Journal of Algal Biomass Utilization
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ISSN: 2229 – 6905

A Morphotaxonomic account of N_2-fixing cyanobacterial diversity in coal field soils of Assam (India)

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Arjun Adhikari and P. P. Baruah. 2015. A Morphotaxonomic account of N_2-fixing cyanobacterial diversity in coal field soils of Assam (India). J. Algal Biomass Utiln. 6 (4): 12 -16

Keywords: N_2-fixing cyanobacteria, acidophilic, Coal fields, Assam

Abstract

Surveys conducted in the coalfields of Assam reported altogether 8 (eight) acidophilic N_2-fixing cyanobacterial species belonging to 5 genera. Of them, 6 species were non-heterocystous filamentous belonging to *Phormidium* (2), *Lyngbya* (1) and *Oscillatoria* (3). The rest two were heterocystous filamentous *Nostoc punctiforme* and *Westiellopsis prolifica*.

Introduction

Coal mining tends to make notable impact on the environment which may vary in severity depending on whether the mine is under operation or abandoned, the method used for the mining and the geological condition of the region (Bell *et al*, 2001). It causes massive damage not only to the landscapes but also it expedites deduction of forest cover, large scale erosion of soil leading to pollution of air, water, soil and concomitant reduction of biodiversity (Down and Stock, 1977). Coal mines are often associated with pyrites which on decomposition forms sulphuric acid thereby making the mining area acidic. In addition to high acidity, these environments exhibit extreme chemical and physical conditions depending on the origin and geochemical nature of the site (Whitton and Satake, 1996).

The Indian sub-continent is rich in coal resources. The mining of coal is actively practiced in many parts of the country including few North Eastern states mainly Meghalaya, Assam and Arunachal Pradesh. In Assam the development of coalfields was initiated in the year 1870 (Akala, 1995). The coal beds in Assam are mainly spread in the easternmost districts of the state and are operated in the name of North Eastern Coal fields, Coal India Limited (NECF-CIL), Margherita. A phenomenal increase of coal mining areas within the state has been recorded during last few decades. The resultant large scale destruction and deterioration of overall environment leads to affect the biodiversity as a whole and the soil micro biota including cyanobacteria in particular in the vicinity of the coal fields.

Cyanobacteria comprise a large group of structurally complex and ecologically significant gram-negative prokaryotes. Their habitat may vary from vast oceanic areas to freshwater ponds, tropical and temperate soils to bare rocks and even extreme habitats like arid deserts, frigid lakes or hot springs (Whitton and Potts, 2000). Though cyanobacteria are highly adapted to different environmental conditions (Wartiainen *et al*., 2008), they are found to be very sensitive to acidic environment (Altermann, 2007). Studies (Brock, 1973; Dominic and Madhusoodanan, 1999) revealed that their numbers are very poor particularly in ecosystems having pH values less than 5. Some of the cyanobacterial taxa are capable of fixing the atmospheric nitrogen (Stewart *et al*., 1987), hence their presence in soil is thought to maintain the nitrogen-level in the soil (Venkataraman, 1993). Considering the potentialities of cyanobacteria in sustaining the fertility of soil (Mandal *et al*, 1998), an understanding on the impact of mining on the soil micro-organisms, particularly on their diversity is a prerequisite. The present study was therefore aimed at isolating and characterizing the cyanobacterial species from acidic soils of different coal fields of Assam.

Materials and Methods

To perform the study, three different coal extracting sites were selected from the easternmost districts of Assam (Figure 1). Open cast coal mining is actively practiced in all the selected coalfields. Soil samples were collected from different sampling sites and were utilized for culturing cyanobacteria using BG-11 medium without nitrogen supplementation under optimal growth condition at $30^0C \pm 2^0C$ temperatures in 2.3 K lux light intensity for 25-30 days at Ecology Laboratory of Department of Botany, Gauhati University. The enrichment flasks were regularly monitored for the growth of cyanobacteria and the organisms were observed microscopically under a Magnus MLX*i* microscope. Identification was carried out mainly based on morphological characteristics such as cell structure and size, number and position of heterocysts following Desikachary (1959), Anand (1989) and Komarek and Anagnostidis (2005, 2013).

