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ISOLATION AND ACCLIMATIZATION OF *ARTHROSPIRA PLATENSIS* FROM TUNGABHADRA RESERVOIR FOR MASS CULTURE IN SEMI-ARID REGION AT BALLARI, KARNATAKA

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ABSTRACT

Spirulina (*Arthrospira platensis*) is a filamentous blue-green alga belonging to Cyanophyta. WHO has called it as the *strongest food on earth*. Since it is rich in bioactive components such as proteins, vitamins, minerals, Poly-Unsaturated Fatty Acids (PUFA), beta-carotene and other pigments having antioxidant activity is generally grown in the freshwater for its nutraceutical compounds, which have applications in health foods, feeds, cosmetics, therapeutics and diagnostics properties. The collection of water sample was done using standard plankton net from Tungabhadra reservoir, a tributary of river Krishna during Mar-Jul 2021.The sample was concentrated for the sake of Spirulina isolation. Manual isolation using fine bristle and subsequent serial dilution for two weeks resulted in the development of impure strain stock. During the isolation process, community of plankton with cyanobacters were sucked out using sterilized micropipette and cultured in the test tubes then in conical flasks using Zarrouk's medium. Different pH conditions were maintained for culture at 5.0, 7.0 and 9.0 respectively. It was found that even at higher temperatures at 36°C at VSK University campus, Ballari, there was neither deformation nor deterioration of the cellular components. The study indicated that mass culture in the laboratory may help in establishment of live-feed market for the local aquaculture sector. The percentage of purity achieved was around 90 % at 9.0 pH value after six weeks of culture.

Key words: Spirulina (*Arthrospira platensis*), isolation, Tungabhadra reservoir, Zarrouk's media.

Introduction

Arthrospira platensis (Spirulina), a Cyanobacterium is a commercial product with high nutritional value, serving a rich source of nutrients for human food, animal food, chemical and pharmaceutical industry. *Spirulina* is a photosynthetic, filamentous, helical-shaped, multicellular and blue-green microalga (La & Ciencias, 2003). It is used as a food supplement for undernourished people in many parts of the world due to its protein content, high digestibility and specific amino acid content (Acreman, 1994; Radhakrishnan et al., 2014). There have been increased efforts of isolation and establishment of phylogenetic relationship among *Arthrospira, Spirulina maxima* and *Phormidium* species in different waterbodies including Kenya and India (Ballot et al., 2004). Commercial utilization of *Spirulina platensis* cyanobacter as a mixed food additive to the shrimp larvae showed promising results than that were fed with *Artemia* nauplii (Colmenares et al., 2005; Matta et al., 2017). Isolation of high hydrogen producing strains of microalgae from the different sources of wild water bodies in China by adopting plating technique was carried out by (He et al., 2012). Different strains of *Spirulina* were even cultivated at different carbon dioxide concentrations using bioreactor to determine the most optimal strain for carbon dioxide mitigation (Zhu et al., 2021). Ancient Mexicans were first in the world to use *Spirulina*. Prasad et al., (2013) isolated edible *Spirulina* from western parts of Mexico and maintained in different states; solid in agar plates at 4°C and in liquid agar at 10°C. Isolation and characterization of *Spirulina platensis* was carried out using different culture media at Mysore by CFTRI nevertheless isolation from the wild freshwater from northern Karnataka region has not been carried out as per the literature survey. The present study is an attempt to isolate and mass culture the *Spirulina* sp from one of the tributaries of River Krishna, Tungabhadra during Mar-June 2021.

Materials and methods

Sample collection

Freshwater samples were collected using plankton net having 50 micron mesh size. The net was hauled along the surface water for 5 minutes having 30 cm diameter and 60 cm long filtering about approximately 1000 liter during early morning hours.

Microscopy and Isolation

The collected water sample was concentrated by filtration method and examined under compound microscope using low power magnification. After repeated drop-by-drop microscopy, the cyanobacters were fixed in the tube culture for multiplication. The isolated plankton community with cyanobacters were cultured in the conical flasks with the Zarrouk's media (Zarrouk C, 1966). Different pH was maintained in three culture containers viz., 5.0, 7.0 and 9.0. Since the pH maintained was alkaline there were gradual eradication of other contaminant plankton as they couldn't thrive in higher pH (Saranraj & Sivasakthi, 2014).

Results and discussion

Spirulina is a planktonic photosynthetic filamentous Cyanobacterium that forms colonies in tropical and sub-tropical water bodies having high levels of

carbonates and bicarbonates. Photosynthetic organisms are capable of fixing Carbon from Carbon dioxide in their metabolic pathways. Reduced amount of Carbon dioxide in the aquatic ecosystem is seldom converted into anaerobic ecosystem.

The standard plankton net was used for the filtration of water along the surface of the reservoir at the coordinates 15.30 N and 76.33 E during early morning at around 08.00 am (Figure-1). The concentration water sample was brought to the Zoology laboratory at Vijayanagara Sri Krishnadevaraya University, Ballari around 65 km from the collection site. The water sample was further concentrated to 100 ml by decanting the water in the darkness.

