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

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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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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 syngeneicity 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 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 Küetz

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 botineri Schmidle showing false branching in untreated specimens.
2A, B. Oscillatoria rasi 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.
Genus—Oscillatoria Vaucher

*Oscillatoria raf* 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 4.4-5.5 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.

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, *Bavinella, 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 Palaeyctonema 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 relics 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. Venkatadhasa, Director, B.S.I.P. for suggesting this problem. Dr Venkatadhasa 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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