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Evaluation and Characterization of Microalgae from Kalaburagi Region as a Potential Source of Biomass Production

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ABSTRACT

Microalgaes are considered for a large number of applications such as biomass and energy production due to their increased capability of biomass production. Various biotic and abiotic factors influence the growth of microalgae. Among the abiotic factors include the temperature, light intensity, pH of the medium, salinity, and nutritional conditions play the major role. Ten samples of microalgae were collected from different sites of water bodies from Gulbarga University campus. The microalgae isolated were identified and characterized using light microscope, SEM and PCR based 18s rRNA. Influence of cultural conditions such as carbon and nitrogen sources, pH, temperature and salinity on biomass production were studied and analyzed. Among the strains identified, a novel species was isolated and identified as Scenedesmus vacuolatus AK1. The maximum growth rate and biomass productivity can be achieved by providing glucose as carbon source and urea as nitrogen sources at an optimum of 0.5gm/l and 0.1mg/l irrespectively. It was also observed that the strain showed a good growth profile and increase in biomass production at an optimum alkaline pH of 8. Maximum biomass productivity of 0.6g/l was observed at an optimum temperature of 30˚C in BG-11 medium. Maximum biomass yield of 1.45g/l was observed at 5mM NaCl concentration.

Keywords : Abiotic Factors, Biomass, Microalgae, Scenedesmus Vacuolatus

I. INTRODUCTION

Microalgae make use of sunlight and fix carbon dioxide in the photosynthesis process which helps in the rapid and efficient production of biomass in comparison to terrestrial plants. Microalgaes are considered for a large number of applications such as biomass and energy production due to their increased capability of biomass production, faster growth rate and its compatibility to be used for different kinds of biofuel production. Most of the stored lipids in the microalgae biomass are accumulated as triacylglycerols (TAG). Microalgae are efficient in removing nutrients from various industrial wastewater and effluents (Dragone et al., 2010, Chisti, 2007). Due to the increased depletion of fossil fuels,

there is an all-time increased demand for finding out an alternative source of energy that is both economically sustainable and environmental friendly. Microalgae isolation from local water bodies such as ponds and lakes might be a useful strategy for isolating potential species that are tolerant to the prevailed geographical and climatic conditions, and can be grown without any extra care or effort thus providing good and cheap biomass productivity. Various biotic and abiotic factors influence the growth of microalgae. Among the abiotic factors include the temperature, light intensity, pH of the medium, salinity, and nutritional conditions play the major role (Mata et al., 2013, Sharma et al., 2012, Krzeminska et al., 2014, Grobbelaar, 2009).

For optimizing biomass productivity, various abiotic factors like temperature, salinity, pH of the growth medium on the growth of the microalgae has been examined in the present research. While studying the effects of the factors in the experiments, only a single 1factor was kept variable at one, keeping all are conditions constant. The effects of the various factors were analyzed and the optimized level of each factor for maximum biomass production was studied.

II. METHODS AND MATERIAL

2.1. Isolation and identification of microalgae

Ten samples of microalgae were collected from different sites of water bodies from Gulbarga University campus and the cultures were grown by regular subculturing in BG-11 medium every 20days. Individual colonies were screened by serial dilution technique and pure cultures were maintained for further studies.

2.2. Characterization of the microalgae by 18Sr RNA

Chromosomal DNA was extracted by using a spin column kit and was amplified using polymerase chain reaction in a thermal cycler and was purified using Exonuclease I -Shrimp Alkaline Phosphatase (Exo-SAP) as described by Darby et al.(Darby et al., 2005). Purified amplicons were sequenced by the Sanger method in ABI 3500x L genetic analyzer (Life Technologies, USA). The editing of the sequencing files (.ab1) were performed using CHROMASLITE (version 1.5) and were then analyzed by the Basic Local Alignment Search Tool (BLAST) with the nearest culture sequences retrieved from the National Centre for Biotechnology Information (NCBI) database that finds the local similarity regions between the sequences (Altschul et al., 1990). The program compares the nucleotides or the protein sequences with the sequence available in the databases and calculates the significance of the matches. The BLAST algorithms were used to deduce the functional and evolutionary relationships between the sequences that help to identify the members of the similar gene families. (i) The initial search was performed to determine potentially closely related type strain using the BLASTN tool (ii) Secondly, pairwise alignment was done to determine the sequence similarity values among the query and targeted sequence identified in the previous step. Further, the multiple sequence alignment followed by the phylogenetic analysis are therefore recommended for the accurate species prediction and the evolutionary relationship. For the nearest and the closest similarity index and sequence(s) text, a phylogenetic tree was determined using MEGA7.0 software(Hall, 2013).

