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SEASONAL BIODIVERSITY OF CYANOBACTERIAL FLORA FROM BILASPUR DISTRICT OF CHHATTISHGARH

Shrivastava A. K. and Shrivastava D. K.*
PG Dept. of Botany and Microbiology, Govt. Danvir Tula Ram College, Durg, Chhattisgarh
*PG Dept. of Botany and Microbiology, Govt. ERRV College, Bilaspur, Chhattisgarh

ABSTRACT :

About 48 cyanobacterial stains were isolated from pasture land; pound and paddy fields of different locality of Bilaspur district which was divided into ten sectors for two successive years and strains were identified as Nitrogen fixer and non-nitrogen fixer. It was interesting to note that there is reoccurrence of cyanobacterial flora throughout the year in a rhythmic way. The thallus organization of non - nitrogen fixture shows rich biodiversity from unicellular to filamentous through colonial form, while nitrogen fixer never seen in unicellular organization rather they are prevalent in colonial and filament form.

KEY WORDS : *Cyanobacteria, Nitrogen fixer, Colonial, Pasture land, Pound and Rice field*

INTRODUCTION:

Nitrogen (N) is building blocks (proteins) and hereditary units (Nucleic Acids) inside an organic body. In atmosphere besides 79% of Nitogen (Kumar, 1991) the capacity to convert them into assemble form like ammonia and nitrate is only present in few bacteria and cyanobacteria. This organism shows oxygenic photosynthesis and oxyphobic Nitrogen fixation like property which make them unique. They are present since rock period and still they are surviving is another peculiar future to them (Fogg, *et al.*, 1973, Doolittle, 1974).

With the exception of water, nitrogen is the greatest limiting factor in agriculture and the nutritional wellbeing of the people depends on it in large areas of the world rather directly dependent on the soil nitrogen of agricultural lands, which support them. Plants generally absorb nitrogenous compounds in the form of NO_3^- (nitrate ion), NH_4^+ (Ammonium ion). Plants and microbes use ammonia as a building block for the synthesis of amino acids and other organic nitrogenous compounds. The organic nitrogen is a major constituent of all the living beings, as integral components of proteins, nucleic acids, Vitamins and several other molecules. In agricultural soil, NO_3^- or NH_4^+ is usually supplied through the application of synthetic N-fertilizers.

In dinitrogen (N_2) molecule, the two atoms are held together by a tight triple bond, when this bond is broken and the single atoms incorporated into another usable molecules, *i.e.*, NH_3 (Ammonia), the

Corresponding Author :
A. K. Srivastava
E-mail : aksbotany@gmail.com
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process is called nitrogen fixation. Industrially, nitrogen is fixed for the manufacture of synthetic N - fertilizers through Haber - Bosch process, requiring hydrogen (from methane and natural gases), very high temperatures and enormous amount of energy (consumed substantially from our limited and valuable fossil fuel, *i.e.*, petroleum reserves). In view of the gross expenditure, the synthetic N - fertilizers becomes rather expensive and beyond the reach of the most small a poor formers in developing countries.

It is here that a need for the search, preservation, protection and development of our natural nitrogen resources, *i.e.*, the self-renewable nitrogen - fixing microorganisms has been realized. This has stimulated vigorous global researches of biological nitrogen fixation during the last 4 - 5 decades offering increasing interest among biologists, about the various fascinating aspects of biological nitrogen fixation, with the focal objective to considerably reduce the unprecedented chemicalization of our cultivable lands with the expensive synthetic N-fertilizers. The scientific concern in this context becomes further grave not only due to the huge cost of the industrially manufactured N - fertilizers, but also because of the truth that heavy dose of a combined nitrogen (nitrate, ammonia or urea fertilizers) is liable to hamper the N_2 - fixing activity of the microbes in soil . This is in response to the self-regulated interest properly of the N_2 - fixers by virtue of which their N_2 - fixing machinery is automatically switches off on sooner the biological production of ammonia exceeds its biological consumption (Gordon and Brill, 1972; Evans and Barber, 1977). The gradual transformation of our natural ecosystem into eutrophic habitats as a result of large scale synthetic fertilizer application has also been shown (Evans and Barber, 1977). These growing realizations have stimulated the recurrently expanding researches on biological nitrogen fixation in a wide variety of N_2 - fixing test materials throughout the world.

