Sub-aerial algal diversity in the biofilms of terracota temples of Bishnupur, West Bengal, India and their Biotechnological potential

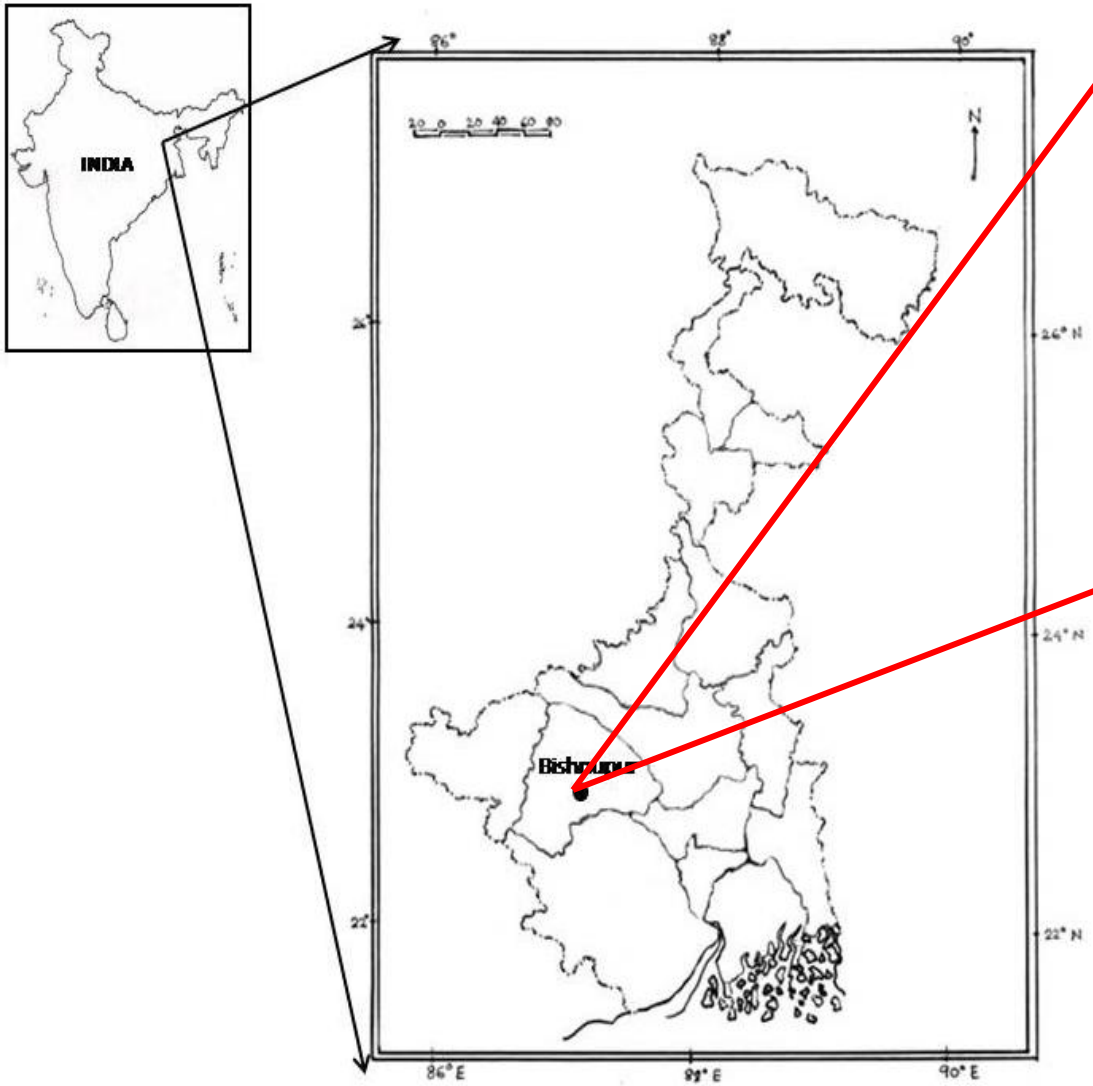
Dr. Jnanendra Rath

Visva-Bharati Culture Collection of Algae (VBCCA, WDCM-931)

Dept. of Botany, Visva-Bharati University

Santiniketan- India

jrath@visva-bharati.ac.in



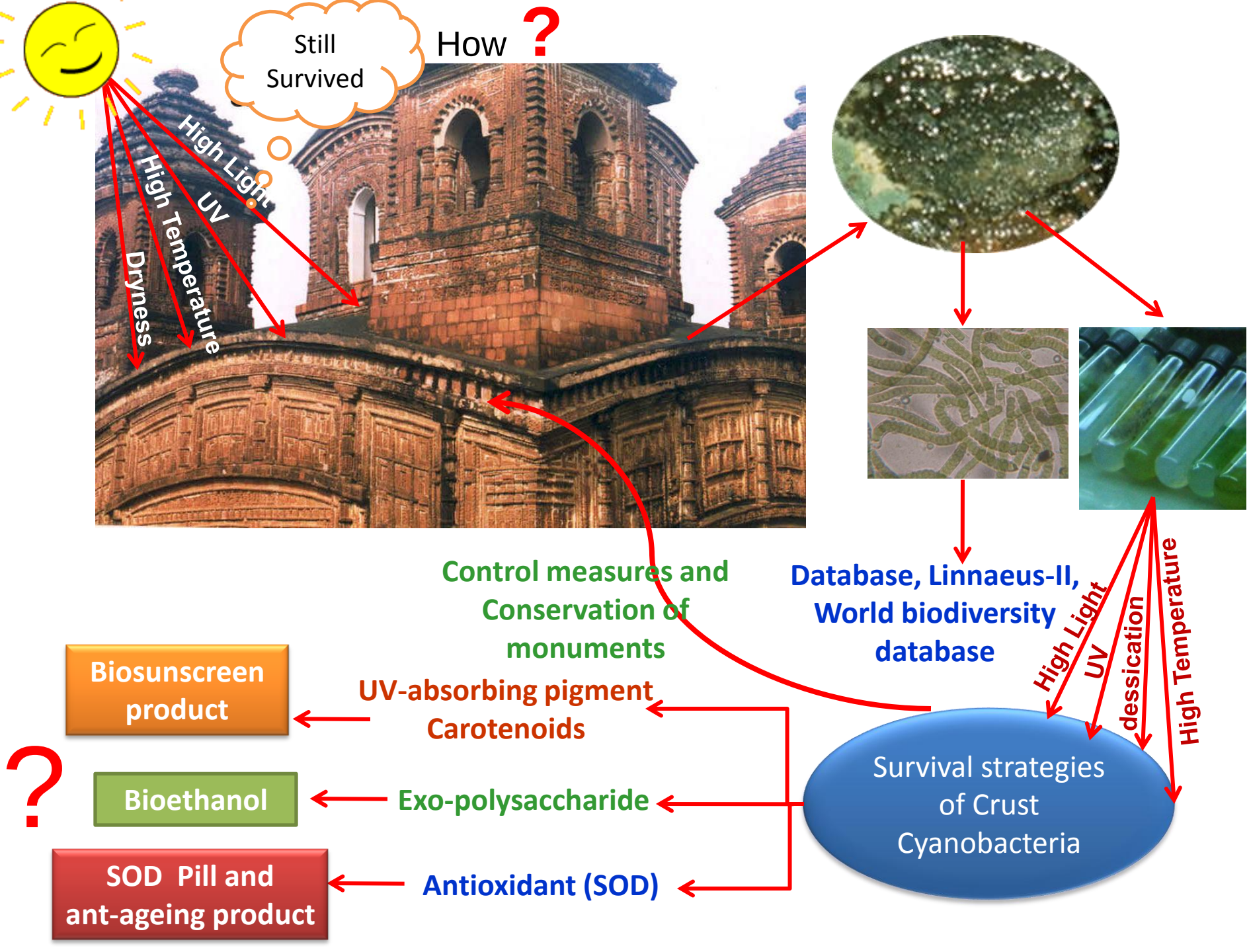
- Bisnupur is famous for its beautiful terracotta temples of 16th century
- Gradually these monuments show alarming symptoms of deterioration due to growth of biological forms in the form of thick mat/crust or biofilms on their surfaces
- During summer the temperature on the monuments rock surfaces goes beyond 60^0 C, coupled with high light intensity and extreme dryness, however in such inhospitable environment certain species of cyanobacteria were found to survive

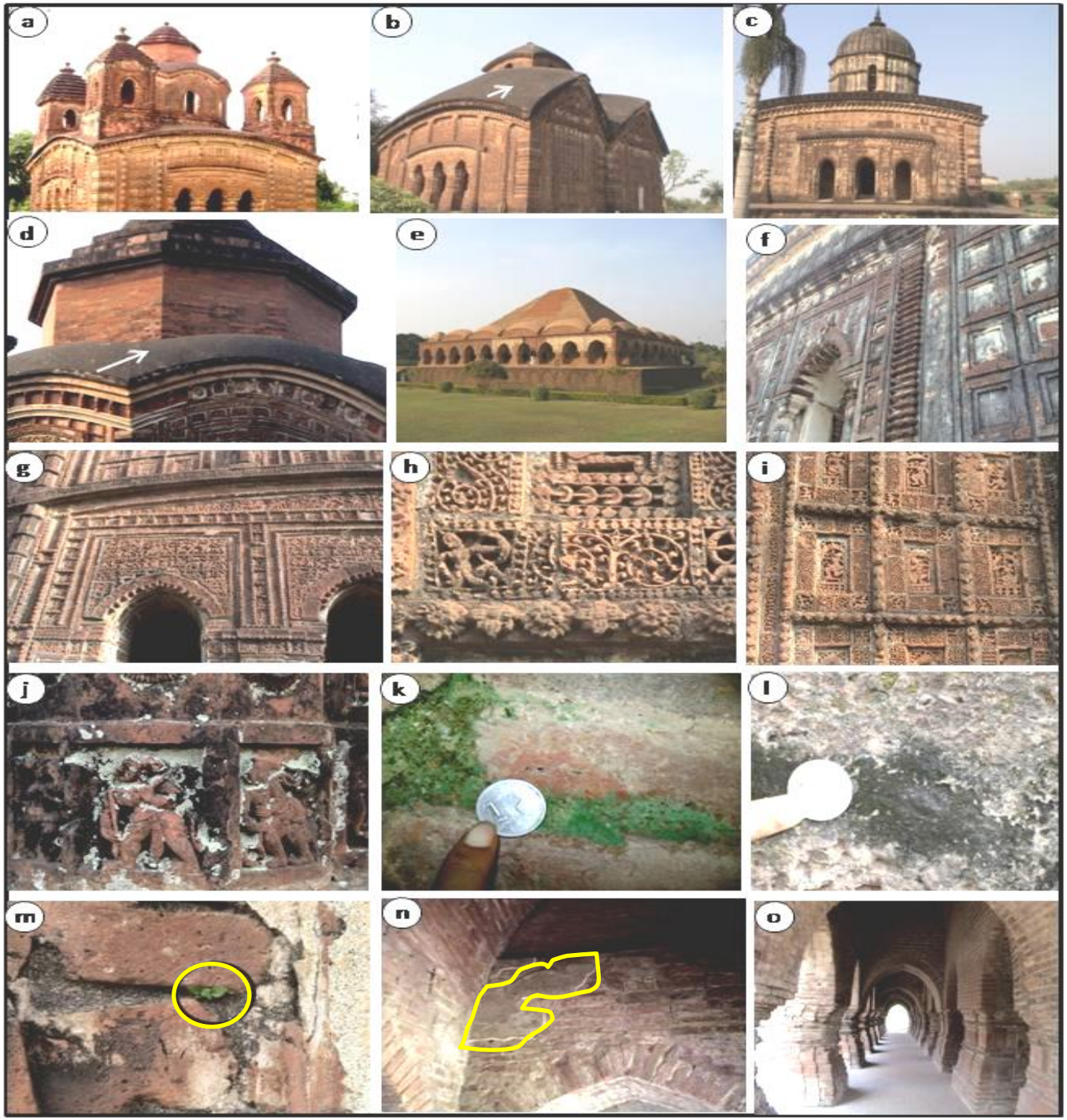
Crusts / mats:

- A crust is a result of intimate association between the substrate and the microorganisms which live within or immediately on the top of the uppermost layer of the substratum know as microbial crust.

Microbial crust in monuments:

- Most of these archaeologically important monuments look blackish-brown due to excessive growth of the Cyanobacteria as crusts/ mats creates problem for conservation of the ancient monuments around the world.
- The organisms in the crust liberated organic matter to the substratum which helps in the growth of other micro-organisms.
- Our work showed that the microorganisms forming the biofilms on sub-aerial surfaces in tropical zones like India (also in Mexico and Brazil) principally composed of filamentous sheathed nitrogen fixing cyanobacteria where as temperate region (North America, UK, France, Germany, Austria, Italy, Spain, Czech Republic, Slovakia and Ukrine) they are principally green micro-algae intermingled with lichens.


