spirulina* has been cultured at pilot plant scale for over 20 years. Bangladesh medium (BD_1) (Jahan *et al.* 1994) was developed in this laboratory for commercial production of *Spirulina*. Later on BD1 medium was further simplified by the *Spirulina* team of BCSIR, reducing the cost of production and developed BD_2, BD_3, BD_4 and BD_5 which can be used as rural technology for *Spirulina* culture (Khatun *et al*., 2006).

Bangladesh is an agro based country. Where the consumption of vegetables is high. But the wastes vegetables are not being used any economic purpose. If the wastes of nutritional vegetable can be used as a source of nutrient for *Spirulina* culture, it would be economically helpful and reduce the use of chemicals. In Bangladesh used for its culture pregnant women, aged persons and children of rural area are badly affected by malnutrition. So this study has considered to find out a crude method to prepare easy and cheap media for *Spirulina* culture whose raw materials are commonly available for rural people, by which they meat up their nutritional demand. Hence the present study is aimed to develop a new culture media at domestic level using cabbage waste leaf extract, which is an excellent source of vitamin, glutamine, amino acid, protein and iron. Antibacterial effect of the *Spirulina* culture in different media is also included in this study.

Materials and methods

Media preparation

The skin of cabbage as waste was collected from the garden of Plant Physiology section, BCSIR Laboratories Dhaka. It was washed with tap water and 30 gm of it was blended by adding 200 ml tap water. After fine blending, extract was sieved with 200 µm mesh cleaned cloth and stored at 4°C

*Corresponding author: E-mail: nasimadaisy5@gmail.com

until further use. For the preparation of different concentrations of cabbage leave extract media, tap water was added separately with stored extract to make 10 , 8 , 6 , 4 and 2 gm/L concentrations and which was thoroughly mixed with 200 mg/L urea, 5 gm/L NaCl and 3 gm/L NaHCO , to increase the concentration of nitrogen, salinity and pH, respectively. BD1 medium (Jahan et.al, 1994) was used as control for this experiment. Each experiment was done with 3 replicates.

Inoculum preparation and maintenance

Culture was carried out in 1L conical flask containing 500 ml of cabbage skin extract medium. Stock culture of S. platensis from Biological Research Division, BCSIR, Dhaka maintained in Zarrouk (Anomymous, 1966) medium was used as inoculums. Equal volume of inoculums (20 ml/L) was added into each flask (initial OD was 0.11). The flasks were manually 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 antibacterial activity

S. platensis cultivated in three different culture media namely Zarrouk (Anomymous, 1966), BD_1 (Jahan *et al.*, 1994) and cabbage skin extract media were used for this purpose. The initial pH of the culture was maintained at 9.5 and temperature 30° ±0.1 °C respectively. S. platensis were grown until the late exponential phase of growth (8 days) was achieved and there after the biomass was collected and dried in three different means namely freeze, sun and shade dried.

Preparation of various extracts of Spirulina platensis

Sun- shade and freeze-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, chloroform and ethanol and kept at room temperature for three days. The extracts were filtered using Whatman filter paper-I and was concentrated by a rotary vacuum evaporator (STUART, RE3022C) at 50°C and concentrated to gummy materials under reduced pressure. The concentrated material was then collected in small vials and then dried at room temperature. These crude extracts were kept at 4°C until use. The concentrated gummy extracts were dissolved in dimethyl sulfo-oxide (DMSO) prior to use in antibacterial activity test.

Determination of antibacterial activity

In vitro antibacterial studies were carried out against four bacterial pathogens viz. Streptococcus pneumoniae ATCC 49619, Bacillus subtilis (received from Prof. Skersman, Australia). Shigella dysenteriae CRL, (ICDDR, B) and Sarcina lutea ATCC 9341. 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 standards. 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 Mueller-Hinton-Agar (MHA) 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 low temperature (4°C) for 2-4 hours followed by incubation at 37°C for 24 hours. After the incubation period, formation of inhibition zones around the wells, confirms the antibacterial activity of the respective extracts.

