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ISOLATION AND TAXONOMY OF THE BLUE-GREEN ALGAE (CYANOBACTERIA), *NOSTOC* AND *ANABAENA* IN KERALA STATE, INDIA

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Blue-green algae (also called cyanobacteria) are ubiquitous, pristine and pioneer photosynthetic microorganisms. Many species of cyanobacteria are capable of fixing atmospheric nitrogen and such species in wet soils are simultaneously augmenting the fertility of the soil, acting as natural bio-fertilizers. *Nostoc* and *Anabaena* are the two important genera of heterocystous cyanobacteria capable of contributing nitrogen to soil, especially in paddy fields. The major objectives of the investigation included survey, collection, isolation and pure culture of nitrogen-fixing species of Cyanobacteria in the soils of Kerala state, India. Altogether, pure cultures of 12 species of *Nostoc* and 5 species of *Anabaena* are prepared.

Key words: *Anabaena*, Cyanobacteria, key to species, *Nostoc*, pure culture

INTRODUCTION

Blue-green algae have a long ancient history of almost 3.5 billion years and diversified extensively to become one of the most successful and ecologically significant organisms on the earth, concerning the longevity of lineage and impact on earth's early environment. Their origin in the Early Pre-cambrian era is considered to be one of the most important steps in evolution (Schopf 1970). They are the primary producers and the first organism to evolve elemental oxygen thus transforming the environment suitable for other forms of life. Among the blue-green algae, the heterocyst bearing ones are economically important due to their ability to fix atmospheric nitrogen and thus augmenting the fertility of soil. These are seen widely distributed in paddy fields, and act as bio-fertilisers contributing nitrogen to the soil and improve crop yield.

The ability of some blue-green algae to grow under nitrogen deficit environments pointed towards their ability to fix nitrogen (Frank 1889). High fertility of the paddy field soils of India are attributed to the occurrence of nitrogen fixing blue green algae (De 1939). The algal growth in paddy fields promotes the paddy crops by nitrogen fixation and secretion of growth promoting substances (Gupta 1966). Nitrogen is the essential nutrient limiting the production of crops and their yield, which is often met with the help of chemical fertilisers. These chemicals are found to have an adverse effect on the environment. The problems caused by the excessive use of chemical fertilisers could be tackled by using bio-fertilisers (Choudhury and Kennedy 2005). Bio-fertilisers are an eco-friendly, effective and economical equivalent to chemical fertilisers (Sahu *et al.* 2012).

The advancement in molecular biological technologies has led to the knowledge that, the blue-green algae are more akin to bacteria in many characters like cell wall material, lack of membrane-bound cell organelles and nucleus, and thus they got the popular name cyanobacteria. The taxonomy of the blue-green algae has always been a confusing task, owing to their morphological plasticity and the simple structure that lacks enough distinguishing characters.

Despite being an important organism having ecological and economic importance; these are among the least explored community. *Nostoc* and *Anabaena* are common genera, among the heterocystous blue green algae, found in the paddy fields. Rice being staple food for millions of populations, the agro-economy of Kerala is related to the paddy cultivation. It was once the major occupation of the majority of the population. The nitrogen-fixing cyanobacterial community plays an important role in augmenting the soil fertility and thus helps in the crop yield. Cyanobacteria contribute about 25–30 kg/ha nitrogen to rice crops (Kaushik 1994).

The common occurrence of *Nostoc* and *Anabaena* in the paddy fields of Kerala was reported earlier itself (Aiyer 1965, Anand and Hooper 1995, John 1963). However, less work is being carried out on this group of microorganisms in the past few years except that of Dominic and Madhusoodanan (1999) and Umamaheswari (2005). A systematic study on the occurrence of *Nostoc* and *Anabaena* from different districts of Kerala is attempted in this study, which will help to know the regionally occurring species, their diversity, and distribution.

