CHARACTERIZATION AND CLASSIFICATIONS OF BLUE-GREEN ALGAE/CYANOBACTERIA*

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INTRODUCTION

Blue-green algae (BGA) resemble both algae and bacteria and have been classified in the kingdom Plantae and in the kingdom Monera (Procaryotae). In kingdom Plantae, BGA are included in division Cyanophyta or class Cyanophyceae or Myxophyceae while in kingdom Procaryotae, they are included in division Cyanobacteria (Murray, 1968) or order Cyanobacteriales (Gibbons and Murray, 1978). Thus BGA classification into taxonomic categories at the levels of kingdom, division, class or order has not been agreed upon, having in fact described as being in a state of chaos (Whitton, 1967, 1969). Furthermore, the morphological simplicity and occurrence of polymophism, aggrevated the problem, making sometimes identification of the organisms difficult. This paper discusses problems encountered in identification and classification of BGA and new approaches that are being explored to overcome taxonomical problems.

CLASSIFICATION BASED UPON MORPHOLOGICAL FEATURES

Traditional classifications

No sexual reproduction has been demonstrated among BGA. Traditional classifications (Geitler, 1932; Fritsch, 1945; Drouet, 1951; Desikachary, 1959; Bourrelly, 1970a) were therefore based almost entirely upon morphological features. The main morphological features used in taxonomy of BGA are: 1) growth form: unicellular, colonial, filamentous 2) compactness and shape of the colonies 3) shape of the filaments 4) sheath: presence, absence, shape 5) cell differentiation: presence or absence of heterocysts and akinetes 6) size and shape of vegetative cells, heterocysts and akinetes 7) polarity: base and apex of filaments distinguishable 8) branching: presence or absence, false or true, when false, "y"- shaped or geminate 9) nature of true branches: uniseriate or multiseriate Combinations of these different characters have been used for designing traditional classification of BGA. Table 1 is an example of one of the many possible combinations of characters showing the morphological diversity of BGA arranged by increasing order of morphological complexity. In the traditional classifications, BGA are placed in the phylum cyanophyta which comprises a single class; Cyanophyceae.

The taxonomic system for BGA was established in the 19th century by Kuetzing (1849), Thuret (1875), Bornet and Flahault (1886-88) and Gomont (1892). In 1875, Thuret considered all filamentous BGA under Hormogonae while the remaining organisms were placed under Coccogonae.

Subsequently several classifications were proposed emphasizing different morphological characters. At one extreme Elenkin (1936) considered that there were 12 orders and 47 families, and at other extreme Drouet (1951) recognized no distinct orders but only 8 families (cf. Fogg et al., 1973).

The classifications proposed by Geitler (1942), Fritsch (1945) and Desikachary (1959, 1973) fall in between these two extremes. Major features of Fritsch's (1945) and Desikachary's (1973) classifications are presented in Table 2.

Desikachary tried to simplify the classification by amalgamating Chroococcales and Chamaesiphonales of Fritsch's classification in the order Chroococcales because of their commonness.

Further, though Fritsch brought most of the species producing true branches under Stigonematales, he included Brachytrichiaceae, a family with organisms forming true but reverse "v"-type branching, in Nostocales. Desikachary did not agree to this view and included all filamentous BGA with or without false branching in Nostocales and all the organisms with true-branching under Stigonematales. He created a new family Mastigocladaceae in Stigonematales and included Brachytrichia, Mastigocladus etc... in it.

Problems encountered in classifying BGA on morphological features.

The difficulty of achieving a satisfactory classification of unicellular BGA belonging to the order Chroococcales in terms of traditional taxonomic criteria has been generally recognized. Their structural simplicity provides the taxonomist with very few useful characters that are determinable by microscopy (Zehnder, 1973). The classifications based on morphological characters sometimes are of little help in identifying BGA which exhibit morphological variations (polymorphism) in response to changes in environmental conditions (Geitler, 1957; Stein, 1963; Gorham et al., 1964; Pearson and Kingsbury, 1966; Sharp, 1969; Komarek, 1971; Sinclair and Whitton, 1977; Jeeji-Bai, 1978; Nikitina et al., 1979; Stam and Holleman, 1979; Rosenchuk and Mikhailo, 1980; Seki et al., 1981) . The ranges of variations of morphological characters of natural populations of the same genus (e.g. Aphanizomenon) were reported to overlap, forming a continuum with no definite hiatus (Horecka and Komareck, 1979; Baker, 1981). The following is a summarization of the problems encountered in utilizing morphological characters for describing BGA.

Shape of the colony

Gloeotrichia echinulata in axenic cultures does not form spherical colonies but rather assume various other forms depending on the developmental stages. In nutrient poor media, spore-like or homocyst-like hormogonia appear. In nutrient rich media, meristematic compact trichomes appear (Chang, 1979). Stanier et al. (1971) reported that Aphanocapsa, in culture, forms colonies similar to Merismopedia. They questioned whether Merismopedia is a growth form of Aphanocapsa.

From comparisons between Nostoc and Anabaena strains, Forest (1968) concluded that the naive notion that Nostoc can be distinguished by a firm sheath and a macroscopic colony form was untenable and that the field practice of labeling all free filaments as Anabaena was bad taxonomically. Forest pointed out that these genera frequently cannot be distinguished by single observation and that their developmental morphology must be followed for determination.

False branching

The character of false branching, is used at the family level in most of the classifications. Fritsch (1945), and Desikachary (1973) included Plectonema, which forms false branching in the Scytonemataceae, while closely related organisms like Lyngbya and Phormidum which do not form false branching, in Oscillatoriaceae. Studies by Rippka et al. (1979) and Stam (1980) have shown that as far as the genus Plectonema is concerned, the taxonomical weight of false branching is over-estimated. Stanier and Cohen-Bazire (1977) recognized Lyngbya-Phormidum- Plectonema as one group (LPP group). But later it was subdivided into two subgroups -- LPP Group A and LPP Group B -- on the basis of the shape of the cell, constrictions and mode of trichome breakage (Rippka et al., 1979) and included in a group comprising filamentous nonheterocystous forms.