Results and discussion

General shape of *Spirulina* is spiral. But straight filaments have better survival capacity. Under less favorable conditions spiral filaments turn straight (Noor *et al.*, 2008). During initial growth period, the filaments were not in normal condition but after 15 days maximum filaments were found to turn in spiral shape. The effect of cabbage skin extracts media on the morphology of *Spirulina* platensis are presented in the Table I and Fig. 1.

Table I: Effect of cabbage skin extract media on morphological view of *Spirulina* platensis.

Days	Microscopic observation
Initial	Filaments are very good and healthy, light blue green in color. Maximum filaments are straight and few are spiral.
After 7 days	Number of filaments is more than initial observation. Conditions of filaments are good, broken filaments present.
After 15 days	Maximum filaments become coil shaped.

In this experiment cabbage skin extract media, pH was maintained from 9.4- 9.8 during entire experimental period (Table II). *Spirulina* required relatively high pH values between 9.5and 9.8 (Bonnin 1992) and values above 10.3 were shown to be harmful for the culture (Richmond *et al*., 1992). The range of temperature of the culture medium was found between 27 and 30°C. It has been, reported that the optimum temperature for the growth of different *Spirulina* strains was in between 30 and 35°C. (Richmond, 1992) Experimental flasks were kept at room temperature near the window and light intensity was 120 Lux to 2500 Lux. Venkataraman (1983) stated that even a short exposure of *Spirulina* cultures to direct intense sunlight would be resulted in bleaching of algal cells. The range of dissolved oxygen level was 1-4 mg/L. Salinity of the culture medium was 10 ppt. Salinity plays a direct role on the purity of the culture (Bonnin, 1992). Fig. 2: shows the growth of S. platensis in the experimental culture media. Despite having started with a similar initial inoculum, the growth of S. platensis was started to change from the second day of cultivation. Optical density

Table II: pH values of the experimental media after different days.

Media conc (gm/L)	Initial pH	Days					
	2	4	6	8	10	12	15
2	9.6	9.6	9.6	9.6	9.6	9.7	9.7	9.8
4	9.5	9.5	9.6	9.5	9.6	9.6	9.6	9.7
6	9.5	9.5	9.5	9.6	9.6	9.6	9.6	9.6
8	9.6	9.4	9.5	9.5	9.6	9.6	9.6	9.6
10	9.5	9.5	9.6	9.5	9.6	9.6	9.6	9.7
Bd1	9.5	9.6	9.6	9.6	9.6	9.7	9.7	9.8

