Artificial chemical degradation of some extant cyanobacteria with special reference to Precambrian contaminants—A cautionary note-II

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Shukla, Manoj, Misra, P. K. & Sharma, Mukund 1992. Artificial chemical degradation of some extant cyanobacteria with special reference to Precambrian contaminants—A cautionary note-II. *Palaeobotanist* **39**(3) : 327-332.

The paper is a cautionary note to all Precambrian palaeobiologists, using palaeopalynological techniques for the extraction of microfossils from Precambrian sediments. It deals with problems of contamination in palaeopalynological preparations. The effect of mineralic acids (HCl and HF) on some common cyanobacteria, *viz., Microcystis, Nostoc, Oscillatoria, Lyngbya* and *Scytonema*, has been discussed. It has been shown that the extant cyanobacteria after treatment with these mineralic acids show morphological similarities with many Precambrian microfossils. Therefore, there is need to exercise more vigil while ascertaining the authenticity and affinity of the microfossils recovered through palaeopalynological techniques specially from Precambrian sediments.

Key-words—Palaeobiology, Microfossils, Cyanobacteria, Precambrian.

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सारांश

कैम्ब्रिय-पूर्व संदूषकों पर विशेष सन्दर्भ सहित वर्तमान सियनोजीवाणुओं का कृत्रिम रासायनिक ह्रास: सावधानीयाँ (भाग-2)

मनोज शुक्ला, प्रदीप कुमार मिश्रा एवं मुकुन्द शर्मा

उक्त शोध-पत्र उन सभी पुराजीवविज्ञानीयों के लिए महत्वपूर्ण है जो कैम्ब्रियपूर्व अवसादों से सूक्ष्मजीवाश्मों के निष्कर्षण में पुरापरागाणविक विधियों का प्रयोग करते हैं। यह शोध-पत्र पुराजैविक निर्मितियों में सम्भाव्य संदूषकों की समस्या से सम्बद्ध है। इसके लिए कुछ सियनोजीवाणुओं—माइक्रोसिस्टिस, नोस्टोक, ओसिलेटोरिआ, लिंगबिआ एवं साइटोनीमा, पर खनिज अम्लों (हाइड्रोक्लोरिक अम्ल एवं हाइड्रोफ्लोरिक अम्ल) के प्रभाव का अध्ययन किया गया है। ऐसा देखा गया है कि वर्तमान सियनोजीवाणु इन खनिज अम्लों के उपचार के पश्चात् अन्य बहुत से कम्ब्रिय-पूर्व सूक्ष्मजीवाश्मों से आकारिकीय समानतायें प्रदर्शित करते हैं अतएव कैम्ब्रिय-पूर्व अवसादों से विभिन्न पुरापरागाणविक विधियों द्वारा उपलब्ध सूक्ष्मजीवाश्मों के अभिनिर्धारण एवं इनकी सजातीयता सुनिश्चित करने में अत्याधिक सावधानी की आवश्यकता है।

KNOWLEDGE and understanding of the sequence of events in the development of earliest ecosystem of earth is markedly incomplete due to paucity of evidences. The meagre records available have undergone substantial diagenetic changes and have often been mutilated beyond recognition. The microfossils of early prokaryotic micro-organisms show simple morphology, similar to the extant members of Kingdom Monera and Fungi. These microfossils have been reported mainly from two lithologies: (i) primary cherts, and (ii) clastic rocks particularly shales. It is easier to prove the indigenous nature and syngenecity of the microfossils found in the thin sections of cherts, since we can observe in the slides if the microfossils are actually embedded in the primary cherts. The different state of their preservation which are result of predepositional degradation and post depositional diagenetic changes have also been largely understood through the works of Awramik *et al.* (1972) on artificial silicification and Knoll *et al.* (1975), Knoll and Barghoorn (1975), Golubic and

