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Taxonomic analysis of the Genus *Tolypothrix* Bornet & Flahault (Desikachary-1959) (Cyanobacteria) and its different species under the family Scytonemataceae and studied in culture

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Abstract

The present work deals with the morpho-taxonomic description and critical analysis of the genus *Tolypothrix* under the family Scytonemataceae (Cyanobacteria or BGA). In this study, most of the pioneer taxonomists from Desikachary (1959), Geitler (1932) and Fritsch (1945) to modern workers, all of them mostly taken or isolated their different strains from natural materials,descriped morphologically and identified very shortly based on traditionally with their research. But Komarek and Anagnostidis (1986, 1988, 1989 and 2013, 2014) suggested morphotaxonomic description need of more intransive study under various cultural conditions and not merely on the basis of the natural material. The morphology of Blue-green algae are much variable under different environmental conditions. In the present study all the isolates strains were favourable for good growth in BG-11 (Stewart et. al.,1972) medium under standard laboratory condition .

The taxonomic review analysis of various taxa of *Tolypothrix* described by Desikachary (1959) in his monograph on Cyanophyta. Most of the earlier workers observed and described their different species of the genus *Tolypothrix* have much similarities or slight difference in several characters to one another and all the description of these forms are based on the observations of collected natural materials from distant places or countries. Due to these reasons, their described characters were very incomplete. Because original work of a particular type of species included their characters which were present or showed in different conditions different types. So, most of the workers couldnot considered its age, soil charecters, temperature, fresh or alkaline water, water lebel conditions, plain or hilly areas ,seasonal effects etc. They considered very few charecters on the basis of their natural materials and only slight differences developed or created new species. But If we make a critical analysis and developed some groups from mentioned characters of different species in his described book Cyanophyta by Desikachary (1959) then we could developed minimum categories of the species. Komarek and Anagnostidis (1988) supported this view also.

Keywords: Cyanobacteria (BGA), Culture, Terminal heterocyst, single Pseudobranch, Scytonemataceae.

1. Introduction

The family **Scytonemataceae** (Cyanobacteria) **Rabenhorst** came into existence in 1865 while the genus ***Tolypothrix*** was described by Bornet and Flahault in 1887.Cyanobacteria have probably displayed a major role throughout the biological history of the earth. Cyanobacterial Multi and diversified characters, different times different scientists gave different names due to its peculiar and flexible characters. The name was given Myxophyceae by Wallr in 1833. Cyanophyta by Schussing in 1925. **Cyanobacteria** by **Stanier in 1977.** Oxygenic photosynthetic bacteria by Castenholz in1989. **Cyanoprocaryota by Komarek & Anagnostidis in 1989.** From 1833 to 2017 about 184 years 16 times name changed Average every ten years changed its name due to its habits and habitats of **Fresh or marine water, free living** or **symbiotic charecters**. They can tolerated **thermal springs up to 73°C.**

Cyanobacteria constitute a monophyletic group within the Bacteria domain (Castenholz, 2001). However, the group taxonomy is traditionally based on morphological characters, according to botanic criteria Komárek & Anagnostidis (1988), and added to ecological data. The high morphological variability and the low number of phenotypic characters used in the cyanobacterial taxonomy leads to serious identification problems. Several studies using cultural or molecular techniques have questioned the use of morphological characteristics for identification of the different species, as well as, at the genus level.

2. Materials and Methods

2.1. Isolation and Maintain of the strains

All the strains belonging to the genera ***Tolypothrix*** of the family **Scytonemataceae** (Cyanobacteria) have been taken for the study which were isolated from their natural places of moist soil, scattered colonies, small cushion like Patches and in submerged rice fields of different districts of Westbengal and Uttar Pradesh.

2.2. Selection of media: In the beginning different culture media have been used but BG11 Medium (Stanier et. al., 1972) supported the better growth and all the experiments performed in BG11 medium (liquid and solid) and maintained in the culture room.