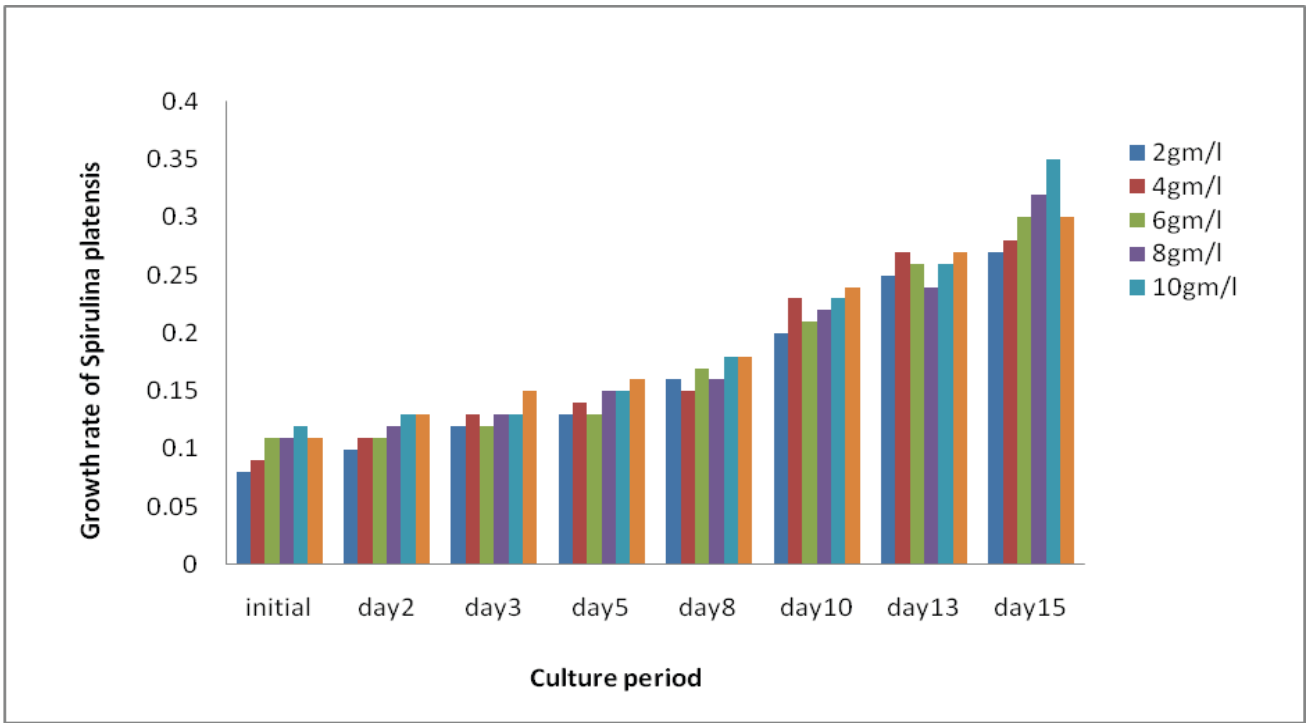

Fig. 2. Bar graph showing optical density of experimental culture media

0.27, 0.28, 0.30, 0.32 and 0.35 were observed in concentration 2, 4, 6, 8 and 10 gm/l of cabbage skin extract media, respectively. Optical density 0.30 was recorded in the BD1 medium. It was observed that, after 1st day of cultivation, optical density was gradually increased in experimental and control medium. Cabbage skin extract culture medium of concentration 10 gm/l yielded 550 gm/L of *Spirulina* platensis, BD_1 yielded 750 mg/L. Whereas, BD_3 and BD_4 media, yielded 664 mg/L and 665 mg/L, respectively (Begum *et al.*, 1998).

***Antimicrobial activity* of *Spirulina* platensis**

Extracts of *Spirulina* platensis with chloroform, ethanol and methanol were screened for their antimicrobial activity. Only freeze dried powder extracts showed antibacterial activity against four pathogenic bacteria viz., Streptococcus Pneumoniae, Bacillus Subtilis, Shigella dysentariae and Sarcina lutea (Table III). Streptococcus pneumoniae and Shigella dysenteriae gave the highest inhibition zone (14

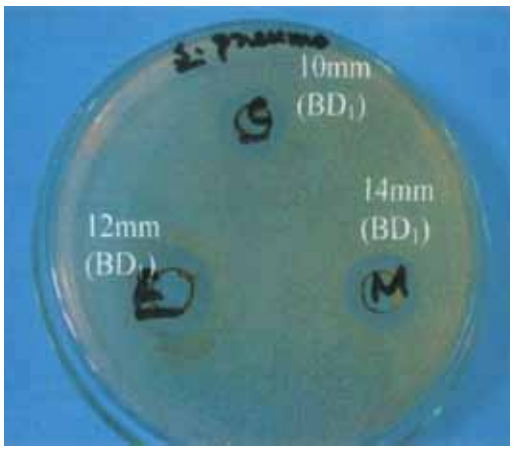

Fig. 2.3. Antimicrobial activity of Spirulina Spirulina Platensis against S.pneumoniae