Still Survived
How ?
High Light
UV
High Temperature
Dryness
Control measures and Conservation of monuments
Database, Linnaeus-II, World biodiversity database
High Light
UV
dessication
High Temperature
Biosunscreen product
UV-absorbing pigment Carotenoids
?
Bioethanol
Exo-polysaccharide
SOD Pill and ant-ageing product
Antioxidant (SOD)
Survival strategies of Crust Cyanobacteria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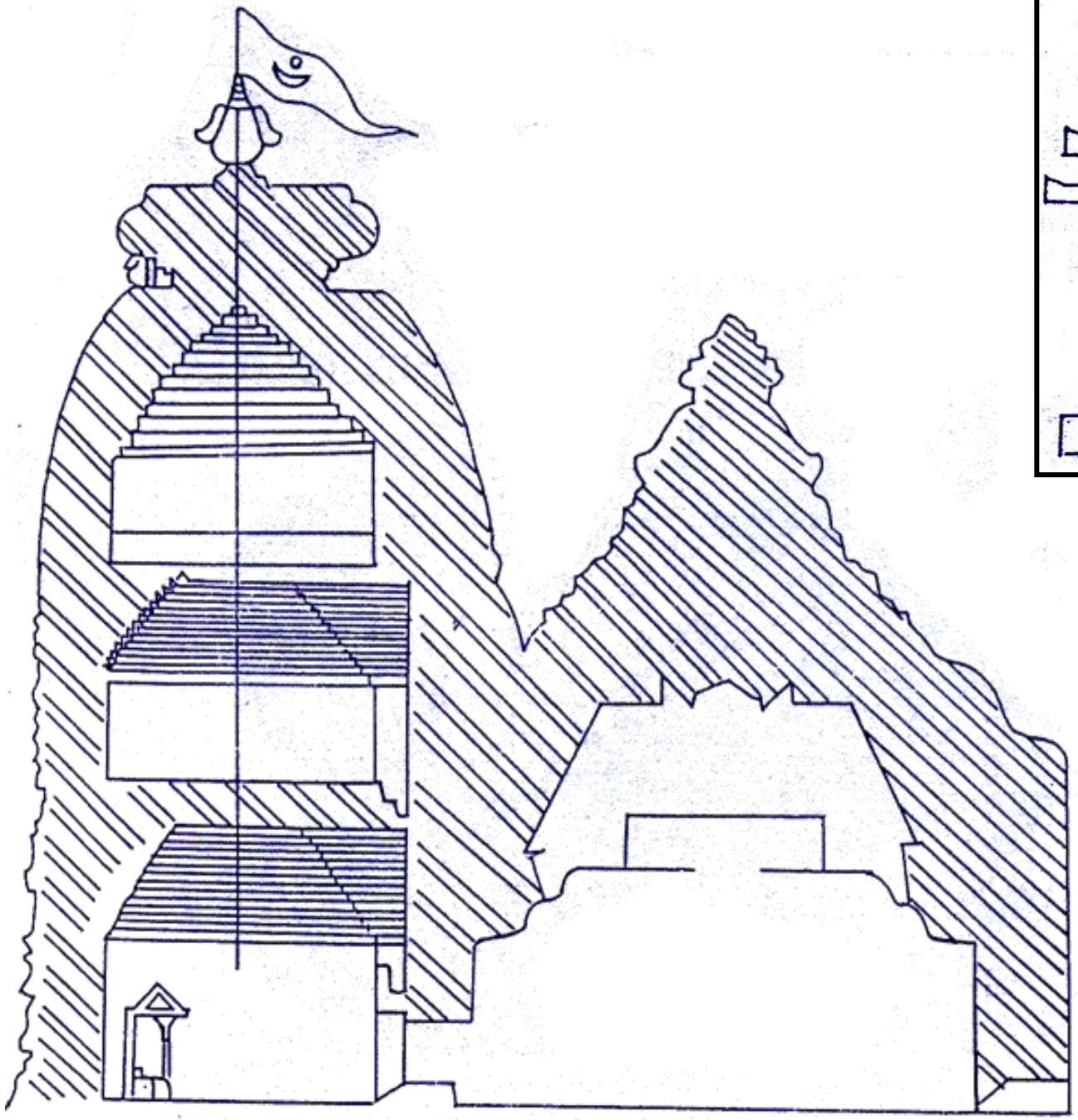
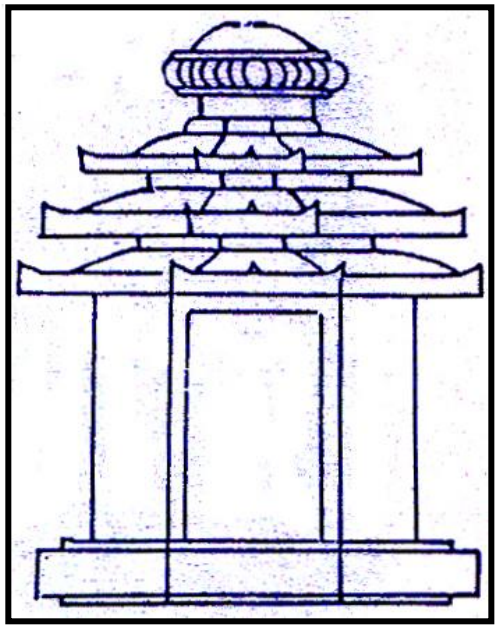
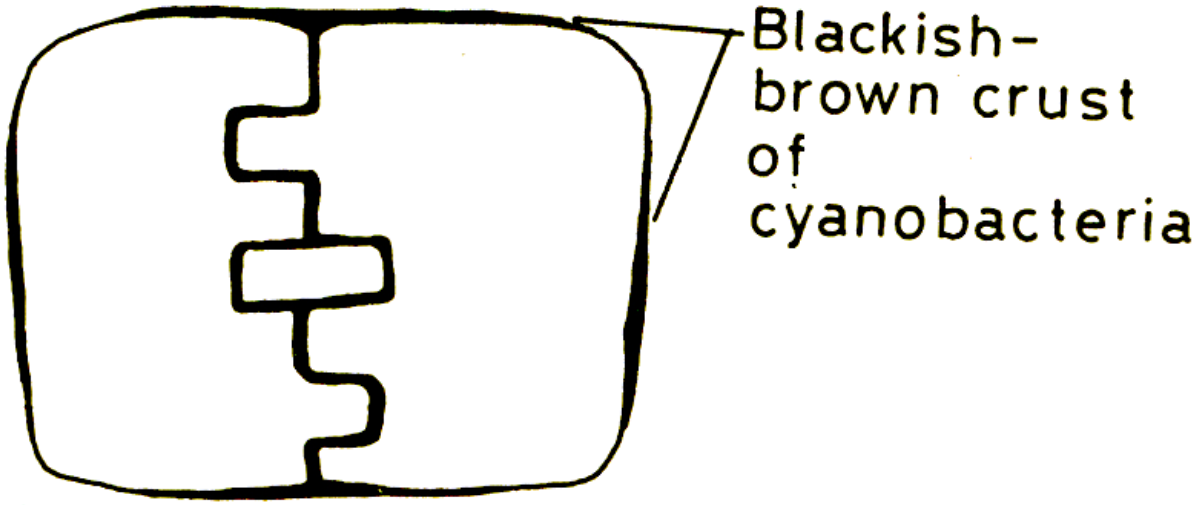
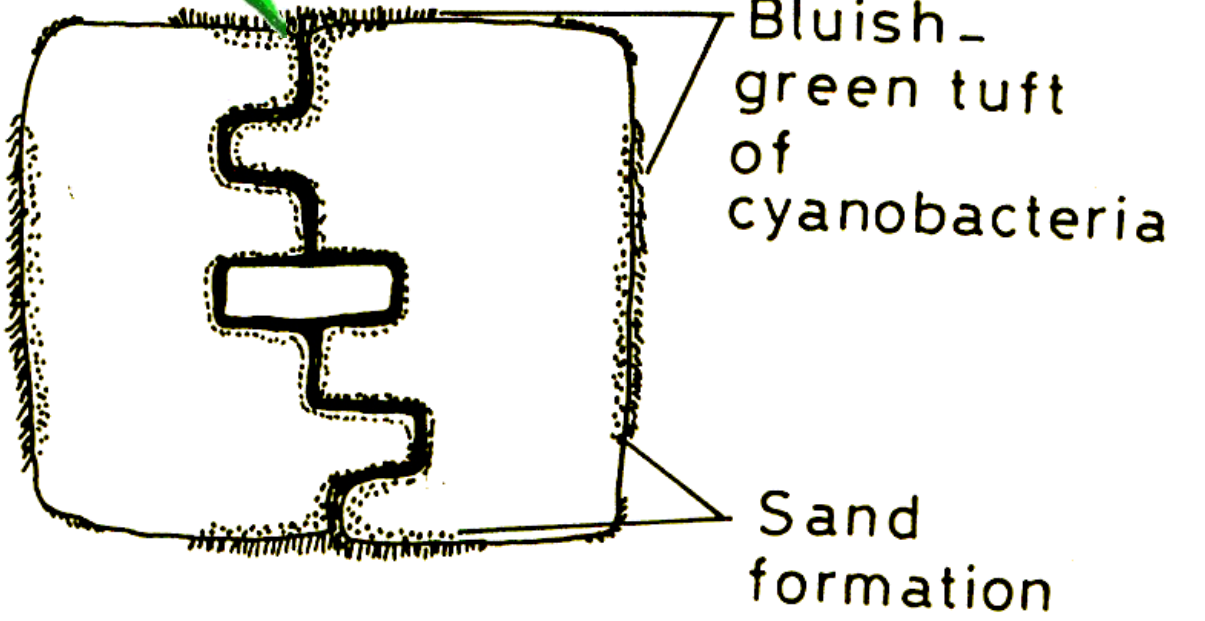




SUMMER



DURING / AFTER MANSOON



Excessive growth of the cyanobacteria in subsequent years provide suitable habitats to support appearance of bryophytes and other higher plants which greatly damage these monuments.

Crusts collected from different sites by non-destructive method

↓

Stored in sterilized bottles having a specific voucher number e. g ICB.1

↓

A pinch of crust were inoculated in the BG-11 medium at 25 ± 1^0 C and 7.5 W/m^2 light intensity up to 60 days.

↓

Species was isolated to unialgal culture after repeated culturing

↓

Unialgal cultures were isolated and kept in Visva-Bharati Culture Collection of Algae having a specific strain number e.g VBCCA010123.

↓

Microphotography diagrams were prepared.

