



RESEARCH ARTICLE

Cyanobacterial association on the roots of epiphytic orchids

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ABSTRACT

The present study is the first report on species level taxonomic enumeration of orchid associated cyanobacteria. Various cyanobacterial species were recorded from the aerial and substrate roots of epiphytic orchids. Aerial roots are having more cyanobacterial population than substrate roots of epiphytic orchids. A total of 10 species of cyanobacteria belonging to 4 families (Chroococcaceae 1; Oscillatoriaceae 4; Nostocaceae 1; Scytonemataceae 4) were recorded. Detailed taxonomic descriptions and microphotographs are also provided.

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INTRODUCTION

Cyanobacterial-plant symbioses are ancient associations believed to have evolved around 500 million years ago (Raven 2002; Bergman *et al.* 2008 a, b). Cyanobacteria form symbiotic partnerships with other plants and they provide host with fixed nitrogen and in return occupy relatively protected environments free from predation and environmental extremes. Photosynthetic hosts can also provide fixed carbon to the cyanobionts and this capacity for heterotrophy enables many cyanobionts to grow in host structures, such as roots of plants that receive little or no light.

Nitrogen fixation by cyanobacteria-orchid association is greatly influenced by existing environmental factors, such as water availability, temperature etc. Prolonged drought may result in a decline in the nitrogen fixation capacity of cyanobacteria-orchid associations. The aerial roots of epiphytes are covered with a velamen, consisted of large dead air-filled cells. It sucks up and retains water with dissolved mineral salts and nutrients and provides mechanical protection, reflects solar radiation and it is permeable to oxygen and carbon dioxide (Pridgeon 1986). The velamen is an econiche suitable for colonization by associative microorganisms both autotrophic and diazotrophic.

The presence of cyanobacteria among associative microorganisms of epiphytic orchids is of special importance, since these bacteria fulfil a wide range of physiological and biochemical reactions and are capable of rapid switching from one metabolic pathway to another (Pankratova 2000). As a rule, associative microorganisms play an important role in the formation and stable existence of symbioses as well as in extension of the ecological range of host plant growth (Hofflich *et al.* 1993).

Studies on epiphytic cyanobacteria for over a century were on understanding their structure, reproduction and several treatises. Cyanobacteria are common epiphytes on the pneumatophores of mangroves (Steinke *et al.* 2003; Shamina *et al.* 2014). Cyanobacterial association on aerial and substrate roots of epiphytic orchids have also been reported (Tsavkelova *et al.* 2001, 2003 a, b). The cyanobacterium, *Dactylococcopsis*

acicularis has been reported from the roots of the orchid, *Spathoglottis plicata* (Untari *et al.* 2009).

There is a very little knowledge on cyanobacteria-orchid association, although it is unclear if the orchids benefit from fixed nitrogen produced by cyanobacteria.

Regarding the taxonomy, no studies on orchid associated cyanobacteria have been reported. In this study, we have analysed the species level cyanobacterial association on the roots of epiphytic orchids of various species. Our study is the first report on the species level taxonomic descriptions of orchid associated cyanobacteria.

MATERIALS AND METHODS

Cyanobacteria are collected from roots of the epiphytic orchids *Acampe praemorsa*, *Dendrobium chrysanthum* and *Dendrobium aphyllum*. Visible cyanobacterial mass were observed on the aerial and substrate roots of epiphytic orchids (Pl. 1, Fig. a, b, c) and they were collected by using scalpels and needles. The collected specimens were deposited in the Plant Diversity Division, University of Calicut.

Microscopic analysis was done in the live condition for the identification of cyanobacteria. Microphotographs were taken using Leica DM 1000 LED Compound microscope. Cyanobacterial identification was done with manuals of Desikachary (1959), Anand (1989) and Prescott (1982).

RESULTS

The present study deals with the species level identification of cyanobacteria. A total of 10 cyanobacteria species belonging to 4 families (Chroococcaceae 1; Oscillatoriaceae 4; Nostocaceae 1; Scytonemataceae 4) were recorded. Detailed taxonomic description of each of these organisms, voucher number, nature of habitat of their occurrence and their microphotographs is also provided.

