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Isolation, identification and characterization of rice field *Calothrix* spp. of Assam

Kiran Gupta* and P.P. Baruah

Department of Botany, Gauhati University, Guwahati-781014, Assam, India. *E-mail: kiran.botany@yahoo.co.in

Abstract

Cyanobacteria are one of the important components of rice field ecosystems. Few of which have the ability to fix atmospheric nitrogen and are therefore, considered as natural biofertilizer which enrich the productivity of rice. *Calothrix* is one of the potent groups of N_2 fixing cyanobacteria and is very common in rice grown areas of Assam. Therefore, the present survey conducted in rice field soils of Assam to study the diversity of *Calothrix* spp. Altogether 5 strains were isolated, identified and characterized based on morphological characters as well as 16S rRNA gene sequence.

Keywords: Cyanobacteria, rice field, nitrogen fixation, Assam

Introduction

Cyanobacteria is a group of cosmopolitan, gram negative, oxygenic, photosynthetic, prokaryotes which grow and multiply at the simple expense of water, light and air (Fay, 1983) and perform two biologically important functions such as carbon and nitrogen fixation and thus enriching the soil fertility with humus and nitrogen content. (Singh *et al.,* 2014). They are ubiquitous in nature and sometimes found in extreme habitats too (Hoffmann, 1989; Whitton and Potts, 2000). During their long and slow evolution, they have achieved huge diversity both in morphology and genetics, ranging from simple unicellular organisms to complex filamentous organisms (Whitton, 1992). A few can fix atmospheric nitrogen in its usable form and have been shown to be agriculturally important as biofertilizer, particularly in tropical rice field soils (De, 1939; Stewart *et al.,* 1987; Singh *et al.,* 2014). Rice fields provide a very congenial condition for abundant growth of N_2-fixing cyanobacteria (Nayak *et al*., 2001; Whitton, 2000). They are also known to maintain the homeostasis of rice field as a sustainable system (Bhattacharya, 2013).

Calothrix is one of the potent N_2 fixing cyanobacterial genera commonly occurring in rice fields of tropics (Huang and Chow, 1992) including India (Habib *et al.,* 2013). They help in maintaining soil fertility by improving the nitrogen status of the rice soils (Singh *et al.,* 2014). Estimation says that *Calothrix* spp. fix 6.8-30.6 kg nitrogen per hactre per year (Burris, 1976). The present endeavor therefore had been taken to study the diversity of *Calothrix* spp. occurring in the rice field soils of Assam.

Materials and Methods

Isolation and culture conditions

For the isolation of strains, 1-g soil samples were inoculated in 100ml sterilized BG-11 medium (pH 7.5) with out nitrogen. Isolation and purification of the organism were performed by serial dilution and plating method (Stanier *et al.,* 1971). Once isolated in pure form, the cultures were maintain in 250 mL flasks containing nitrogen free BG-11 medium (pH 7.5) at 25±2ºC under 2000 Lux light incubation with 16/8 hour photo period. The culture vessels were hand-shaken four to five times daily to to maintain homogeneity. To maintain the axenic culture, repeated sub culturing of each strain was done by transferring into fresh medium in every 20 days. Stock cultures of the test organisms were maintained in the Ecology laboratory of Department of Botany, Gauhati University.

Identification of Calothrix isolates

Isolated *Calothrix* species were morphologically identified following the keys given by Desikachary (1959) and Koma´rek and Anagnostidis (1989) and their identification were confirmed on the basis of partial 16S rRNA gene sequence. Genomic DNA isolation and 16S rRNA amplification and sequencing were done in Xcelris Labs Limited, Ahmedabad (India). The sequences were analyzed using BLASTn for 16S rRNA gene. The nucleotide sequences have been deposited in the NCBI Genbank data base and the accession numbers were acquired accordingly (Table.

2). The isolated strains were *Calothrix* sp. (GUEco 1001), *Calothrix* (GUEco 1002), *Calothrix* (GUEco 1003), *Calothrix* (GUEco 1004), *and Calothrix marchica*. Since it is not a phylogenetic study, we think partial gene sequencing is sufficient for identification. Phylogenetic tree (Fig. 1) was constricted by the Neighbour joining (kimura) method using software MEGA 7 (Kumar *et al.,* 2016).