Hoffman (1976) and Golubic and Barghoorn (1977) on comparative studies of Precambrian Cyanobacteria and extant degraded micro-organisms. But in the case of microfossils, recovered through palynological maceration of shales (acid resistant organic residue recovered by digestion of rocks in HF, HCl and other mineralic acids), the rock matrix, the very evidence necessary to prove the syngenecity of the microfossils, is destroyed. This has resulted in inadvertently describing extant microflora as Precambrian microfossils. These contaminants are introduced in the samples in the field, during maceration process through water or at the time of preparation of slides (aeroflora). Several Precambrian workers have discussed these problems and have suggested methods to overcome them (Maithy & Pflug 1978; Venkatachala, 1987). Recently, Manoharachary *et al.* (1990) have demonstrated that extant soil fungi can withstand the effects of mineralic acids and retain their shape and brown colour which provides a false notion of advanced thermal alteration. The present attempt to study the effect of mineralic acids on some known forms of extant cyanobacteria is a continuation of our earlier work.

MATERIAL AND METHOD

Five extant cyanobacterial genera, viz., *Microcystis* Küetz, *Nostoc* Vaucher, *Oscillatoria* Vaucher, *Lyngbya* Agardh and *Scytonema* Agardh were selected for the study. Morphologically similar analogues of these specimens are common constituents of Precambrian microfloral assemblages. These cyanobacteria members were collected either from field and washed or taken from living cultures. As the morphology of algae does not alter after preserving the material in 4 per cent formaline (Robin, South & Whittick, 1987), same treatment can be given to treated material as well. These micro-organisms were treated with mineralic acids as detailed below:

1. 50 ml of water containing micro-organisms of each genera were put in separate plastic bottles.
2. The sample was treated with HCl (36.5 wt%) for two days to observe its effect. HCl treatment is a routine process for carbonate rocks to isolate fossils. After washing it with distilled water the residue was further treated with HF treatment which is also a routine process for silicious sedimentary rocks. This was carried out to observe the effect of HF on fossils.
3. The sample was treated with LR HF of 40 per cent. The acid was removed with decantation and centrifugation with distilled water.
4. Temporary slides of these organic remains were prepared in glycerine and sealed with wax.

The experiment was conducted at room temperature (23°-17°C). Another set of slides of untreated cyanobacteria was also prepared for reference and comparison.

OBSERVATIONS

Genus—*Microcystis* Kuetz

Microcystis aeuroginosa Küetz

Pl. 1, fig. 3A, B

Description—Colonies spherical, ovoid or irregular in shape with densely packed and evenly distributed cells in common mucilage; cells dark blue-green to black in colour, each with numerous gas vacuoles. Cells 3.5-9 μm in diameter (cf. Desikachary, T. V., 1959, p. 93, pl. 17, figs 1, 2, 6; pl. 18, fig. 10).

Observations—After acid treatment colonies become irregular, mucilaginous covering around the cells are not distinguishable, individual cell wall remains prominent; pigments, vacuoles and other cellular contents disappear. Shape and size of cells, however, remain same.

→

PLATE 1

(Bar in each figure represents 20 μm)

1A. *Scytonema bottneri* Schmidle showing false branching in untreated specimens.
1B, C, D. After acid treatment.
2A, B. *Oscillatoria raoi* De Toni, J.—untreated specimen showing prominent sheath and cell structure.
2C, D. After treatment sheath is lost and cells became disjointed.
3A. *Microcystis aeuroginosa* Küetz—clusters of cells without acid treatment.
3B. After acid treatment cells became hyaline.
4A. *Nostoc* sp.—untreated specimens showing distinct heterocysts and vegetative cells.
4B. Shows the swollen cells, lacking differentiation between heterocysts and vegetative cells.
5A. *Lyngbya majuscula* Harvey ex Gomont—untreated specimen showing colourless filament.
5B. After treatment filaments become dark brown in colour. Folding of mucilaginous sheath appears as septation of trichome in some filaments.