Microscopy and Isolation

The sample was then mounted for microscopic analysis. This process was repeated until the cyanobacters were found. Other methods followed by Acreman, 1994 were consulted to avoid contamination. The strains of *Spirulina* were identified taxonomically using the key Tomaselli, 1997 and Ciferri, 1983. One of the other methods of species identification is by using universal 18S rDNA (Kasan et al., 2020). The slide mounted with the drop containing spiral microalgae were sucked using sterile micropipette and transferred onto another slide containing water sample so as to have maximum dilution. After repeated serial dilutions, individual filaments were picked up by fine bristles made out of '0' number painting brush with having retained only one bristle intact and transferred to 10 ml test tubes with culture media for multiplication.

The mother culture was maintained in the 50 ml vials with the Sterilized Zarrouk's media. The composition of Zarrouk's culture media is shown in the table-1, where sl. No. 1 and 2 macronutrients were taken as standard solution-A and sl.no 3 to 9 macronutrients were taken as standard solution-B. The remaining chemicals act as trace element solutions. Additional micronutrients were added to the culture. Standard solution A and B were autoclaved separately and mixed just before use under laminar flow.

In addition parallel test tube culture and conical flask cultures were maintained for two weeks. The change in the concentration was recorded on daily basis by microscopy are depicted in the figure-2. At the same time culture was streaked in agar plates for pure strain maintenance (figure-3). The optical density recorded in Spectrophotometer after two weeks of culture at 560 nm was 0.7. Although the present method of isolation was cumbersome, yet after serial dilution pure culture line was established after 6 weeks of sub-culturing on a weekly basis (Figure-4). The percentage of purity achieved was around 90 %. The cultures that were maintained at 9.0 pH had less contamination than that are maintained at 7.0 and 5.0 pH respectively. It indicated alkaline medium did not facilitate the growth of other contaminating algae, Arulmoorthy et al., 2017 observed similar findings as a bloom of cyanobacter along Muttukadu backwaters of South India having 7.11 to 8.42 pH.

Sl. No	*Chemical name*	*gm / liter*	*medium*
1	Sodium bi-carbonate	16.80	Dist.Water
2	Di-potassium hydrogen phosphate	0.500	Dist.Water
3	Ferrous sulphate	0.010	Dist.Water
4	Sodium nitrate	2.500	Dist.Water
5	Potassium Sulphate	1.000	Dist.Water
6	Magnesium sulphate	1.200	Dist.Water
7	Sodium chloride	1.000	Dist.Water
8	Calcium chloride	0.040	Dist.Water
9	EDTA	0.100	Dist.Water
10	Manganese chloride	1.810	Dist.Water
11	Zinc sulphate	0.222	Dist.Water
12	Sodium molybdate	0.039	Dist.Water
13	Copper sulphate	0.079	Dist.Water
14	Cobalt nitrate	0.049	Dist.Water
15	Boric acid	2.860	Dist.Water

Table.1. Zarrouk's media compostion (Source: CSIR-CFTRI)


a)


b)


c)

Fig.1. a, b: Sample collection at the Tungabhadra reservoir using standard plankton net during Mar and June 2021, c: Green algae concentrated in one liter pet bottle.

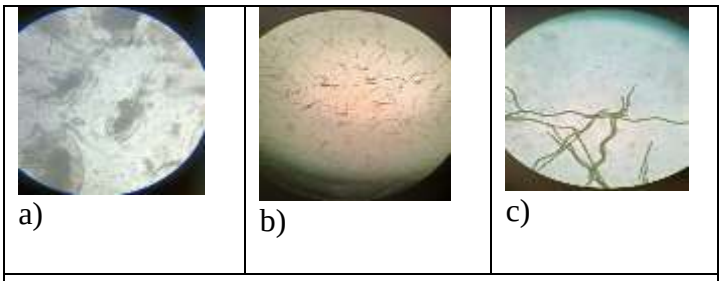

Fig.2. Microscopic view of the samples. a: with more contaminants, b and c: *Spirulina* filaments with least contaminants.

Fig.3. Mother cultures are maintained in agar plates and flask

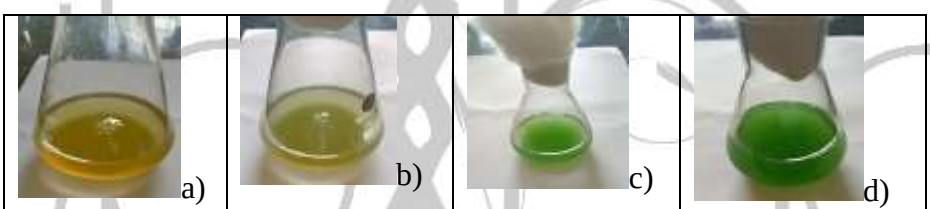

Fig.4. Subcultures of *Arthrospora platensis* a: one week old; b: two weeks old; c: three weeks old; d: six weeks old.

Conclusion

Owing to the high inputs from the neighbouring agricultural runoff, the nutrient load rich in carbonates and bicarbonates especially during early monsoon must have resulted in the provision of suitable environmental conditions along the impoundment site of Tungabhadra reservoir which facilitated the occurrence of Arthrospira platensis (Spirulina platensis). Maintenance of alkaline pH was found to be suitable for the growth of Spirulina cultures.

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