2.3. Preparation of media: All the mentioned macro and micronutrients have been weighed according to their precise quantity dissolved into 1 litre distilled water one by one. The final pH was adjusted at 7.5. The media have been used of Nitrate nitogen and ammonical nitrogen. The quantity of $NaNO_3$ and NH_4Cl for different concentrations are taken: (1) Nitrogen deficient medium (-N; without nitrogen). (2)Normal concentration of nitrate nitrogen and ammonical nitrogen (+N; 20 mM). (3)More concentration of nitrate nitrogen and ammonical nitrogen (+1.5N; 30 mM). (4)Rich (+2N; 40 mm) and high (+4N; 80 mM) concentrations of nitrate nitrogen and ammonical nitrogen respectively. For nitrogen deficient medium used either $NaNO_3$ or NH_4Cl, this medium was divided into two parts : one for solidified medium (Agar slant) by adding agar (15 gm/l litre) and other for liquid medium. The cultures were maintained and observed in ideal laboratory conditions and incubated for 25-30 days at 32 ± 2°C and at 3000-3500 Lux light intensity under 14/10 LD cycle .

Experiments were performed by three replicates.

2.4. Identification of the isolates : Our present observations and the identification of the selected isolates for morphological and physiological studies have been made using standard monographs of Geitler, (1932), Desikachary (1959); Starmach (1966); Komarek and Anagnostidis (1986, 1988) ,Tiwari et.al. and Tiwari (1972,1975 and 1979).

2.5. Morphological observation and identifications: By our microscopes Motic and Nikon with attaching photosystem the morphological observations were recorded.

3. Results

The different parameters i.e. chemical or environmental may induce development of branching in the different strains of the family Scytonemataceae which are as below:

Effect of nitrogen ($NaNO_3$ and NH_4Cl): In total 6 different concentrations i.e., 0.0 mM, 20 mM, 30 mM, 40 mM, 80 mM were developed solid as well as liquid medium and grown in our ideal laboratory conditions. Both the species. In the nitrogen deficient medium Pseudo branches i.e. geminate and single branches are normally more and heterocytes are also

present in all the strains of the family Scytonemataceae. But in the normal nitrogenous medium heterocytes are absent and branching frequency may be 15-45% decrease in different strains of *Tolypothrix*. (0.0 mM, 20 mM, 30 mM, 40 mM, 80 mM) increase the geminate branches and in tolerable rich nitrogen (40 mM; $NaNO_3$) medium branches may be increased upto 60%. And it interesting that in case of different strains of *Tolypothrix*, there 75-95% are decrease of the single branching with terminal or basal heterocytes in the increasing concentration of nitrogen (20 mM, 30 mM, 40 mM, 80 mM). If there are any kind of branching present, they are mostly geminate branching. But even up to normal nitrogenous, ammonium chloride (+N; NH_4Cl) medium increase the development of branching.

Table-1. ***Tolypothrix byssoidea*** and ***Tolypothrix distorta*** showed the development of heterocytes different concentrations (00 mM, 20 mM, 30 mM, 40 mM, 80 mM) of nitrate nitrogen medium.

Concentrations	Heterocytes	
	Tolypothrix byssoidea	***Tolypothrix distorta***
00 mM	+++	++++
20 mM	++	+++
30 mM	-	++
40 mM	-	+
80 mM	-	-

• maximum = ++++++; • more = +++++; • very common = ++++;• common = +++;• rare = ++;• absent like = +;• absent = -.

In case of Heterocysts ***Tolypothrix distorta*** developed more heterocysts as compare to ***Tolypothrix byssoidea*** in nitrogen free medium. Increasing concentrations of nitrogen decreasing the heterocysts and in ***Tolypothrix byssoidea*** upto 20 mM concentrations developed heterocysts but **Tolypothrix distorta *can develop heterocysts*** upto 40 mM concentrations on nitrogenous medium.

Table-2. ***Tolypothrix byssoidea*** and ***Tolypothrix distorta*** showed the development of **Tolypotricoid** branching in different concentrations (00 mM, 20 mM, 30 mM, 40 mM, 80 mM) of nitrate nitrogen medium.