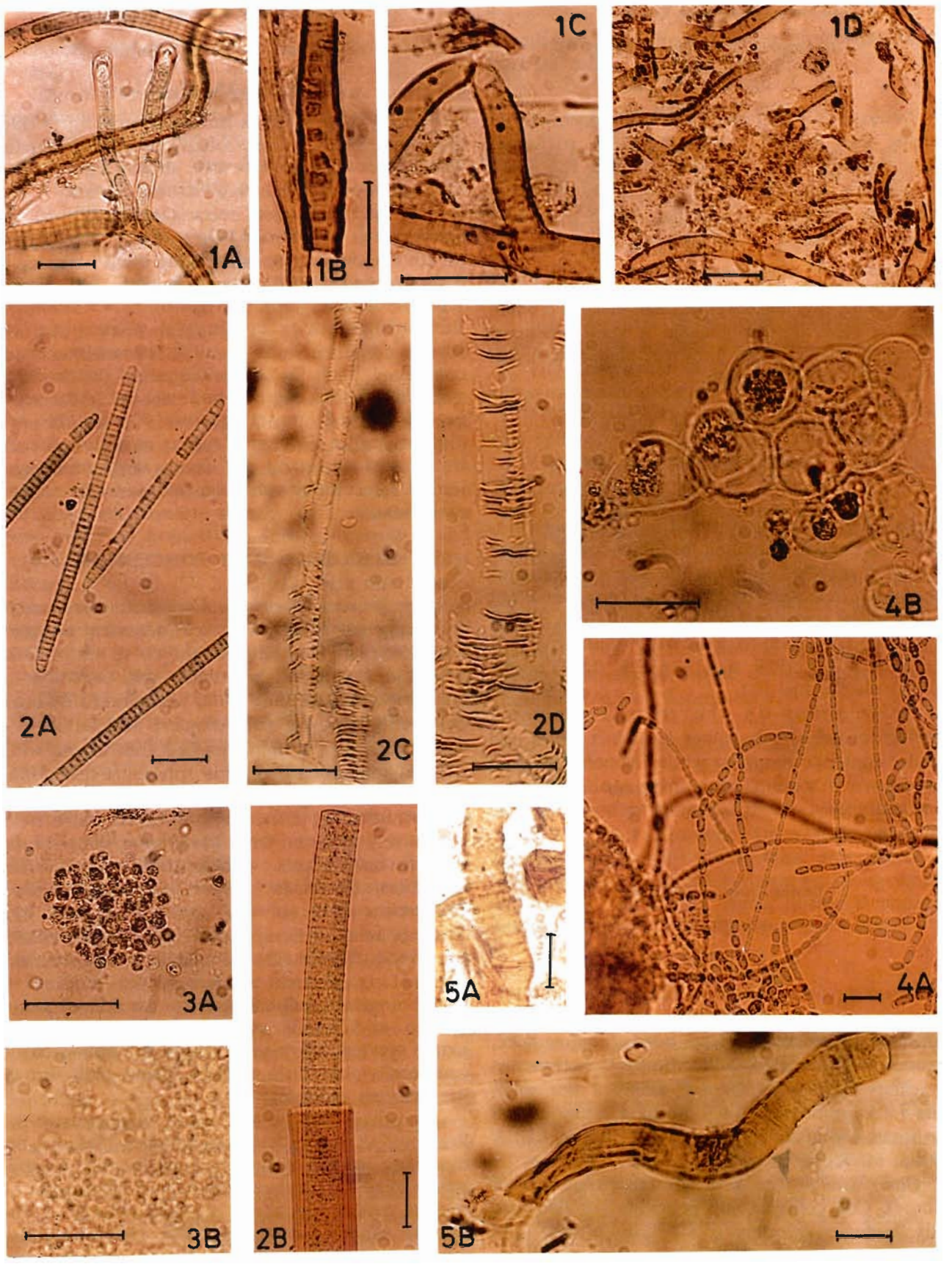

PLATE 1

Genus—*Oscillatoria* Vaucher

Oscillatoria raoi De Toni, J.

