Survey and Documentation of Brackish Water Algal Diversity from East Coast Region of Odisha, India

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Abstract A total of 150 algal samples were collected from 14 sampling sites of various fresh water and brackish water habitats of east coast region of Odisha, India during the period 2009-2011. About 41 algal strains were isolated and cultured into their pure forms. Out of the collected strains 5 taxa of Bacillariophyta, 12 taxa of Cyanobacteria/Cyanophyta and 24 taxa of Chlorophyta have been identified using relevant monographs and are being screened to evaluate their potential for biofuel production.

Keywords Algal diversity, Cyanobacteria, Bacillariophyta, Chlorophyta

1. Introduction

Odisha, located in the east coast of India (Lat. 17°48'-23°34' N & Long. 81°24'-87°29’ E) has an area of 1,55,842 km² which is surrounded by West Bengal to the north-east, Jharkhand to the north, Chattisgarh to the west and north-west and Andhra Pradesh to the south. In order to explore the algal biodiversity various field surveys was carried out during the year 2009-2011. Several water bodies which drain into the Bay of Bengal, provides a variety of ecological niches and a good habitats for the growth of algal species. Though there are several studies on algal biodiversity assessment in India have been carried out in the past[1-4], very comprehensive survey and seasonal collection of algal species in this typical location is few[5-7]. In the present study field trips were organized to carry out detail survey and collection of algal samples for screening and characterization of potential micro-algae for biodiesel production.

2. Materials and Methods

2.1. Study Area

Algal samples were collected randomly from 14 sampling sites of 8 estuaries of rivers and distributaries from coast of Odisha during 2009-2011 through several collection trips as shown in Table 1. In the southern part of the state, the estuaries of Rushikulya river along with its two major distributaries at Bahuda and Gopalpur were surveyed, along with its distributaries viz., Kushhabhadra, Nua nai, Devi along with its distributaries viz., Kushhabhadra, Nua nai, Devi and Musa nai were visited for algal sample collection. Site map and different sampling sites for collection of microalgal samples are shown in Figure 1 and Figure 2.

2.2. Sampling and Observation

Samples were collected using forceps and needle and/or plankton net (45µm pore size). Epilithic samples were scraped using a tooth brush. All algal samples were assigned voucher numbers and were preserved in a pre-sterilized specimen bottle with 4% formaldehyde solution. Planktonic samples were fixed with Lugol's Iodine on the spot and brought to the laboratory for analysis. Simultaneously the replica of each sample was kept in the culture media and brought to the laboratory and incubated. Under microscope, single cells were isolated using glass capillary and put in the culture slants and incubated under light of 3000-4000 lux intensity. Cellular dimension measurement was carried out by micrometry and microphotograph of each specimen was taken using a Meiji ML-TH-05 Trinocular research microscope fitted with Nikon Coolpix 4500 digital camera. The organisms were identified using relevant monographs for various algal groups[8-11].

2.1.1. Culture Conditions for Growing Microalgae

• Temperature: All the cultures were maintained in an air conditioned culture room at a temperature of 25 ± 2°C.
• Intensity of Light: Light is the most vital factoring for the development of the photosynthetic algae both in in-vitro and in-vivo condition. The growth of algae is influenced by wave length, flux density and duration of light exposure. Hence the culture was maintained at light intensity 3000 - 4000 lux provided by cool white fluorescent tubes, with
14:10 hr. of light / dark period.

- **pH:** pH of cultures were maintained in the range of 6.8-7.0.
- **Growth Medium:** The vital nutrients that enable the algae to grow through the culture medium are Macronutrients, Micronutrients and Agar-Agar (a solidifying agent).

The media used in the present study were Bold Basal for green algae, Walne and BG11 for cyanobacteria and modified BG11 for the diatom cultures, the details of which are presented in Table 2.

**Table 1.** Details of collection from Odisha, East coast of India, in the year 2009-2011 showing place of collection, date of collection, habitat and voucher numbers