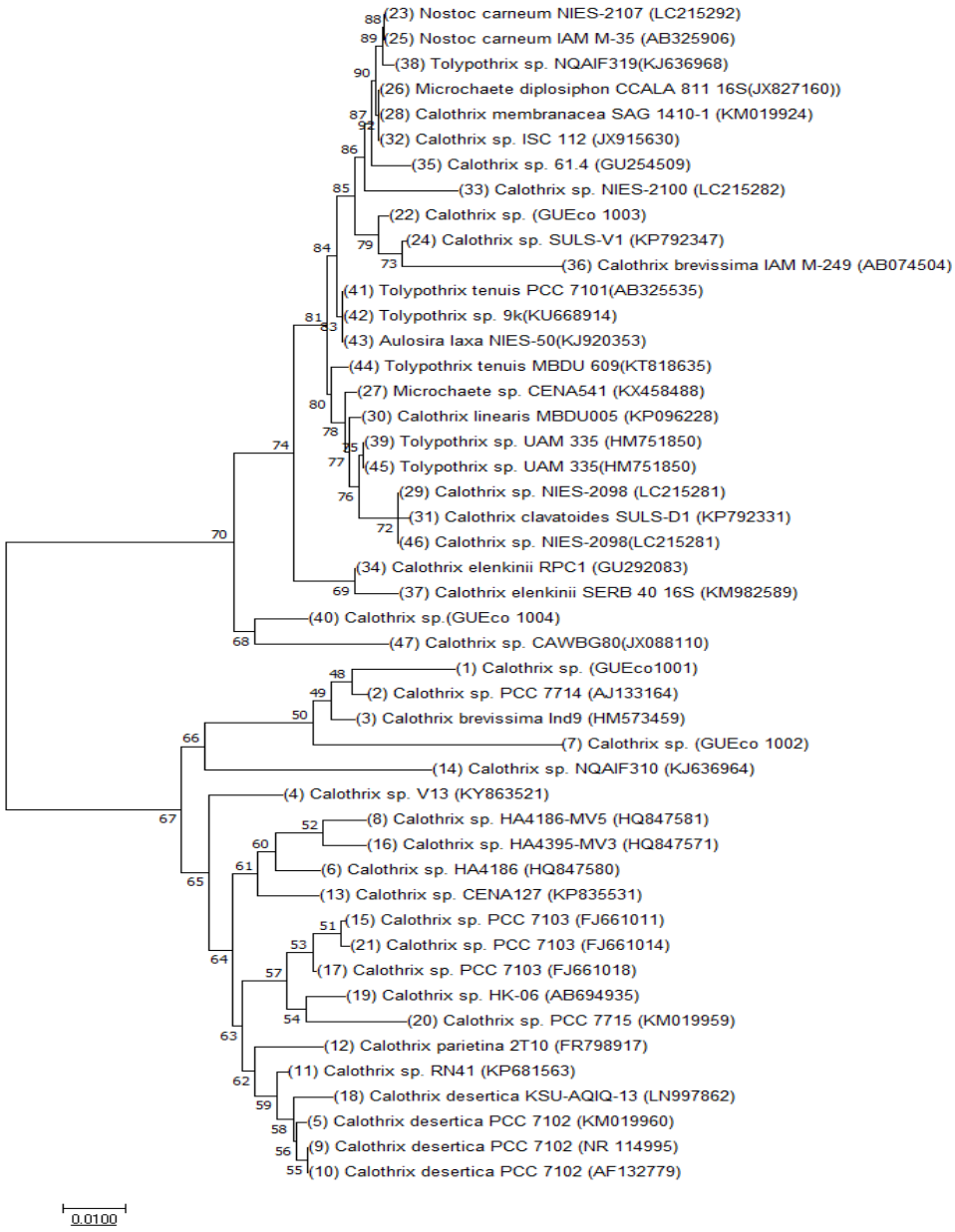

Fig. 1 Neighbour- joining (kimura) tree based on partial 16S rRNA gene sequence. The Genbank accession number of each taxa is in parentheses. The scale bar indicates 0.0100 substitutions per nucleotide positions. Evolutionary analyses were conducted in MEGA 7 software