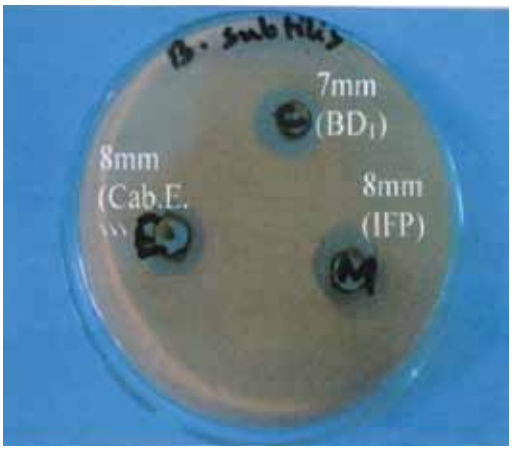

Fig. 2.4. Antimicrobial activity of Platensis against B. subtilis

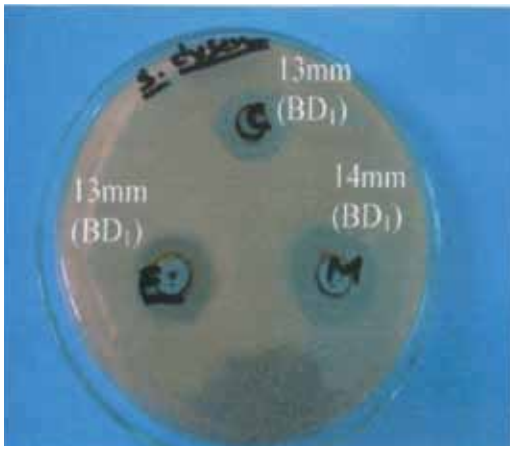

Fig. 2.5. Antimicrobial activity of Spirulina Spirulina Platensis against S. dysenteriae

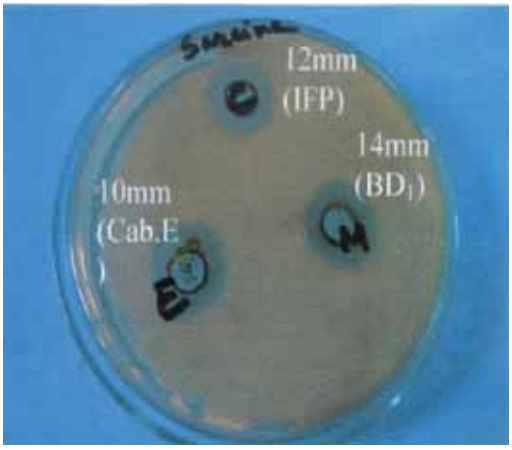

Fig. 2.6. Antimicrobial activity of Platensis against Sarcina lutea

Table III: Inhibition zone activities of freeze dried *Spirulina* platensis cultured in different

Bacteria	Methanol Chloroform			Ethanol					
	BD1 Cab.E	Zarrouk		BD1 Cab.E	Zarrouk		BD1 Cab.E	Zarrouk	
Sarcina lutea	7	12	9	7	11	10	7	12	8
Streptococcus pneumoniae	14	7	7	12	7	7	10	7	10
Bacillus subtilis	8	8	8	7	7	8	7	7	8
Shigella dysenteriae	14	8	10	13	8	7	13	7	7

mm) against methanol extract (cultured in Bd1 medium Fig. 3.1 and Fig. 3.3). Sarcina lutea gave 12 mm inhibition zone (Fig. 3.4) against methanol and chloroform extracts (cultured in Zarrouk medium). In case of Bacillus subtiles maximum inhibition zone was found in methanol extracts (8 mm) and minimum was 7 mm against ethanol and chloroform extracts (3.2). This result was observed by agar cup method. The diameter of the inhibition zone dependent mainly on types of the powder, type of solvents, amount of extracts used and the tested bacterial organisms (Ozdemir *et al*., 2004; Kaushik and Chauhan, 2007; Bhowmik *et al.*, 2009). Antimicrobial activity shown by Spirulina platensis is because they produce certain biological active substances that could be be intracellular or extracellular metabolites having diverse biological activity.