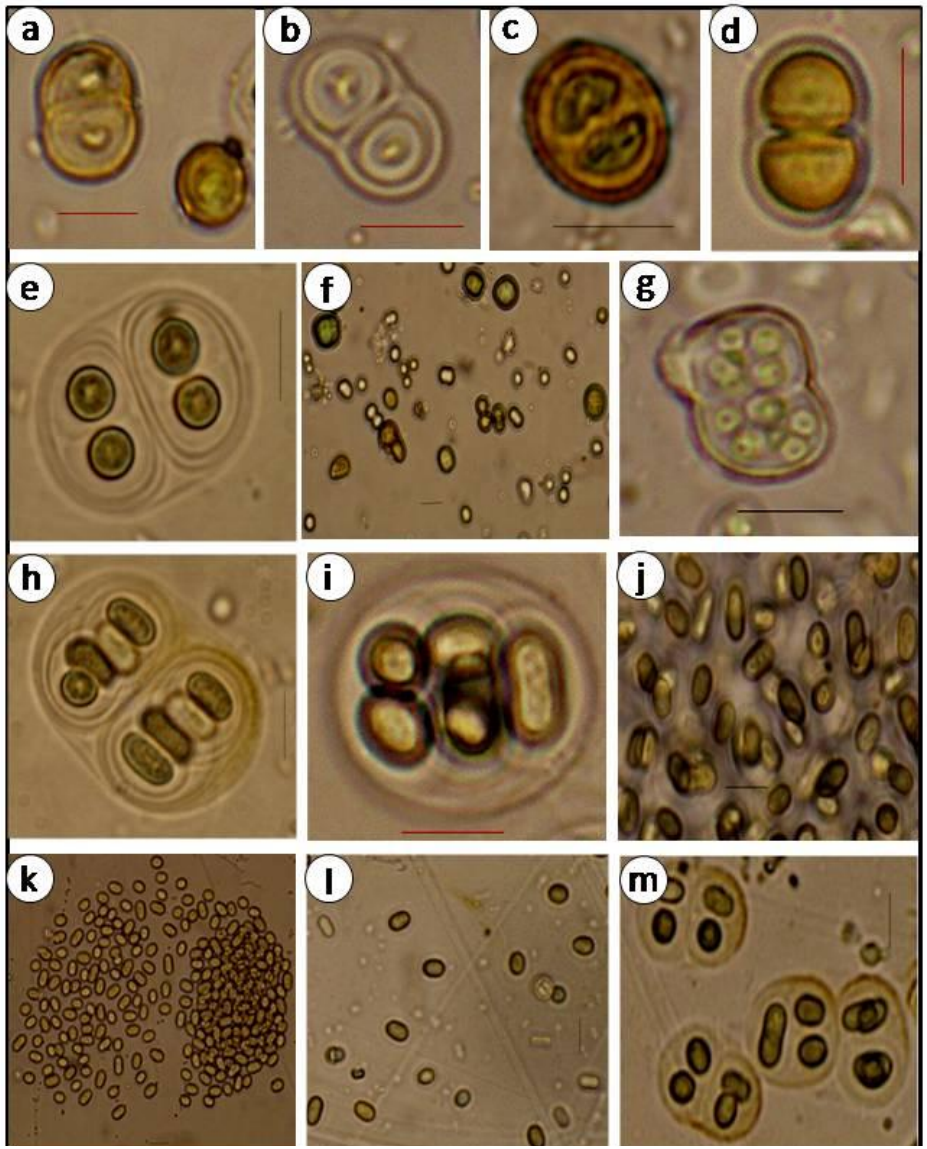
↓

Identified following standard monographs

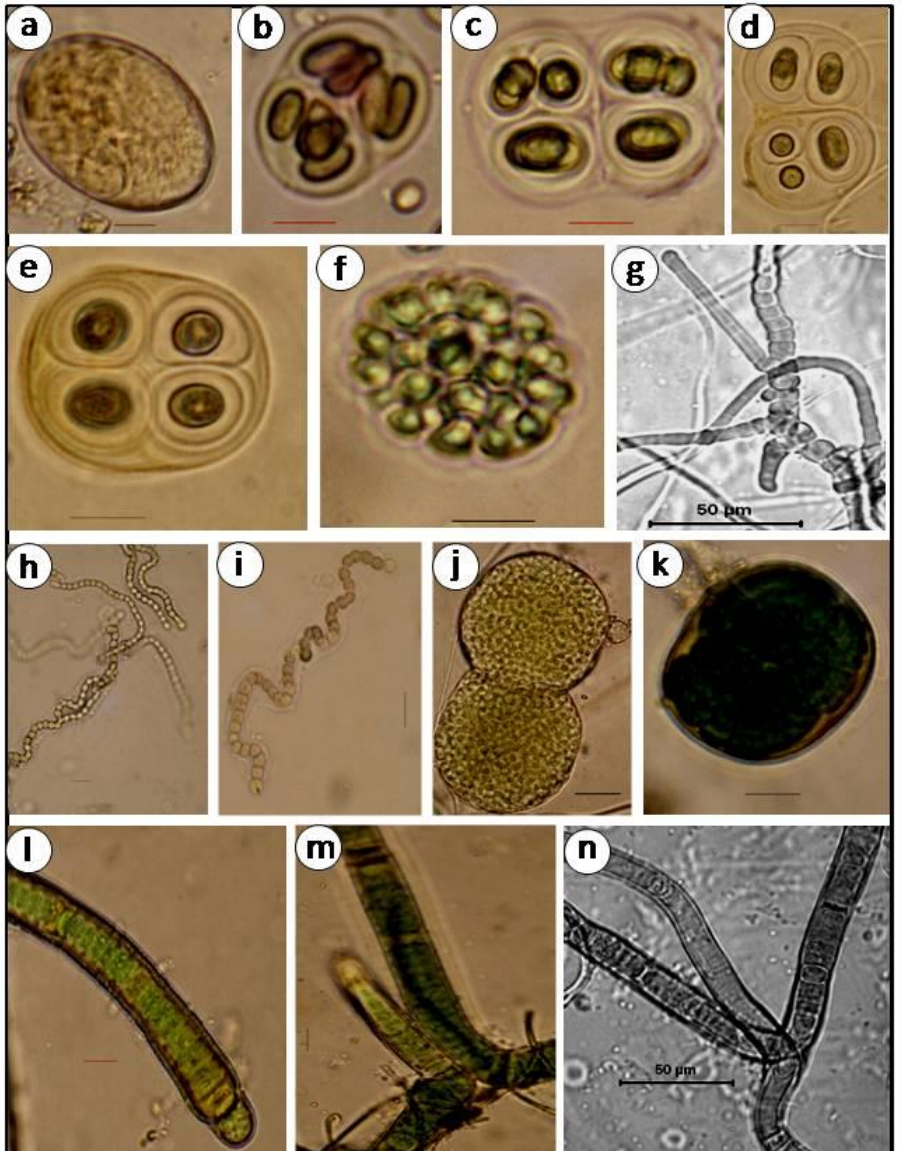
↓

Generation time and physiological characteristics of each species was worked out

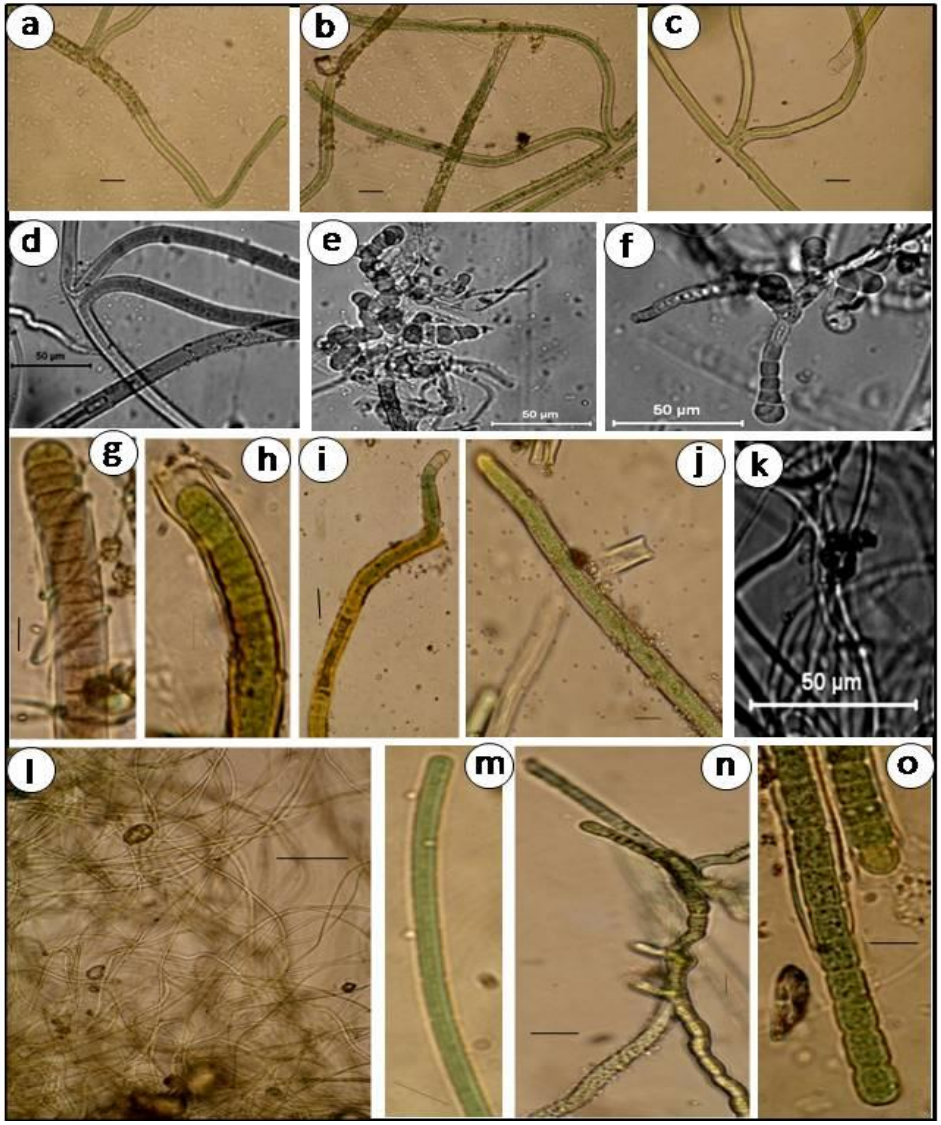
45 isolates of algae were isolated and deposited in VBCCA in the current study



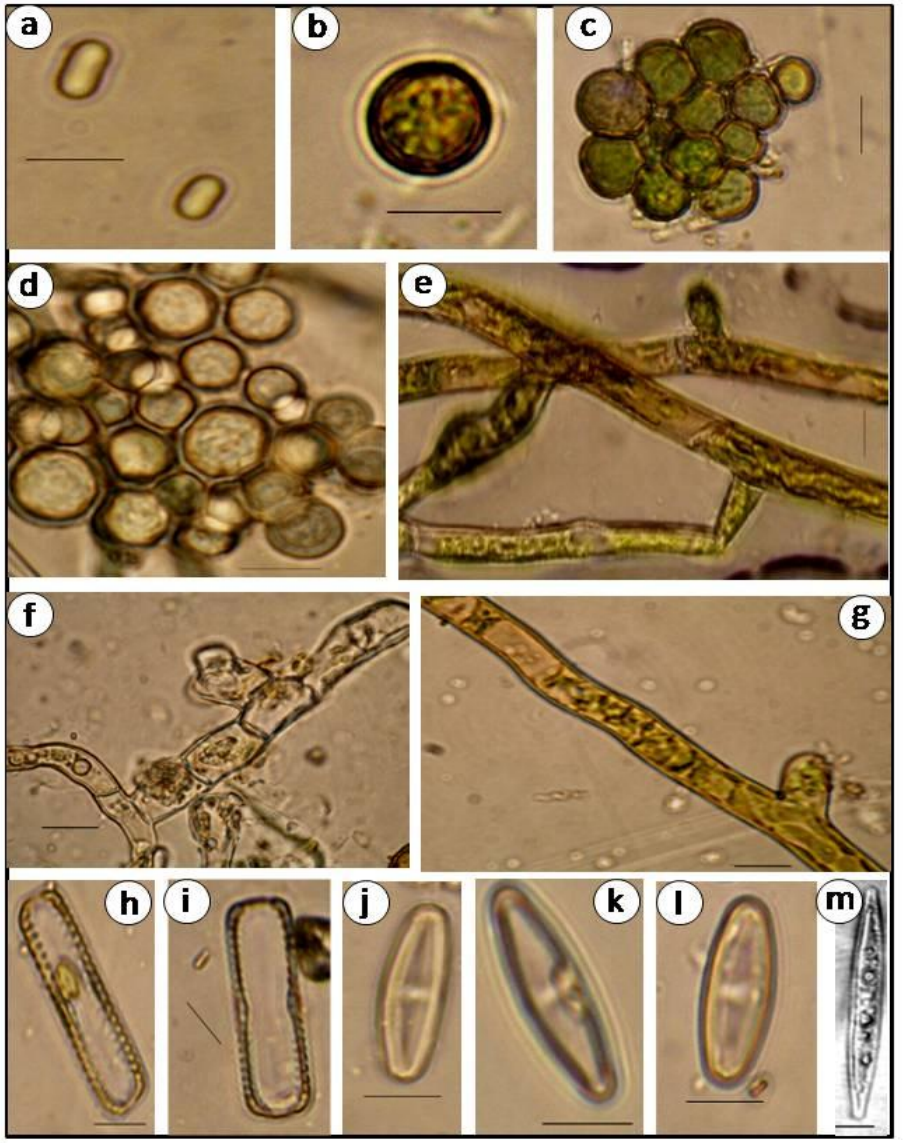
Light microscopic photographs of micro algae colonizing on the surface of terracotta temples of Bishnupur. a. *Chroococcus indicus*; b, c. *Chroococcus minor*; d. *Chroococcus turgidus* var. *maximus*; e. *Chroococcus varius*; f. *Cyanosarcina burmensis*; g. *Cyanosarcina parthenonensis* ; h, i. *Cyanosarcina spectabilis*; j. *Aphanothece castagnei* ; k. *Aphanothece microscopica* ; l. *Aphanothece pallid*; m. *Aphanothece stagnina*. Scale bar = 10 µm



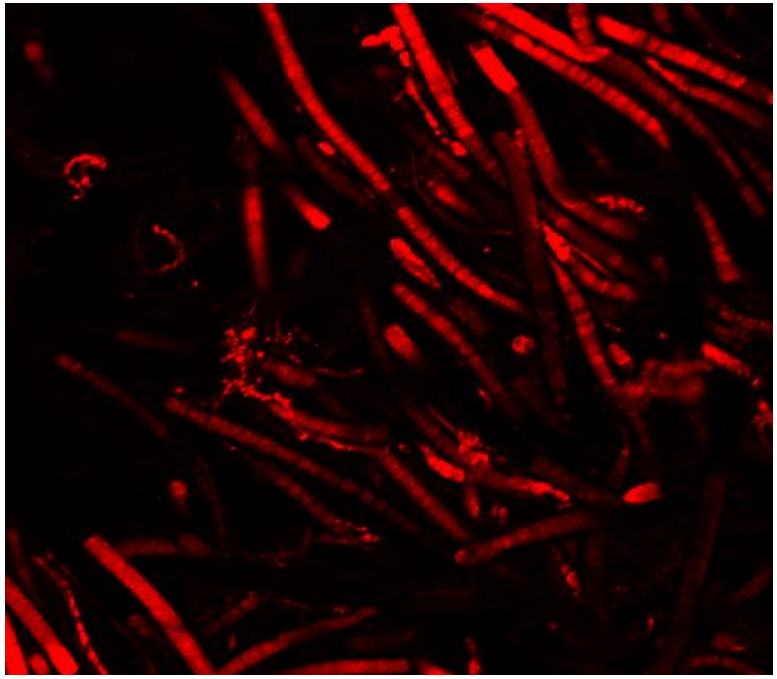
Light microscopic photographs of micro algae colonizing on the surface of terracotta temples of Bishnupur. a. *Cyanothece aeruginosa*; b-d. *Gloeothece rupestris*; e. *Gloeocapsa sanguinea*; f. *Chroococcidiopsis kashayi*; g. *Westiellopsis prolifica*; h. *Nostoc linckia*; i. *Nostoc microscopicum*; j. k. *Nostoc muscurum*; l. *Calothrix clavatoides*; m. *Calothrix marchica*; n. *Scytonema bohneri*. Scale bar a- f, h-m = 10 µm ; g, n = 50 µm

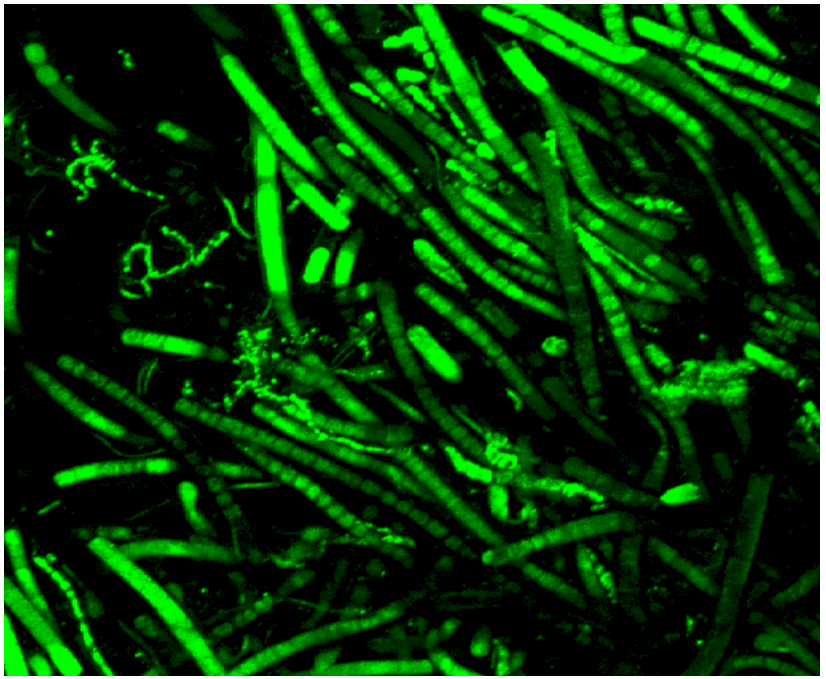


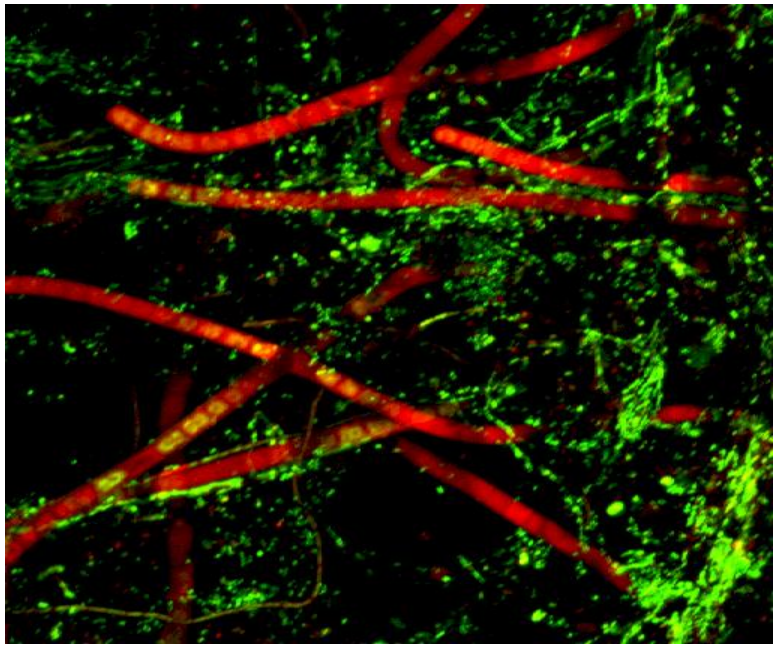
Light microscopic photographs of micro algae colonizing on the surface of terracotta temples of Bishnupur. a. *Scytonema geitleri*; b. *Scytonema multiramosum*; c. *Scytonema rivulare*; d. *Scytonema schmidtii*; e, f. *Stigonema tomentosum*; g. *Lyngbya arboricola*; h. *Lyngbya major*; i. *Lyngbya palmar*um; j. *Phormidium aerugineo-coeruleum*; k,l. *Phormidium ambiguum*; m. *Phormidium retzii*; n. *Leptolyngbya boryana*; o. *Leptolyngbya polysiphoniae*. Scale bar a-c, g-j, l-o = 10 µm; d- f, k = 50 µm