Taxonomic Enumeration

Order: Chroococcales

Family: Chroococcaceae

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***Chroococcus turgidus* (Kutz.) Nag. (Pl. 1, Fig. d)**

Cells spherical, groups of 4, without sheath 8.340 µm to 9.830 µm broad, with sheath 18.474 µm - 25.656 µm diameter, sheath colourless and distinct.

Occurred as brownish mat on the aerial and substrate roots of *Acampe praemorsa* (Voucher Number: CU 139309).

Order: Nostocales
Family: Oscillatoriaceae

***Oscillatoria obscura* Bruhl et Biswas (Pl. 1, Fig. e)**

Trichomes straight, blue-green, not constricted at the cross walls, cross walls granulated, 4.704 µm - 5.0 µm broad, cell length 0.9 µm - 1.2 µm, end cells rounded.

Occurred as brownish mat on the aerial and substrate roots of *Acampe praemorsa* (Voucher Number: CU 139308).

***Oscillatoria annae* van Goor (Pl. 1, Fig. h)**

Trichome straight, dark blue-green, slightly constricted at the cross walls, 6.811 µm - 7.355 µm broad, end cells rounded.

Occurred as blackish mat on the aerial roots of *Dendrobium chrysanthum* (Voucher Number: CU 139310).

***Oscillatoria formosa* Bory ex Gomont (Pl. 1, Fig. f)**

Thallus aggregated to form a dark blue-green plant mass, trichome straight, curved and slightly tapering toward the apex, apical cell conical, cells nearly quadrate, 2.828 µm - 3.352 µm broad, 1.970 µm - 4.563 µm long,

Occurred as blackish mat on the substrate roots of *Dendrobium chrysanthum* (Voucher Number: CU 139310).

***Phormidium tenue* (Menegh.) Gomont (Pl. 1, Fig. i)**

Thallus pale blue green, thin, trichome straight or slightly bent, densely entangled, constricted at the cross walls, attenuated at the ends, 1.563 µm broad, not granulated, end cell conical.

Occurred as brownish mat on the substrate roots of *Dendrobium aphyllum* (Voucher Number: CU 139311).

Order: Nostocales
Family: Nostocaceae

***Nostoc muscorum* Ag. ex Born. et Flah. (Pl. 1, Fig. g)**

Colony brown, trichome 4.40 µm broad, 15.008 µm long, cells barrel shaped, constricted at the cross walls, heterocyst spherical, 6.537 µm broad, 6.811 µm long.

Occurred as brownish mat on the aerial roots of *Dendrobium chrysanthum* (Voucher Number: CU 139329).

Order: Nostocales
Family: Scytonemataceae

***Scytonema hofmanni* Ag. ex Born. et Flah. (Pl. 1, Fig. k)**

Thallus blue-green, Filaments 10.854 µm - 12.278 µm broad, firm sheath, filament long, false branches aggregated, heterocysts oblong, cells unequal in length, 7.999 µm - 10.275 µm long, 9.679 µm - 10.428 µm broad.

Occurred as greenish mat on the substrate roots of *Dendrobium chrysanthum* (Voucher Number: CU 139328).

***Scytonema bohneri* Schmidle (Pl. 1, Fig. j)**

Filaments blackish green, false branched, end cell rounded, branches mostly single, filaments 8-11 µm broad, sheath

colourless, firm, trichome bluish-green, trichomes 6.426 µm - 8.376 µm broad, 7.160 µm - 8.38 µm long, not constricted at the cross walls, granulated at the cross walls, heterocysts intercalary, ellipsoidal to cylindrical, yellowish, 7.999 µm - 8.247 µm broad, 5.235 µm - 7.263 µm long.

Occurred as blackish mat on the aerial roots of *Dendrobium chrysanthum* (Voucher Number: CU 139332).

***Scytonema stuposum* (Kutzing) Bornet ex Born. et Flah. (Pl. 1, Fig. l)**

Thallus broadly expanded, blackish violet to reddish, false branched, false branches nearly resembling the main filaments, sheath thick, gelatinous, trichome 12.711 µm - 18.029 µm broad, 8.074 µm - 12.278 µm long, heterocysts 15.634 µm broad, 10.216 µm long, heterocysts as long as the vegetative cells, heterocyst olive-yellow appearance.

Occurred as blackish mat on the aerial roots of *Dendrobium chrysanthum* (Voucher Number: CU 139332).