Sheath

It is doubtful whether the morphology of the sheath, and even its presence or absence, can be used as a taxonomic character. Schwabe (1962) pointed out that the presence or absence of a sheath is influenced by environmental factors. Gomont (1892) used sheath morphology or its absence as a taxonomic character at subtribe level to differentiate Oscillatoria, Lyngbya, Phormidium and Plectonema. But the sheaths of homocystous BGA are often difficult to visualize or appeared in aged cultures only, causing conflictuous identifications (cf. Stam, 1980)). Unification of Lyngbya and Phormidium in one genus distinguisable from Oscillatoria by the presence of sheath was proposed by Bourrelly (1970b). Stam (1980) based on DNA-DNA hybridization experiments indicated that characters such as false branching and sheath morphology cannot be used at the generic level but at the specific or lower levels only and proposed that Lyngya, Phormidium and Plectonema should be merged into one genus.

Shape of the filaments

In the case of heterocystous BGA with tapered trichome, several authors have reported the loss of the typical trichome-polarity in the presence of combined nitrogen. This has been described for Calothrix and Gloeotrichia (Fay et al., 1968), Calothrix membranacea (Pearson and Kingsbury, 1966), Fremyella diplosiphon (Wyatt et al., 1973), and several species of Rivulariaceae (Sinclair and Whitton, 1977). Fay et al. (1968) suggested that tapering might have resulted due to increasing gradient of N-deficiency between the basal heterocyst and the apical region. However, we have frequently observed the disparition of tapering in Gloeotrichia sp. filaments when grown on solid N-free medium (unpubl.). Coiling is used as a taxonomic feature for Spirulina spp. However, Lewin (1980) reported that in a pure clonal culture of S. platensis spontaneous variants arose in which coiling was more lax than in the original strain or the filaments were uncoiled. These features persisted in subcultures. On the other hand, normally straight filaments of G. echinulata transformed into double helices when a critical culture density has been attained (Lange, 1975). Similar observations and their taxonomical implications were presented by Jeeji Bai and Seshadri (1980). Among the taxa of Anabaenopsis described, A. magna, A. arnoldii and A. elenkinii which possessed constricted and spirally coiled filaments were recognized as valid species while other taxa represented either synonyms or morphological forms of these species (Jeeji-Bai et al., 1977). However, it is not known how far this spiral nature of the filaments is stable under different environmental conditions.

Apical cell

Shape of the tips of the trichome has been used as a taxonomic character at species level, especially in Oscillatoria. However, while studying polymorphism in natural samples of this genus, De Bicudo and Senna (1977) concluded that the shape of the tip of the trichome has no taxonomic significance when based on examination of isolated trichomes. Form and size of the apical cells of O. redekei was reported to change depending on age, light intensity and growth stage of the strains (Meffert et al., 1981).

Size of the cells

In the taxonomy of unicellular BGA, cell size plays an important role at the species level, but in nature numerous morphological deviations which are not genetically stable exist within specific algal taxa. The cell size of most species is influenced by environmental conditions. Kondratyeva et al. (1974) measured cell size of Microcystis aeruginosa and found that larger cells occurred under less favorable culture conditions. They pointed out that underestimation of the effect of environment on cell size may cause errors in recognition of intraspecific taxa. Kruger et al. (1981) reported a wide range of cell diameters of Microcystis isolates observed at different irradiance levels (1.8 to 7.2 um) and concluded that the use of cell size as a taxonomic character without careful considerations of environmental conditions is questionable. Similarly, cell size of Synechococcus lividus is also influenced by temperature, light intensity and dissolved solids (Kullberg, 1977). Variation in size of the cells of filamentous BGA in response to variation in environmental conditions have also been reported. Statistically significant differences in cell width were observed between natural and cultured samples of O. jenensis (Prykhod'kova, 1980).

Separation of species according to classical conventions is based largely on cell width even though the width limits often appear arbitrary. That is the reason why Whitton et al. (1979) deliberately avoided the use of a binomial in some genera of BGA (especially Chroococales) in their computer coding code for algae although it might be easy to allocate one with a classical flora. Whitton et al. (1979) coded records of cells within categories based on the geometric series 2, 4, 8, 16, 32, 64, 128, 256 and 512 um. However, they pointed out that some species are so variable that it occupies several different width categories. Field populations of Lyngbya were quoted as an example of such a variability.

Mutants

Anacystis nidulans is a rod shaped unicellular alga belonging to Chroococcales. However, Drouet (1968) classified it as a form of Schizothrix calcicola because it forms short chains of cells (which appear like filaments) during cell division and included it in family Oscillatoriaceae. Kunisawa and Cohen-Bazire (1970) obtained stable filamentous mutants of A. nidulans. Similarly, Ingram and Van Baalen (1970) have also obtained filamentous mutants of another unicellular BGA, Agmenellum quadruplicatum on treatment with N-methyl-N'-nitro-N-nitrosoguanidine (NTG). Commenting on these mutants Fogg et al. (1973) questioned whether these filamentous mutants should be regarded as ecophenes of a unicellular alga. Singh and Tiwary (1969) obtained ultraviolet induced mutants of Nostoc linckia which produced 3-pored heterocysts, each pore subtending a branch as in Brachytrichia and Mastigocladus (cf. Fogg et al., 1973). Position of akinetes, whether adjacent to or away from the heterocysts, was considered as one of the most important characters in determining many species belonging to Nostocaceae. However, a number of mutants of BGA unable to form akinetes have been reported, some occurring spontaneously (Singh and Sinha, 1965) others induced by ultraviolet treatment (Singh, 1967) or NTG (Singh, 1978). Several spontaneous heterocyst minus (Het-) mutants were discovered when the cultures of heterocystous BGA were maintained for a long time in the presence of a source of combined nitrogen (Rippka et al., 1979). The mutant, sometimes, outgrows the wild type after several transfers, becoming the dominant member of the population. Such a spontaneous Het- mutants may create confusion in identification of some heterocystous BGA.