Concentrations	Tolypotricoid Branching	
	Tolypothrix byssoidea	***Tolypothrix distorta***
00 mM	+++++	+++++
20 mM	+++	++++
30 mM	++	++
40 mM	-	**(+)-**
80 mM	-	-

• maximum = ++++++; • more = +++++; • very common = ++++;• common = +++;• rare = ++;• absent like = +;• absent= -.

In case of **Tolypotricoid Branching** *i.e.* single pseudobranching ***Tolypothrix distorta*** developed more single pseudobranching as compare to ***Tolypothrix byssoidea*** in nitrogen free medium.Increasing concentrations of nitrogen decreasing the pseudobranching and in ***Tolypothrix byssoidea*** upto 30 mM concentrations dedeloped single pseudobranching but ***Tolypothrix distorta*** can develop single pseudobranching upto 40 mM concentrations on nitrogenous medium.

Table-3. ***Tolypothrix byssoidea*** and ***Tolypothrix distorta*** showed the development of **Scytonemoid** branching in different concentrations (00mM, 20 mM, 30 mM, 40 mM, 80 mM) of nitrate nitrogen medium.

Concentrations	Scytonemoid Branching	
	Tolypothrix byssoidea	***Tolypothrix distorta***
00 mM	+++	+++
20 mM	++	++
30 mM	++	++
40 mM	**+(-)**	**+(-)**
80 mM	**+(-)**	**+(-)**

• maximum = ++++++; • more = +++++; • very common = ++++;• common = +++;• rare = ++;• absent like = +;• absent= -.

In case of **Scytonemoid Branching** *i.e.* geminate pseudobranching, ***Tolypothrix distorta*** *and* ***Tolypothrix byssoidea*** developed more geminate pseudobranching in nitrogen free medium. Increasing concentrations of nitrogen decreasing the pseudobranching and in ***Tolypothrix byssoidea*** upto 80 mM concentrations developed geminate pseudobranching but ***Tolypothrix distorta also can developgeminate*** pseudobranching upto 80 mM concentrations on nitrogenous medium.

4. Discussion

Fritsch (1945) also pointed out that segregation of these two genera on the basis of relative frequency of single or paired branches were not satisfactory. Bharadwaja (1934) concluded that in ***Scytonema,*** formation of single or geminate false branches, occurs by the side of dead cells, two-pored heterocytes or dead cells adjoining such heterocytes and that in ***Tolypothrix,*** formation of single branches occurs by the side of one pored heterocytes or dead cells adjoining such heterocytes. In ***Tolypothrix*** false branches similar to ***Scytonema*** are also present.

Desikachary (1948) studied ***Camptylonema indicum*** and ***Camptylonemopsis lahorense*** and found that ***Camptylonema indicum*** with true branching as well as false branching is a member of Stigonemataceae. But ***Camptylonema lahorense*** with only false branching is a member of Scytonemataceae, he renamed ***Camptylonema lahorense*** **as** ***Camptylonemopsis lahorensis*****. Pandey and Mitra (1965) and Pandey and Mitra** (1959a, 1959b) made a comparative study of ***Scytonemapraegnaus, Scytonema ocellatum, Tolypothrix nodosa, Tolypothrix tenuis, Tolypothrix aerenophila*** and ***Camptylonemopsis lahorensis*** and they found that young stages of different members of Scytonemataceae show crescent-shaped young stages comparable to the genus ***Camptylonemopsis*****.** They further suggested that ***Camptylonema lahorensis*** may be the young stage of ***Tolypothrix aerenophila*****.** Prasad and Srivastava (1965) studied two species of ***Tolypothrix*** viz. ***Tolypothrix magna***and ***Tolypothrix limbata*** and found that several false branches were formed at one place or one after other in uniparous and biparous arrangement.