***Scytonema ocellatum* Lyngbye ex Born. et Flah. (Pl. 1, Fig. m)**

Thallus cushion-like, false branched, trichome 7.381 µm - 10.726 µm broad, 5.060 µm - 7.877 µm long, olive green, not torulose, cells quadrate, heterocysts sub-quadrate, 8.426 µm - 9.436 µm broad, 6.66 µm - 9.79 µm long, yellowish.

Occurred as blackish mat on the aerial roots of *Dendrobium chrysanthum* (Voucher Number: CU 139332).

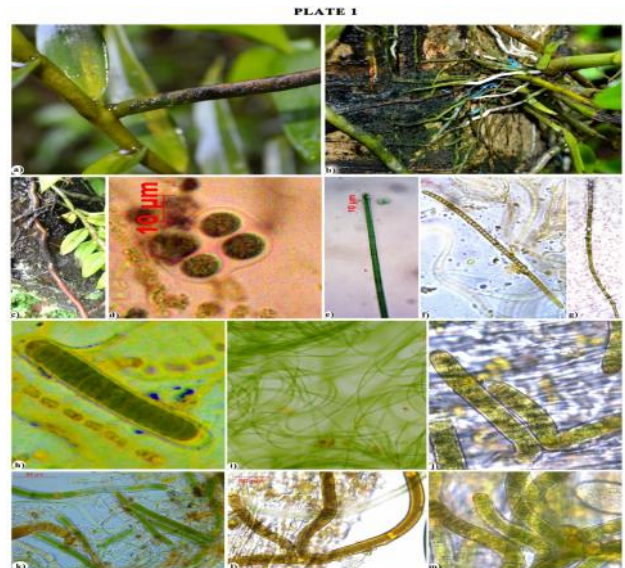


Fig. a),b),c) Visible cyanobacterial mass on the roots of epiphytic orchids; d) *Chroococcus turgidus*; e) *Oscillatoria obscura*; f) *Oscillatoria formosa*; g) *Nostoc muscorum*; h) *Oscillatoria annae*; i) *Phormidium tenue*; j) *Scytonema bohneri*; k) *Scytonema hofmanni*; l) *Scytonema stuposum*; m) *Scytonema ocellatum*

DISCUSSION

The results obtained from the study reveals that orchid roots are well populated by cyanobacteria. Cyanobacteria differed in their population density on the surface of aerial and substrate roots of epiphytic orchids. Most of the cyanobacterial species are occurred as mixed population. We have observed that aerial roots are having more cyanobacterial population than substrate roots of epiphytic orchids.

Chroococcus turgidus and *Oscillatoria obscura* were found on the aerial and substrate roots of *Acampe praemorsa*; *Oscillatoria annae*, *Nostoc muscorum*, *Scytonema bohneri*, *Scytonema stuposum* and *Scytonema ocellatum* were found

at the aerial roots and *Oscillatoria formosa* and *Scytonema hofmanni* were found at the substrate roots of *Dendrobium chrysanthum*; *Phormidium tenue* is found at the substrate roots of *Dendrobium aphyllum*.

Previous studies revealed that various cyanobacteria had been found on aerial roots of epiphytic orchids *Acampe papillosa*, *Phalaenopsis amabilis* and *Dendrobium moschatum* and the substrate roots of *A.papillosa* and *D.moschatum* (Tsavkelova *et al.* 2001, 2003 a, b), but a comprehensive identification of cyanobacteria up to species level has not yet been performed.

Cyanobacterial association greatly depends on the conditions of plant growth. Cyanobacterial mat can be observed on the epiphytic roots of orchid when there is persistent moisture avail in the environment. The aerial and substrate root surface of epiphytic orchids covered with dark blue-green mass of organisms. Many cyanobacterial species identified in the present study are well known nitrogen fixers. The species of *Nostoc*, *Oscillatoria* and *Scytonema* were identified from the aerial and substrate roots of epiphytic orchids confirm these organisms are having a strong symbiotic association with the orchids. The pigments of cyanobacteria isolated from the aerial roots of *Dendrobium* may protect these bacteria from the harmful action of ultraviolet radiation, as this epiphytic orchid lives under direct sunlight (Tsavkelova *et al.* 2001).

The problem of taxonomic assignment of orchid associated cyanobacteria has not yet been sufficiently studied and hence our study is the first report on the species level taxonomic enumeration of orchid associated cyanobacteria.

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