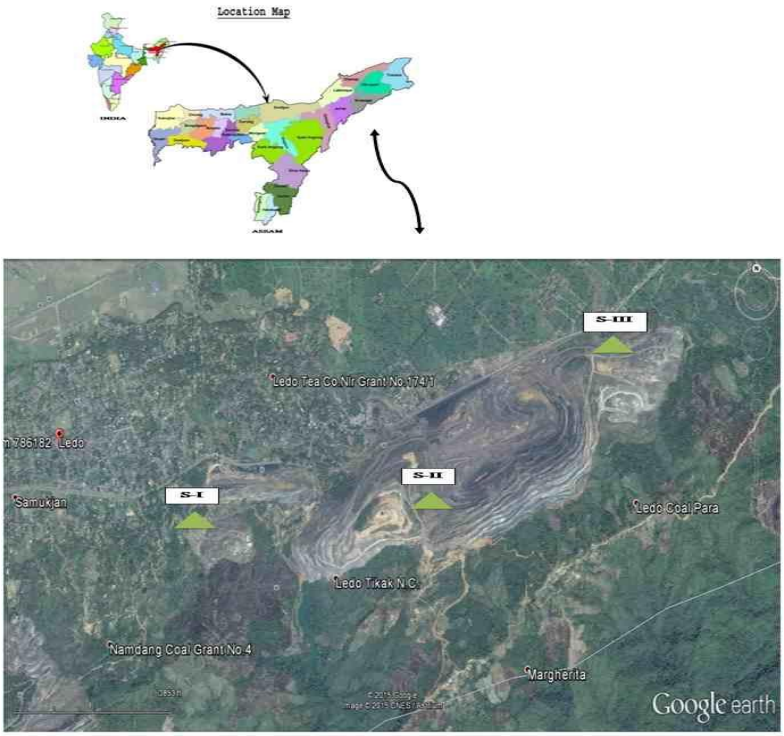

Fig. 1. Map showing different Sampling Sites in Coalfields of Assam.

Results

Coal mine soil represents a physically disturbed habitat for the existence of soil micro-organism due to internal high temperature and low pH (Johnson, 2003). In spite of such extremities, the coal mine soil is not a microbiologically sterile habitat and often harbours specific group of thermoacid tolerant micro-organisms including cyanobacteria (Belly and Brock, 1974). During the present investigation, low cyanobacterial diversity was observed in the coalfields of Assam. N_2-fixing cyanobacterial population in the study area was represented by 8 species belonging to 5 different genera. Non-heterocystous genera like *Oscillatoria* and *Phormidium* appeared as the dominant taxa, whereas the heterocystous members were represented by two species only. They were *Nostoc punctiforme* and *Westiellopsis prolifica*. Microscopic photograph of all the recorded taxa is given in Plate 1. Morpho-taxonomic descriptions of the isolated species are as follows:

Systematic account of Cyanobacteria recorded from the coalfields of Assam (Komarek et Anagnostidis, 2005)

Cyanoprokaryota-II
Order- Oscillatoriales
Family- Phormidiacae

1. *Phormidium tenue* (Menegh.) Gomont (Pl. 1, Fig. 5)
Desikachary 1959, page 259, plate 43, figure 13-15; Komarek & Anagnostidis 2005, page 219, figure 272.

Thallus pale blue-green, thin, trichome straight or slightly bent, slightly constricted at the cross-walls, attenuated at the ends, 1-3 µm in diameter and 2-4.5 µm long.
Collection No. CF-3/06; Date: - 26/04/2014.
pH:- 4.3; Temperature- 32°C; Place:- Site-I, Tinsukia, Assam.

2. *Phormidium fragile* (Meneghini) Gomont (Pl. 1, Fig. 6)
Desikachary 1959, page 253, plate 44, figure 1-3; Komarek & Anagnostidis 2005, page 197, figure 241.

Thallus brownish blue-green, lamellated, trichomes more or less flexuous, distinctly constricted at the cross-walls, attenuated at the ends, 1.5-2.5 µm in diameter and 1-3 µm long.
Collection No. CF-3/05; Date: - 26/04/2014.
pH:- 4.2; Temperature- 32°C; Place:- Site-I, Tinsukia, Assam.

Family- Oscillatoriaceae
3. *Lyngbya major* Menegh. ex Gomont 1892 (Pl. 1, Fig. 1)
Desikachary 1959, page 320, plate 51, figure 11; Komarek & Anagnostidis 2005, page 619, figure 941.
Filaments long, straight, becoming dark-green, sheath thick, lamellated, cells 10–15 µm broad and 2-4 µm long, slightly constricted at cross-walls, end cell rounded.
Collection No. CF-1/03; Date: - 8/04/2015.
pH:- 4.6; Temperature- 28°C; Place:- Site-II, Tinsukia, Assam.

4. *Oscillatoria princeps* Vaucher ex. Gomont 1892 (Pl. 1, Fig. 4)
Desikachary 1959, page 210, plate 37, figure 1, 10, 11, 13, 14; Komarek & Anagnostidis 2005, page 590, figure 883.

Thallus blue-green, slightly curved, constriction absent at the cross-walls, cells 3-5 µm in diameter and 12-16 µm long, end cell slightly capitates.
Collection No. CF-1/09; Date: - 26/04/2014.
pH:- 4.3; Temperature- 32°C; Place:- Site-I, Tinsukia, Assam.

5. *Oscillatoria formosa* Bory ex. Gomont 1892 (Pl. 1, Fig. 2)
Desikachary 1959, page 232, plate 40, figure 15; Komarek & Anagnostidis 2005, page 421, figure 602.