The majority of Cyanobacteria that fix atmospheric nitrogen utilizing photosynthetic energy produced by their own photo-pigment apparatus have been attractive organisms as low cost N_2 fixers in wet fields (Venkataraman, 1981).

India is basically a rice-growing country and there is, thus a prevalence of paddy fields in larger parts of the country. Growing paddy fields have slightly alkaline in water-logged condition. Enlodge the luxuriant flora of N_2 - fixing Cyanobacteria (Fogg, *et al.*, 1973), Which contribute significantly to the soil nitrogen economy of rice agriculture in India (Venkataraman, 1972, 1981) and other tropical countries (Roger and Kulasooriya. 1980; Singh and Singh, 1989; Watanabe and Yamamoto, 1967). On the basis of above facts this study offers a good scope to identify the rich Cyanobacterial flora of many of the unexplored fields for using them as a viable bio fertilizer.

MATERIALS AND METHODS:

The Bilaspur District territory, as well as those belonging to adjoining parts of the neighbor districts and state have been surveyed for the collection of the various nitrogen-fixing and non-nitrogen-fixing Cyanobacterial species. The entire survey area has been divided in to ten different zones in accordance to the three different types of sampling sites. In

general all the ten zones i:e., Zone - I, Zone-II, Zone-III, Zone-IV, Zone-V, Zone VI, Zone VII, Zone VIII, Zone IX and Zone-X were subdivided into three different sites each, i.e., (a) faddy fields (b) Ponds & tanks (c) Small ditches and pasture fields.

The cyanobacteria were collected throught the year round from three sites each of the ten different survey zones, and repeated in the subsequent year. However, data on temperature, relative humidity and rainfall has been recorded for the first year of survey. Collections were invariably made in separate screw cap slant tubes. The native water and soil were collected for preparing soil extracts for the culture of the respective Cyanobacterial forms, in case the organisms did not respond well to the synthetic media. Identification of Nitrogen fixer and Non Nitrogen fixer strains:

The collected samples of Cyanobacterial species were separated manually and prepared as homogeneous suspension with help of glass beads. The homogeneous Cyanobacterial suspensions were diluted with the suitable medium/soil extracts/ pond water and 0.1ml of this dilute suspension was streaked on the same medium/soil extract/pond water, gelled with 1.2% agar-agar, poured in Petri dishes. For the isolation of N_2 - fixing forms, the medium was left unsupplemented with any combination in organic nitrogen source in order to force a situation where non - N_2 - fixing contaminant (green or blue green other algae, or fungi) should automatically be weeded out. But for the isolation of non N_2 fixing cyanobacterial forms supplementation with the prescribed combined inorganic nitrogen source was essential in the selected medium. Those non - N_2 - fixing forms being isolated on soil extract or pond water (gelled with agar) were over layered with the thin layer of the same gelled medium containing 5 mM KNO_3 as a combined nitrogen source. The Petri dishes were incubated for a fortnight in the growth chamber for the growth of the visible cyanobacterial colonies.

The individual colonies coming up after about a fortnight of incubation were located on the surfaces of agar-medium. These colonies were sucked up into sterile fine glass capillaries (One Colony by each) whose tips were broken into tubes (one colony in one individual tube) containing 5ml of the sterile growth medium/soil extract/ pond water and incubated for their culture in liquid medium. Every individual culture was observed under microscope aseptically to trace out the contaminants, if any. So long as contaminants were not removed completely, the process of streaking the dilute suspension of the immediate previous culture on fresh agar medium and sucking up the clean colonies for their growth in fresh liquid medium was repeated time and again.

Morphological Characterization of strains:

By regular transferring the exponential phase cultures to fresh media, the clonally cultures were maintained in the axenic state. Morphological observation of isolated cyanobacteria forms were made through Compound light microscope and Micro imaging photography system (MIPS), Model No.- ML - TR (Olympus). Based On morphological characterization, the systematic identification of the cyanobacterial species were characterised.