Anand and Gunaseeli (1978) studied effect of inorganic nitrogen source on the taxonomy of the genus ***Tolypothrix*** and they found the amount of combined NO_3 nitrogen in the medium is directly related with the occurrence of geminate (Scytonemoid) branches and inversely related with the occurrence of single (Tolypotricoid) branches. Anand and Gunaseeli (1978) further studied both the genera ***Scytonema*** and ***Tolypothix*** in different culture media. They found that ***Tolypothrix*** strain could be identified as ***Tolypothrix distorta*** or ***Tolypothrix bouteillei*** or ***Tolypothrix tenuis*** or ***Tolypothrix limbata*** depending on growth in different media and in the same way Scytonema strain could be identified as ***Scytonema cincinnatum*** **or** ***Scytonema coactile*****.**

Komarek and Anagnostidis (1988) emphasized the mode of germination of hormogonium either it is isopolar or it is heteropolar. On this basis, ***Scytonema***has been retained in the family **Scytonemataceae and Tolypothrix and Camptylonemopsis** have been transferred into Microchaetaceae along with the genus Microchaete. The distinction of the family Scytonemataceae is on the basis of isopolar germination and that of Microchaetaceae is on heteropolar germination of hormogonia. They further emphasized that branching is obligatory for Scytonemataceae but facultative in case of Microchaetaceae (***Tolypothrix, Camptylonemopsis*** and ***Microchaete*****)**.

Komarek and Anagnostidis (1988) referred the activities of the International Association for cyanophyte Research (IAC) and suggested the need of more intransive study under various cultural conditions and not merely on the basis of the natural material. Thus nobody had attempted to study under cultural conditions and described the only those characters which were present in their collected materials. According to Komárek in (1992) Generic name of ***Tolypothrix*** Kützing ex Bornet et Flahault 1888. Ann. Sci. Nat. Bot., ser. 7, 5: 118 and developed **Synonymous strains :** SCLEROTHRIX Kützing, 1833. Alg. Aq. dulc., Dec. 2. no. 17. [TOLYPOTHRIX Kützing, Phycologia generalis, p. 227, 1843 ex Bornet et Flahault, 1888: ***Conferva, Oscillatoria, Scytonema, Calothrix, Sclerothrix, Hypheothrix, Lyngbya*** spec **And his observations of Taxonomic position and higher hierarchy were Cyanophyceae, Nostocales, Microchaetaceae, Tolypotrichoideae. At last his critical analysis about the genus *Tolypotrx* and concidered only 44 species an 17 unclear taxa.**

J.Komarek etall. (2014) defined recently all cyanobacterial genera (some still invalid) are listed in the family to which they are likely to belong and an indication is given of their taxonomic validity and level of polyphasic characterization of each genus. Inspite of their observations under cultural conditions, only characters present in natural materials and not convencing to distinguish them to each other.

Thus the most of the described species are similar to measurements and in other so many characters. In case of ***Tolypothrix lophopodeophila*** West and ***Tolypothrixceylanica*** Schmidle, the size of filaments, vegetative cells, trichomes and heterocyte has not been mentioned. It is interesting that these two species have not been reported second time from India. It indicates that most of the later workers were much particular about the measurements and it also indicates that characters other than measurements are not well defined. The whole classification of cyanobacteria (species, genera, families, orders) has undergone extensive restructuring and revision in recent years with the advent of phylogenetic analyses based on molecular sequence data. Several recent revisionary and monographic works initiated a revision and it is anticipated there will be further changes in the future. Taxonomy has been transformed from a system that simply placed morphologically similar taxa into a hierarchical system of classification that ideally reflects evolutionary relationships and creates a network of hypotheses about evolutionary history.