Thallus blue-green to blackish-green, trichome straight, slightly constricted at the cross-walls, cells 3-6 µm in diameter and 2-5 µm long, attenuated at the at the ends and bent.
Collection No. CF-2/05; Date: - 24/09/2014.
pH:- 4.8; Temperature- 31°C; Place:- Site-II, Tinsukia, Assam.

6. *Oscillatoria tenius* C. Agardh ex. Gomont 1892 (Pl. 1, Fig. 3)

Desikachary 1959, page 222, plate 42, figure 15; Komarek & Anagnostidis 2005, page 587, figure 878.
Thallus blue green, trichome straight, slightly constricted at the cross-walls, granule present, cells 4-8 µm in diameter and 2.5-4 µm long.
Collection No. CF-2/04; Date: - 23/09/2014.
pH:- 4.5; Temperature- 31°C; Place:- Site-II, Tinsukia, Assam

Cyanoprokaryota-III, Heterocystous Genera
Order- Nostocales
Family- Nostocaceae

7. *Nostoc punctiforme* (Kutz) Hariot (Pl. 1, Fig. 7)
Desikachary, 1959, page 374, plate 69, figure 1; Komarek & Anagnostidis 2013, page 963, figure 1253.

Thallus greenish to yellowish, colonial, mucilaginous, filaments short, compactly arranged in the colony, heterocystous, 4.8–6.5 µm in diameter and 2.5–6.5 µm long, heterocyst on each side of the colony, cells small, spherical to sub-spherical, 2–4.5 µm in diameter and 4.5–6.5 µm long.
Collection No. CF-3/02; Date: - 24/09/2014.
pH:- 4.6; Temperature- 29°C; Place:- Site-III, Tinsukia, Assam.

Order- Stigonematales
Family- Fischerellaceae

8. *Westiellopsis prolifica* Janet
Desikachary, 1959, page 596, plate 131, figures 1-12; Komarek & Anagnostidis 2013, page 580, figure 707.

Thallus filamentous with true branching, main filament flexuous with short, barrel-shaped cells, 7-12 µm in diameter and 8-13 µm long, heterocysts oblong-cylindrical, 5-6 µm in diameter and 10 to 23 µm long.
Collection No. CF-2/03; Date: - 23/09/2014.
pH:- 4.6; Temperature- 29°C; Place:- Site-II, Tinsukia, Assam.

Acknowledgement
The authors are thankful to Assam Science Technology and Environmental Council (ASTEC), Government of Assam for providing financial assistance in terms of a major research project under 'Assam Vikash Yojana'.

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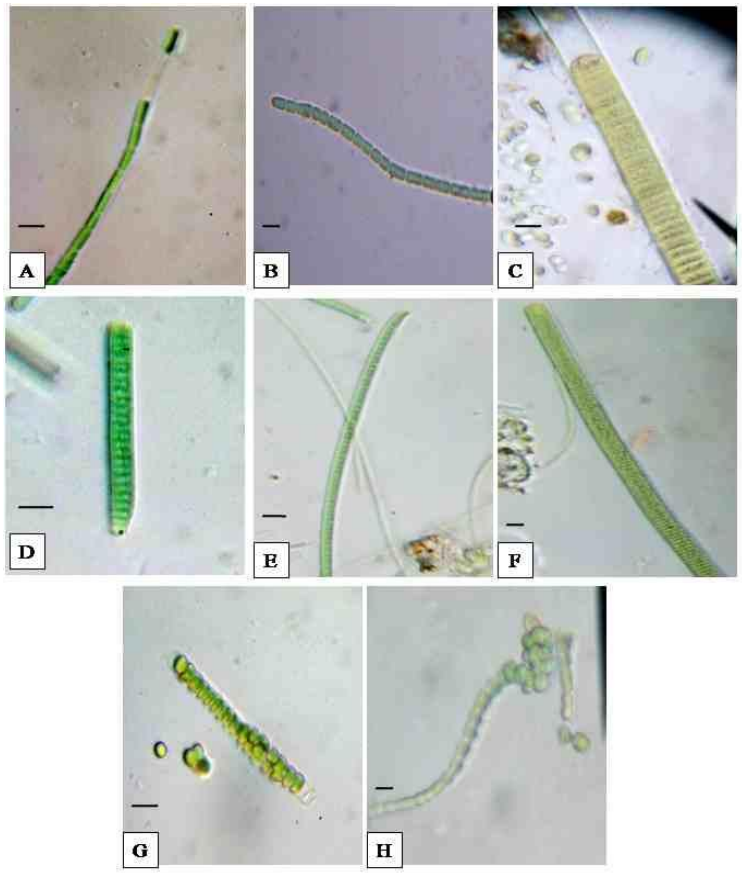

Plate 1. Microphotographs of **A**. *Phormidium tenue*, **B**. *Phormidium fragile*, **C**. *Lyngbya major*, **D**. *Oscillatoria princeps*, **E**. *Oscillatoria formosa*, **F**. *Oscillatoria tenius*, **G**. *Nostoc punctiforme*, **H**. *Westiellopsis prolifica*. (Scale bar=10 μm)