MATERIALS AND METHODS

Study area

Kerala lies between 8° 18′–12° 48′ N latitude and 74° 52′–77° 22′ E longitude. The state is a small strip of land 560 km long on the west coast of India and a maximum width of 132 km and a geographical area of 38,863 km^2. Kerala has 3 distinct geographical formations like lowland, midland, and highland. The state is bounded on the north and the northeast by Karnataka, the east by the Western Ghats and the south by Tamil Nadu, and the west by the Arabian Sea. Kerala lies in the tropic region. The region has a humid tropical wet climate, with an average temperature of 27–32 °C, and an annual rainfall of 3,107 mm. Kerala experiences two monsoons *i.e.,* the Southwest monsoon and the Northeast monsoon. Though small in size, Kerala is affluent in water sources, with 44 rivers and many brackish water bodies, and streams. The soil is mainly lateritic in mid and high lands, and alluvial in lowlands. Rice is the most important food crop grown in the lowlands and midlands of Kerala. In the high land, plantation crops like rubber, tea, pepper, etc are cultivated.

Collection, isolation, and identification of samples

Algal samples from different districts of Kerala were collected during 2015–2016. The samples were collected randomly as soil samples and direct planktonic mass. The collected samples were enriched initially in BG-11 (Rippka *et al.* 1981) agar medium (without $NaNO_3$) poured in petri plates, and incubated at 25± 2 °C under 3000 lux illumination provided by cool white fluorescent lamps. After 10–15 days freshly grown individual colonies were picked out and transferred to BG_0-11 liquid medium for pure culture in 250 ml conical flask. The purity of the culture was monitored by regular observation under a microscope. The morphological examination of the cyanobacterial strains was carried out under a Labomed microscope; the microphotographs were taken with the help of a camera attached to the microscope (Progres) and the nature of filaments and the shape and size of vegetative cells, heterocyst and akinetes were analysed. Identification was made using the taxonomic literature of Desikachary (1959).

RESULTS AND DISCUSSION

In this study 30 isolates of nitrogen fixing blue green algae, belonging to 17 morphotypes were identified. The vegetative and reproductive characters such as shape, colour and size of the thallus; width and length of trichome; entangled or not entangled form of trichome; shape, colour and thickness of the mucilaginous envelope; shape, size and colour of vegetative cells, heterocysts and akinetes as well as colour and ornamentation of cell walls of the akinetes were used in the taxonomic determination. A taxonomic key for the identification of *Nostoc* and *Anabaena* occurring in Kerala was attempted and a detailed description of the taxa is given below.

Key to the genera

1a	Trichome highly coiled and in a definite colony	2
1b	Trichome not so coiled and seen as planktonic mass	*Anabaena*
2a	Colonies usually a mucilaginous mass	*Nostoc*

Nostoc Vaucher ex Bornet et Flahault, Ann. Sci. Nat. Bot., Ser. VII, 7: 181 (1888)

Type species**.** *Nostoc commune* Vaucher ex Bornet et Flahault

Key to the identification of *Nostoc* species of Kerala

1a	Cells barrel-shaped	2
1b	Cells not barrel-shaped	10
2a	Colonies spherical to globose	3
2b	Colonies not spherical	7
3a	Colonies brownish in colour	4
3b	Colonies blue-green	6
4a	Akinetes barrel-shaped	5
4b	Akinetes oblong	*N. punctiforme*
5a	Trichome more than 5 μm broad	*N. piscinale*
5b	Trichome 3.5-4 μm broad	*N. linckia*
6a	Trichome pale blue-green, loosely arranged	*N. paludosum*
6b	Trichome dark green tightly entangled	*Nostoc* sp. (14848)
7a	Spores compressed; epispore brown and smooth	*N. entophytum*
7b	Spores not compressed	8
8a	Spores seen away from heterocyst	*N. spongiaeforme*
8b	Spores seen adjacent to heterocyst	9
9a	Spores spherical barrel-shaped	*N. muscorum*
9b	Spores elongate and curved	*Nostoc* sp. (14849)
10a	Vegetative cells cylindrical	11
10b	Vegetative cells spherical	*N. humifusum*
11a	Akinete ellipsoidal	*N. ellipsosporum*
11b	Akinete spherical to oblong	*N. carneum*

