ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(6): 1518 - 1523

ORIGINAL RESEARCH ARTICLE

In vitro Cultivation of *Spirulina platensis* using Rice Mill Effluent

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Received 26 Aug 2012; Revised 28 Nov 2012; Accepted 04 Dec 2012

ABSTRACT

The water samples were collected for the isolation of strains of alga *Spirulina platensis* from three different locations and the strain *Spirulina platensis* was isolated and it was, designated as ANS - 1 strain. The characteristics of *Spirulina platensis* ANS -1 were compared with reference CAS -10. The waste water rice mill effluent was collected and its pH was adjusted to 9-11 by using sodium bicarbonate @ 800 mg l^{-1} and it was used as a medium. The isolated strain ANS -1 and reference strain CAS 10 were grown in substrates 1/6 diluted Zarrouk's medium (control) and rice mill effluent. The well performed strain CAS 10 under *in vitro* condition was selected as efficient one. The growth of *Spirulina platensis* was measured both in laboratory and outdoor condition by using the parameters *viz*., optical density, population, dry weight, protein and chlorophyll content. The high growth and dry weight were recorded in 1/6 diluted Zarrouk's medium when compared to rice mill effluent medium. Maximum protein and chlorophyll content were noticed in 1/6 diluted Zarrouk's medium than rice mill effluent.

Key words: *Spirulina platensis*, Zarrouk's medium, Protein content and Rice mill effluent

1. INTRODUCTION

Industrial pollution has been and continues to be a major factor causing the degradation of the environment around us, affecting the water we use, the air we breathe and the soil we live on. The exponential increase in industrialization is not only consuming large areas of agriculture lands, but simultaneously causing serious environmental degradation as well as to sail. Water originating from various industries is finding their place in agriculture. The challenge is to properly incorporate the disposal of the wastes in a controlled management programme so that the applied industrial solid wastes do not contribute any problem of pollution to soil, soil microbes and environment [1].

Blue-green algae (Cyanobacteria) are among the most primitive life forms on Earth. Their cellular structure is a simple prokaryote. They share features with plants, as they have the ability to perform photosynthesis. They share features with primitive bacteria because they lack a plant cell wall. Interestingly, they also share characteristics of the animal kingdom as they contain on their cellular membrane complex sugars similar to glycogen. Among blue-green algae, both edible and toxic species adapted to almost any of the most extreme habitats on earth. Edible blue-green algae, *including Nostoc*, *Spirulina*, and *Aphanizomenon* species have been used for food for thousands of years [2, 3].

The current environmental conditions deteriorations, mental and physical stress, changes in the diet have been serious risk factors for the humans, increased the death rate and civilization diseases. These are the obvious reasons why new progressive trends are being extensively developed in modern medicine, pharmacology and biotechnology and more effective harmless medicaments are being sought for to treat and prevent various diseases. One of the trends in biotechnology is associated with Blue green microalgae *Spirulina platensis* which have been widely employed as food and feed additives in agriculture, food industry, pharmaceuticals, perfume making, medicine and science [4].

Spirulina are multicellular and filamentous blue green algae that has gained considerable popularity in the health food industry and increasingly as a protein and vitamin supplement to aquacultures diets. It grows in water, can be

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harvested and processed easily and has very high macro and micro nutrient contents. It has long been used as a dietary supplement by people living close to the alkaline lakes where it is naturally found for instance those living adjacent to lake chad in the kanem region have very low levels of malnutrition despite living on a Spartan millet base diet. This traditional food, known as dihe, was rediscovered in chad by a European scientific mission and is now widely cultured throughout the world. In many countries of Africa, it is still used as human food as a major source of protein and is collected from natural water, dried and eaten. It has gained considerable popularity in the human health food industry and in a many countries of Asia, it is used as protein supplement and as health food [3].

The mass cultivation of *Spirulina* is achieved both in fresh water and waste water. *Spirulina* grown in clean waters and under strictly controlled conditions could be used for human nutrition. The micro alga grown in waste water is used as animal feed and provide a source of the fine chemicals and fuels. The waste water system is highly applicable in populated countries like India where wastes are generated in high quantities and pose environmental problem. Large scale production of *Spirulina* is feasible in tropical conditions in developing countries, where land costs and labour are comparatively cheaper. The microalga can be exploited as a potential source of food, feed and fuel [5].