The divergence of opinion which has resulted from the use of morphological criteria is enormous (Fogg et al., 1973) and has led to a superfluity of taxa.Whitton (1969) tried to keep account of the new species of BGA created between 1959 and 1969. In the majority of the approximately 110 cases recorded, the new species were not so very different from the ones described by Geitler (1932) and might have been accommodated within that system. The same author also pointed out that in earlier years a single character difference was sometimes all that separated a new species from one previously described.

Numerous cases are known in which the phenotype changes drastically because of a base pair change in just one gene (Stam, 1980). The question arises whether a difference in just one phenotypic character justifies a division in a taxon. Revisions of some taxa have lead to drastic decrease of the species. In coccoid forms, Drouet and Daily (1956) recognized only nine genera while Desikachary (1959) recognized thirty genera. Drouet's revision (1968) of the Oscillatoriaceae recognizes only six genera as against sixteen genera by Desikachary (1959). Though the reduction of number of taxa, to some extent, was in proper direction, revision of coccoid and filamentous forms by Drouet and Daily (1956) Drouet (1968) was not generally accepted (cf. Stanier et al., 1971 and Desikachary, 1973). At present Desikachary (1959, 1973) recognizes about 1500 species in 150 genera. The aim of taxonomy is not only the elaboration of the existing representatives of one group of organisms but also the summarization of knowledge on their morphological, physiological and ecological diversity. An ideal taxonomy should be a tool for ecological, physiological and biochemical studies. It should take in account the genetic relatedness of organisms and emphasize phylogenic relationships. It is clear, in case of BGA, that morphological features alone are not enough to summarize such information.

THE CONCEPT OF CYANOBACTERIA, IMPLICATIONS FOR TAXONOMY

Blue-green algae have been traditionally included in the major group algae according to the classification developed by phycologists, working under the provisions of International Code of Botanical Nomeclature (Stafleu et al. 1972). According to Botanical Code, generic and specific discriminatory properties are either structural or ecologial, these being virtually the only characters determinable in the field. Types are represented by herbarium specimens or for algae by a latin diganoses together with illustrations. The concept of "type specimen" has not been applied consistently in the taxonomy of BGA and even now descriptions of new species are published without specimen being placed on permanent deposit (Forest, 1968). Since many BGA exhibit polymophism, the identification under different environmental conditions may sometimes become difficult.

To overcome such problems, Whitton (1969), Desikachary (1970, 1973) and Stanier et al. (1971) recommended that BGA may be characterized by culturing them in axenic state under defined conditions. Under the circumstances, since Botanical Code does not recognize cultures as valid type materials, they may have to be dealth under Bacteriological Code which recognizes cultured organisms as holotype in place of herbarium specimen, description or illustration.

Based on the prokaryotic nature of BGA and their resemblances with bacteria, Stanier et al. (1971) coined the name Cyanobacteria for BGA. Classification of BGA alongside the bacteria was considered as a truer representation of their phylogenic position (Fogg et al., 1973). In 1977, Stanier and Cohen-Bazire stated that the only logical taxonomic treatment of BGA was to place them in the superkingdom Prokaryotae as a division, class or order of bacteria: the Cyanobacteria.

A proposal to place the nomeclature of the Cyanobacteria under the rules of the International Code of Nomeclature of Bacteria was published (Stanier et al., 1978) and a taxonomic treatment for the genera was presented according to this new concept (Rippka et al., 1979) . Though the concept of cyanobacteria was not universally accepted (Bourrelly, 1979; Geitler, 1979; Golubic, 1979; Lewin, 1979; Kondra'eva, 1981), Gibbons and Murray (1978) proposed the sub-division of kingdom procaryotae into 4 divisions based on the cell wall properties and placed cyanobacteria in the division Gracilicutes which is reserved for all bacteria with gram-negative cell wall (Table 3). During the International Botanical Congress in Sydney (1981) and during the International Microbiological Congress in Boston (1982), a compromise solution was evolved whereby both the Codes may coexist and recognize each other for the nomeclature of cyanobacteria or blue-green algae (Friedman and Borowitzka, 1982; J. B. Waterbury cf. Rippka and Cohen-Bazire, 1983). This dual status provides taxonomists with a wider range of criteria for the classification of BGA/Cyanobacteria and a more flexible system for type specimen deposition.

NEW TRENDS IN CLASSIFICATION

The classical conventions used in the classification of BGA frequently proved unsatisfactory and a symptom of this undesirable stage of affairs is that conventional taxonomy frequently appears to have been ignored by physiologists and biochemists working with BGA (Whitton, 1969). Hence, the latest trends in classification of BGA are directed towards evolving a system based on morphological and non-morphological (viz., dynamic, physiological, biochemical and genetic) characters.

Possible non-morphological characters for taxonomic treatment

Dynamic characters.

Stanier et al. (1971) classified unicellular blue-green algae into six typological groups (Table 4) by considering plane or planes of successive cell division as a primary determinative character. The examples given in Fig. 3 indicate the extent of variability

with regard to cell division and other characters in the same genera identified according to conventional taxonomical keys.

Physiological properties.
In their comparative study of the fatty acids and lipids of axenic strains of filamentous BGA, Kenyon et al. (1972) assigned the strains to typological groups. They found three physiological characteristics that could be used to subdivide these groups: 1) they ability to grow heterotrophically on glucose in the dark; 2) the ability to grow on glucose in the light in the presence of 10-5M DCMU; and 3) the ability of nonheterocystous forms to fix N2 in anaerobiosis. Based on these physiological characteristics, the strains of Oscillatoria, Lyngbya, Anabaena and Calothrix were divided each into two groups while the strains of Plectonema, Spirulina, Microchaete and Chlorogloeopsis were considered each as one group. Attempts are also being made to explore the relationships between individual unicellular species as well as filamentous species based on their sensitivity to antibiotics and increased concentrations of sodium chloride (Avilov et al., 1979).