RESULTS AND DISCUSSION:

48 strains of cyanobacteria were isolated from different habitat of Bilashpur district of Chhattishgarh state (Table -1). This shown their occurrence in the district as well as their neighboring areas. The survey was done regoursely throughout the year (Table -2) at regular and frequent interval shown a clear cut diversity and location in specific area make it very interesting. As per their morphological thallus organization it has shown three common diversities (Table - 3). The identification and differentiation between nitrogen fixer and non-nitrogen fixer strains by supplementing with nitrogen with or without nitrogen supplement give a clear cut differentiation make physiological characterization of nitrogen fixation.

Table - 1 : A comprehensive list of Cyanobacteria collected from different study sites of the ten survey zone.

Survey area		Cyanobacteria collected and identified
Survey zones	Study sites	
I	**a**	*1. Aphanothece microscopica; 2. Gloeothece samoensis; 3. Anabaena variabilis; 4. Anabaena spherica; 5 Nostoc hatei; 6. Phormidium stagnina; 7. Oscillatoria splendida;*
	b	*8. Aphaenocapsa crassa; 9. Microcystis aeruginosa; 10. Oscillitoria princeps*
	c	*2.; 8.; 10; 11. Chroococcus micrococcus*
II	**a**	*3.; 5.; 10; 12. Aphanocapsa banariensis; 13. Anabaena iyengarii; 14. Anabaena oryzae; 15. Synecoccus aeriginesus; 16. Oscillitoria okeni*
	b	*1.; 6.; 7.; 9.; 10.; 15.; 16.; 17. Aphaenocapsa koordersi*
	c	*1.; 4.; 7.; 10.; 11.; 15.; 17.; 18. Phormidium retzii*
III	**a**	*2.; 3.; 6.; 8.; 11.;13.;14.;15.;18.;19.Aphaenothece saxicola;20. Anabaena ambigua; 21. Scytonema bohneri;*
	b	*6.;8.;11.;15.;19.; 22. Microcystis pseudofilamentosa.*
	c	*1.; 3.;5.;8.;9.;10.;15.;16.;19.;21.;22.; 23. Lyngbya martenciana;*
IV	**a**	*2.; 6.; 8.; 11.; 14.,15.;19.;21.;24.Aulosira prolifica; 25. Anabaena doliolum; 26. Gloeotrichia echinulata; 27. Nostoc ellipsosporum; 28. Nostoc spongiaeforme;*
	b	*6.; 8.; 9.; 16.; 19.; 23.;*
	c	*2.; 7.; 12.;15.;18.;23.;27.;29. Synechocystis aquatilis;*
V	**a**	*3.; 6.;10.;11.;14.;15.;17.;19.;21.;27.; 30. Gloeocapsa luteo-fusca; 31. Cylindrospermum indentatum;*
	b	*7.; 9.;19.;22.;23.;32. Phormidium calcicola;*
	c	*6.; 8.;16.;23.;29.;33. Lyngbya shackletoni;*
VI	**a**	*2.; 14.;17.;18.;19.;20.;24.;26.;32.;34. Anabaena cariabilis var. kashiensis ; 35. Calothrix elenkinii;*
	b	*7.; 22.;29.;33.;36.;33.;36 Microcystis robusta*
	c	*8.; 11.;19.;21.;33.;36.;*
VII	**a**	*3.; 5.; 15.;19.;20.;25.;26.;28.;29; 37.Lyngbya birgii; 38. Anabaena anomala;*
	b	*6.; 23.;33.;36.;*
	c	*1.; 4.;6.;11.;16.;39.Nostoc carneum;*
VIII	**a**	*2.; 8.;15.;16.;21.;24.;32.;35.;39.; 40. Nostoc calcicola; 41. Scytonema stuposum 42. Tolypothrix robusta*
	b	*6.; 7.;9.;22.;29.;33.;43. Oscillatoria prolifica;*
	c	*1.; 5.; 19.;36.;39.;44. Chroococcidiopsis indica;*
IX	**a**	*4.; 10.;14.;25.;26.;31.;34.;35.;42.; 45;Aphaenothece naegelii; 46. Gloeothece rhodochlamys; 47. Nostoc muscorum; 48. Rivularia aquatica;*
	b	*6.; 16.; 19.;22.;29.;33.;36*
	c	*1.; 7.; 12.; 23.;29.;33.;44.;46.;*
X	**a**	*2.; 6.; 11.;14.;19.;24.;25.;27.;32.;34.;40.;46.;48.;*
	b	*9.; 1.; 15.;19.;22.;43.;*
	c	*1, 4.; 6.;11.;16.;23.;29.;44.;46.;*

Table - 2 : Numbers of Cyanobacteria available in the different months of the first year (2012-2013) of the survey.