5. Conclusion

Taxonomic classification is the primary method used to evaluate the diversity of all biological groups of organisms. Criteria for classification have continually changed over the years since Linnaeus conceived his scientific system. Traditional taxonomy based on morphologic criteria has been questioned by several authors because of the gap lack in the variations observed in the nature and in laboratory conditions. According to our present study, need cultural study for proper identification upto species lebel but in past very much confused all the researchers due to previous descriptions. Thallus growth of ***Scytonemataceae***, has been designated by a number of terms e.g. caespitose, thin gelatinous, mucilaginous, crustaceous, cushion-shaped, floccose, papyraceous, spongy, woolly, caespitose globose, spongy, free floating, cushion, cushion soft and wrinkled in various combinations etc. It is well known and recorded that these various species of ***Tolypothrix*** genus were collected from various habitats on moist soil, submerged wall, rocks, stones, sand stones, damp soils, paddy fields, side of tanks, side of walls, cultivated soil, aquatic plants ,on trees with moss, free floating in stagnant water or fresh water etc. Some species which has special type of thallus is mostly reported from all types of habitats. But according to the present observation under special circumstances alga forms a special type of thallus otherwise its thallus shape varies according to variation in their habitats**.** Therefore, except a few species, all the described species have differences like present observations which certainly change according to change in different medium composition and age of alga. So, need of more intransive study under various cultural conditions and not merely on the basis of the natural material. Thus nobody had attempted to study under cultural conditions and described the only those characters which were present in their collected materials. The present observations prove that similar alga has more broad filament and trichomes, less hormogones, more geminate and less single branching, less heterocytes, more constriction at the cross walls and discoid cells in nitrogen rich medium as compared to nitrogen deficient medium. It also proves that they show further different characters under solid condition than liquid condition. It is observed that all strains have 20- 60% (-80%) narrower filaments and trichomes in young growth than old growth. Finally a

Table-4: Segregation of Species Desikachary (1959) of the Genus *Tolypothrix* based on the Present Analysis

Gr.	Name according to previous workers	Thallus		Filaments		Trichome	Sheath		False Bran-ches	Anyspeci-al featur-es	Place of collection
		Texture	Colour	Length	breadth («m)	breadth («m)	Texture	Colour			
I	***Tolypothrix bouteillei* (Breb,et Desm) Forti (1907)**	**Caespitose, circular**	**Blackish brown**	**1 mm**	**5 – 7**	**4 – 5**	**Thin close to the trichome**	**Hyaline to golden yellow**	**Single with basal heterocytes**	**Heterocytes mostly basal**	**Paddy fields, Bombay**
”	***T .fragilis* (Gardner) Geitler(1932)**	Thin, gelatinou		Short	5.5 – 7	4 – 5.5	Thin	Hyaline			On walls, Banaras
”	***T. nodosa* Bharadw.(1934)**	Mucilagin-ous	blue green to yellow brown		5.2 – 7.3 (–8.4)	4.2 – 9.4	Thin	Hyaline	more single with basal, heterocytes		in ponds, Banaras
II	***Tolypothrix conglutinata* Borzi ex Born. et., Flah. (1879)**	Crustaceous	Blue green or brownish		14 – 18	8 – 10	Thick irregular broadness	Hyaline			Type not recorded
”	***T. limbata* Thuret. (1887)**	Floccose, Caespitose	Blue green	2 – 3 mm	10 – 15	6 – 9	Thick lamellated	Hyaline & yellowish brown		Heterocytes single or in row	Madras
”	***T. Phillophila* west et west (1897)**		Olive green to blackish green	1mm	12.5 – 18	8.5 – 10	thick with lamellated			Basal heterocytes	On pond, Ceylon
”	***T. byssoidea* (Berk) kirchner (1932)**	Woolly, cushion	Brownish or blackish	1mm	10 – 15 (– 17)	9 – 11	Thin	Yellowish to brownish		Spore seen ones	On most soil, Lahore