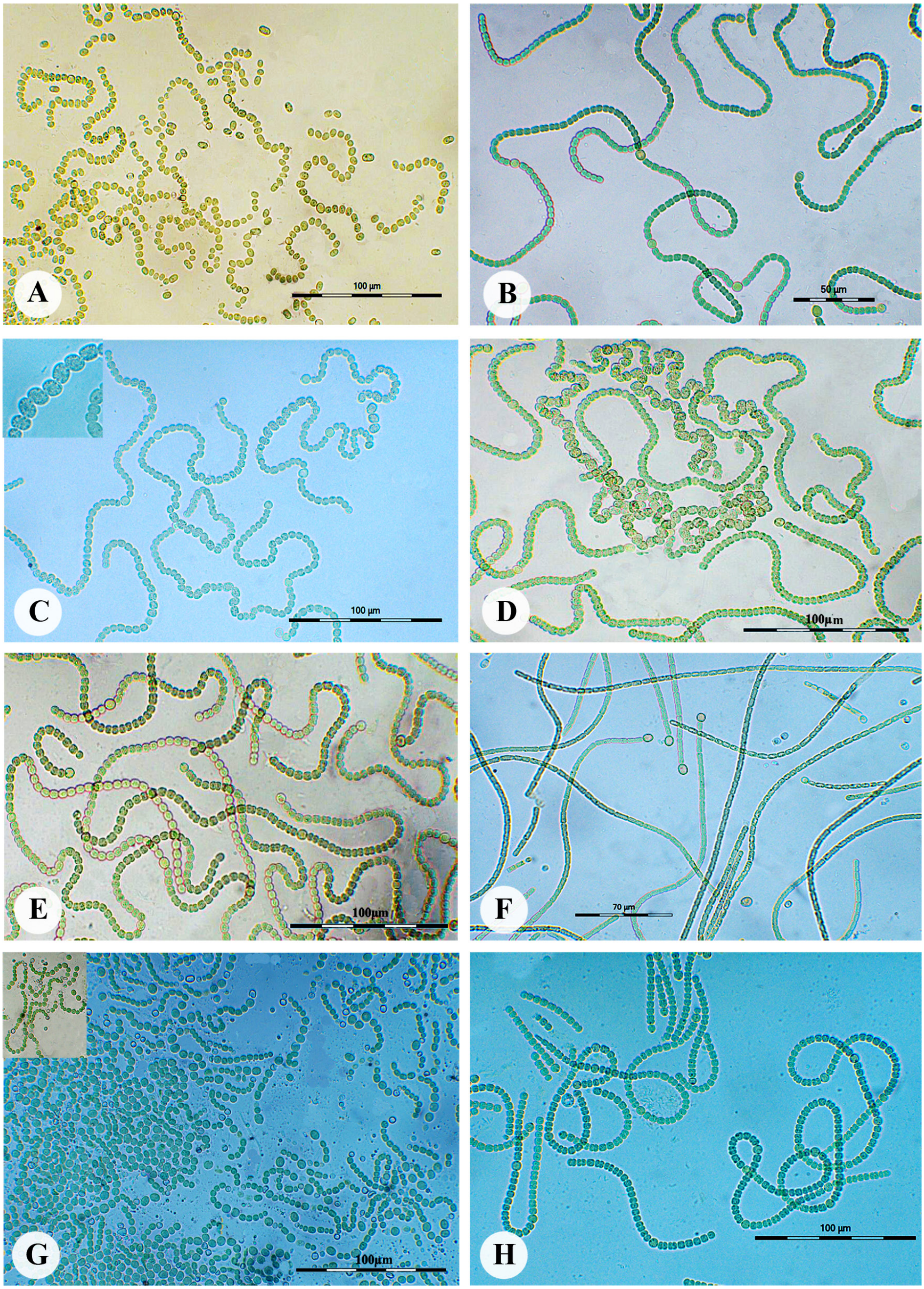

Fig. 1. A = *Nostoc entophytum*; B = *N. paludosum*; C = *N. linckia* (Akinetes in inset); D = *N. piscinale*; E = *N. carneum*; F = *N. ellipsosporum*; G = *N. humifusum* (Akinetes in inset); H = *N. muscorum*

Nostoc entophytum Born. et Flah. (Fig. 1A): Thallus is macroscopic, leathery, and yellowish-brown. Filaments densely entangled; sheath distinct, brownish. Trichome 3–4 μm broad, short barrel-shaped. Heterocyst intercalary and terminal, broader than vegetative cells (5–6 μm broad), spherical; akinetes compressed 7–8 μm long and 5–6 μm broad as a chain near the heterocyst, epispore smooth. – Habitat: isolated from paddy field soil. – Specimen examined: MBGH 14847 (Kuruvattoor, Kozhikode).