Fatty acid composition.
In 1968, Holton et al. speculated that the patterns of fatty acid composition among BGA could be of phylogenic significance, increased morphological complexity being accompanied by increased physiological abilities, the fatty acid composition being an indication of this. Analysis by Holton and Blecker (1970) showed that some BGA have a fatty acid composition of the bacterial type, some of the chloroplast type, and some of a unique type. Bacterial type of fatty acid composition is relatively common among unicellular BGA whereas the presence of large quantities of polyenoic fatty acids is characteristic of most filamentous forms. More highly unsaturated acids were found in the morphological more complex BGA. Parker et al. (1967) grouped eleven species into three categories according to their fatty acid composition. A taxonomic treatment of unicellular and filamentous BGA was done by Kenyon and Stanier (1970), Kenyon (1972) and Kenyon et al. (1972) according to the major fatty acid with the highest degree of unsaturation found in each strain. Four groups were recognized: 1) those in which there is little or no desaturation of oleate; 2) those in which linoleate is desaturated towards the omega end of the molecule, to give alphalinoleate; 3) those in which linoleate is desaturated towards the caboxyl end of the molecule to give gammalinoleleate; 4) those in which octadecatetraenoate is synthesized.

Pigments.
Correlation analysis was applied to a study of taxonomic similarities of nine species of BGA using distribution of biliproteins electrophoretic fractions. Results corresponded to the taxonomic distribution by morphological characters (Sud'yina et al., 1980). Carotenoid composition of twenty one isolates of unicellular BGA was analysed with an aim to use it as taxonomic character (Smit et al., 1983). Although there were not large qualitative differences between the carotenoids of the Microcystis aeruginosa isolates, quantitative differences were apparent. Analysis of the data led to the clustering of these isolates into 7 groups. According to Smit et al. (1983), quantitative carotenoid composition may be valuable tool in the taxonomy of the unicellular BGA.

Isozymes.
Studying isozymes of 8 unialgal cultures of BGA (Microcystis aeruginosa; Anabaena cylindrica; A. hassalii; A variabilis; Nostoc punctiforme; N. muscorum; Spirulina platensis; Oscillatoria subbrevis), Tupyk (1978) found a variability suggesting that isozymes of dehydrogenase may be used in taxonomic researches. Further studies (Tupyk, 1980 a, b, c) on the seasonal and age-related variability of glutamate dehydrogenase, malate dehydrogenase and succinate dehydrogenase of the same strains showed that in all seasons and period of algal growth, the stable and low-

variable forms of the enzymes prevailed. Variable isozymes, peculiar to the definite age and season were also observed.

Genome size.
Herdman et al. (1979b) studied the genome size of 128 strains of BGA representative of all major taxonomic groups. The range was from 1.6 x 109 to 8.6 x 109 daltons. The sizes were discontinuously distributed into 4 distinct groups which had means of 2.2 x 109, 3.6 x 109, 5.0 x 109 and 7.4 x 109 daltons. The majority of unicellular strains contained genomes of 1.6 x 109 to 2.7 x 109 comparable in size to those of other bacteria, whereas most pleurocapsalean and filamentous strains possessed larger genomes. From these results it appears that genome size may be a taxonomic character permitting the subdivision of some groups.

Deoxyribonucleic acid base composition.
A summary of all data available on deoxyribonucleic acid (DNA) base composition of BGA was presented by Herdman et al. (1979a) and the taxonomic and evolutionary implications discussed. In this work, assignment of strains to sections and genera followed the taxonomic proposal of Rippka et al. (1979). Results confirmed a wide span of mean DNA base composition in BGA (35.7 to 71.4 mol% GC) and revealed that major DNA base compositional divergence are confined to two sub-groups: unicellular forms that reproduce by binary fission (35 to 71 mol % GC) and filamentous, nonheterocystous forms (40 to 67 mol% GC). For all other BGA including heterocystous and pleurocapsalean forms the overall range was only 38 to 47 mol% GC. The authors pointed out the paradoxical situation where the mol % GC range for the unicellular genus Synechococcus (39% to 71%) is wider than that of two large subgroups such as heterocystous and pleurocapsalean BGA (38% to 76%) which are entirely diverse and very different with respect to structure and development. They concluded that DNA base composition is not a useful taxonomic character among pleurocapsalean and heterocystous BGA but that it emerges as a most important differential property among unicellular forms where the two major genera Synechococcus and Synechocystis can be subdivided by virtue of marked discontinuities delimiting three ranges for Synechoccoccus (39 to 43, 47 to 56 and 66 to 71 mol% GC) and two ranges for Synechocytis (35 to 37 and 42 to 48 mol% GC). The distinction between Spirulina and Arthrospira has long been a subject of controversy. Traditionally, members with regular helical septate trichomes have been placed in the genus Arthrospira and those with helical nonseptate trichomes have been included in genus Spirulina. Generic separation based on the presence or absence of cross-walls was questioned by some workers since some species of so called Spirulina were shown to possess cross-walls. However, DNA base composition of both the genera were found to differ substantially thereby suggesting genetic basis for recognition of two genera (Rippka et al., 1979).

Sequence-specific deoxyribonucleases.
Studies of the value of sequence-specific deoxyribonucleases as taxonomic markers for BGA (de Waard and Duyvesteyn, 1980) have led to some provisional conclusions. First, the presence of a common endonuclease does not give an indication as to the taxonomic affinity of a set of strains. Second, closely related strains such as Anabaena subcylindrica and Anabaena cylindrica (93% homology, as evidenced by hybridization studies) can have different endonucleases. This property can be used to advantage to conclude whether two strains having many properties in common are probably two isolates of the same speices or different species within a genus.

DNA-DNA hybridization.
Stam and Venema (1977) showed that the genotypic relationship between BGA can be determined by a DNA-DNA hybridization method and that this relationship could be used for taxonomic purposes. Stam (1980) found a high similarity between strains of

the genera Lyngbya, Plectonema and Phormidium which supported the creation of a "LPP" group. These results showed that the characters of false branching and sheath morphology cannot be used at the generic level but at the specific or lower levels only. A similar technique was used by Lachance (1981) to study heterocystous BGA. The members of the genera Nodularia, Cylindrospermum, Chlorogloeopsis and Fischerella formed discrete clusters with an intrageneric value of relative binding higher than 55% and an intergeneric relatedness lower than 40%. The genus Nostoc was heterogenous. The genus Calothrix comprised four clusters with various degrees of internal homogeneity and two strains which showed low relatedness to any others. The general relatedness of heterocystous forms to various nonheterocystous ones (unicellular and filamentous) was on the order of 10-20%.