		Months (March -2012- feb.2013)											
Survey zone	Study site	March	April	May	June	July	August	Sep	Oct	Nov	Dec	Jan	Feb
I	a	-	-	-	-	1,6,7	1,4,6,7	1,2,3,4,	1,2,3,4,	2,3,4,5,	2,3,6,	6,7	6,7
	b	6,9,22,	6,9,10,22	9,22	9,22	9,10,22	6,8,9,10,22	6,8,9,10, 22	6,8,9,10, 22	6,9,10,22	9,10,22	9, 10, 22	6,9,1 0,22
	c	--	--	--	--	10,12	10,11,12	2,8,10,11,	2,8,10,12	2,8,10	8,10	8, 10	-
II	a	-	-	-	-	-	14,15,	,14,15,16	5,10,12, 13,14,16	5,10,12,	5,10	10,	-
	b	9,10,22	9,22	9,22	9,22	9,22	1,6,7, 9,22	9,15,16,17 ,22	1,6,7,9,10, 22	9,10 ,22	9,10,22	9, 10, 22	9,10, 22
	c	-	-	-	-	-	11,15,17,18	,10,11,15,	1,10,11	1,10,11	-	-	-
III	a	-	-	-	-	-	15,19,20	2,3,6,13	14,15,18,	8,14,15,	,20,21	6,18	6
	b	9,11,22	9,11,22	9,6,22	9,6,22	9,6, 19,22	6,8,9,15, 19,22	6,8,9,11, 15,19,20, 22	6,8,9,11, 15,19,22	9,11,19, 15,22	9,6,19 22	6,9 11,2 2	2,9,2 2
	c	9,10 ,23	9,22	-	-	10, 19	1,3,5,815,1 6,19,21,23	1,3,5,815, 16,19,21,	1,3,5,815, 16, ,21	5,815,16,1 9,21,23	10	9	-
IV	a	6	-	-	-	-	2,11,14,15, 19,21,24,25	3,10,11,14 ,15	11,14, 15,19,27,	11,14,15,1 9,27,	19	6	6
	b	6,9,22	6, 9,22	6, 9,22	9,22	9,22	8,9,16, 19,22	8,9,19,22 23	9,16,19,22 23	9,19,22,23 ,	9,22	6, 9,22	6, 9,22
	c	-	-	-	-	-	2,27,29	7,12, ,27,29	,7,12, 1518,23	1518,23,2 7,29	-	-	-
V	a						2,8,11, 14,15,19,21						
	b	,9,22	9,22	9,22	9,22	9,22	7,9,19,22,3 2	7,9,19,22,	7,9,19 ,22,32	9,22,23,32	7,9,19, 22	9,22	9,22
	c	9	9,23	9	-	-	7,9,19,22,	7,9,19,22,	7,9,19, 22,23,	19,22,23,	19,22,2 3,32	9	9
VI	a	-	-	-	-	--	2,14,17,19, 20,24,32	2,14,17,19 ,20,24,	17,19,20,2 4, ,32,34	24,26,32,	20,24,	-	-
	b	9,22	9,22	9,22	9,22	9,22	9,22,33,	7,9,,22,29, 33,	7,9,22,29, 33,	7,9,22,29, 33,	7,9,22	9,22	9,22
	c	11,19	-	-	-	-	8,19,21,33,	,19,21,33,	8,,19,21	8,19,21,33	11,19	11	,11
VII	a	-	-	-	-	-	19,	15,33,37,3 8	3,5,15 20,25,	3,19,20,2, 28,33,	3,5,15, 19,20	-	-
	b	6, 9,22	6, 9,22	9,22	9,22	9,22	9,22,23,33,	9,22,23,33	9,22,23	9,22,23,33	9,22,33	6,9,2 2	6,9,2 2
	c	6,	-	-	-	-	1,4, 11,16,	1,4, 16,29	1,4,16, 29	1,4,11, 16,29	1,4,6,	6	6
VIII	a	8	8	-	-	-	2,8,15,21,2 4,32,35,39,	2,8,15,21, 24,32,35,	24,32,35, 39,40,41,	24,32,35,3 9,40,41,42	21,24,3 2,	8	8
	b	6, 9,22	6, 9,22	6, 9,22	69,22	9,22	6,7,9,22,29, 33,43	9,22,24,32 ,35,39,40, 41,42	9,22,24,32 ,35,39,40, 41,42	9,22,24,32 ,35,39,40, 41,	9,21,22 ,24,	6,8, 9,22	6,8,9, 22
	c	-	-	-	-	-	39,44	5,19,,39	19,,39,4	1,5,19,	1,5,19	-	
IX	a	-	-	-	-	-	25,26,31,34 ,35,42,45	4,10,14,25 ,26,31,34, 35,42,45	4,10,14,25 ,26,31,34, 35,42,45	4,10,14,25 ,26,31,34,	25,26,3 1,34,	-	-
	b	9,22	9,22	9,22	9,22	9,22	9,22,46,47, 48	9,22,46,47 ,	9,22,46,47 ,	9,22,46,47 ,	9,22	9,22	9,22
	c	-	-	-	-	-	1,7,12,	1,7,12,23, 29,33,44	1,7,12,23, 29,33,44,	1,7,12,23, 29,33,	1,7,12, 23,29	-	-
X	a	-	-	-	-	-	-	2,6,11, 19,	27,34,40,4 6,48	2,6,1114.; 19,24	11,14,	-	-
	b	9,22	9,22	9,22	9,22	9,22	9,22	9,15,19,22	9,15,19,22 43	9,15,19,22 ,43	9,15,19 22,	9,22	9,22
	c	-	-	-	-	-	-	1,4,6,11,1 6,23,	1,4,6,11,1 6,23,29,44	16,23,29,4 4,46	1,4,6, 11,16,	-	-