2. MATERIALS AND METHODS

2.1. Isolation and purification of *Spirulina platensis*

Individual colonies from the enrichment culture flasks were lifted with the help of an inoculation needle and suspended in 5 ml of distilled water in test tubes. The tubes were uniformly shaken manually to make a homogenous suspension. 0.5 ml of this suspension was inoculated into 50 ml sterile Zarrouk's medium in 100 ml Erlenmeyer flask. The inoculated flasks were incubated; the isolated colonies were picked up and examined under compound microscope. Among the water collected from three different locations, there was algal growth only in the sample from Annamalai Nagar temple pond in Zarrouk's medium. The isolated strain was purified and designated as ANS1 (Annamalai Nagar *Spirulina* 1). The isolated strain (ANS -1) was compared with reference strain (CAS-10) for its characters.

2.2. Cultivation of *Spirulina platensis* in Zarrouk's medium under laboratory condition

The pure culture of algal strains ANS-1, CAS-10 were grown in Zarrouk's medium and used as the standardized inoculum for comparing their growth rate. The standardized inoculum was measuring 0.91 optical density at 320nm and used in the growth studies to measure various growth parameters. The standardized inoculum 5ml was inoculated in to 50ml Zarrouk's medium in 100ml Erlenmeyer flasks and incubated for 21 days in light chamber. Growth of *spirulina platensis* two strains were measured through optical density, population, dry weight, protein and chlorophyll content to select the better stains for mass production.

2.3. Collection of Rice mill effluent

Rice mill effluent was collected from Ambiga Rice mill, Annamalai Nagar and used for the growth of *Spirulina platensis* strains. The following were the characteristics of Rice mill effluent.

2.4. *In vitro* cultivation of *Spirulina platensis* using Rice mill effluent

The *Spirulina platensis* strain CAS-10, ANS-1 were grown under laboratory condition in various substrates *viz*., 1/6 diluted Zarrouk's medium and Agro industrial effluent *viz*., Rice mill effluent medium and sago industrial effluent. The 1/6 diluted Zarrouk's medium was used as a standard medium to compare the growth. 40 ml of standardized algal inoculum was inoculated into 400 ml of the various substrates in 500 ml Erlenmeyer flasks separately. After inoculation, the substrates in 500 ml Erlenmeyer flasks were incubated under illuminated light chamber (3 to 4 k lux light intensity) at 28 ± 2°C temperature for 21 days. Three replications were maintained for each medium.

2.5. Estimation of growth parameters in *Spirulina platensis*

The growth parameters of *Spirulina platensis* strains ANS 1 and CAS 10 in various media were measured at three different periods (7^{th}, 14^{th}, 21^{st} day).

2.5.1. Optical density

Optical densities (O.D) of the algal strains were measured in spectrophotometer (Spectronic 1001). The O.D value was observed at different wavelength (300 to 650 nm) and the higher O.D values were recorded at 320 nm wavelength. Hence, the growth of *Spirulina platensis* strains

was measured at 320 nm wavelength after the incubation days.

2.5.2. Population estimation

The population was estimated by direct microscopic count method. 0.1 ml of the culture was taken and spread over the marked 1 sq. cm area of the clean glass slide. Then it was observed under compound microscope after covering with cover glass. Numbers of cells were counted per microscopic field and the populations were estimated by using conn's direct microscopic count method.

2.5.3. Dry weight estimation

The dry weights of the strains were estimated in standard bottle. 50 ml of the cultures was taken and centrifuged at 6000 rpm in the centrifuge. The cell debris obtained was transferred to standard bottles (known weight), air dried and kept in hot air oven at 60°C to a constant weight. Then it was cooled in desiccators and weighed. The difference in weight (culture bottle–empty bottle) was expressed as dry weight in mg.

2.5.4. Protein estimation

The protein content of *Spirulina platensis* cultures were estimated by the Lowry's method [6].

2.5.5. Chlorophyll estimation

One gram of *Spirulina platensis* was homogenized in 20 ml acetone (80%) and allowed to stand overnight in dark at 4EC for complete extract followed by centrifugation at 10,000 rpm for 5 minutes. The contents of total chlorophyll (T-Chl), chlorophyll a (Chl-a) and chlorophyll b (Chl-b) in the supernatant were determined spectophotometrically according to Lichtenthaler [7] method.

2.6. Statistical analysis

The experimental data were analysed as per the procedure suggested by Panse and Sukhatme [8].

3. RESULTS AND DISCUSSION

Cyanobacteria are the photoautotrophic microorganisms largely distributed in various aquatic environments. Some of them have been used as human food for many years because of their high protein content (35-65 per cent) and nutritional value. *Spirulina* is the best known genus and it was consumed by the Aztecs in Mexico valley and by the Chand lake population in Africa. At present some countries including Argentina are culturing it on a large scale. *Spirulina* is beneficial to health due to the presence of compounds like essential amino acids, vitamins, natural pigments and essential fatty acids.