The definition of genera

Many researchers recognize the imperfection and weaknesses of classifications based only on morphological features. As reported above, many non-morphological characters can be used to complement the morphological features. However achievements in this field are still inadequate to evolve a "modern taxonomy" of BGA. The only taxonomic treatment of BGA proposed on the bases of the concept of Cyanobacteria is the one by Rippka et al. (1979). It was designed from a comparative study of 178 axenic strains. Revised definitions of 22 genera were proposed. Differential characters are both constant and readily determinable in culture material. The authors attempted to maintain the system of generic nomenclature and the generic definitions now used by phycologists. However when the discriminatory characters that nominally distinguished two genera were not determinable on cultures or within the range of variation of a single strain, the existing genera were combined. The twenty two genera recognized were placed in five sections, each distinguished by a particular pattern of structure and development (Table 5).

The fact that the only discriminatory characters retained are the ones observed in culture is certainly convenient for laboratory studies of axenic strains but lead to a drastic reduction of the number of genera which is hardly acceptable and may, in some cases, make the definition of a genus more dependant upon the composition of the culture medium than upon the nature of the BGA itself.

The genus Calothrix in Rippka's classification is an example of the limitations of a taxonomic treatment based on character observable in culture only. Because axenic strains of Rivularia and Gloeotrichia do not produce mucilaginous colonies on GO medium (BG 11 medium, without mineral nitrogen) they have been included by Rippka et al. in the genus Calothrix. From the point of view of morphology and ecology, no doubt there is a clear difference between Calothrix and Gloeotrichia. The former grows most frequently adpressed on soil or other substrata as velvet like patches, while the latter develops characteristic mucilaginous colonies. Studies conducted in rice fields of South East Asia (Roger unpublished) have shown that representatives of Calothrix are present in most rice soils but a massive growth of it was never observed. On the other hand, Gloeotrichia is less frequent than Calothrix but when present develops large blooms of ecological significance. Such a luxuriant growth of Gloeotrichia is most probably due to the fact that mucilaginous strains are resistant to grazing by invertebrates. This mucilaginous character of Gloeotrichia is ecologically very important. In our studies, when soil suspensions containing BGA were plated on agarized GO medium, we observed that Gloeotrichia rarely formed characteristic, radiated, rounded, and pigmented mucilaginous colonies, but most frequently developed translucent and irregular mucilaginous colonies in which the tapering of the filaments was strongly altered when compared with field specimens. These colonies were mucilaginous and completely different from the characteristic dark velvet like, patches developed by Calothrix growing on the same solid medium. Subculturings of these "first generation" Gloeotrichia colonies on GO medium lead to loss of tapering and colony forming ability. It is certainly questionable whether the fact that alteration of morphology of Gloeotrichia upon subculturing in GO medium,

making it indistinguishable from Calothrix is enough to merge the two genera into one. Further, utilization of a different culture medium may induce different changes (and lead to a different definition of the genera?).

The classification developed by Rippka et al. (1979) refers to a limited number of BGA strains that are in axenic culture and to the observation of their growth in one culture medium only. It is worthwhile to assess the stability of the morphological characters considered by Rippka et al. in other media before arriving at a definite conclusion .

Rippka's classification is definitely a milestone in BGA taxonomy but it should be considered as one of the possible starting points for the definition of large taxa and not as a final product.

The definition of species

As pointed out by Ravin (1963), "species" is not a fixed concept. The species definition is closely related to the investigator's concept. Microbial geneticist lacking adequate morphological criteria resort to genetic crosses for defining species. A botanist based his species primarily on gross morphology, not on genetics.

Three categories for bacteria species suggested by Ravin (Nomenspecies, Taxospecies and Genospecies) gives an example of the possible ways for the definition of BGA species.

Genospecies are based on the observation of genetic events in the laboratory. When genetic transfer and recombination can be demonstrated within a population it constitutes a genospecies. Genetic recombination was demonstrated in Anacystis nidulans and Cylindrospermum majus (Kumar, 1962; Singh and Sinha, 1965 and Kumar and Ueda, 1984), but information is still too scarce to the possibility of establishing genospecies in BGA.

Nomenspecies applies to a population which shows a common group of characteristics selected for operation usefulness such as for identification or economic value. Nomespecies would be synonymous with most legitimate species under the International Rules. Species of BGA in traditional taxonomy are nomenspecies based on morpholoigcal features. As pointed out by Forest (1968) such species are limited in that, aside from the morphological diagnostic characteristics, the organisms may have little in common. This is especially obvious for unicellular BGA. Taxospecies refers to populations having a high "coefficient of similarity" within them. Numerical methods would be useful for determining taxospecies in BGA. Potentialities of such methods in BGA taxonomy were reviewed by Komareck (1973). Numerical evaluation requires as comprehensive a set of characters as possible worked up into a form capable of being coded. Numerical classification involves the polythetic formation of taxonomic groups without any preliminary definition of the distinguishing features characterizing the superior taxon. But as pointed out by Komareck, result of this procedure do not necessarily differ substantially from the result obtained by traditional methods and does not exclude the necessity for observing the rules of the Code of Botanical Nomenclature.Data should be collected from natural and clonal laboratory grown populations, including the limits of natural and induced variability. Current trends in BGA taxonomy is to look for taxospecies taking in account morphological, dynamic, physiological and biochemical characters. However, whereas numerical taxonomy is currently used for bacteria, a similar system has still to be developed for BGA.

CONCLUSION

The aim of taxonomy is not only the elaboration of the list of existing representatives of one group of organisms but also the summarization of the knowledge on their morphological, physiological and ecological diversity. In the case of BGA, it is clear that morphological features alone are not enough to identity and classify taxa. Hence, actual trends in BGA taxonomy is to evolve a system which is a synthesis of many available criteria paying more attention to the criteria which are stable in different

environmental conditions and can be easily determinable. So far the work done in different aspects of BGA taxonomy has established only a route to such a taxonomy. Presently, though inadequacies exist in conventional taxonomy, it would be premature to reject classical system until a concrete system is evolved embodying fool-proof distinguishing characteristics of different taxa for easy identification. An ideal taxonomy should be a common tool for ecological, physiological and biochemical studies, such a classification has still to be designed for BGA.