Nostoc paludosum Kutzing ex Born. et Flah. (Fig. 1B): Thallus is microscopically small, pale blue-green colour; gelatinous colonies spherical later become lobed, sheath colourless. Trichome 4 μm broad; cells barrel-shaped; 3–4.5 μm long. Apical cell dome shaped; heterocysts intercalary and terminal, spherical; broader than vegetative cells, 5–6 μm broad. Akinetes oval, 4 μm broad, 5–8 μm long. Akinetes were not observed in cultures. – Habitat: paddy field soils. – Specimen examined: MBGH 14841 (Anthikad, Trissur).

Nostoc linckia (Roth) Bornet ex Born. et Flah. (Fig. 1C): Thallus is gelatinous blackish green to brown in colour, tuberculate, at first globose later irregularly expanding. Filaments densely entangled; sheath colourless; trichome 4.5–5 μm broad; cells short barrel-shaped. Heterocyst sub spherical; both terminal and intercalary; akinetes sub spherical, 7–7.5 μm broad and 6–7 μm long; seen as a chain away from heterocyst. – Habitat: paddy field as gelatinous mass. – Specimen examined: MBGH 14809 (Kakathara, Palakkad).

Nostoc piscinale Kutzing ex Born. et Flah. (Fig. 1D): Thallus is at first globose, gelatinous; brown, filaments loosely entangled; sheath distinct at the periphery; trichome 5–7 μm broad, cells shorter than broad; heterocyst sub-spherical, 6–7 μm broad; spore in long chains; globose 6–7 μm broad. – Habitat: isolation from paddy field soil. – Specimen examined: MBGH 14831 (Periyangad, Kozhikode).

Nostoc carneum Ag. ex Born. et Flah. (Fig. 1E): Thallus is globose first later expands, gelatinous, flesh coloured, reddish brown, filaments loosely entangled; trichome 4–5 μm broad, cells cylindrical; heterocyst 5–6 μm broad, spherical to oblong; apical cell rounded; akinetes ovate 5.5–6 μm broad, away from heterocyst, epispore smooth. – Habitat: on rocks. – Specimen examined: MBGH 8883 (Mukkali, Palakkad).

Nostoc ellipsosporum (Desm.) Rabenh. ex Born. et Flah. (Fig. 1F): Thallus is gelatinous, irregularly expanded, attached by lower surface; reddish brown. Trichome straight or slightly curved, with the same diameter along the filament. Filaments loosely entangled; trichome 4–5 μm broad; cells cylindrical 6–10 μm long, olivaceous. Cells with dark brown granules and gas vesicles. Heterocyst intercalary and terminal; sub-spherical; 7–8 μm broad and 10–12 μm long. Akinetes ellipsoidal 5–8 μm broad; 12–18 μm long, epispore smooth. Akinete formations are very rare in cultures. – Habitat: found as gelatinous mass on rocks. – Specimen examined: MBGH 8882 (Mukkali, Palakkad).

Nostoc humifusum Carmicheal ex Born. et Flah. (Fig. 1G): Thallus is dark green in colour, rounded, later become expanded, and attached to the periphery. Trichome 2–3.5 μm broad; cells spherical. Heterocyst intercalary and terminal. Akinetes spherical; away from heterocyst 5 μm broad and 6 μm long and epispore smooth. – Habitat: in stagnant water. – Specimen examined: MBGH 14839 (Kothamangalam, Ernakulam).