Pl. 1, fig. 2 A, B, C, D

Description—Trichomes unbranched, solitary or forming thin masses, sheath indistinct, trichomes uniformly thick without constrictions at the septa, and slightly tapering at the ends, hormogones prominent, cell contents granular. Cell 3-5 μm long and 4.5-7 μm broad (cf. Desikachary, T. V., 1959, p. 223, pl. 42, figs 16-19).

Observations—After 3-4 days of acid treatment cell contents, pigment and mucilage disappear while transverse septa are disorganized. A week later many septa are displaced and hollow trichomes with septal marking on walls are seen.

Genus—*Lyngbya* C. A. Agardh

Lyngbya majuscula Harvey ex Gomont

Pl. 1, fig. 5A, B

Description—Filaments long, unbranched, solitary or densely entwined into flat masses. Sheath lamellated usually colourless but in some filaments light brown in colour, trichome dull blue-green, not constricted at septa, end cells rounded. Cells 17-23.5 μm broad, 3.0-4.5 μm long. Sheath 6-9 μm thick (cf. Desikachary, T.V. 1959, p. 313, pl. 48, fig. 7; pl. 49, fig. 12; pl. 52, fig. 10).

Observations—In the treated filaments mucilaginous sheath becomes indistinct, septa and cellular contents disappear while filaments become dark brown in colour. Mucilaginous sheath forms folds and appears as septations of trichome in some filaments.

Genus—*Nostoc* Vaucher

Nostoc sp.

Pl. 1, fig. 4A, B

Description—Colonies macroscopic with irregular outline, sheath firm and gelatinous, filaments unbranched having beaded appearance, flexuous and entangled, cells cylindrical, heterocysts intercalary and barrel-shaped, akinetes not seen. Cells 4-4.5 μm broad, 7.5-9.5 μm long, heterocysts 6 μm broad and 10-12.5 μm long (cf. Tiffany, L.H. & Britton, M. E., 1952, p. 364).

Observations—The acids dissolve mucilage of colonies. Cell contents and pigments are also lost. Cells become swollen (5.5-7 μm broad) and heterocysts can not be distinguished from vegetative cells of filaments.

Genus—*Scytonema* C.A. Agardh

Scytonema botineri Schmidle

Pl. 1, fig. 1A, B, C, D

Description—Filaments showing false branching, branches are solitary or arising in pair, usually between heterocysts, sheath light brown homogeneous, trichome bluish green, cells rectangular, heterocysts rectangular to ellipsoidal. Hormogones frequently observed. Cells 6-8.5 μm broad, 4-9 μm long, sheath 1.2 μm thick, heterocysts 7.5-8 μm broad, 10-14.5 μm long (cf. Desikachary, T.V., 1959, p. 457, pl. 87, fig. 1).

Observations—The filaments become segregated at many places and false branching is not commonly seen as a consequence of chemical degradation. Cell contents and majority of the septa get dissolved, mucilaginous sheath is not distinguishable. Filaments appear as hollow sheath. Heterocysts withstand the chemical treatment and remain intact alongwith polar nodule in the treated specimens.

DISCUSSION AND CONCLUSIONS

The present observations bring out that many of the changes occurring after acid treatment in extant cyanobacteria are similar to those in fossils. Mucilage cover, cell contents and septation of trichomes in increasing order are affected by acids commonly used for palynological maceration.

The clusters of tightly packed, individually well-defined micrometric organic spheroids resembling certain modern chroococcacean cyanobacteria (eg., *Gomphosphaeria, Microcystis, Eucapsis*) have been extensively reported from the marine shales under various names, *viz.*, problematica, controversial structures, *Bavlinella, Sphaerocongregus variabilis* (Moorman, 1974, p. 535; pl 1, figs 1-4, 7-9), *Pyritosphaera barbaria* (Love, 1957, p. 443; pl. 33, figs 3-5). Dissolution of mucilaginous covering, typical in extant *Microcystis*, makes it morphologically vulnerable to be considered as any of the microfossils mentioned above. However, extant treated *Microcystis* becomes colourless and can easily be distinguished from dark brown Precambrian microfossils.