| Sl. no | Place of collection                  | Date of collection | Habitat                                                                 | Voucher numbers |
|--------|-------------------------------------|--------------------|-------------------------------------------------------------------------|-----------------|
| 1      | Chilika lake, Balugaon, Khurda      | 09.12.2009         | Planktonic, epilithic (Temp. 25°C, pH – 8.5)                           | 1 - 4           |
| 2      | Temporary pool, Pathara, Ganjam    | 09.12.2009         | Planktonic (Temp. 33°C, pH – 8.7)                                      | 5               |
| 3      | Palur canal, Ganjam                | 09.12.2009         | Planktonic, floating (Temp. 29°C, pH – 8.9)                            | 6 - 9           |
| 4      | Bahuda estuary, Sonapur, Ganjam    | 21.01.2010         | Epilithic, planktonic, epizoic, filamentous (Temp. 26°C, pH – 8)       | 10 - 40         |
| 5      | Gopalpur estuary, Ganjam           | 04.02.2010         | Planktonic, epizoic, benthic, filamentous (Temp. 27°C, pH – 9)         | 41 - 47         |
| 6      | Rushikulya river, Gopalpur, Ganjam | 04.02.2010         | Benthic, epizoic, planktonic (Temp. 34°C, pH – 7.5)                    | 48 - 55         |
| 7      | Rushikulya estuary, Ganjam         | 04.02.2010         | Benthic, epilithic, floating (Temp. 28°C, pH – 8)                      | 56 - 60         |
| 8      | Daya river, Bhubaneswar             | 19.03.2010         | Floating filamentous mat (Temp. 29°C, pH – 6.5)                        | 61-62           |
| 9      | Kushabhadra estuary, Chandrabhaga, Puri | 25.03.2010  | Benthic, epilithic, epizoic, Planktonic, floating (Temp. 28°C, pH – 7.5) | 63 - 74         |
| 10     | Nua nai estuary, Puri              | 25.03.2010         | Benthic, epilithic, epizoic, Planktonic, floating (Temp. 32°C, pH – 8.5) | 75 – 82        |
| 11     | Musa Nai estuary, Puri             | 25.03.2010         | Benthic, epilithic, epizoic, Planktonic, floating (Temp. 33°C, pH – 7.5) | 83-88          |
| 12     | Mahanadi river estuary, Paradeep   | 16.07.2010         | Benthic, epilithic, epizoic, Planktonic, floating (Temp. 33°C, pH – 7.5) | 89-99          |
| 13     | Subarnarekha estuary, Budhabalanga, Kasafal, Dhamara | 3-6. 01. 2011 | Benthic, epilithic, epizoic, Planktonic, filamentous (Temp. 18°C, pH – 6.8) | 99-137         |
| 14     | Devi estuary, Astaranga             | 07.07.2011         | Benthic, epilithic, epizoic, Planktonic, floating (Temp. 27°C, pH – 6.7) | 137-150        |

**Figure 1.** Site map of collection sites of Odisha. Black dots indicate the sampling sites

**Figure 2.** Respective photographs of collection sites of Odisha. 1. Chilika Lake, 2. Algal bloom in Chilika Lake, 3. Temporary Pool (Pathara), 4. Bahuda Estuary, 5. Rusikulya Estuary, 6.Kushabhadra estuary, 7. Nuanai river estuary, 8. Musa river estuary, 9. Mahanadi river estuary, 10. Devi estuary
Table 2. Different growth medium were used for micro algal culture (a) Macronutrients (b) Micronutrients

| Media composition | BBM(g/l) | WALNE(g/l) | BG -11(g/l) |
|------------------|----------|------------|-------------|
| NaNo3            | 0.25     | 0.1        | -           |
| CaCl2.2H2O       | 0.025    | -          | -           |
| MgSO4.7H2O       | 0.075    | -          | 0.075       |
| K2HPO4           | 0.075    | -          | 0.04        |
| KH2PO4           | 0.175    | -          | -           |
| FeSO4.7H2O       | 0.005    | -          | -           |
| Na2EDTA          | 0.005    | 4.5        | 0.001       |
| NaCl             | 0.175    | -          | -           |
| MgCl2.6H2O       | 2        | -          | -           |
| K2HPO4.3H2O      | -        | -          | 0.02        |
| KCL              | -        | -          | -           |
| Na2CO3           | -        | -          | 0.006       |
| Citric acid      | -        | -          | -           |
| Ferric Ammonium  | -        | -          | 0.006       |
| Citrate          | -        | -          | -           |
| MgEDTA           | -        | -          | -           |
| NaH2PO4.2H2O     | -        | 0.02       | -           |
| H3BO3            | -        | 0.0336     | -           |
| MnCl2.4H2O       | -        | 3.6        | -           |
| FeCl3.6H2O       | -        | 0.013      | -           |
| NaSiO3           | -        | 6          | -           |
| Micronutrients   |          |            |             |
| H3BO3            | 2.86     | -          | 2.86        |
| MnCl2.4H2O       | 1.81     | -          | 1.81        |
| ZnSO4.7H2O       | 0.222    | 0.044      | 0.222       |
| CuSO4.5H2O       | 0.079    | 0.02       | 0.079       |
| Na2MoO4.2H2O     | 0.390    | -          | 0.390       |
| Co(NO3)2.6H2O    | 0.0494   | -          | 0.0494      |
| CoCl3.6H2O       | -        | 0.02       | -           |
| (NH4)Mo7O24H2O   | -        | 0.009      | -           |