2.3. Identification and Characterization of S. vacuolatus AK1 using light microscopy and Scanning Electron Microscopy (SEM)

2.3.1. SEM analysis

Isolated microalgae species was examined under light microscope followed by scanning electron microscope (SEM) for cell morphology. For SEM the sample preparation includes the smearing of the sample on a small adhesive carbon tape piece that is glued to a brass stub. The sample is then gold coated with the

help of sputtering unit (model: JFC1600)at 10mA of current for 10 sec. The sample coated with gold was then placed in the SEM chamber (Jeol, JSM 6390LA) and the secondary electron/Back Scattered electron images of the sample were recorded. Elemental analysis was carried out in the scanned area/point /line using the EDAX detector. A drop of the algal suspension was taken on acoverslip and air-dried. It was fixed using 4% (w/v) glutaraldehyde and kept overnight, and then it was rinsed with distilled water followed by dehydration by 95% ethanol and air drying. The sample after air drying was coated with gold-palladium of 90 Å thickness in polaronSc 7640 sputter coater for 30 min and this coated sample was observed by scanning electron microscope at 15 kV.

2.4. Influence of cultural conditions

The strategy of the various cultural conditions like nitrogen and glucose sources, pH, temperature, and NaCl were studied for the high productivity of biomass by the microalgae. The microalgae growth was determined using a spectrophotometer by measuring the optical density at 680 nm wavelength (Hitachi U-2900). The microalgal biomass was obtained by centrifugation and dried at 105℃ in an oven to get the dry weight of the microalgae.

2.4.1 Effect of pH

Experiments to observe the effect of pH were carried out in Erlenmeyer flask containing BG11 medium and of culture was inoculated by regulating the pH from 5-11 with a difference of 1 pH, incubated at 30℃ for 20 days at an rpm of 120 in a rotary shaker and the optical density (OD) was measured.

2.4.2. Effect of temperature

Effect of temperature was assessed under different temperatures ranging from 15 to 40℃ with a range of 5 ℃. The culture was inoculated in BG-11 medium with optimum pH 8 and incubated for 20 days and the biomass obtained was analyzed for the lipid content by measuring the OD.

2.4.3 Effect of salinity

The effect of salinity on the biomass production was observed in the microalgal cultures in BG11 medium by varying the concentrations of NaCl from 5 mM, 25 mM, 50 mM and 100 mM with controls at optimum pH - 8, the temperature of 30℃ by incubating for 20 days in a rotary shaker and the optical density was measured.

2.4.4 Effect of nitrogen source

The experiments were performed in Erlenmeyer flask containing BG11 medium, with the addition of various nitrogen sources such as sodium nitrate, urea, ammonium, and yeast extract for studying the effect on the biomass production. Different concentrations from 0.02 g/l to 0.2 g/l at a difference of 0.02 g/l were used and incubated at optimum pH-8, temperature 30 ℃, for 20 days in a rotary shaker and the optical density was observed.

2.4.5 Effect of carbon source

Effect of different carbon sources such as fructose, glucose, maltose, and sucrose was studied on biomass production and lipid accumulation. The experiments were carried out in Erlenmeyer flask containing BG11 medium with different concentrations in the range of 0.1g/l to 0.5 g/l at optimum pH of 8, temperature 30℃ and incubated for 20days.

III.RESULTS AND DISCUSSION

3.1 Isolation and identification of S. vacuolatus AK1

It has been found that out of the ten samples of microalgae collected from different freshwater bodies from Gulbarga University campus, one novel strain isolated was visualized under the light microscope as shown in (Figure 1A). The cells were spherical in shape, green in color and non-motile and by scanning electron microscopy (SEM) the cells were observed as

spherical and the size ranged from 5µm- 10 µm in diameter as shown in (Figure 1B).

3.2 Molecular identification by 18SrRNA sequencing

Identification of the microalgae at species level was carried out by 18S rRNA sequencing by outsourcing from NCIM Pune, India. The complete sequenced data of 1500 base obtained by PCR amplification was subjected to BLAST (Basic Local Alignment Search Tool) to check the nearest culture sequence which showed 99% similarity with Scenedesmus sp. as shown in. The strain was identified as ScenedesmusvacuolatusAK1 and the accession number was obtained by depositing in NCBI. The multiple sequence alignment of the most related species was generated through NCBI and a phylogenetic tree was constructed by using MEGA 7.0 software (Figure 2).

3.3 Influence of cultural conditions

S. vacuolatus AK1 was grown in Erlenmeyer flask containing BG11 medium for 20days under optimized laboratory cultural conditions. Growth parameters such as pH, nitrogen sources, carbon sources, temperature, and salinity were studied at specific time intervals. The growth was determined by the OD at 680nm wavelength (Figure 3).