Results

Altogether 5 strains of *Calothrix* were isolated from the rice field soils of Assam. The morphological characteristics of the isolated strains were listed in Table 1. Since classification of cyanobacteria based on morphological characteristics is not always reliable due to some variations that may happen for different culture and environmental conditions (Nayak *et al.,* 2007) and lead to misidentifications (Komarek and Anagnostidis, 1989), sequencing data (Table. 2) is presented in support of the strains.

Table 1 Morphological characteristics of the selected strains

***Calothrix* strains**	**Morphological characteristics**
Calothrix sp. (GUEco 1001)	The filaments are single with a distinct colourless and unlamellated sheath, ending gradually to a pointed apex, base not swollen; trichome are 4-6µ broad ending in a thin hair. Collection site: Mirza (26.3002ºN, 91.6931ºE)
Calothrix sp. (GUEco 1002)	The filaments are single with a distinct colourless and unlamellated sheath, ending gradually to a pointed apex, base not swollen; trichome are 4-6µ broad ending in a thin hair. Collection site: Dharapur (26.1417ºN, 91.6202ºE)
Calothrix sp. (GUEco 1003)	Filaments are 80-250µ long, bent at the base, and 6-9µ broad, in the middle 4.5-6µ broad; colourless, trichomes, at the base 5-7µ broad, in the middle 3.5-4.5µ broad, apical hair not formed. Collection site: Suwalkuchi (26.1703º N, 91.5703º E)
Calothrix sp. (GUEco 1004)	Filaments are single, free floating or attached, unbranched, bent, about 8µ broad at the base, ending in a hair, 2-2.5µ broad; trichome 5.1µ broad; cells cylindrical, heterocysts basal. Collection site : Chandrapur (20.2095º N, 79.6931ºE)
Calothrix marchica Lemmermann	Filaments are straight, or slightly bent, single, at the base 5-6µ broad, with a close thin colourless sheath; gradually attenuated into a hair, heterocyst single, basal nearly spherical or hemispherical, 4-5.5µ broad. Collection site : Sonapur (26.1172ºN, 91.9802ºE)

Table 2 Sequence identities of *Calothrix* strains in this study based on nucleotide- nucleotide BLAST (blastn) of NCBI

***Calothrix* strains**	**Query coverage %**	**NCBI Accession no.**	**Closet BLAST match**
Calothrix sp. (GUEco 1001)	100	KT232077	*Calothrix* sp. PCC7714
Calothrix sp. (GUEco 1002)	99	MF109999	*Calothrix brevissima* Ind9
Calothrix sp. (GUEco 1003)	99	MF110000	*Calothrix* sp. SULSV1
Calothrix sp. (GUEco 1004)	98	MF109988	*Calothrix* sp.CAWBG80
Calothrix marchica	Yet to assigned		

Discussion

The rice fields constitute one of the convenient habitats for the growth of cyanobacteria and their role in rice field soils is well established (Metting, 1981, Singh and Bisoyi, 1987). Out of several genera of cyanobacteria found in the rice fields, *Calothrix* have been recorded predominantly worldwide (Huang and Chow, 1992; Habib *et al.,* 2013). The occurrence of 5 strains of Calothrix from the rice field soil of Assam is in conformity with the work done by the earlier workers (Choudhury, 1999, Bharadwaj and Baruah, 2013). Further, indepth study on their nitrogen fixing ability as well as possible formulation of biofertilizer could be suggested.

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References

Bharadwaj, N. and Baruah, P.P. 2013. Diversity and abundance of N2- fixing cyanobacterial population in rice field soil crusts of Lower Brahmaputra Valley agro-climatic zone. *J. Algal Biomass Utilization* **4**(4): 23–33.