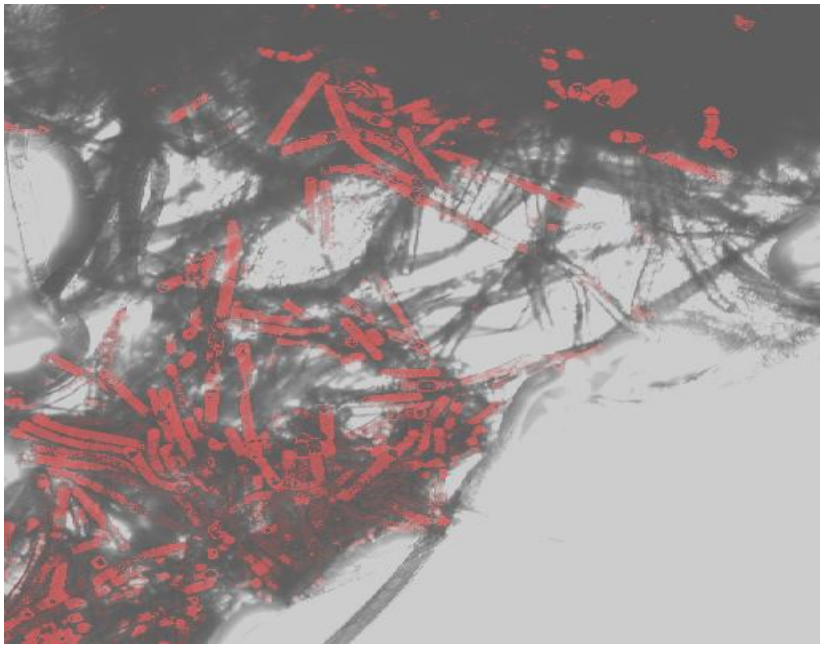


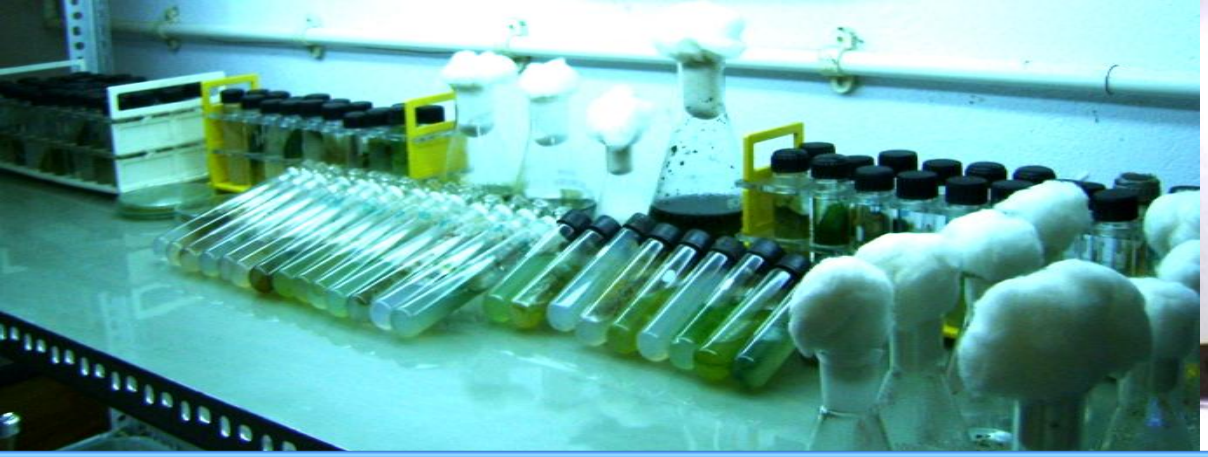
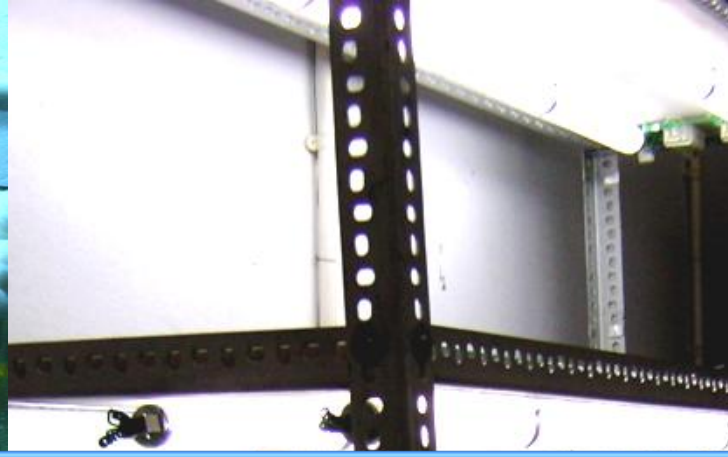
Light microscopic photographs of micro algae colonizing on the surface of terracotta temples of Bishnupur. a. *Synechococcus aeruginosus;* b. *Bracteacoccus minor;* c. *Chlorococcum infusionum* d. *Radiococcus nimbatus* e. *Printzina effuse*; f, g. *Trentepohlia aurea*; h, i. *Gomphonema lanceolatum*; j. *Navicula gracilis* k. l. *Nitzschia amphibia*; m. *Nitzschia palea*. Scale bar = 10 µm







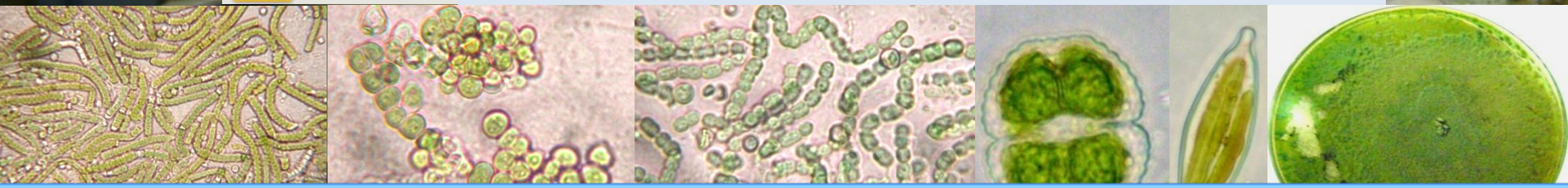


VISVA-BHARATI CULTURE COLLECTION OF ALGAE (VBCCA, WDCM-931)


Affiliated to

WFCC **World Federation for culture collection**



Director: Prof. Samit Ray
Curator: Dr. Jnanendra Rath

Contact: vbcca@visva-bharati.ac.in

WFCC

World Federation for Culture Collections

India		
ABM	WDCM934	APEX BIOSCIENCES CENTRE FOR MICROORGANISMS
ABRC	WDCM912	Anaerobic Bacterial Resource Centre
CCDMBI	WDCM119	Culture Collection, Department of Microbiology
CIPDE	WDCM462	Collection of Insect Pathogens, Dept. of Entomology
DBV	WDCM173	Division of Standardisation
DMSRDE	WDCM166	DMSRDE Culture Collection
DUM	WDCM40	Delhi University Mycological Herbarium
ITCC	WDCM430	Indian Type Culture Collection
MCC	WDCM930	Microbial Culture Collection
MCM	WDCM561	MACS Collection of Microorganisms
MPKV	WDCM448	Biological Nitrogen Fixation Project College of Agriculture
MTCC	WDCM773	Microbial Type Culture Collection & Gene Bank
NCDC	WDCM775	National Collection of Dairy Cultures
NCIM	WDCM3	National Collection of Industrial Microorganisms
NFCCI	WDCM932	National Fungal Culture Collection of India
NTCCI	WDCM107	Culture Collection, Microbiology and Cell Biology Laboratory
RRJ	WDCM846	RRL , Jammu INDIA
UMFFTD	WDCM562	Food and Fermentation Technology Division, University of Mumbai
VBCCA	WDCM931	VISVA-BHARATI CULTURE COLLECTION OF ALGAE
VPCI	WDCM497	Fungal Culture Collection

In India 20 culture collections are registered with WFCC and VBCCA is the only one registered culture collection of Algae from India

VISVA-BHARATI CULTURE COLLECTION OF ALGAE

(VBCCA, WDCM931)
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WFCC
WORLD FEDERATION FOR CULTURE COLLECTIONS

This is to certify that Sikha Mandal, PhD student of Department of Botany, Visva-Bharati, Santiniketan has submitted 12 strain of algae isolated from East Calcutta Wetlands in our culture collection VBCCA. The strain has been checked and assigned strain no – VBCCA 01101, VBCCA 01102, VBCCA 01103, VBCCA 01104, VBCCA 01105, VBCCA 01106, VBCCA 01107, VBCCA 01108, VBCCA 01109 VBCCA 01110, VBCCA 01111, VBCCA 01112.

Jnanendra Rath
(Curator, VBCCA)

Samit Ray
(Director VBCCA)

Commercially available Sunscreen Products

Ombrelle (with Mexoryl) SPF 30 Lotion by by L'Oreal

- Avobenzone
- Mexoryl
- Titanium dioxide

- U.S. Food and Drug Administration hasn't yet approved it for use in the U.S

Lakme Skin Brightening Gel

Sun Block

Sunscreen Lotion

Tan Protection Index 70 (TPI 70)

Lakme Sun Expert : Total Sun Control

Sunscreen Soufflé

Impact of available sunscreens on Human skin

- 40 % of the population is sensitive to it, experiencing red, itchy skin.

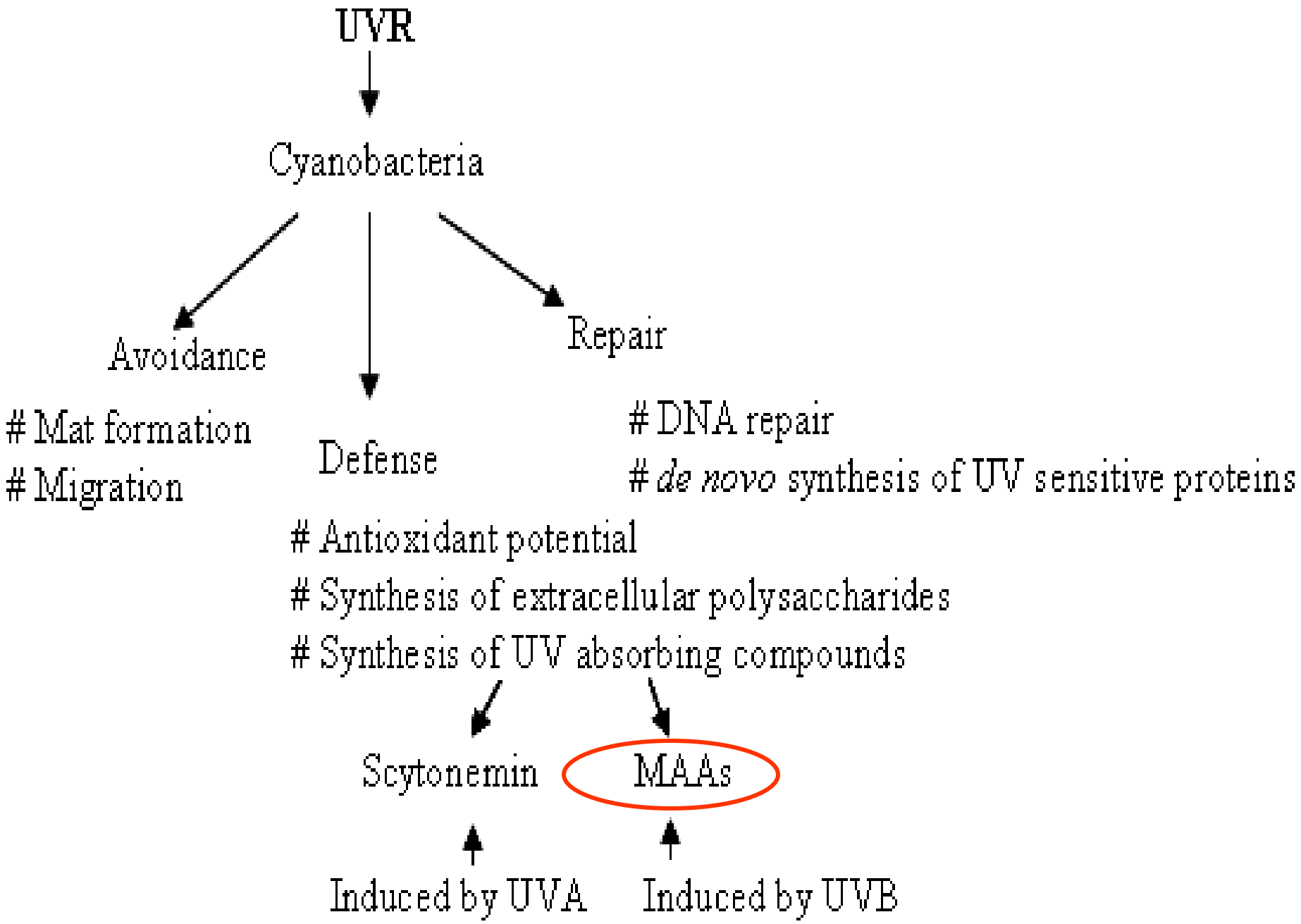
- Avobenzone increases the formation of a particular DNA defect in human cells. This defect occurs when two adjacent molecules of thymine, one of the four bases of DNA, link together chemically to form a dimer, causing skin cancer.

- Titanium dioxide and zinc oxide oxidize DNA, and it produces free radicals that break DNA strands and suppress the immune system.

- Skins dose absorb these chemicals, since detected in urine sample.