REFERENCES CITED

1. Avilov, I. A., L. P. Efremova, and E. S. Sidorenkova. 1979. Comparative study of the sensitivity to antibiotics and increased concentrations of sodium chloride in blue-green algae of different taxonomic position. Vestn Leningr. Univ. Biol. 0(2):86-91.
2. Baker, K. K. 1981. Ecology and taxonomy of 5 natural populations of the genus Aphanizomenon (Cyanophyceae). Arch. Hydrobiol. 92(2):222-251. 3. Bornet, E., and C. Flahault. 1886-88. Revision des Nostocacees heterocystees contenues dans les principaux herbiers de France. Ann. Sci. Nat. Bot. 3:323-81; 4:343-73; 5:51-129; 7: 177-262. (in French)
4. Bourrelly, P. 1970a. Les Algues d'eau douce. III. Les algues bleues et rouges, les Eugleniens, Peridiniens et Cryptomonadines, 512 p. N. Boubee and Cie (Paris) pub.
5. Bourrelly, P. 1970b. Note sur la famille des Oscillatoriacees. Schweitz. Z. Hydrol. 32:519-522.
6. Bourrelly, P. 1979. Cyanophyceae, algae or bacteria? Rev. Algol. 14(1):5-10. (in French)
7. Chang, T. S. 1979. Zur morphologie von Gloeotrichia echinulata (Smith) Ritcher in axenischer Kultur. Nova Hedwigia 31(l-2):265-283.
8. De Bicudo, C. E. and P. A. C. Senna. 1977. Morphological variations in Oscillatoria (Cyanophyceae) in the field and its taxonomical implications. Arch. Hydrobiol. Suppl. 51(1):25-32.
9. Desikachary, T. V. 1959. Cyanophyta monograph, Indian Council for Agricultural Research, New Delhi, India pp. 686.
10. Desikachary, T. V. 1970. Taxonomy of blue-green algae - problems and prospects. Schweiz. Z. Hydrol. 32:490-494.
11. Desikachary, T. V. 1973. Status of classical taxonomy. Pages 473-481 in N. G. Carr and B. A. Whitton, ed. The biology of blue-green algae. Blackwell Publications, Oxford.
12. de Waard, A., and D. Duyvesteyn. 1980. Are sequence-specific deoxyribunuclease of value as taxonomic markers of Cyanobacteria species? Arch. Microbiol. 128:242-247.
13. Drouet, F. 1951. Cyanophyta. Pages 159-166 in G. M. Smith, ed. Manual of phycology. Chronica Botanica Co., Waltham, Mass.
14. Drouet, F. 1968. Revision of the classification of the Oscillatoriaceae. Monogr. Acad Nat. Sci. Philad. 15.
15. Drouet, F., and W. Daily. 1956. Revision of the Coccoid Myxophyceae. Butler Univ. Bot. Studies 12.
16. Elenkin, A. A. 1936. Monographia algarum Cyanophycearum aquidulcium et terrestrium Infinibus URSS Inventarum. Vol. 1 Acad. Nauk URSS, Moscow and Leningrad.
17. Fay, P., W. D. P. Stewart, A. E. Walsby, and G. E. Fogg. 1968. Is the heterocyst the site of nitrogen fixation in blue-green algae? Nature 220:810-812.
18. Fogg, G. E., W. D. P. Stewart, P. Fay, and A. E. Walsby. 1973. The blue-green algae. Academic Press, London. 459 p.
19. Forest, M. 1968. The approach of a modern algal taxonomist. Pages 185-200 in D. F. Jackson, ed. Algae, man and the environment. Syracuse Univ. Press Publ.

20. Friedman, E. I., and L. Borowitzka. 1982. The symposium on taxonomic concepts in blue-green algae: towards a compromise with the bacteriological code? Taxon 31:673-683.
21. Fritsch, F. E. 1945. The structure and reproduction of the algae. Vol. 2. Univ. Press, Cambridge.
22. Geitler, L. 1932. Cyanophyceae. Vol. 14 in L. Rabenhorst, ed. Kryptogamenflora von Deutschland, Osterreich und der Schweiz. Akademische Verlagsgesellschaft, Leipzig.
23. Geitler, L. 1942. Schizophyta: Klasse schizophyceae. Pages l-232 in A. Engler and P. Prantl, ed. 2nd ed. Vol.lb. Die Naturlichen Pflanzenfamilien. 24. Geitler, L. 1957. Zur morphologischen variation und Oekologie auf kleinsten Raum von Cyanophanon mirabile. Oesterr. Bot. Z. 104:100-l14. 25. Geitler, L. 1979. Einige kritische Bemerkungen zu neuen zusammenfassenden Darstellungen der Morphologie und Systematik der Cyanophyceen. Plant Syst. Evol. 132:153-160.
26. Gibbons, N. E., and R. G. E. Murray. 1978. Proposals concerning the higher taxa of bacteria. Int. J. Syst. Bact. 28:l-6.
27. Golubic, S. 1979. Cyanobacteria (blue-green algae) under the Bacteriological Code? An ecological objection. Taxon 28:387-389.
28. Gomont, M. 1892. Monographie des Oscillatoriees (Nostocacee s homocystees). Ann. Sci. Nat. VII. Bot. 15:265-368; 16:91-264. Reprint 1962. J. Crammer, Weinheim.
29. Gorham, P. R., J. McLachlan, U. T. Hammer, and W. K. Kim. 1964. Isolation and culture of toxic strains of Anabaena-flos-aquae(end-un d) (Lyngb.) de Breb. Int. Ver. Theor. Angew. Limnol. Verh. 15:796-804.
30. Herdman, M., M. Janvier, R. Rippka, and R. Y. Stanier. 1979a. Genome size of cyanobacteria. J. Gen. Microbiol. 111:73-85.
31. Herdman, M., M. Janvier, J. B. Waterbury, R. Rippka, and R. Y. Stanier. 1979b. Deoxyribonucleic acid base composition of cyanobacteria. J. Gen. Microbiol. 111:63-71.
32. Holton, R. W., and H. H. Blecker. 1970. Fatty acids of blue-green algae. Pages l15-127 in J. E. Zajic, ed. Properties and products of algae. Plenum Press, N.Y.
33. Holton, R. W., H. H. Blecker, and T. S. Stevens. 1968. Fatty acids in blue-green algae: possible relation to phylogenic position. Science 160:545-547.
34. Horecka, M., and J. Komarek. 1979. Taxonomic position of 3 planktonic blue-green algae from the genera Aphanizomenon and Cylindrospermopsis. Preslia (Prague) 51(4):289-312.
35. Ingram, L. O., and C. Van Baalen. 1970. Characteristics of a stable filamentous mutant of a coccoid blue-green alga. J. Bact. 102:784-789. 36. Jeeji-Bai, N. 1978. Morphological variations of some species of Calothrix and Fortiea. Arch. Protiskend. 119(4):367-387.
37. Jeeji-Bai, N., E. Hegewald, and C. J. Soeder. 1977. Revision and taxonomic analysis of the genus Anabaenopsis. Arch. Hydrobiol. (Suppl.) 51(1):3-24.
38. Jeeji-Bai, N., and C. V. Seshadri. 1980. Coiling and uncoiling of trichomes in the genus Spirulina. Arch. Hydrobiol. Suppl. 60(l):32-47. 39. Kenyon, C. N. 1972. Fatty acids composition of unicellular strains of blue-green algae. J. Bact. 109(2):827-834.
40. Kenyon, C. N., R. Rippka, and R. Y. Stanier. 1972. Fatty acid composition and physiological properties of some filamentous blue-green algae. Arch. Microbiol. 83:216-236.
41. Kenyon, C. N., and R. Y. Stanier. 1970. Possible evolutionary significance of polyunsaturated fatty acids in BGA. Nature 227:l164-l166. 42. Kirkby, S. M., and B. A. Whitton. 1976. Uses of coded data in study of Calothrix and Rivularia. Br. Phycol. J. ll:407-416.
43. Komarek, J. 1971. Benuetzung von Kulturen in der Blaualgen Taxonomie. Schweig. Z. Hydrol. 33:553-565.