Nostoc muscorum Ag. ex Born. et Flah. (Fig. 1H): Thallus is gelatinous, membranous, irregularly expanded. Blue-green in colour. Filaments entangled; trichome 4–5 μm broad. Cells short barrel-shaped 3–5 μm long; apical cells dome shaped. Heterocyst intercalary and terminal; spherical to barrel in shape, 5–7 μm broad. Akinetes produced adjacent to heterocysts; many in a series, 4–6 μm broad and 5–7 μm long; epispore smooth. Akinete formation is not observed in cultures. – This name is currently regarded as a taxonomic synonym of *Desmonostoc muscorum* (C. Agardh ex Bornet et Flahault) Hrouzek et Ventura. – Habitat: moist soils of stream banks and paddy field soil. – Specimens examined: MBGH 8885 (Ambenkunnu, Palakkad), MBGH 8894 (Malampuzha, Palakkad), MBGH 14850 (Chakkulath, Alapuzha).

Nostoc sp. (Fig. 2I): The colonies at first appear globose, later expanding; Thallus is pale blue-green, less coiled and microscopically small. Trichome are 3–5 μm broad; vegetative cells barrel to cylindrical, 3.5–5 μm long; end cells rounded. Heterocyst intercalary and terminal, terminal ones are very rare, spherical, 5.5–7 μm broad and as long as broad. Akinetes seen as a chain and scattered, elongate, 3–4 μm broad and 6–8 μm long sometimes curved. – Habitat: from cultures of paddy field soil. – Specimen examined: MBGH 14849 (Kuruvattoor, Kozhikode).

Nostoc punctiforme (Kutz) Hariot (Fig. 2J): Trichome is brownish-green in colour, filaments slightly coiled. Vegetative cells barrel-shaped 4–5 μm broad and 3–4 μm long. Apical cells rounded; heterocyst both intercalary and terminal. Spherical in shape, 5–6 μm broad and 4.5–6 μm long. Akinetes seen away from the heterocyst, compressed barrel-shaped 7–8.5 μm broad and 5-6 μm long. – Habitat: from the cultures of paddy field soil. – Specimen examined: MBGH 14860 (Jhandamuk, Trissur).

Nostoc spongiaeforme Agardh ex Born. et Flah. (Fig. 2K): Thallus is expanding ribbon like, gelatinous, blue-green to brownish, filaments flexuous, loosely entangled, sheath yellowish-brown; trichome 4–5 μm broad, cells barrel to cylindrical in shape, apical cell dome shaped; heterocyst sub spherical, both intercalary and terminal, 6–7 μm broad; akinetes oblong, seen away from the heterocyst, 6–8 μm broad and 8–10 μm long, epispore yellowish. – Habitat: seen as a gelatinous mass on the surface of the laterite soil in the wetlands. – Specimen examined: MBGH 14852 (Barker fuel station, Nellikatte, Kasargod).

Nostoc sp. (Fig. 2L): Thallus is dark green, leathery, highly coiled and microscopically small, trichome 2–3 μm broad; vegetative cell barrel to cylindrical, 3–3.5 μm long, end cell rounded. Heterocyst is both terminal and intercalary, spherical, 3–5 μm broad and as long as broad. Akinetes away from the heterocyst, barrel to spherical. – Habitat: from cultures of paddy field soil. – Specimen examined: 14848 (Kuruvattoor, Kozhikode).

Anabaena Bory

Type species *Anabaena oscillarioides* Bory

Key to the identification of *Anabaena* species of Kerala

1a	Akinetes adjacent to heterocyst	2
1b	Akinetes away from heterocyst	*A. oryzae*
2a	Akinetes on both sides of the heterocyst	3
2b	Akinetes formed on one side	*A. aphanizomenoides*
3a	Apical cell rounded or dome shaped	*A. sphaerica*
3a	Apical cell conical	4
4a	Vegetative cells cylindrical	*A. orientalis*
4b	Vegetative cells barrel-shaped	*A. torulosa*

Anabaena sphaerica Born. et Flah. (Fig. 2M): Thallus is floccose, blue-green; straight. Cells spherical to barrel, 5 μm broad; apical cells rounded or dome shaped. Heterocyst intercalary; sub-spherical, 6–7 μm broad; akinetes on both sides of the heterocyst, oval, 8 μm broad and 10–12 μm long, epispore smooth. – Habitat: found as a mat on the soil surface. – Specimen examined: MBGH 14837 (Thalappara, Malappuram).