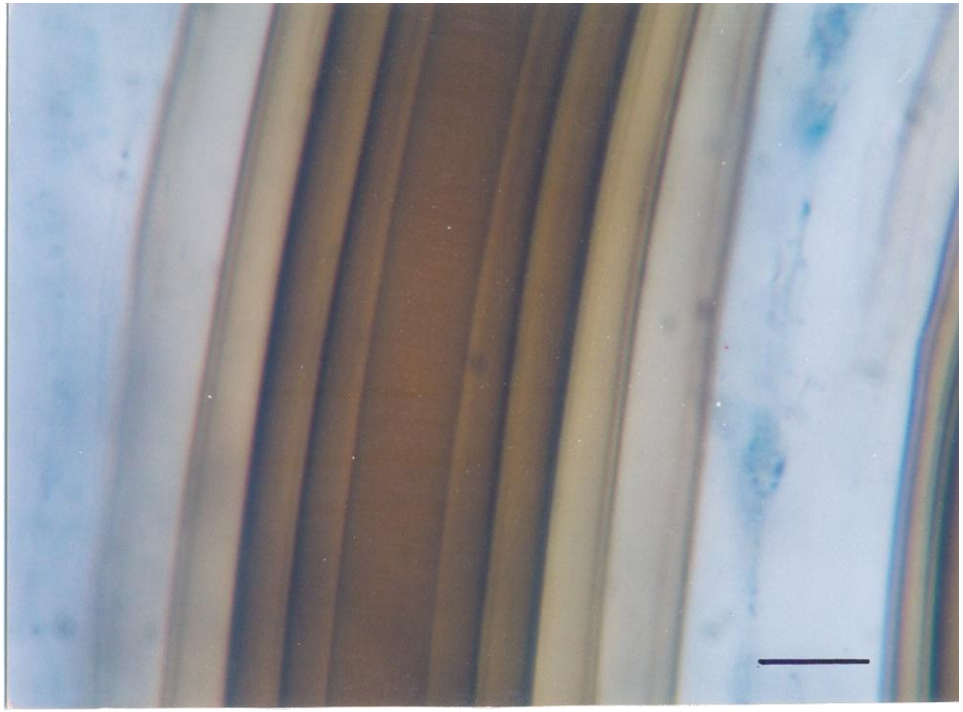
Bio-Protective Sunscreen Lotion

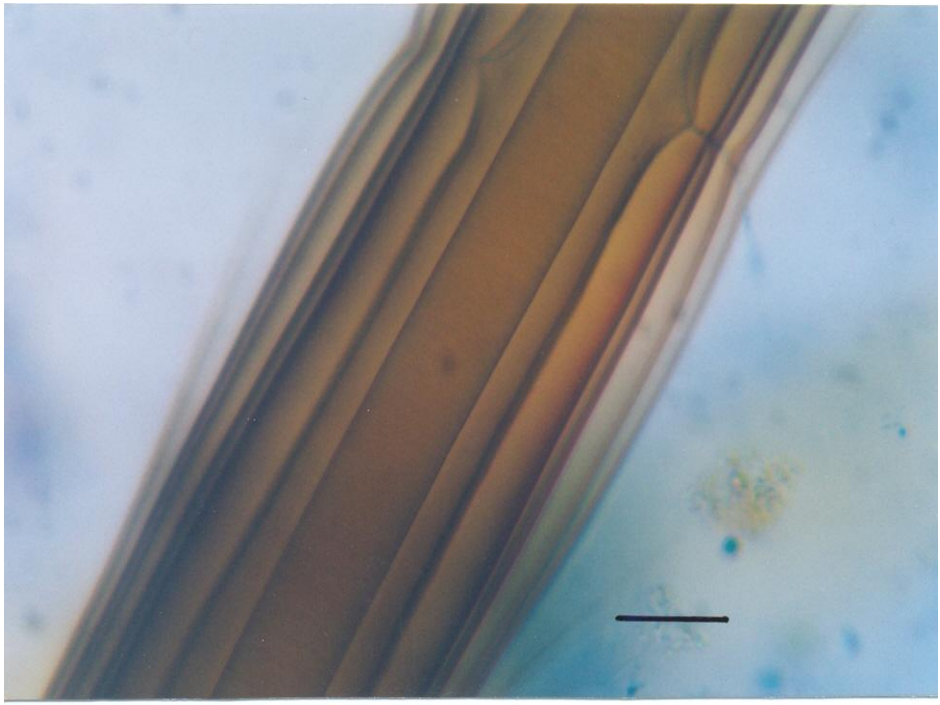
?

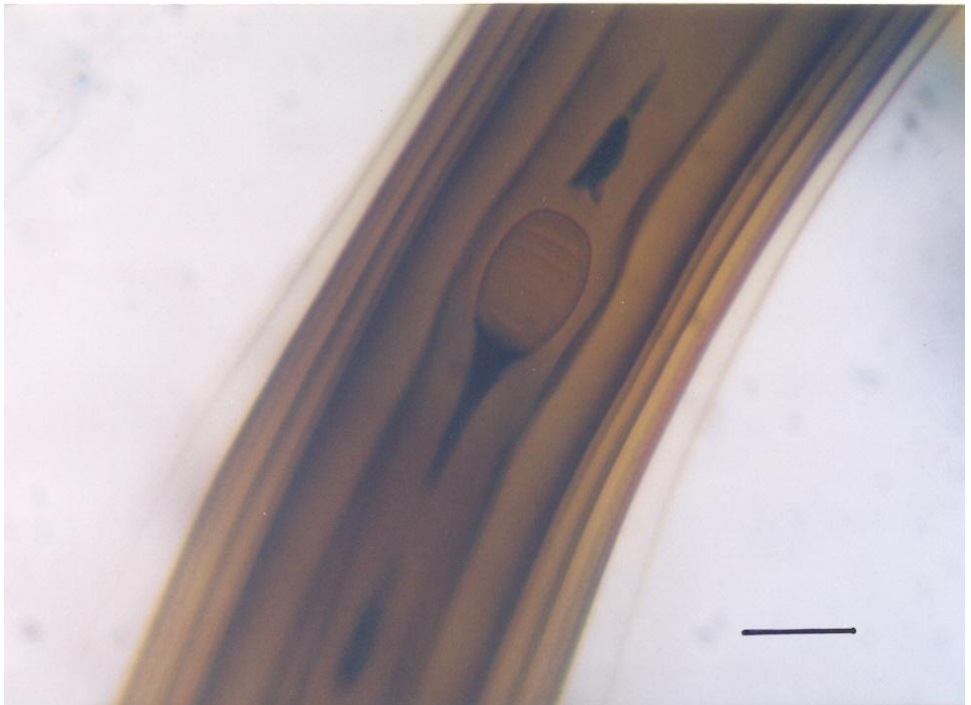

UVR
Cyanobacteria
Avoidance
Mat formation
Migration
Defense
Repair
DNA repair
de novo synthesis of UV sensitive proteins
Antioxidant potential
Synthesis of extracellular polysaccharides
Synthesis of UV absorbing compounds
Scytonemin
MAAs
Induced by UVA
Induced by UVB

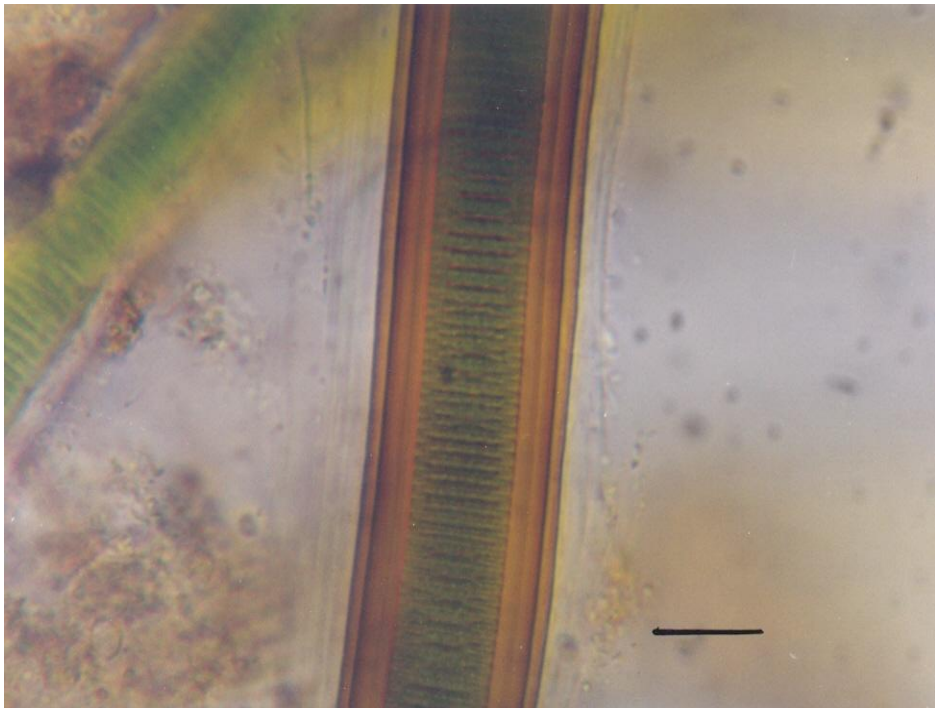
Why *Lyngbya* ?

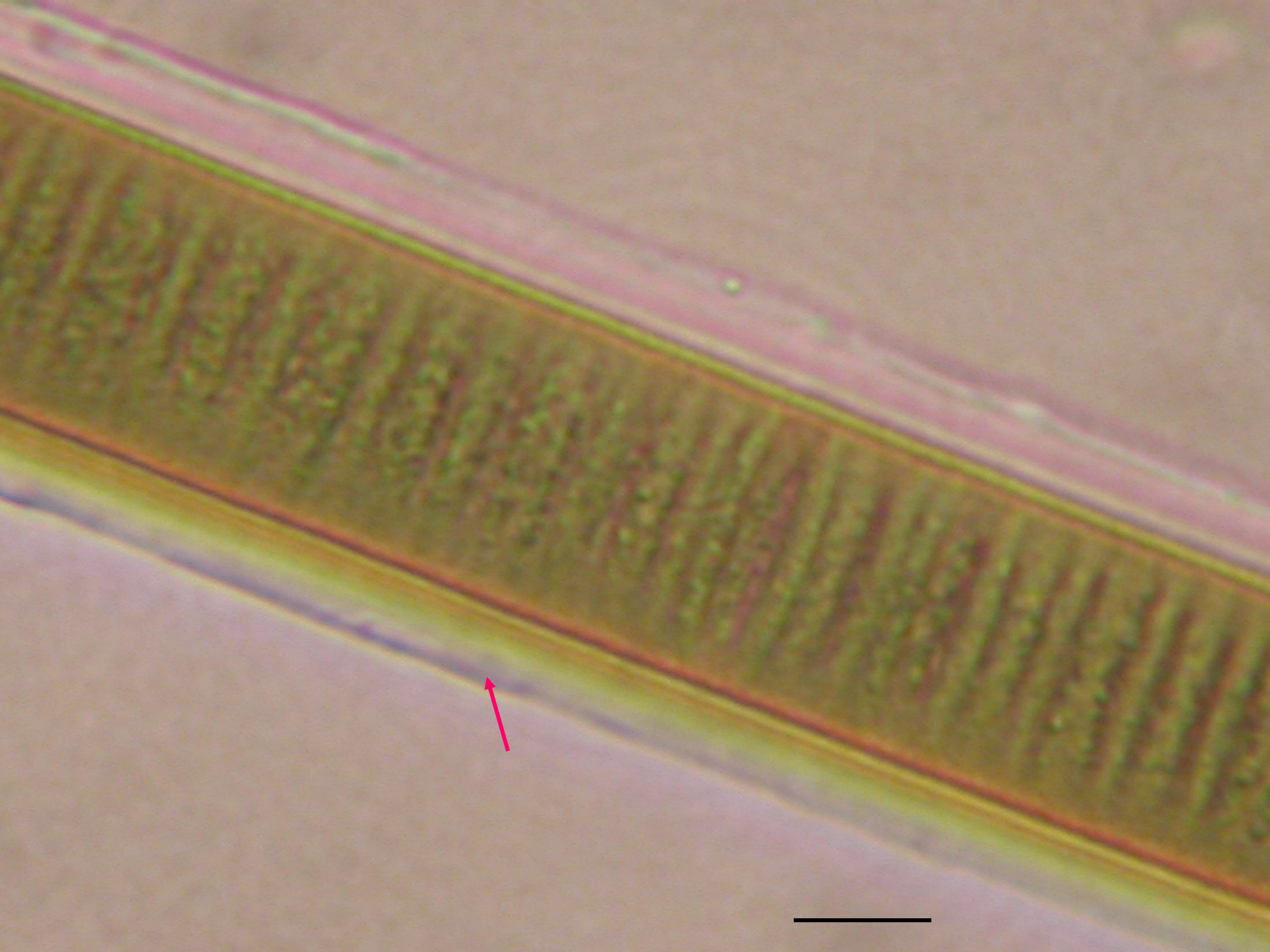
- It is having a wide multilayered sheath and having high MAAs content.
- Have high SOD (Antioxidant) activity (80 % SOD scavenging activity)
- Rich Allophycocyanin content
- Though it is difficult to culture terrestrial cyanobacteria, media composition and pure culture of the organism was established in our lab.
- Protocol for mass culture of this organism was on the process.
- Terrestrial cyanobacteria have proven to be the most versatile produces of secondary metabolites.
- *Lyngbya* produces several secondary metabolites including barbamid, Curacin A & B, antillatoxin B and malyngamide H and thus this one strain provides a treasure trove of biosynthetic machinery to produce secondary metabolites with many unique structural features.