The genus *Spirulina* belongs to the Oscillatoriaceae family contains the group of filamentous Cyanobacteria characterized by spiral shaped chains of cells enclosed in a thin sheath. In the present investigation, the isolate was identified based on the microscopic and cultural studies. The isolated *Spirulina platensis* strain ANS -1 resembled the characters of the reference strain CAS 10. The strains were characterized based on the parameters *viz*., average number of spirals, distance between spirals, length, width of trichome, percent of long and short trichome. The characteristics of the two strains CAS 10, and ANS -1 were similar to the characters reported by Deore [9], Gupta and Chagwal [10] who carried out observations in *Spirulina gigantean* var *schmidle* and *Arthrospira platensis.*

The characters of *Spirulina platensis* strain ANS-1 were compared with the reference strain CAS-10 and the results are presented in Table - 1. Morphologically they were found to be similar, multicellular, filamentous, unbranched and helicoidal trichomes. The characters measured were spiral number, distance between spirals, length and width of trichome, number of long trichome and short trichome. The *Spirulina platensis* strain CAS-10 and ANS-1 were preserved as calcium alginate beads for 60 days and their viability was tested by estimating the population per ml on 30^{th} and 60^{th} days after beads formations. The viable cell populations are presented in (**Fig 1**).

Spirulina requires a medium of high alkalinity with pH values not less than 8.5 and a steady supply of bicarbonate ions (Ciferri, 1983). In the present investigation, the *Spirulina platensis* strains CAS - 10 and ANS -1 were grown in Zarrouk's medium and the growth was analyzed based on the parameters like optical density, population, dry weight, protein and chlorophyll content. All the growth parameters were high in CAS - 10.

The growth of *Spirulina platensis* strains CAS -10 and ANS -1 was estimated in Zarrouk's medium under laboratory condition through various parameters like optical density. Cell population per ml, dry weight, protein and chlorophyll content and the data are presented in Table - 2. The various growth parameters *viz*., optical density (O.D), cell population per ml, dry weight,

protein and chlorophyll content were higher in the *Spirulina platensis* strain CAS -10 compared to ANS -1.

The *Spirulina platensis* strains CAS-10 and ANS-1 were grown under laboratory condition i.e., rice mill effluent with 1/6 diluted in Zarrouk's medium. The growth was observed through the parameters *viz*., optical density, cell population, dry weight, protein and chlorophyll content. The growth of *Spirulina platensis* strains CAS -10 and ANS -1 under laboratory condition was recorded by estimating O.D value on 7th, 14th, 21st day of growth and the data are furnished in Table - 3. The growth of both the algal strains was higher in 1/6 diluted Zarrouk's medium compared to other substrates.

The growth of *Spirulina platensis* strains CAS -10 and ANS -1 in rice mill effluent under laboratory condition was recorded by estimating cell population, dry weight, protein content and chlorophyll content on 7th, 14th, 21st day of growth and the data presented in Table – 4 to Table - 7. The growth parameters, protein content and chlorophyll content of Spirulina platensis were maximum in the Zarrouk's medium when compared to rice mill effluent. This study is in line with the earlier findings of Clement *et al*. [11], Venkatraman [12], AnusyaDevi *et al*. [13], Tasneem [14], and Po Chung *et al*. [15] on using diluted media for SCP production.

Table 1: Comparison of the characters of *Spirulina platensis* strains

S. No	Characters	CAS-10	ANS-1
1	Average number of spirals	3-4	2-3
2	Distance between spirals	45-50 µm	45-50 µm
3	Width of trichome	12.0 µm	11 µm
4	Length of trichome	260 µm	250 µm
5	Number of short trichomes	27 per cent	24 per cent
6	Number of long trichomes	66 per cent	58 per cent

Fig 1: Survival of *Spirulina platensis* strains in calcium alginate beads

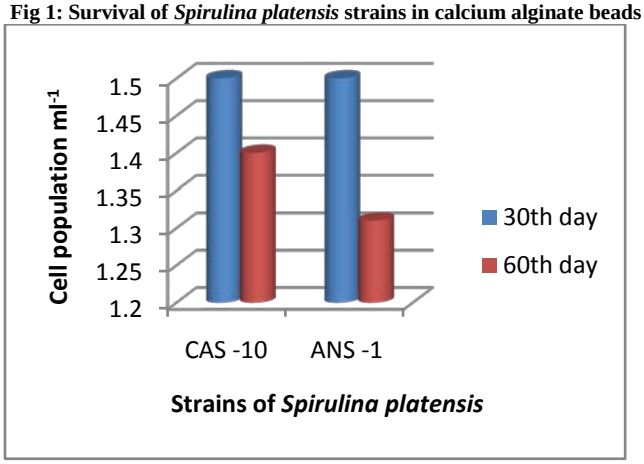

Table 2: Growth parameters of *Spirulina platensis* under laboratory condition in Zarrouk's medium