Anabaena aphanizomenoides Forti (Fig. 2N): Trichome is single, straight, 4–5 μm broad; constricted at the cross walls. Cells barrel to cylindrical with gas vacuoles. Heterocyst is mostly intercalary; sub spherical to barrel, 5–5.5 μm broad, 5–6 μm long. Akinetes adjacent to the heterocyst single; elongate, 5–6 μm broad and 8.5–10 μm long with smooth wall. – This name is currently regarded as a taxonomic synonym of *Sphaerospermopsis aphanizomenoides* (Forti) Zapomelova, Jezberova, Hrouzek, Hisem, Rehakova et Komarkova. – Habitat: from cultures of paddy field soil. – Specimens examined: MBGH 14843 (Anthikad, Trissur), 14829 (Vazhakad, Kozhikode).

Anabaena oryzae Fritsch (Fig. 2O): Plant mass is mucilaginous, pale blue-green in colour; Thallus soft, membranous; trichome straight or slightly curved and 3–4 μm broad, cells more or less barrel-shaped as long as or 11/2

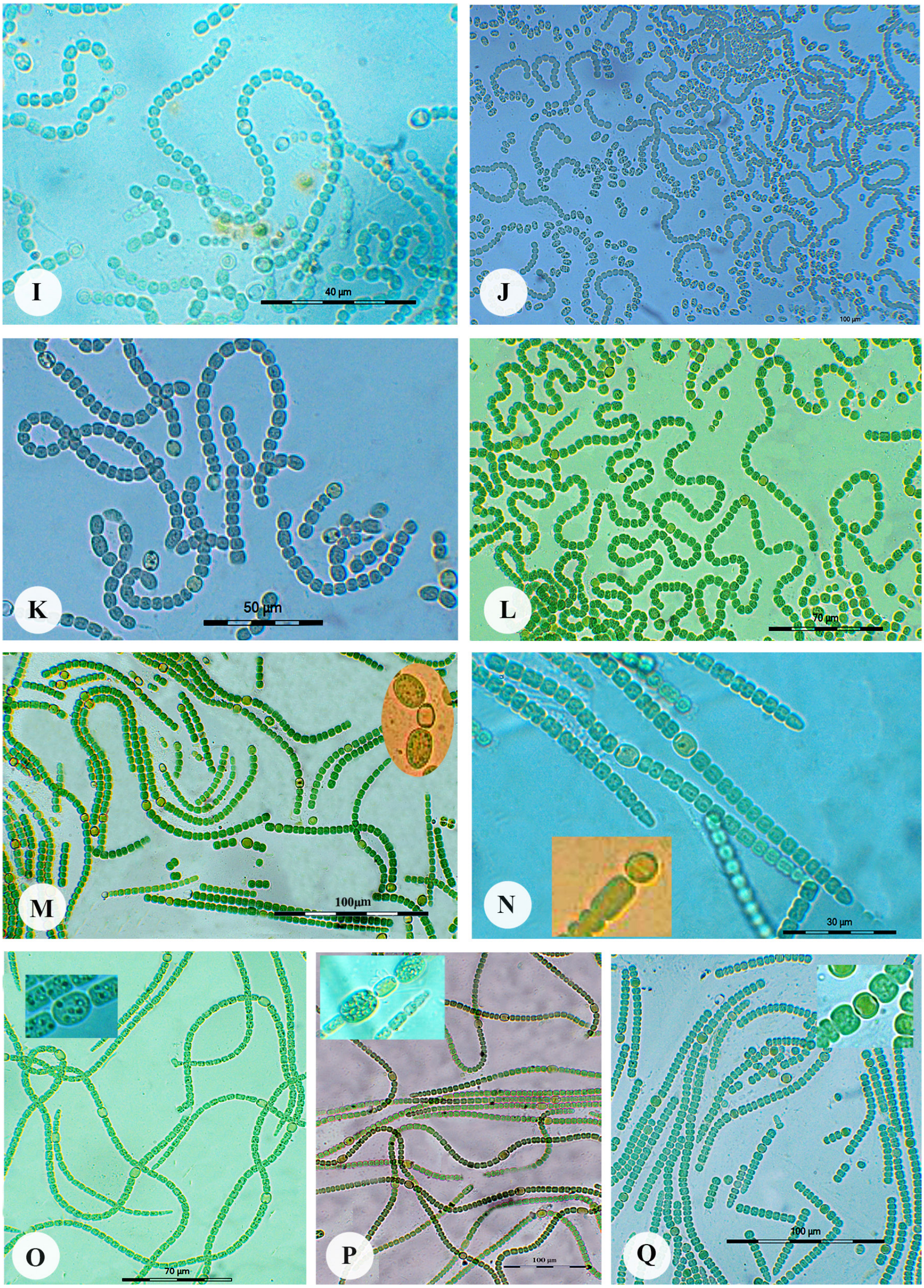

Fig 2. I = *Nostoc* sp.; J = *N. punctiforme*; K = *N. spongiaeforme*; L = *Nostoc* sp.; M = *A. sphaerica* (Akinetes in inset); N = *A. aphanizomenoides* (Akinetes in inset); O = *A. oryzae* (Akinetes in inset); P = *A. torulosa* (Akinetes in inset); Q = *A. orientalis* (Akinetes in inset)

times long as broad; end cells conical with pointed apex, heterocyst terminal and intercalary, terminal ones conical, barrel-shaped, broader than vegetative cells, 6–7 μm broad and 7–8 μm long; spores away from heterocyst, single, short ellipsoidal, 5–6 μm broad and 7–8 μm long epispore thick. – Habitat: paddy field. – Specimen examined: MBGH 14840 (Irinjalakuda, Trissur).

Anabaena torulosa (Carm.) Lagerh. ex Born. et Flah. (Fig. 2P): Thallus is thin, pale blue-green; trichome 3–4 μm broad, cells barrel-shaped; apical cell acutely conical. Heterocyst sub-spherical; 5 μm broad and 6–7 μm long; akinete on both the sides of the heterocysts, cylindrical to barrel with rounded ends, 7–8 μm broad, up to twice as long as broad, epispore smooth. – Habitat: attached to the culms of the paddy plant. – Specimen examined: MBGH 14846 (Kuruvattoor, Kozhikode).