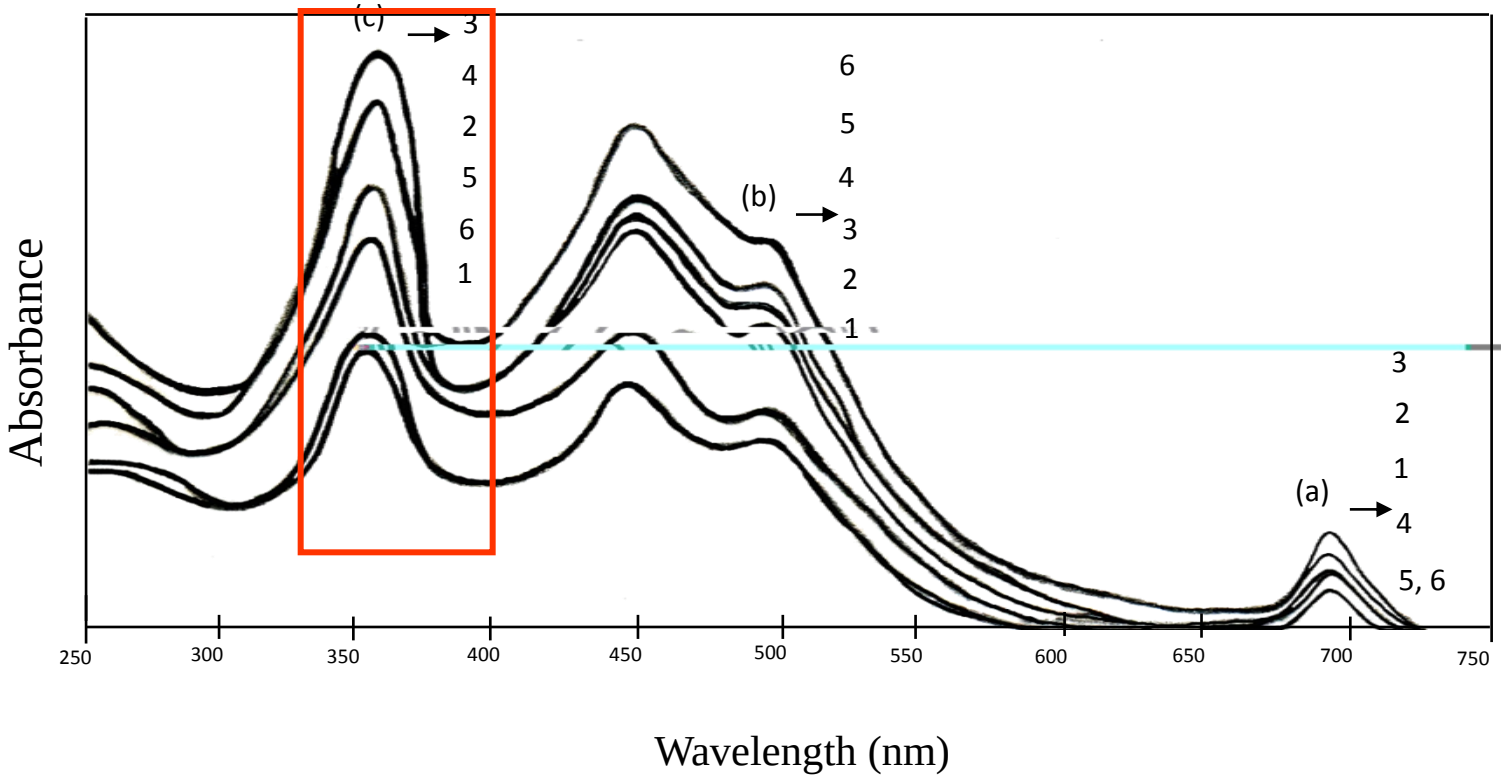
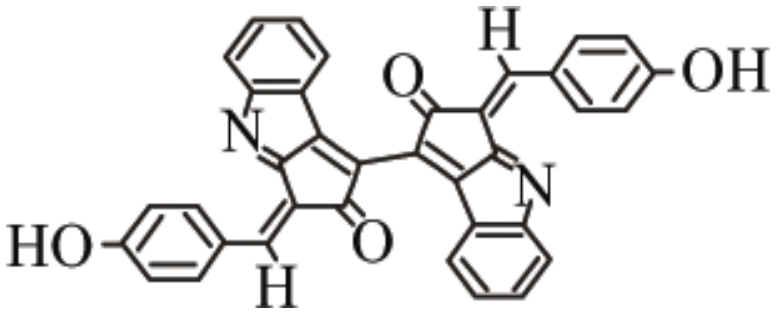
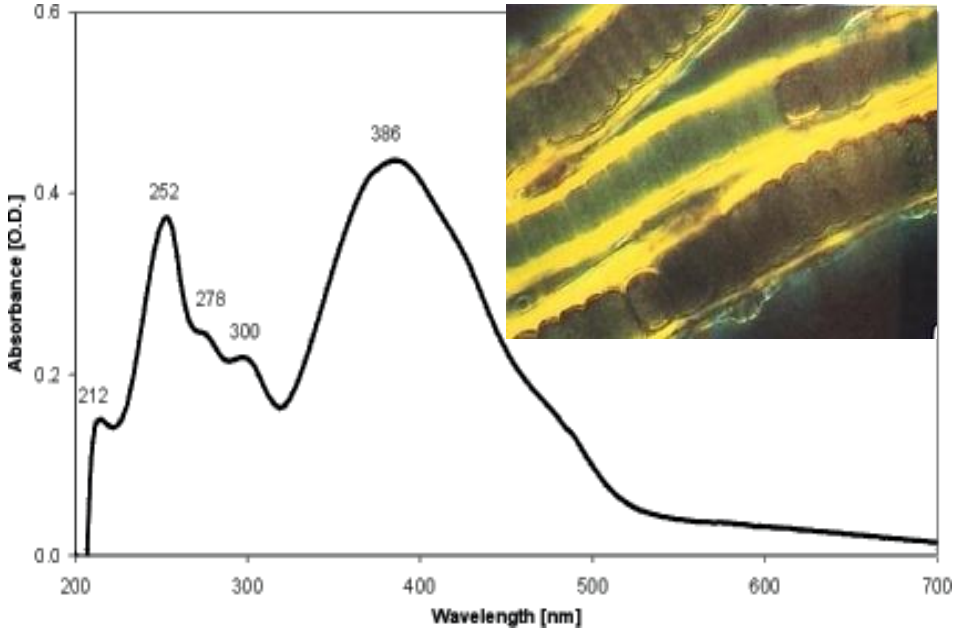


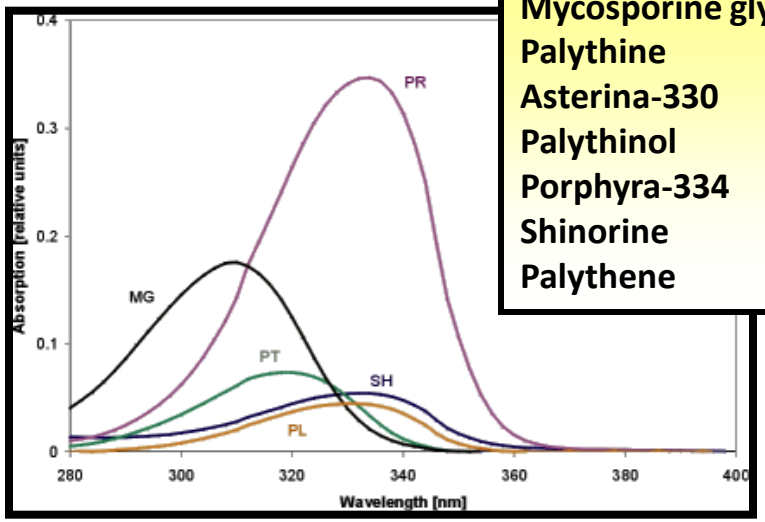
Fig. Absorption spectra of methanol extract (90% v/v, 45^0 C, 1 min) of *Lyngbya aestuarii* from Chilika lake after irradiation with UV-B for different durations. 1-0 h (Control), 2- 3 h, 3- 6 h, 4- 12 h, 5- 24 h, 6 - 48 h. (a) Chlorophyll-*a*, (b) carotenoid, (c) MAAs.

UV-protecting Pigments

- Scytonemin is a yellowish brown, lipid soluble dimeric pigment located in the extracellular polysaccharide sheath having molecular weight of 546 and a structure based on indolic and phenolic subunits. It has an absorption maximum at 386 nm.







Mycosporine glycine	**310**
Palythine	**320**
Asterina-330	**330**
Palythinol	**332**
Porphyra-334	**334**
Shinorine	**334**
Palythene	**360**

Mycosporine amino acid like substances (MAAs) are watersoluble substances Characterized by a cyclohexenone on cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid on its imino alcohol having absorption maxima ranging from 310 to 360 nm and an average molecular weight of 300.

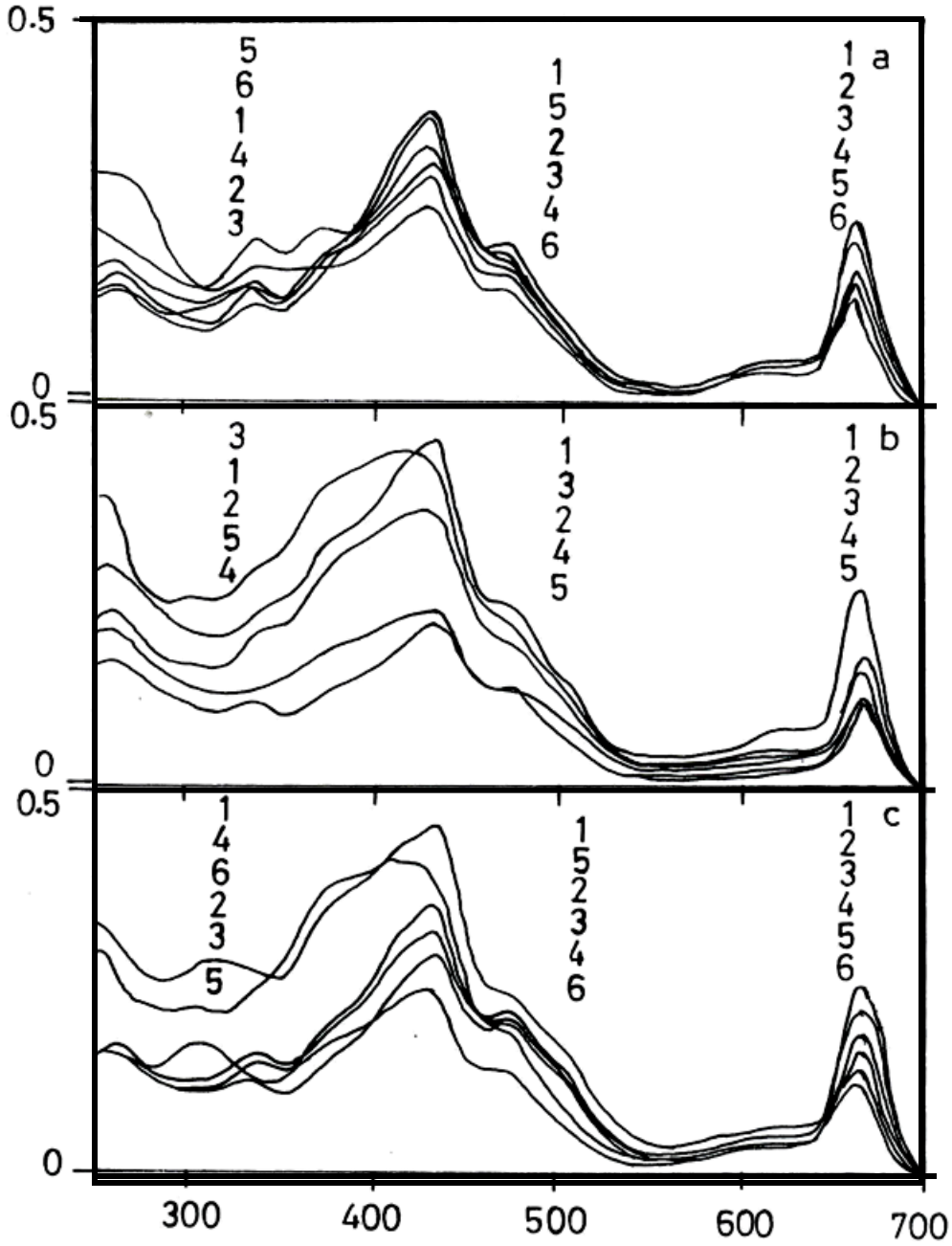


Fig. 3: Absorption spectra of methanolic extract (90% (v/v) 45^0 C, 1 h) of a. different Isolates of *Nostoc commune*, b. different isolates of *Nostoc Sp.* , *Calothrix sp.* and *Fischerella sp.* c. different isolates of *Tolypothrix sp.*