3.3.1 Effect of pH and Temperature

The effect of pH on the growth media is one of the important factors affecting the growth of microalgae; the pH ranging from 5-11 was studied to observe the growing influence on microalgae. The strain showed a good growth profile in alkaline pH with an increase in biomass production and the optimum pH was 8. The yield of the biomass displayed an increasing trend with an increase in pH up to 11 and the culture accumulated and settled down as the pH increased which induced auto flocculation. This result is in agreement for Scenedesmus dimorphus reported by Vidyashankar et al. (Vidyashankar et al., 2013). Similar observations were also reported by Wang et al. (Wang et al., 2013) where it was reported that the pH slightly affected the medium for the algal growth and lipid accumulation. Study by Tripathi et al., was also consistent with our findings where it was reported that the microalgae survived at a higher pH(Tripathi et al., 2015). Temperature is an important abiotic factor that affects the growth of microalgae directly by influencing the biochemical process. The growth of S. vacuolatus AK1 was observed under different ranges of temperature. Maximum biomass productivity of 0.6g/l was observed at an optimum temperature of 30˚C in BG-11 medium. The strain could tolerate the temperature up to 35˚C and the growth of the microalgae gradually decreased as the temperature increased due to the reduction in the size of the cells and respiration, which are in line with our observations as reported by Muhammad Imran Khan et al. (Khan et al., 2018). In another study by Xin et al., it was reported that Scenedesmus sp. grown at 30˚C had higher cell density and good population growth when compared with low temperature (Xin et al., 2011).

3.3.2 Effect of salinity

The immediate response of the cultures to the salinity or osmotic shock was the change in the morphology of the species which turned the cells to spherical and brown color as the incubation time increased. The effect of the salinity on the production of the biomass studied was as shown in (Figure 4A). The biomass increased with the addition of NaCl and the optimum was 5mM NaCl/l. As the concentration of the salt increased the growth of the microalgae declined. Maximum biomass yield of 1.45g/l was observed at 5mM NaCl concentration which is in agreement with the results reported by Luo et al., where the optimum biomass growth for chlorella sp. Was found to be at 1.6g/l in 5mM salt concentration (Luo et al., 2019).

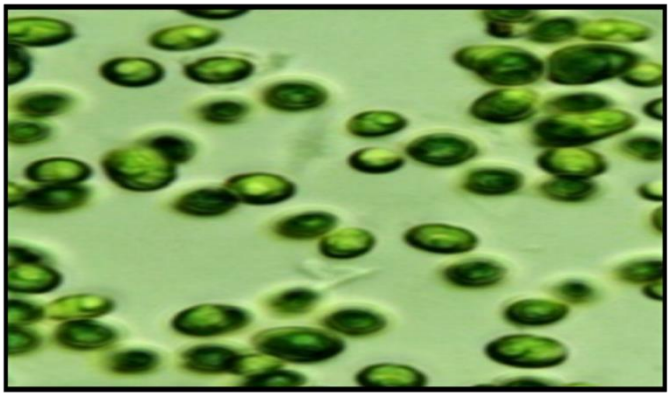
3.3.3 Effect of nitrogen

Different nitrogen sources were used for the study in BG11 medium for the biomass production (Figure 4B). Predominantly nitrogen source plays a major role in determining the quality of the lipids produced by the microalgae. Urea was an appropriate nitrogen source that increased the biomass of the strain. At an optimum of 0.1mg/l of urea showed 1.2g/l of biomass production. Our findings are similar to the report presented by who showed that urea was the best nitrogen source under starvation conditions with the production of higher biomass of 1.3g/l. A similar observation was observed by Goswami et al but on a different species (S.dimorphus)where the biomass growth was found to be nitrogen dependent (Goswami and Kalita, 2011).

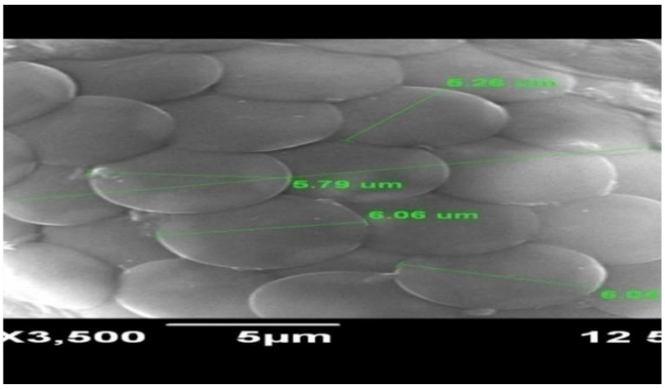
3.3.4 Effect of carbon sources

The different carbon sources were used to examine the influence of the strain AK1 for the growth in BG11 medium shown in (Figure 4C). A highly positive correlation was obtained between the lipid content and the biomass, with an increase in the supplement of carbon source. Glucose was proved to be the best carbon source at an optimum concentration of 0.5g/l with the maximum production of 1.5g/l biomass. Increase in the carbon source induced accumulation of lipids and biomass production these findings are similar to who reported the maximum yield of lipid (58mg/l) in 1.5% glucose. Our results were in agreement with the report of showed that glucose (0.5g/l)was an efficient trigger to increase the biomass production of 2.08g/l. Ren et al. showed similar results which corresponded to glucose as the significant carbon source with maximum biomass of 3.4g/l in Chlorella Vulgaris(Ren et al., 2013).