”	***T. Camptylonemoides*** **(Ghose) (1924)**	Thin, popyraceous			10 – 12	8 – 10	Thin	Yellowish to brownish	More single		On tree trunk, Lahore
”	***T. rechingeri*** **(wille) Geitler (1925)**	Cushion, soft	Brownish olive green	1 – 2 mm	10 – 16	7 – 11		Yellowish to brownish	Basal part single branches		
III	***Tolypothrix arenophila*** **(west et west) (1897)**	Membrane-ous, thin			14.5 – 15 (-18)		Thick lamellated	Yellow to brown	Less	Attenuated at the ends	On damp soil, Lahore
”	***T. menuis*** **(Kuetz) johs.Schmidtm. (1899)**	Caespitose, or cushion	blue green or brown	2cm	(4) 6 – 17 (– 18)	(4) 5– 13	Thin often lamellated	Colourless to yellow-ish brown	Repeatedly	Heterocytes in row	Fresh water N.India
”	***T. Distorta kuetzinge*****x Born et. Flah.**	Caespitose, or cushion + ca incrustation	Blue green or brown	3cm	10 –15	9 – 12	Thin	Hyaline to Brownish	More	heterocytes in row	In water, Lahore
IV	***Tolypothrixforeaui*****. (Fremy1931)**	Caespitose globose	Brown or blackish	1mm	14 – 21	5 – 7	Lamellated and divergent	Golden yellow	More	heterocytes in row	In water
”	***T. magna*** **Bharadwaja (1934)**	Thin	Dirty blue green		23.1–26.7	9.4–13.6	Lamellated and divergent		Short & more single		In tank, Ceylon
V	***Tolypothrix crassa*** **west and west (1907)**	Spongy, free floating	Blackish green		25–27	11.5 – 14.5	Very thick	Dirty yellow	Sparse and short		Ceylon, on bark
”	***T. robusta Gardner*** **(1927)**	Flexuous		1–2mm	22 – 30	12 – 18	Lamellated at oldage	Hyaline brown		Older part narrower	Type not recorded

considerable differences in branching have also been observed in different medium (liquid or solid and nitrogenous or nitrogen deficient medium). More branches were seen in liquid and nitrogenous medium than solid and nitrogen deficient medium respectively.

Most of the earlier described species of the genus ***Tolypothrix*** in Desikachary (1959) have much similarities in several characters to one another and all the description of these forms are based on observations of collected natural materials from distant places or countries. Due to this reason, their described characters are also incomplete. Because original work of a particular type of species included the characters which were present in his material created new species and did not mention other characters. If we make a analysis of mentioned characters of described species in Desikachary (1959), we find only five catagories of the species which are as follows in groups (Table 4).

GROUP-I: With 5-8 µm broad filaments, and thin and unlamellated sheath without attenution at terminal ends. (*Tolypthrix* nodosa Bharadwaja, *T. bouteillei* (Breb. et Desm.) Forti, *T.fragilis* (Gardner) Geitler and *Tolypothrix nodosa* Bharadwaja).

GROUP-II: With 10-18 µm broad filaments, thick and lamellated sheath (*T. conglutinate* Borzi ex Born. et Flah., *T. limbata* Thuret with its variety *cylindrica* Ghose, *T. phylophila* West et West, *T. byssoidea* (Berk.) Kirchner, (*T. Camptylonemopsis* Ghose, *T. rechingeri* (Wille) Geitleri and *T. conglutinata* var. *colorata Ghose*).

GROUP-III: With 10-18 µm broad filaments, thick lamellated sheath and attenuated terminal parts (*T. arenophilla* West et West *T. tenuis* kuetz. Johs. Schmidt. em.,*T. distorta* Kuetz ex. Born. et Flah. including its variety and *penicillata* (Ag.) Lemmermann).

GROUP-IV: With 14-26.7 µm broad filaments and divergent lamellation in sheath (*T. foreaui* Fremy and *T. magna* Bharadwaja) and

GROUP-V: With 14-30 µm broad filaments, thick and parallel lamellated sheath (*T. crassa* West and West.,*T. robusta* Gardner). Except these species *T. lophopodellophila* West, *T. ceylenica* Schmidle and *T. binata* Zeller are with incomplete description due to this reason they could not segregate according to above grouping of these species.

Conflict of Interest

The authors of this paper have no conflict of interest.

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