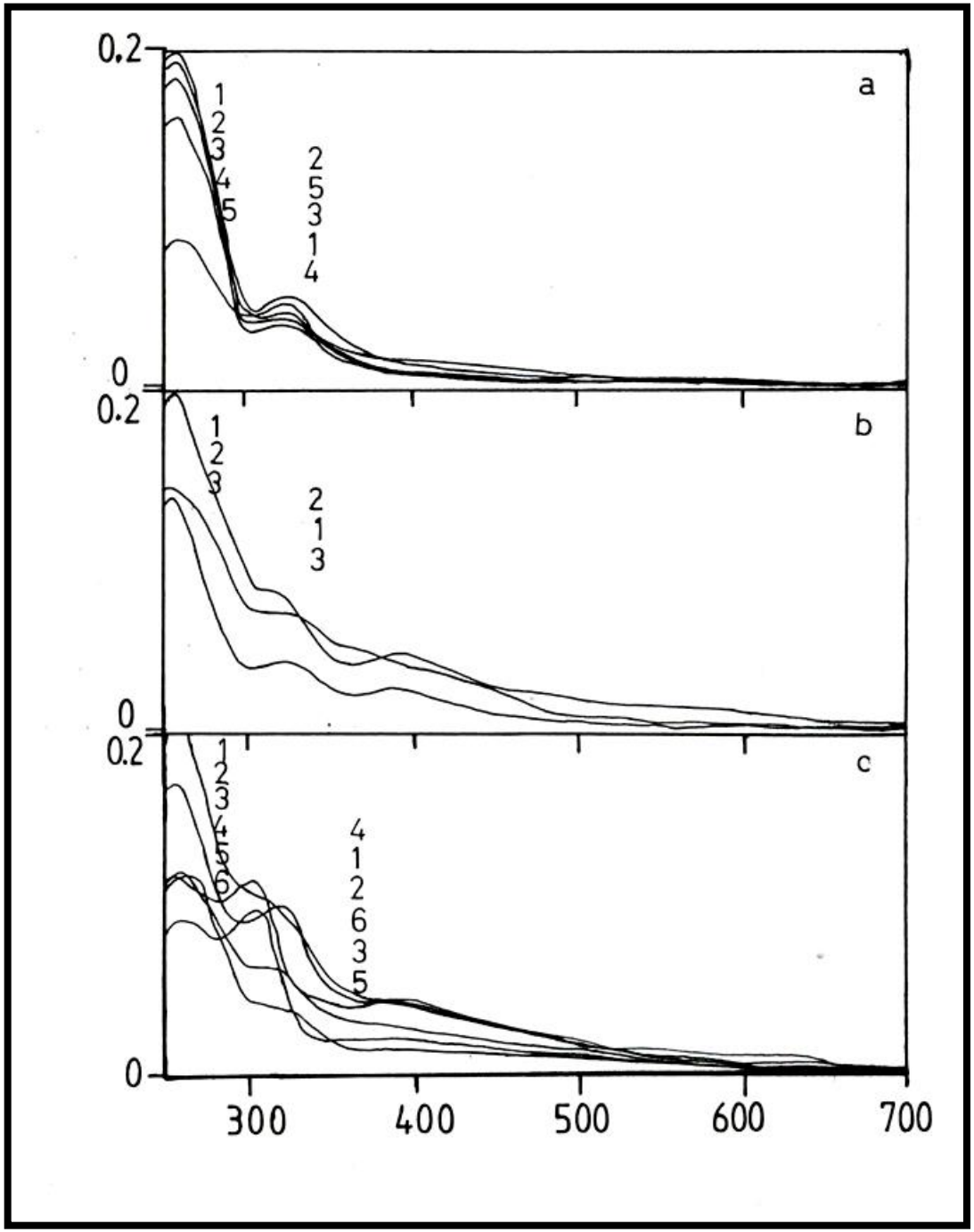
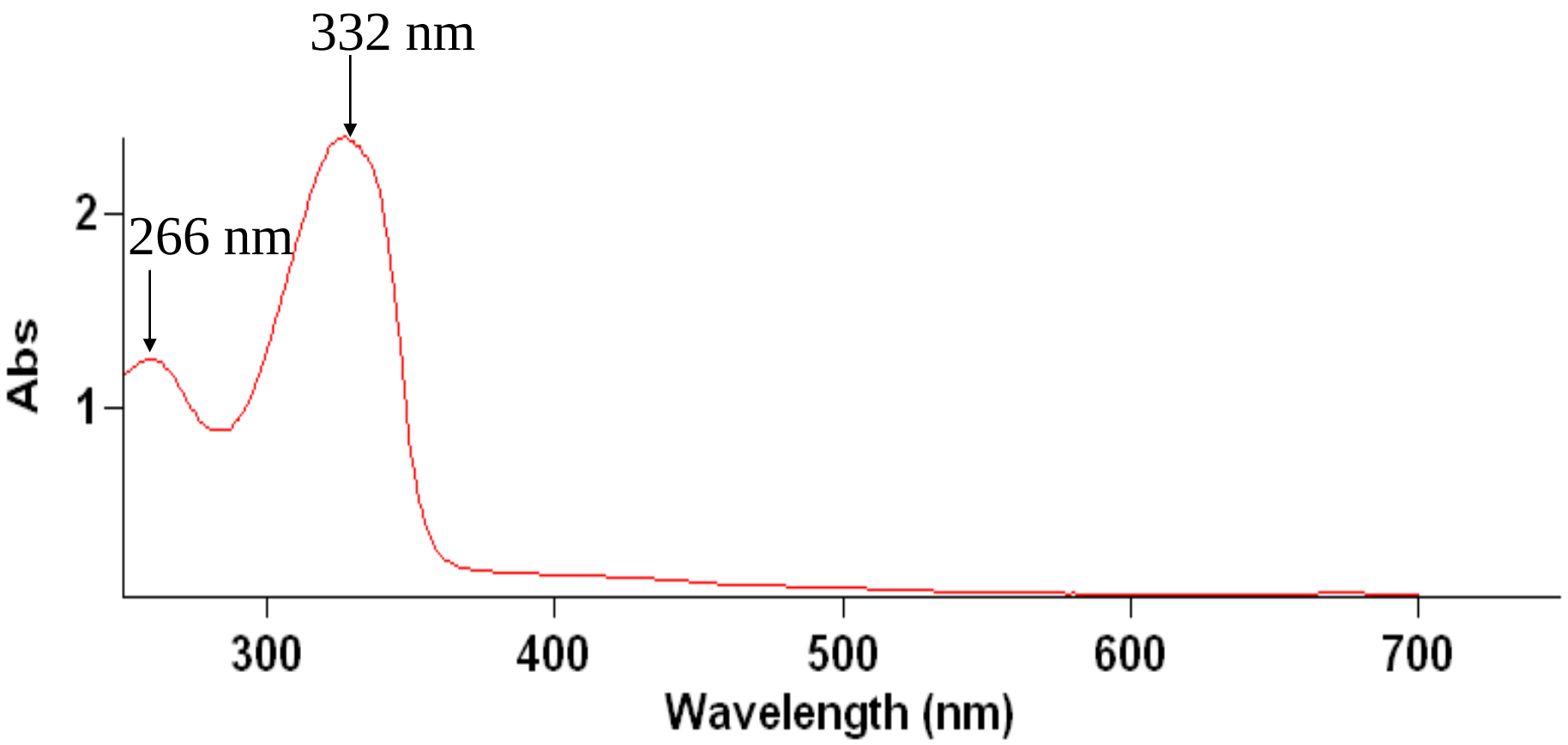
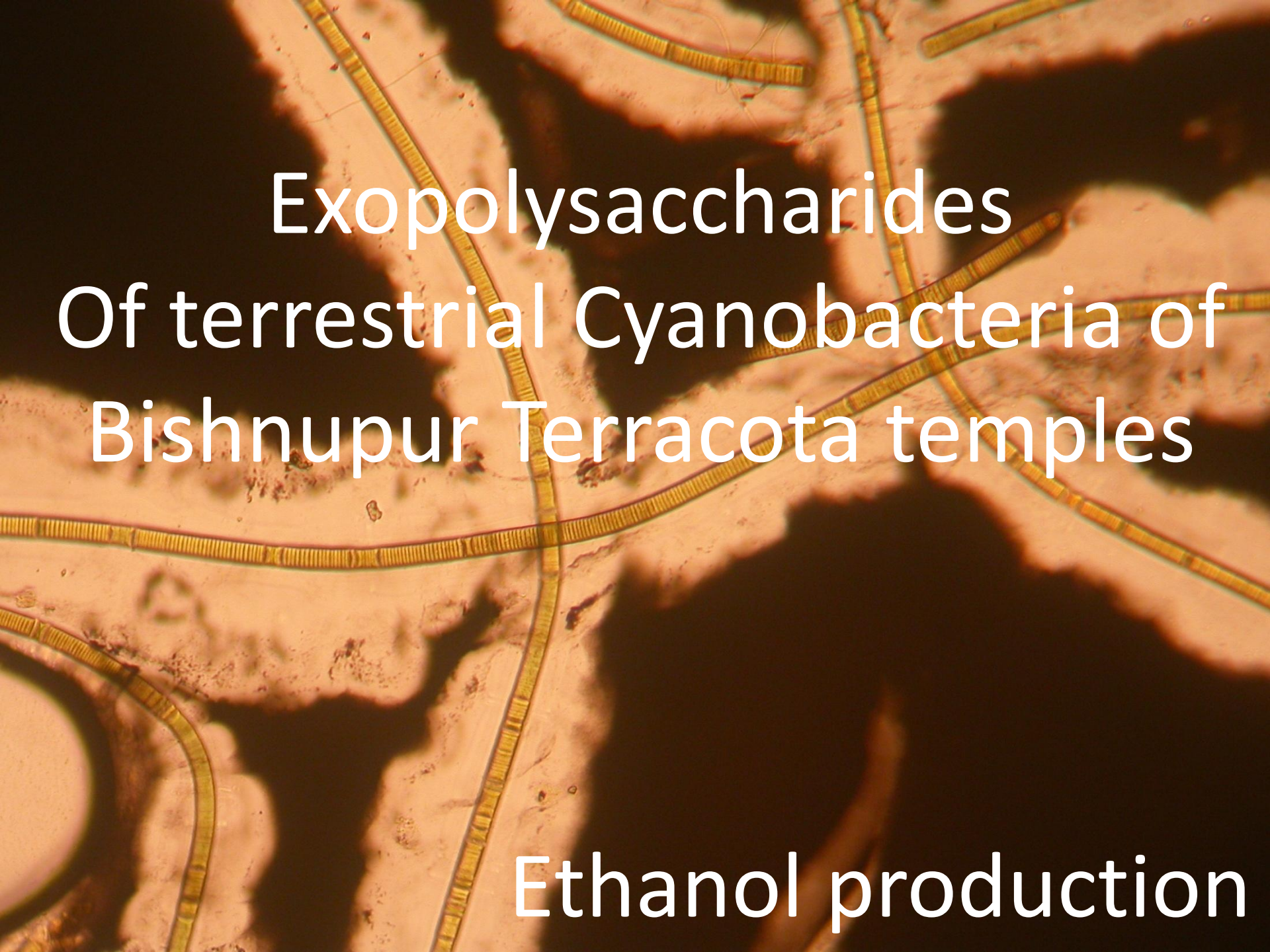


Fig. 3: Absorption spectra of methanolic extract (20% (v/v) 45^0 C, 1 h) of a. different isolates of *Nostoc commune*, b. different isolates of *Nostoc sp.* , *Calothrix sp.* and *Fischerella sp*. c. different isolates of *Tolypothrix sp.*

Partially Purified UV-absorbing pigment in the cyanobacteria *Lyngbya*

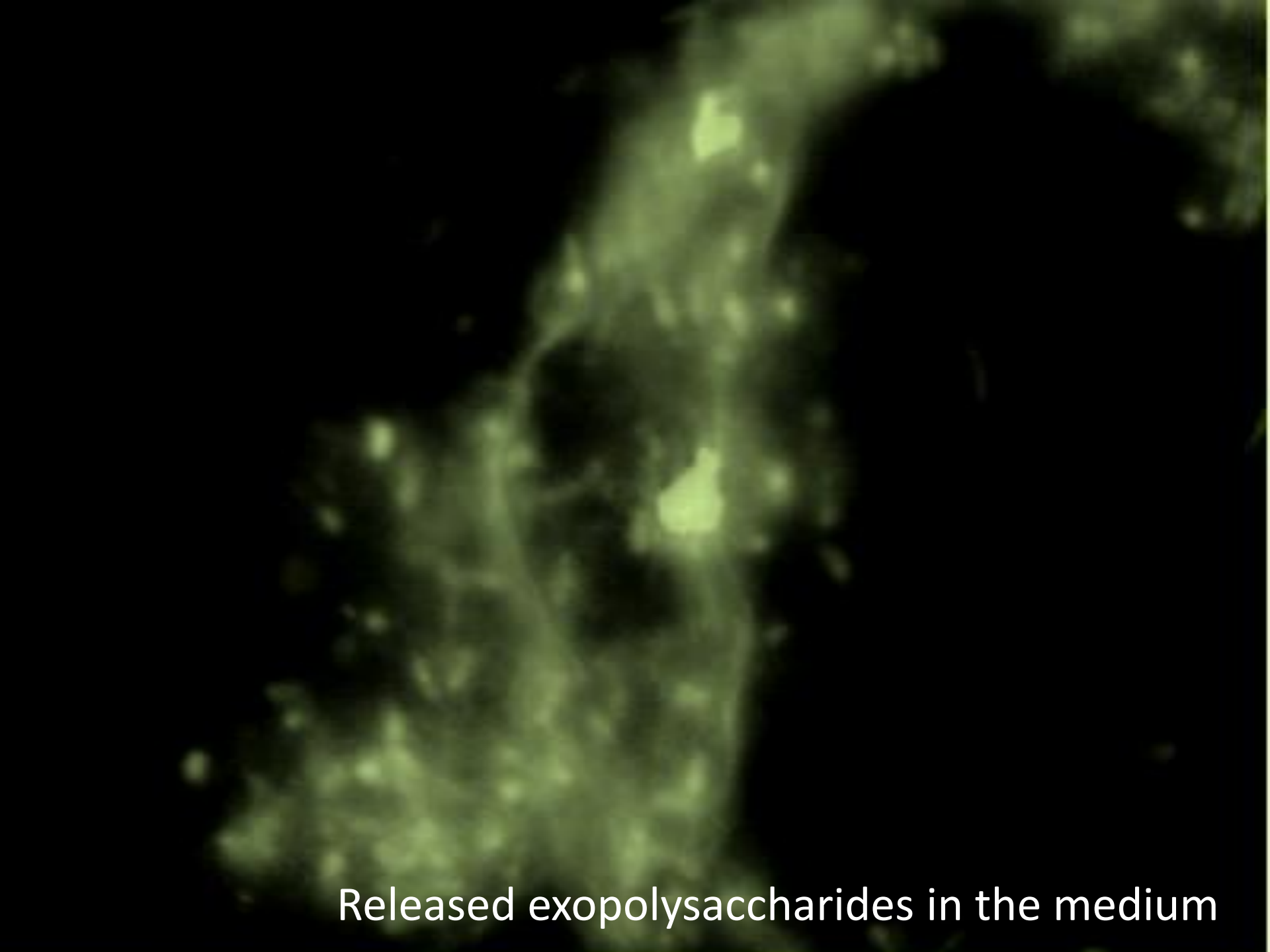


Funding CSIR (2010-2-13): **Prospecting Chemical components from Cyanobacteria to Deal with UV Radiation Hazards**



Exopolysaccharides Of terrestrial Cyanobacteria of Bishnupur Terracota temples

Ethanol production


Released exopolysaccharides in the medium

Exopolysaccharides: Ethanol products:

- With response to UV-radiation *Lyngbya* produced thick sheath layer which composed of simple sugars released to the medium, which is the very inexpensive source to use for ethanol and other designer fuels.

- Glucose, and sucrose can be continually harvested without harming or destroying the cyanobacteria (harvesting cellulose and sugars from true algae or crops, like corn and sugarcane, requires killing the organisms and using enzymes and mechanical methods to extract the sugars).

- *Lyngbya* produce a relatively pure, gel-like form of exopolysaccharides that can be broken down easily into glucose

- The huge expense in making cellulosic ethanol and biofuels is in using enzymes and mechanical methods to break cellulose down, so Using the cyanobacteria escapes these expensive processes

Nano-Biotechnology

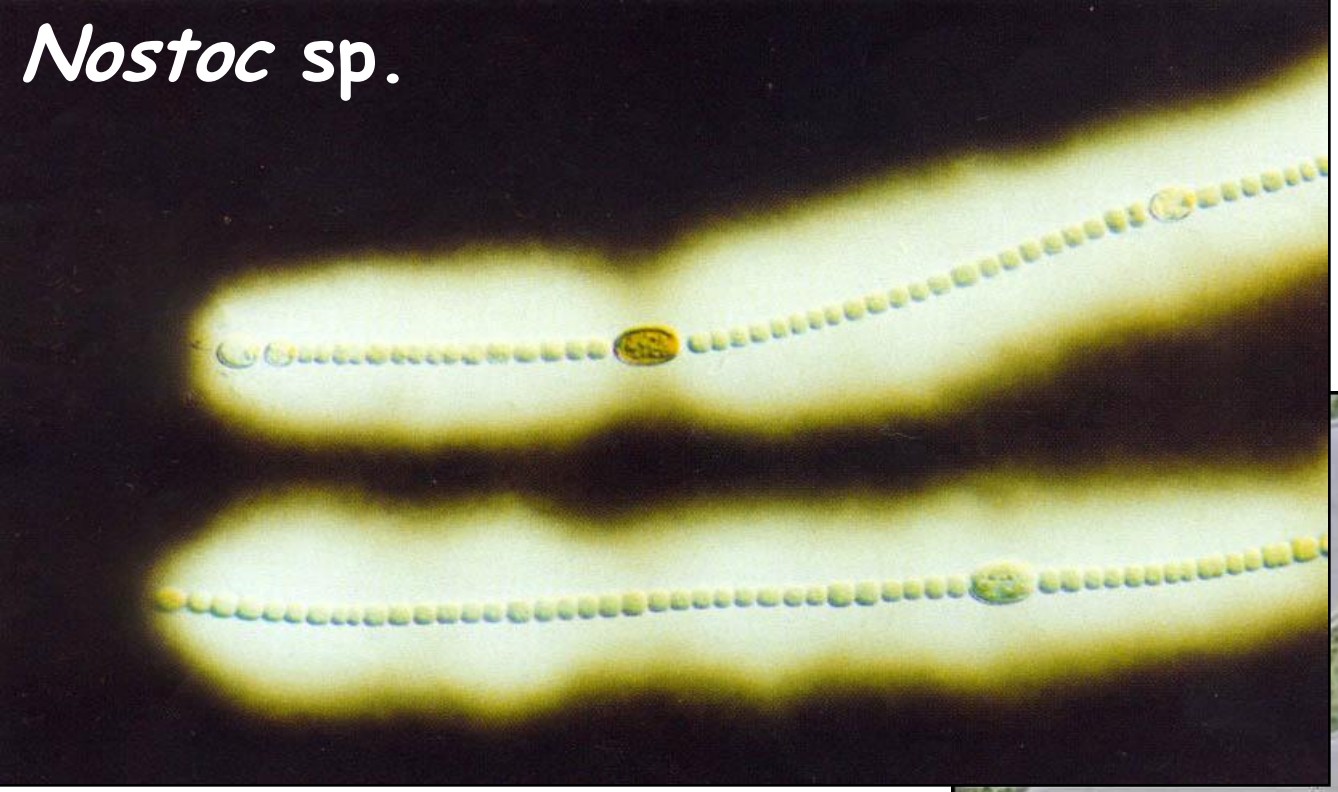
New Biofuel Production Technique Developed

Cyanobateria makes cellulose for biofuel conversion

By **Gabriel Gache**, Science News Editor
24th of April 2008, 11:12 GMT

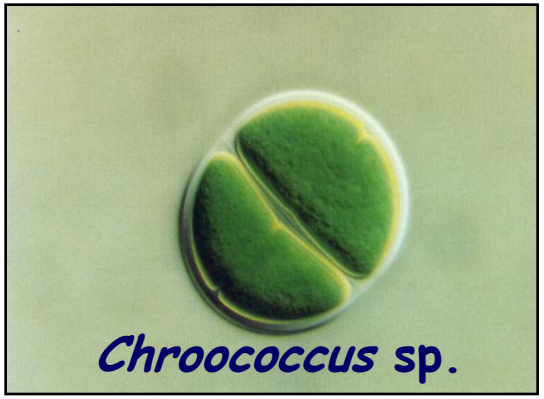
- **Professor R. Malcolm Brown Jr. and Dr. David Nobles at the University of Texas at Austin, developed a genetically engineer cyanobacteria which produce more carbohydrate.**

Nostoc sp.