Anabaena orientalis Dixit (Fig. 2Q): Thallus is seen as a suspension in liquid culture, dark green, trichome single, straight or slightly curved; vegetative cells cylindrical, 3.5–4.5 μm broad and 3–4.5 μm long, end cells conical with rounded ends. Heterocyst cylindrical, intercalary, 4.5–5 μm broad and 7–8.5 μm long; akinetes on one side of the heterocyst; at first single, barrel-shaped later elongate 10–14 μm long and 8–10 μm broad. – Habitat: from culture of paddy field soil. – Specimen examined: MBGH 14807 (Bidunampallam, Palakkad).

CONCLUSIONS

The present study was aimed at the survey of *Nostoc* spp. and *Anabaena* spp. from different districts of Kerala. The results indicated the ubiquitous distribution of these two genera in all the localities studied. The majority of the collected specimens were either found among the culms of the rice plant or are isolated from the soil collected from the fields.

From the observations, the cultivated soil, especially the paddy fields appears to support the growth of both *Nostoc* and *Anabaena*; this result was in support of Tiffany, 1951. This abundance could be due to lower nitrogen status in the rice fields and these Cyanobacteria contribute and compensate for the nitrogen deficiency of the crop to some extent. The present work documented the occurrence of 12 species of *Nostoc* and 5 species of *Anabaena*. The results confirm the dominance of *Nostoc* species; earlier studies also supported the predominance of *Nostoc* in the rice fields of Kerala, Tamil Nadu, West Bengal, Assam and Haryana (Venkataraman, 1972). In contrast, Vijayan and Ray (2015) reported a higher number of *Anabaena* sp. compared to *Nostoc* sp. from Kuttanadu paddy fields, Alappuzha. The cyanobacteria generally grow well in a higher pH, the same was noticed in the present study also, where all the isolates were grown in BG_0-11 medium with a pH of 7.5±3. There are only a few reports on the occurrence of cyanobacteria at low pH (acidic range) as they are in general intolerant to low pH conditions (Hunt *et al.* 1979, Dominic and Madhusoodanan 1999).

The understanding of native species of cyanobacteria in a region helps in selecting appropriate cyanobacterial inocula to be applied as bio-fertilisers to crops like paddy.

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