Conclusion

Our country will be benefited if nationwide use this nutritive and cheap unused cabbage skin extract for *Spirulina* culture at rural level. This study also noticed that only freeze dried *Spirulina* powder extract showed anti bacterial activity against specific bacteria

References:

Balloni, W., Tomaselli, L., Giovannettl, L. and Margheri, M.C.(1980), Biologia fondamentale del genere *Spirulina*. In: Cantarelli C., Ciferri, O., Florenzano, G., Kapsiotis, G., Malerassi, R., Treccani, Progetto finalizzato "*Ricerca di nuove fonti Proteiche e di nuov formulazioni alimontari*" Atti del Convegno: Prospettive della cottura di *Spirulina* in India.Consiglie Nazionale della Richerche. Firenze-Academia dei Georgofili CNR Tipografia Coppini; pp. 49-82.

Barry. (1980). Procedure for testing antimicrobial Agents in Agar media. In: Antibiotics in Laborator Medicine. Lorin V (eds), Williams Wilkins Co. Baltimore: USA, pp. 1-23.

Begum S, Noor P, Akhter N and Majid FZ (1998), Spirulina culture in Bangladesh VI. Domestic production of *Spirulina, Bang. J.Sci. and Ind. Res*, **33 (3)**: 473-478.

Bhowmik Dola, Dubey Jaishree and Mehra Sandeep (2009), *Probiotic Efficiency of Spirulina platensis*- Stimulating Growth of Lactic Acid Bacteria. *World Journal of Dairy & Food Science,* **4(2)**: 160-163.

Bonnin G (1992), *Spirulina* Production Engineering Handbook, B.E.C.C.M.A., A-5 place Roger Salengro 4400 Nantes France. 79.

Henrikson R (1989), Earth food of *Spirulina* Ronore Entirreprises Inc. Laguna beach, California p.15.

Hayashi, O., T. Katoh and Y. Okuwaki. (1994), Enhancement of antibody production in mice by dietary *Spirulina platensis*. J. Nutr. Sci. Vitaminol **40**: 431-441.

Jahan MAA, Noor P, Akhtar N (1994), Spirulina culture in Bangladesh 111.Developement of a Medium, suitable for commercial cultivation of *Spirulin* in Bangladesh, *Bang. J. Sci. Ind. Res*. **2(4)**: 149.

Kaushik P and Chauhan A (2007), *In vitro* antimicrobial activity of laboratory grown culture of *Spirulina platensis, Indian J. Microbiology*, **48**: 348-352.

Khatun R, Noor P, Akhtar N, Jahan MAA, Hossain M and Munshi JL (2006), Spirulina Culture in Bangladesh XI Selection of a Culture Medium, Suitable for Culturing a Local Strain of *Spirulina, Bang. J. Sci. Ind. Res*, **41(3-4)**: 227-234.

Noor P, Akhtar N, Begum S and Munshi JL (2008), Spirulina Culture in Bangladesh XII. Effects of Different Culture Media, Different Culture Vessels and Different Cultural Conditions on Coiled and Straight Filament Characteristics of *Spirulina, Bang. J. Sci. Ind. Res*, **43(3)**: 369- 376.

Ozdemir G, Karabay NU, Dalay MC, Pazarbasi B (2004), Antibacterial activity of volatile components and various extracts of *Spirulina platensis, Phytotherapy Research*, **18**: 754–757.

Richmond A (1992), Mass culture of cyanobacteria. In: Mann, N., Carr, N., Eds. *Photosynthetic* prokaryotes. 2nd ed. Plenum Press, New York and London. pp. 181-210.

Switzer L (1982), *Spirulina*, The Whole Food Revolution Bantam Books, London, Sydney. PP. 49-126.

Venkataraman LV (1983), Blue green alga *Spirulina*. Central food technological research institute, Mysore India; pp. 3-74.

Anomymous, Zarrouk (1966), Contribution a I "etude dune cyanophycee; influence de divers facteurs physiques et chimiques sur Ia croissance et la photosynthese de *Spirullina maxima* (Setch et Gardner) Geitler Ph. D thesis, University of Paris.

Received: 27 November 2013; Revised: 14 July 2014
Accepted: 25 August 2014