2.1.2. Modified BG11 for Diatom Culture

The required nutrient composition for the Diatom culture was essentially same as that of the BG11 used for cyanobacterial culture, except for supplementing with double the concentration of NO3−, PO43− and Na2SiO3. Instead of distilled water, sea water was used for the Diatom culture.

3. Results and Discussion

3.1. Isolation and Documentation of Algal Strain

Forty one different algal strains were isolated and cultured into their pure forms and deposited in form of slants in the Culture Collection Centre & Repository for brackish water algae at IMMT (CSIR), Bhubaneswar assigning with a strain number (Table 3). The microphotographs of the isolated algal samples are shown in Figure 3.
Table 3. List of algal strains maintained in IMMT Culture Collection / Repository

| Sl.No. | No. of cultures collected so far | Strain Number |
|--------|---------------------------------|---------------|
| 1      | Chlorococcales sp.               | IMMTCC-01     |
| 2      | Chlorella sp.                    | IMMTCC-02     |
| 3      | Scenedesmus sp.                  | IMMTCC-03     |
| 4      | Chlorococcales sp.               | IMMTCC-04     |
| 5      | Chlorella sp.                    | IMMTCC-05     |
| 6      | Scenedesmus sp.                  | IMMTCC-06     |
| 7      | Scenedesmus sp.                  | IMMTCC-07     |
| 8      | Chlorella sp.                    | IMMTCC-08     |
| 9      | Chlorella sp.                    | IMMTCC-09     |
| 10     | Phormidium sp.                   | IMMTCC-10     |
| 11     | Chlorococcales sp.               | IMMTCC-11     |
| 12     | Chlorococcales sp.               | IMMTCC-12     |
| 13     | Scenedesmus sp.                  | IMMTCC-13     |
| 14     | Oscillatoria sp.                 | IMMTCC-14     |
| 15     | Bracteococcus minor              | IMMTCC-15     |
| 16     | Chlorella sp.                    | IMMTCC-16     |
| 17     | Chlorococcales sp.               | IMMTCC-17     |
| 18     | Chlorella sp.                    | IMMTCC-18     |
| 19     | Phormidium sp.                   | IMMTCC-19     |
| 20     | Phormidium sp.                   | IMMTCC-20     |
| 21     | Oscillatoria sp.                 | IMMTCC-21     |
| 22     | Oocystis sp.                     | IMMTCC-22     |
| 23     | Chlamydomonas sp.                | IMMTCC-23     |
| 24     | Scenedesmus sp.                  | IMMTCC-24     |
| 25     | Scenedesmus sp.                  | IMMTCC-25     |
| 26     | Chlorococcales sp.               | IMMTCC-26     |
| 27     | Chlorella sp.                    | IMMTCC-27     |
| 28     | Chrococodiopsis sp.              | IMMTCC-28     |
| 29     | Phormidium sp.                   | IMMTCC-29     |
| 30     | Phormidium sp.                   | IMMTCC-30     |
| 31     | Chlorella sp.                    | IMMTCC-31     |
| 32     | Chlorella sp.                    | IMMTCC-32     |
| 33     | Phormidium sp.                   | IMMTCC-33     |
| 34     | Cymbella                         | IMMTCC-34     |
| 35     | Nitzschia sp.                    | IMMTCC-35     |
| 36     | Diatom                           | IMMTCC-36     |
| 37     | Diatom                           | IMMTCC-37     |
| 38     | Nostoc                           | IMMTCC-38     |
| 39     | Aphanothece                      | IMMTCC-39     |
| 40     | Nostoc                           | IMMTCC-40     |
| 41     | Navicula                         | IMMTCC-41     |

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**4. Conclusions**

In the present field survey a total of 41 algal taxa have been identified from collection of 150 algal samples. Of these 12 taxa of Cyanobacteria/Cyanoprokaryota, 24 taxa of Chlorophyta and 5 taxa of Bacillariophyta are being and maintained in pure cultures. The collection of green algal species is being evaluated for their potentiality of biofuel production.