Bhattacharya, S., Deep, P.R., Sahu, J.K. and Nayak, B. 2013. Diversity of Rice Field Cyanobacteria from Tropical Rice Field of Western Odisha. *Internat. J. Sci. Res*. 4(8): 121-124.

Burris, R.H. 1976. Nitrogen fixation by blue-green algae of the Lizard Island area of the Great Barrier Reef. *Australian J. Plant Physiol*. 3: 41-51.

Choudhary, K.K. 1999. Ex-situ conservation of cyanobacterial germplasm of North Bihar, Life Science Botany India, PhD Thesis,B.R.A. Bihar University, Muzaffarpur, Bihar, India.

De, P.K. 1939. The role of blue green algae in nitrogen fixation in rice fields. *Proc. R. Soc. Lond. Serb.* 127: 121-139.

Desikachary, T.V. 1959. Cyanophyta, Monographs on Algae, Indian Council of Agricultural Research, New Delhi, India, pp. 656.

Fay, P. 1983. The blue green (Cyanophyta- Cyanobacteria), (Arnold-Heinemann, London).

Habib, K., Manikar, N., Ansari, S. and Fatma, T. 2013. Carbaryl stress induced cellular changes in *Calothrix brevissima. Environ. Sci. Pollut. Res*. 20: 862-871.

Hoffmann, L. 1989. Algae of terrestrial habitats. *The Botanical Review* **55**: 77-105.

Huang, T.C. and Chow, T.J. 1992. Characterzation of the *Calothrix* isolates from rice fields. *Bot. Bull. Academia Sinica.* 33: 23-31.

Komarek, J. and Anagnostidis, K. 1989. Modern approach to the classification system of Cyanophytes 4-Nostocales. *Algol. Stud.* 56: 247-345.

Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: Molecular evolutionary Genetic Analysis version 7.0 for bigger datasets. *Molecular Biol. Evolution.* 33: 1870-1874.

Metting, B., 1981. The systematics and ecology of soil algae. *The Botanical*. Rev. 47: 195–312.

Nayak, S., Prasanna, R., Dominic, T.K., Singh, P.K. 2001. Floristic abundance and relative distribution of different cyanobacterial genera in rice field soil at different crop growth stages. *Phykos* **40**: 14-21.

Nayak, S., Prasanna, R., Prasanna, B.M. and Sahoo, D.B. 2007. Analysing diversity among Indian isolates of *Anabaena* (Nostocales, Cyanophyta) using morphological, physiological and biochemical characters. *World J. Microbiol. Biotechnol*. 23: 1575-1584.

Singh, D. P., Khattar, J. I. S., Gupta, M. and Kaur, G. 2014. Evaluation of toxicological impact of cartap hydrochloride on some physiological activities of a non-heterocystous cyanobacterium *Leptolyngbya foveolarum*. *Pest. Biochem. Physiol.* 110: 63-70.

Singh, P.K. and Bisoyi, R.N. 1989. Blue green algae in rice fields. *Phyko.* 28: 181–195.

Stanier, R.Y., Kunisawa, R., Mandel, M. and Cohen-Bazire, G. 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol. Rev*. 35(2):171-205.

Stewart, W.D.P., P. Rowell, N.W. Kerbly, R.H. Reed and G.C. Machray. 1987. N2–fixing cyanobacterioa and their potential applications. *Phil. Trans. R. Soc. Lond.* 17**:** 245-258.

Whitton, B.A. 1992. Diversity, ecology and taxonomy of the cyanobacteria. In: Mann N.H., Carr N.G. (eds) Photosynthetic prokaryotes, Plenum press, New York, pp. 1-51.

Whitton, B.A., 2000. Soils and rice fields. In; *The Ecology of Cyanobacteria* Ed. Whitton, B.A. and M. Potts, Kluwer Academic Publishers, Dordrecht, pp. 233-255.

Whitton BA, Potts M 2000. Introduction to cyanobacteria. In Whitton BA, Potts M (eds), The ecology of cyanobacteria: Their diversity in time and space (pp. 1–11). *Dordrecht: Kluwer Academic*