Sheath genera are the main constituents of the Precambrian microfossil assemblages. Seldom the transverse septae are seen in trichomes. Various stages of septal displacement from trichome can be seen in treated specimens of *Oscillatoria* (Pl. 1, fig. 2C, D). Schopf and Walter (1980, 1982) have also reported *Oscillatoria*-like filamentous cyanobacteria from Fortescue Group (Thumbiana Group), Western

Australia where several cells have been found separated from each other and at some places transverse septum between the adjacent cells is absent.

Due to diagenetic effects the granular nature is a prominent feature of true microfossils which helps in differentiating them from smooth-walled extant contaminants. The true fossils also have clear impression of shale minerals (see Hoffmann, 1984; pl. 32.3, fig. H, Q).

Several species of *Oscillatoria, Lyngbya* and *Scytonema* grow luxuriantly on rocks in xerophytic conditions (Bold & Wynne, 1985; Robin South & Whittick, 1987). These organisms can be the field contaminants in Precambrian rocks and if sustain acidic treatment, they can cause problem for the Precambrian palaeobiologists.

Several species of *Lyngbya* are the main constituents of chert microbial fossil assemblages. On the contrary, the shale microbiota mainly includes sheath genera. These remains include the cellular partings which are probably held by the indistinct and hyaline sheath. Such remains have been reported from Bitter Springs Formation and several other Precambrian localities of the world (Schopf, 1968; Pl. 77, figs 1-5). These microfossils with clear cell structure can easily be distinguished from the extant acid treated specimens where cell contents have been lost. However, some specimens described as *Palaeocytonema* from shales (Mandal *et al.*, 1984; pl. 2, fig. 12) do show morphological and colour comparison with treated specimens of *Lyngbya*. The authenticity of such microfossils needs reassessment.

Poor records of heterocysts in Precambrian sediments may be attributed to the general swelling of cell wall during preservation which makes it difficult to differentiate a vegetative cell from heterocyst. The ultimate reasons for this swelling are not known. However, drawing comparison from treated extant specimen (Pl. 1, fig. 4B), possibly high acidic or pH conditions for long durations may have been responsible for the changes brought about in the morphology of fossil *Nostoc*. In the present experiment it has been observed that treatment with mineralic acids (HCl and HF) results in loss of cell contents and poor incidence of false branching in *Scytonema*. A treated extant *Scytonema* can be compared to any Precambrian sheath genera. It also retains its brown colour, thus making very difficult to differentiate it from true fossils.

The frequency of occurrence of sheaths are higher than the septate filamentous forms in the Precambrian fossil assemblages. This low frequency of septate filaments may be attributed to the effect of chemicals during post depositional diagenesis. There are possibilities that some of the sheath genera of Precambrian microfossils reported may be relicts of extant *Scytonema*.

PRECAUTIONS

The present experimental data suggest the need of extra vigil while ascertaining the affinity and authenticity of Precambrian microfossils obtained through acid maceration. Some general precautions during sample collection and maceration can help to avoid the unintentional mistakes. The precautions are:

(i) Collection of samples from unweathered zones avoiding fissures and joints.

(ii) Knowledge of the soil and hydroflora of sampling locality.

(iii) Thorough cleaning of samples crushed to 1 mm size with the help of ultrasonic vibrator.

(iv) Use of double distilled water in all maceration work.

(v) Contamination free laboratory.

(vi) Knowledge of aeroflora of the place of maceration.

(vii) Fluorescence test of the organic residue easily differentiates the extinct and extant microremains in the organic residue of macerated material. The extant material generally emits a yellow fluorescence while those extinct have lost their property of fluorescence.

ACKNOWLEDGEMENTS

We are thankful to Professor T. V. Desikachary and to Dr B. S. Venkatachala, Director, B.S.I.P. for suggesting this problem. Dr Venkatachala gave valuable suggestions during the progress of the work. We also thank the constant encouragement of Professor C. P. Sharma, Head, Botany Department, Lucknow University during the progress of this work.

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