Table3 : Morphological dissimilar Cyanobacteria found during the survey

	Unicellular	Colonial	Filamentous
Non – Nitrogen fixing	*1. Aphaenocapsa banariensis* *2. Aphaenocapsa crassa* *3 Aphaenocapsa koordersi*] *4 .Aphaenothece microscopica* *5. Aphaenothece naegelii* *6. Aphaenothece saxicola* *7. Chroococcus macrococcus* *8. Chroococcidiopsis indica* *9. Gloeothece rhodochlamys* *10. Gloeothece samoensis* *11. Synechocystis aquatilis* *2 Synecoccus aeriginesus*	*1..Microcystis aeruginosa* *2..Microcystis pseudofilamentosa* *3.Microcystis robusta*	*1. Lyngbya birgei* *2. Lyngbya shackletoni* *3 .Lyngbya martenciana* *4. Oscillitoria okeni* *5. Oscillitoria princeps* *6. Oscillatoria prolifica* *7. Oscillatoria splendida* *8. Phormidium calcicola* *9. Phormidium retzii* *10.Phormidium stagnina*
Nitrogen fixing	NIL	*1.Gloeocapsa luteo-fusca*]	*1.Anabaena ambigua* *2.Anabaena anomala* *3. Anabaena cariabilis* *4 .Anabaena doliolum* *5. Anabaena iyengarii* *6. Anabaena oryzae* *7. Anabaena spherica* *8. Anabaena variabilis* *9. Aulosira prolifica* *10.Calothrix elenkinii* *11. Cylindrospermum indentatum* *12. Gloeotrichia echinulata* *13. Nostoc calcicola* *14. Nostoc carneum* *15. Nostoc ellipsosporum* *16. Nostoc hatei* *17. Nostoc muscorum* *18. Nostoc spongiaeforme* *19. Rivularia aquatica* *20. Scytonema bohneri* *21. Scytonema stuposum* *22. Tolypothrix robusta*

CONCLUSION:

Above work reveals the rich biodiversity of Nitrogen fixer and Non Nitrogen fixer strains of cyanobacteria in this area. So this is the need of microbiologist to preserve these germ plasm for future.

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