Figures and Tables



A



B

Figure 1 : (A) Microscopic examination and **(B)** Scanning electron micrograph of Scenedesmus vacuolatus AK1.

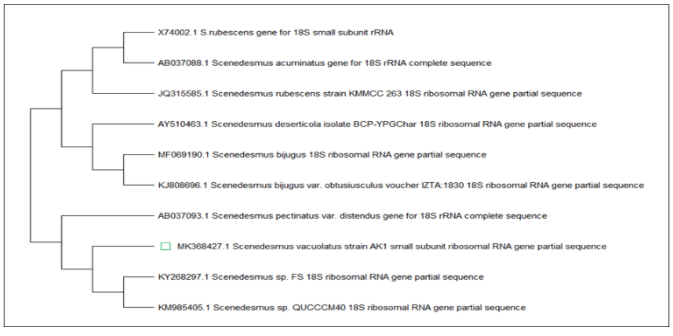


Figure 2 : Phylogenetic tree of strain Scenedesmus sp.AK1 using MEGA 7.0.

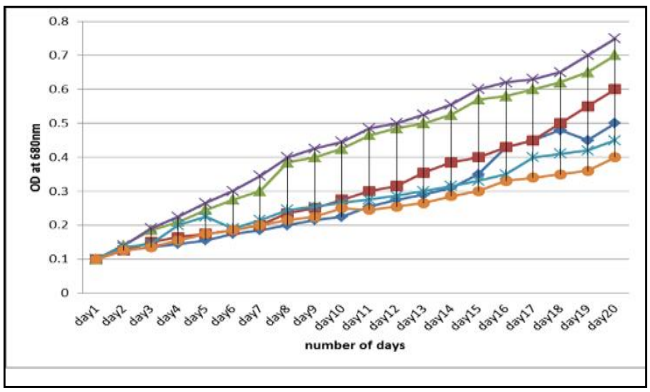


Figure 3 : Growth of Scenedesmusvacuolatus AK1 as measured by optical density (OD) at 680nm wavelength.

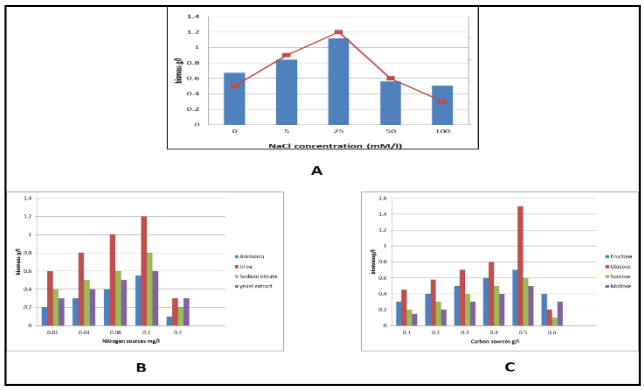


Figure 4 : Effect of **(A)** Nacl, **(B)** Nitrogen and **(C)** Carbon sources on the biomass production.

IV. CONCLUSION

By observing the morphology and by molecular characterization, the isolated microalgae were identified as Scenedesmus vacuolatus. The effects of different growth conditions on the growth of microalgae isolate Scenedesmus vacuolatus has been identified from which it was concluded that the maximum growth rate and biomass productivity can be achieved by providing glucose as carbon source at an optimum concentration of 0.5g/l respectively. Among the nitrogen sources urea was an appropriate nitrogen source that increased the biomass of the strain at an optimum of 0.1mg/l. It was also observed that the strain showed a good growth profile in alkaline pH with an increase in biomass production and the optimum pH was 8. The yield of the biomass displayed an increasing trend with an increase in pH up to 11.The effect of temperature on the biomass production was also observed. Maximum biomass productivity of 0.6g/l was observed at an optimum temperature of 30˚C in BG-11 medium. The strain could tolerate the temperature up to 35˚C. Lastly, it was observed that the biomass increased with the addition of NaCl. Maximum biomass yield of 1.45g/l was observed at 5mM NaCl concentration.

The study was primarily focused on the isolation, characterization of microalgae (Scenedesmus vacuolatus) followed by the effect of various factors on the growth of the biomass. However, the lipid production of the isolated microalgae, important for the evaluation of the organism for biofuel production, was not considered for the study which is a limitation of the study undertaken.

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