Gloeothece sp.

Sheath


Chroococcus sp.

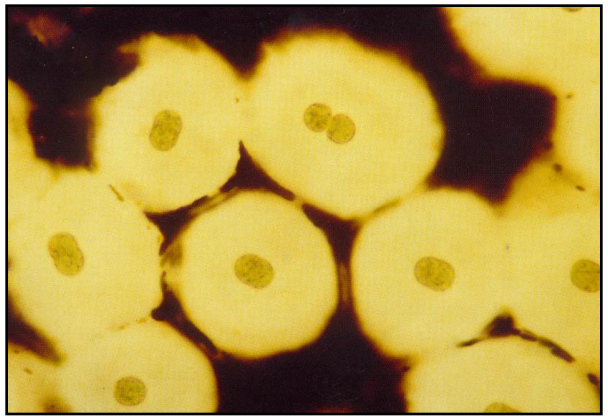

Gloeothece membranacea


Phormidium sp.

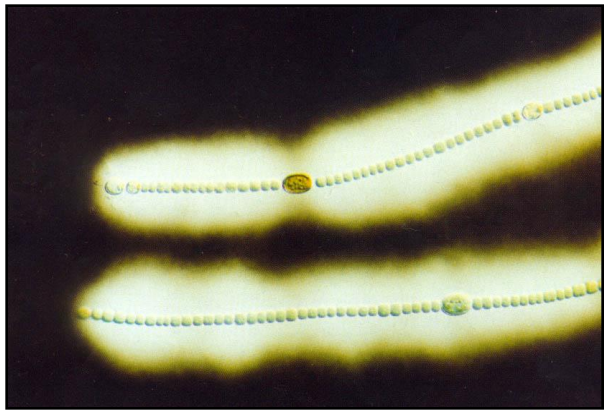

Scytonema sp.

(thin layer surrounding cells or cell groups)

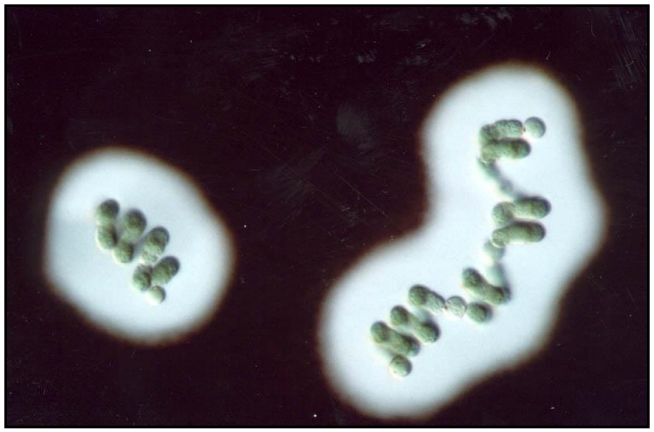
Capsule



Cyanothece sp.



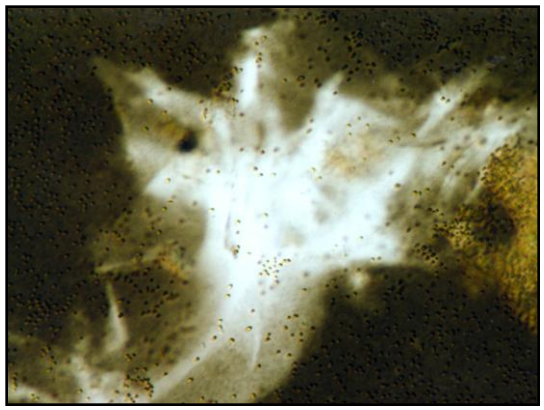
Nostoc sp.



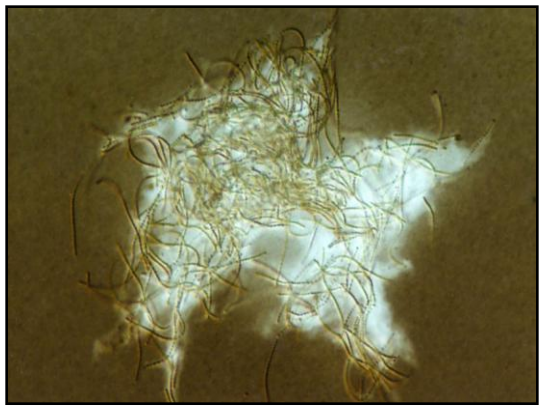
Cyanospira capsulata

(thick and slimy layer, intimately associated with cell surface)

Slime



Cyanothece VBCCA 1008

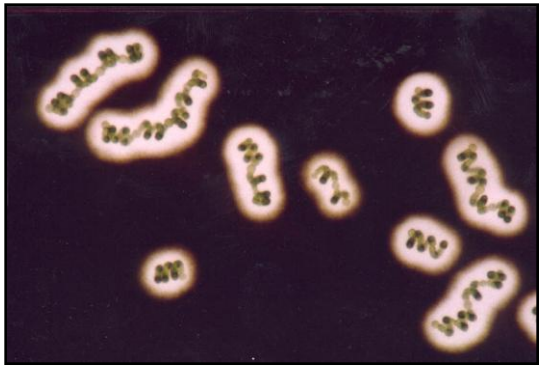


Nostoc VBCCA 1036

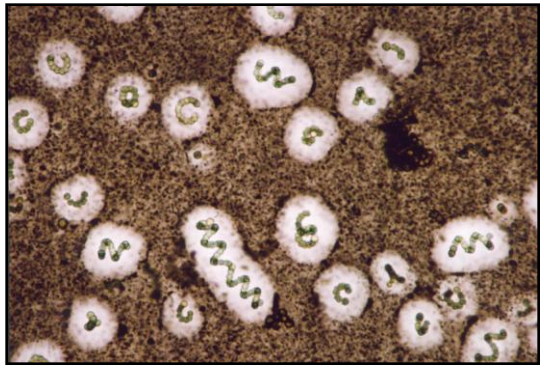
Nostoc VBCCA 10103

(mucilaginous material dispersed around the organism)

Released polysaccharide (RPS)

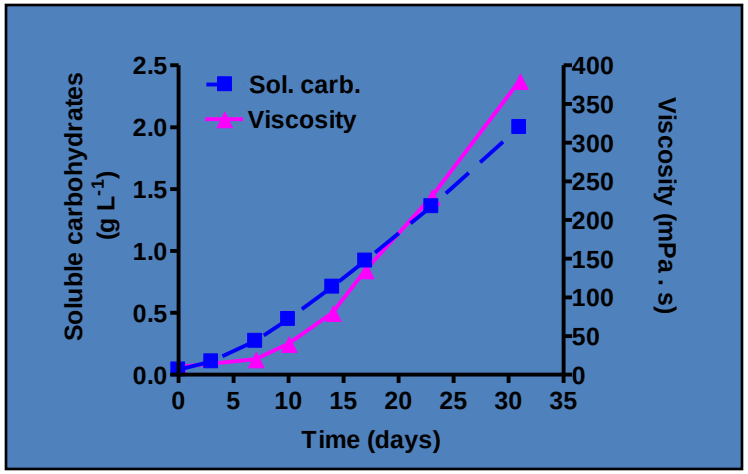


3 days old colture



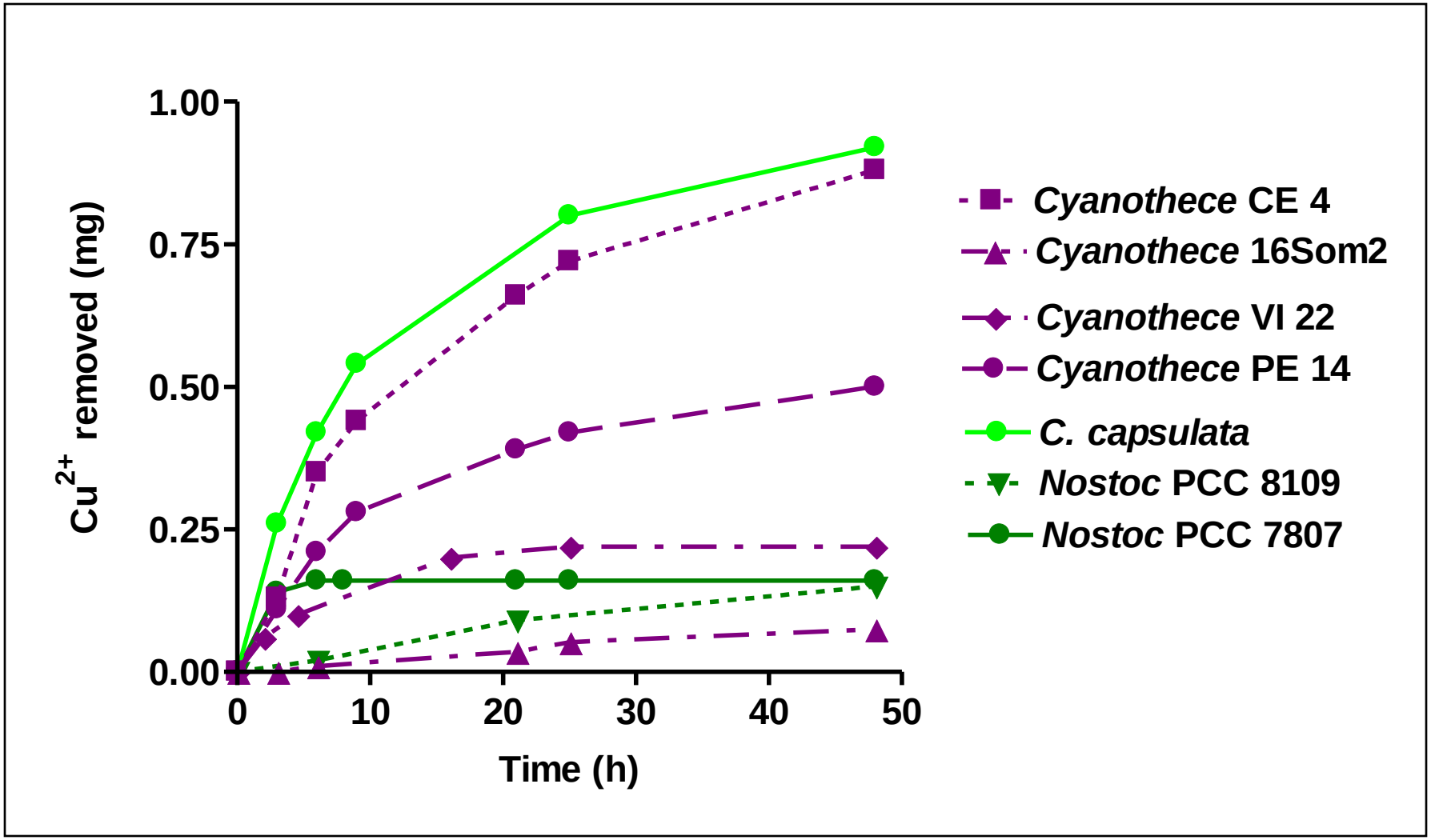
20 days old colture

Cyanospira capsulata



(water-soluble polysaccharide, released into the culture medium)

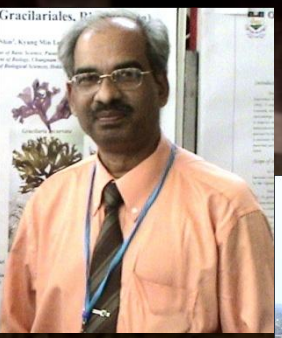
Selection of EPS-producing cyanobacteria for Cu removal



Funding: University Grant Commission, New Delhi, India (2009-2012)

Assessment of bioerosion of exposed surfaces of monuments and hypogea and development of preventive strategies for their conservation

INDO-ITALIAN POC, CHCRT-1



Indian group:

Prof. S.P. Adhikary

Dr. J. Rath

Mr. M. Jena, SRF

Utkal University, Bhubaneswar

Italian group: Prof. Roberto De Philippis (PI)

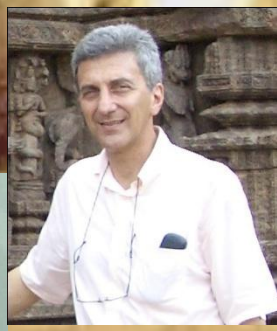
Univ. of Florence

Prof. Patrizia Albertano

Univ. of Rome

Prof. Clara Urzi

Univ. of Messina



The Cyanobacterial Desiccome: Structural and Molecular Basis of Anhydrobiosis

- Dying without dying is a remarkable ability, called anhydrobiosis (life without water) is a characteristic feature of many cyanobacteria.

- In desiccation susceptible systems the complete water removal causes irreversible damage to cells where in desiccation tolerant ones such damages are either prevented or repaired upon wetting.

- The proposal is to dissect out the basis of desiccation tolerance by using few cyanobacterial anhydrobiotes isolated from Indian environments and maintained at our laboratory.

Funding: DST, Govt. of India (2010-2013)

Thank You






UGC DST CSIR