44. Komarek, J. 1973. Prospects for taxonomic developments. Pages 482-486 in N. G. Carr and B. A. Whitton, ed. The biology of blue-green algae. Blackwell Publications, Oxford.
45. Kondrat'eva, N. V. 1981. Inadmissibility of placing the nomeclature of blue-green algae (cyanophyta) under the rules of the International Code of Nomeclature of Bacteria. Bot. Zh. (Leningr.) 66(2):215-226.
46. Kondrat'eva, N. V., O. V. Kovalkenko, and O. A. Kysolva. 1974. Variability of cell width of Microcystis aeruginosa Kutz emend. Eluek. and Oscillatoria agardhii Com. (Cyanophyta) under cultivation conditions. Biol. Abst. 58: No. 29821, 3194.
47. Kruger, G. H. J., J. N. Eloff, and J. A. Pretorius. 1981. Morphological changes in toxic and non-toxic Microcystis isolates at different irradiance levels. J. Phycol. 17:52-56.
48. Kuetzing, F. T. 1849. Species Algarum. Leipzig.
49. Kullberg, R. G. 1977. The effect of some ecological factors on cell size of the hot spring alga Synechococcus lividus (Cyanophyta(end-un d)). J. Phycol. 13:lll-l15.
50. Kumar, H. D. 1962. Apparent genetic recombination in a blue-green alga. Nature (London) 196:1121-1122.
51. Kumar, H. D., and K. Ueda. 1984. Conjugation in the cyanobacterium Anacystis nidulans. Mol. Gen. Genet. 195:356-357.
52. Kunisawa, R., and G. Cohen-Bazire. 1970. Mutations of Anacystis nidulans that affect cell division. Arch. Mikrobiol. 71:49-59.
53. Lachance, M. A. 1981. Genetic relatedness of cyanobacteria by DNA-DNA reassociation. Int. J. Syst. Bact. 31(2):139-147.
54. Lange, W. 1975. Transformation of non polar filaments of the blue-green alga Gloeotrichia echinulata U. Wisc. 1052 into double helices. J. Phycol. ll:75-79.
55. Lewin, R. A. 1979. Formal taxonomic treatment of Cyanophyta. Int. J. Syst. Bact. 29:411-412.
56. Lewin, R. A. 1980. Uncoiled variants of Spirulina platensis (Cyanophyceae Oscillatoriaceae). Arch. Hydrobiol. Suppl. 60(l):48-52. 57. Meffert, M. E., R. Oberhauser, and J. Overbeck. 1981. Morphology and taxonomy of Oscillatoria redekei (Cyanophyta). Br. Phycol. J. 16:107-l14. 58. Murray, R. G. E. 1968. Microbial structure as an aid to microbial classification and taxonomy. Spisy Prirodoved. Fak. Univ. J. E. Purkyne Brne 43:245-252.
59. Nikitina, K. A., T. G. Yudina, and M. V. Gusev. 1979. Morphological heterogeneity of the Cyanobacterium Anabaena variabilis under different conditions of its growth and destruction. Mikrobiol. 48(5):873-879.
60. Parker, P. L., C. Van Baalen, and M. Larry. 1967. Fatty acids in eleven species of blue-green algae. Geochemical significance. Science l15:707-708.
61. Pearson, J. E. and J. M. Kingsbury. 1966. Culturally induced variations in four morphologically diverse blue-green algae. Am. J. Bot. 53:192-200. 62. Prykhod'kova. 1980. Studies of variability in width and length of Oscillatoria jenensis (Cyanophyta) cells. Ukr. Bot. Zh. 37(2):16-21.
63. Ravin, A. W. 1963. Experimental approaches to the study of Bacterial Phylogeny. Amer. Nat. 97:307-18.
64. Resenchuk, N. V., and A. A. Mikhailo. 1980. Morphological variability of trichomes of the clone culture Spirulina platensis strain 603 k under various conditions of cultivation. Bot. 2 h. (Leningrad) 65(6):805-815. (in Russian)
65. Rippka, R., and G. Cohen-Bazire. 1983.The Cyanobacteriales: A legitimate order based on the type strain Cyanobacterium stanieri. Ann. Microbiol. (Inst. Pasteur) 134B:21-36.
66. Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman, and R. Y. Stanier. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. lll:l-61.
67. Schwabe, G. H. 1962. Blaualgenprobleme. Schweitz. Z. Hydrol. 24:207-222.
68. Seki, H., H. Ozawa, and S. Ichimura. 1981. Temperature dependence of filament length of Anabaena spiroides var. crassa. Hydrobiol. 83(13):419-424.