S. No	Growth parameters	*Spirulina platensis* strain	
		CAS -10	ANS -1
1	O.D (at 320nm)	2.305	2.256
2	Cell population/ml	1.5×10^4	1.3×10^4
3	Dry weight (mg^{-1})	30	25
4	Protein (mg^{-1})	22	17.5
5	Chlorophyll (mg^{-1})	14	11

Table 3: Growth pattern of *Spirulina platensis* under laboratory condition

S. No	Substrate	O.D value at 320 nm					
		CAS -10			ANS -1		
		7th day	14th day	21st day	7th day	14th day	21st day
1	Zarrouk's medium	2.375	2.426	2.586	1.875	2.135	2.204
2	Rice mill effluent medium	1.635	1.726	1.814	1.062	1.193	1.313
	SED	0.0042	0.1114	0.0040	0.0023	0.0012	0.0032
	CD (p=0.05)	0.0084	0.0238	0.0080	0.0046	0.0025	0.0064

Table 4: Cell population of *Spirulina platensis* under laboratory condition

S. No	Substrate	Cell population ml^{-1}					
		CAS -10			ANS -1		
		7th day	14th day	21st day	7th day	14th day	21st day
1	Zarrouk's medium	1.35×10^4	1.44×10^4	1.52×10^4	1.30×10^4	1.32×10^4	1.41×10^4
2	Rice mill effluent medium	1.13×10^4	1.10×10^4	1.16×10^4	1.08×10^4	1.03×10^4	1.16×10^4
SED		135.26	127.62	42.82	20.32	25.62	72.48
CD (p=0.05)		270.52	255.24	84.74	40.64	51.24	154.87

Table 5: Dry weight of *Spirulina platensis* under laboratory condition

S. No	Substrate	Dry weight $mgl^{-1}d^{-1}$					
		CAS -10			ANS -1		
		7th day	14th day	21st day	7th day	14th day	21st day
1	1/6 diluted Zarrouk's medium	19	23	27	18	22	26
2	Rice mill effluent medium	15	19	22	17	18	20
SED		0.421	0.3921	0.5326	0.3523	0.4260	0.3436
CD (p=0.05)		0.8426	0.7244	0.1652	0.7046	0.8520	0.7973

Table 6: Protein content of *Spirulina platensis* under laboratory condition

S. No	Substrate	Protein content $ml^{-1}d^{-1}$					
		CAS – 10			ANS – 1		
		7th day	14th day	21st day	7th day	14th day	21st day
1	1/6 diluted Zarrouk's medium	12	15	17	10	15.5	17.5
2	Rice mill effluent medium	8.5	11	13	9	12.5	13
SED		0.2013	0.2201	0.2432	0.1022	0.1425	0.1894
CD (p=0.05)		0.416	0.4412	0.4874	0.2144	0.2850	0.3788

Table 7: Chlorophyll content of *Spirulina platensis* under laboratory condition

S. No	Substrate	Chlorophyll content $ml^{-1}d^{-1}$					
		CAS -10			ANS -1		
		7th day	14th day	21st day	7th day	14th day	21st day
1	1/6 diluted Zarrouk's medium	8.79	9.15	9.82	8.24	8.95	9.20
2	Rice mill effluent medium	7.86	8.31	8.91	7.19	8.00	8.56
SED		0.0409	0.067	0.0775	0.0291	0.0312	0.0358
CD (p=0.05)		0.0821	0.1347	0.1559	0.0189	0.0254	0.0300

4. CONCLUSION

From the present study, it was concluded that the *Spirulina platensis* was cultivated on different concentrations of Zarrouk's medium with agro industrial waste supplementation yield better growth than the control Zarrouk's medium. The growth of *Spirulina platensis* was high in Zarrouk's medium containing agro industrial waste. This work clearly showed the capability of agro industrial waste for the cultivation of *Spirulina platensis*. This present research improves the production of the Blue green algae *Spirulina platensis* in future and prevents the environmental pollution emerging from the agro industrial waste.

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