69. Sharp, J. H. 1969. Blue-green algae from Bermuda waters: ecologically selected variations of a single species. J. Phycol. 5:57-
70. Sinclair, C., B. A. Whitton. 1977. Influence of nitrogen source on morphology of Rivulariaceae (Cyanophyta). J. Phycol. 13:335-380.
71. Singh, H. N. 1967. Genetic control of sporulation in the blue-green alga Anabaena doliolum. Planta (Berl.) 75:33-38.
72. Singh, V. P. 1978. Mutations defective in nitrogen fixation, heterocyst and spore production in the blue-green alga Anabaena variabilis. Biochem. Physiol. Pflanz. 173:365-372.
73. Singh, R. N., and R. Sinha. 1965. Genetic recombination in a blue-green alga, Cylindrospermum majus Kuetz. Nature (London) 207:782-783.
74. Singh, R. N., and D. N. Tiwary. 1969. Induction by ultraviolet irradiation of mutation in the blue-green alga Nostoc linckia (Roth.) Born. et Flah. Nature (London) 221:62-64.
75. Smit, E. J., G. H. Krueger, and J. N. Eloff. 1983. Carotenoid composition as taxonomic character for Microcystis isolates. J. Limnol. Soc. South Afr. 9(l):43-48.
76. Stafleu, F. A., C. E. B. Bonner, R. McVaugh, R. D. Meikle, R. C. Rollins, R. Ross, J. M. Schopf, G. M. Schulze, R. de Vilmorin, and E. G. Voss. 1972. International Code of Botanical Nomenclature. A. Oasth
oek, Utrecht.
77. Stam, W. T. 1980. Relationships between a number of filamentous blue-green algal strains (Cyanophyceae) revealed by DNA-DNA hybridization. Arch. Hydrobiol. Suppl. 56(4):351-374.
78. Stam, W. T., and H. C. Holleman. 1979. Cultures of Phormidium, Plectonema, Lyngbia and Synechoccus (Cyanophyceae) under different conditions: their growth and morphological variability. Acta Bot. Neerl. 28(l):45-66.
79. Stam, W. T., and G. Venema. 1977. The use of DNA-DNA hybridization for determination of the relationship between some blue-green algae (Cyanophyceae). Acta Bot. Neerl. 26(4):327-342.
80. Stanier, R. Y., and G. Cohen-Bazire. 1977. Phototrophic prokaryotes: the cyanobacteria. Ann. Rev. Microbiol. 31:225-274.
81. Stanier, R. Y., R. Kumizawa, M. Mandel, and G. Cohen-Bazire. 1971. Purification and properties of unicellular blue-green algae (order Chroococcales) Bact. Rev. 35:17l-205.
82. Stanier, R. Y., W. R. Sistrom, T. A. Hansen, B. A. Whitton, R. W. Castenholz, N. Pfenning, V. N. Gorlenko, E. N. Kondratieva, K. E. Eimhjellen, R. Whittenbury, R. L. Gherna, and H. G. Truper. 1978. Proposal to place the nomeclature of the cyanobacteria (blue-green algae) under the International Code of Nomeclature of Bacteria. Int. J. Syst. Bacteriol. 28(2):335-336.
83. Stein, J. R. 1963. Morphological variations of a Tolypothrix in culture. Br. Phycoll. Bull. 2:6-9.
84. Sud'yina, O., V. D. Symechayevs'kyi, and S. I. Los. 1980. Correlation analysis of blue-green algae (Cyanophyta) similarity by electrophoretic spectra of biliproteins (taking in account the age and seasonal variability). Ukr. Bot. Zh. 37(4):16-21.
85. Thuret, G. 1875. Essai de classification des Nostochinees. Annls. Sci. Nat. (Bot.) 6:372-382.
86. Tupyk, N. D. 1978. Species specificity of malate dehydrogenase multiple forms in blue-green algae. Ukr. Bot. Zh. 35(6):602-606 (in Ukr. with Russian and English summary).
87. Tupyk, N. D. 1980a. Malate dehydrogenase of Cyanophyta. Ukr. Bot. Zh. 37(3):63-37. (in Ukr. with Russian and English summary)
88. Tupyk, N. D. 1980b. Seasonal and age-related variability of blue-green algae (Cyanophyta) glutamate dehydrogenase multiplicity of molecular forms. Ukr. Bot. Zh. 37(4):27-31. (in Ukr. with Russian and English summary)

89. Tupyk, N. D. 1980c. Seasonal variability of succinate dehydrogenase isozymes in representative of Cyanophyta. Ukr. Bot. Zh. 37(5):72-76. (in Ukr. with Russian and English summary)
90. Whitton, B. A. 1967. Some comments on the taxonomy of blue-green algae. Br. Phycol. Bull. 3:412.
91. Whitton, B. A. 1969. The taxonomy of blue-green algae. Br. Phycol. J. 4(l):121-123.
92. Whitton, B. A., B. M. Diaz, and N. T. Holms. 1979. A computer oriented numerical coding system for algae. Br. Phycol. J. 14:353-360.
93. Wyatt, J. T., T. C. Martin, and J. W. Jackson. 1973. An examination of three strains of the blue-green algal genus Fremyella. Phycologia 12:153-16l.
94. Zehnder, A. 1973. 6 Symposium ueber Fragen der Cyanophytentaxonomie in Kastanienbaum, 3-6 August 1972. Schw. Zeit. Hydrol. 35(l):12l-135.