Current taxonomic issues with planktonic representatives of the genus *Anabaena* (Cyanobacteria) with special reference to their morphological features; literary review

Problémy současné taxonomie planktonních zástupců sinic rodu *Anabaena* se zvláštním zřetelem na jejich morfologické vlastnosti; literární rešerše

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Abstract

Planktonic representatives of nostocacean genus *Anabaena* belong to the group of important water-bloom-forming cyanobacteria. Their populations develop mostly in summertime, especially in eutrophicated water bodies. Hence, they can strongly influence the quality of various kinds of surface waters. For clear identification, unambiguous species definition and a complete knowledge of the range of morphological variability of single morphospecies are necessary.. This review of the literature summarizes studies connected with the taxonomy of the above mentioned cyanobacterial group, mainly with respect to phenotypic features and morphological variability in relation to changing growth conditions.

1. Current taxonomy of cyanobacteria

It is generally accepted that the current system of cyanobacterial taxonomy should use the Bacteriological Code of Nomenclature (Rippka et al. 1979, Castenholz & Warterbury 1989). However, the botanical name based on morphological characteristics remains the initial step in the new bacteriological approach (Li & Watanabe 1999).

The main difference between the botanical and the bacteriological approach is practical. The botanical system emphasizes a study of natural material, and so it reflects the knowledge of scientists working with field samples. On the other hand, bacteriological systematics is based on genome information gained from clonal cyanobacterial cultures. It allows an approximation of the real phylogenetic relationships among cyanobacteria, but it does not solve the question of the classification of such cyanobacteria, that have not been successfully cultured yet (LAAMANEN 2002).

Nevertheless, the two "schools", botanical and bacteriological, have approached one another (LAAMANEN 2002) over the last decade. In the framework of "the botanical school", a revised cyanobacterial system has been created (ANAGNOSTIDIS & KOMÁREK 1985), which represents a compromise between the botanical and bacteriological approaches. The classification is based predominantly on morphological and ultrastructural criteria, but information gained by biochemical and molecular methods applied on cyanobacterial isolates are also taken into account.

Currently, the application of so-called polyphasic (also combined or complex) approach within the bacteriological classification has become more and more standard. The theme was reviewed by VANDAMME (1996). It combines knowledge obtained by the application of both phenotypic and genetic methods on axenic cyanobacterial cultures. The approach is widespread in present works, and it is possible to say that it has become the norm (CASTENHOLZ 1992, KAŠTOVSKÝ 2002, MOLLENHAUER et al. 1994, PALINSKA et al. 1996, SUDA et al. 1998, LEHTIMÄKI et al. 2000, KAŠTOVSKÝ 2002).

2. Ecology of bloom-forming representatives of the genus *Anabaena*

As mentioned in the abstract of this review, *Anabaena* and also closely related *Aphanizomenon* belong to the cyanobacterial genera that often create mass occurrences (water-blooms), especially in eutrophicated water bodies (UEHLINGER 1981, KOMÁRKOVÁ 1983, REYNOLDS 1984, PECHAR & FOTT 1989, KOMÁREK 1996, PAERL 1996). Numerous field observations confirm that representatives of these genera are common components of water blooms not only in the temperate zone (HINDÁK & MOUSTAKA 1988, KOMÁRKOVÁ-LEGNEROVÁ & ELORANTA 1992, WILLAME & HOFFMANN 1999, BUCKA & WILK-WOŹNIAK 1999). The main factors influencing their occurrence and growth are, similarly to other cyanobacterial genera, relatively high water temperature, high intensity of light and enhanced nutrient concentration (PAERL 1996, KRIVTSOV et al. 2001). Various environmental factors can also influence the toxicity of single strains (RAPALA et al. 1997, RAPALA & SIVONEN 1998).

Previous statements show that rapid development of water blooms can be observed mostly in summer. Blooms of *Anabaena* are normally mixed (consisting of several species) and usually occur during the first part of summer (CMIECH et al. 1984), when *Anabaena* tends to dominate the phytoplankton community (CMIECH et al. 1984, KRIVTSOV et al. 1999). Nevertheless, a smaller amount of *Anabaena* or *Aphanizomenon* can appear in phytoplankton all year round (FJERDINGSTAD 1966, CMIECH et al. 1984) and the dominance of *Aphanizomenon* in winter phytoplankton has been recorded as well (HORNE & GOLDMAN 1974).

However, most *Anabaena* or *Aphanizomenon* filaments usually overwinter as single akinetes in sediments of water bodies and, at the end of spring, the first filaments develop at the bottom during the spring phytoplankton maximum, and later are spread throughout the water column by wind-induced turbulence (HORNE & GOLDMAN 1974, KOMÁRKOVÁ 1983, KRAVCHUK et al. 2002, KANOSHINA et al. 2003, KARLSSON-ELFGREN & BRUNBERG 2004). Goal-directed prevention of akinete germination seems to be a possible method to inhibit bloom formation (BAKER & BELLIFEMINE 2000, TSUJIMURA 2004) and this is why the mechanism of akinete development and its relation to various factors is a popular topic of numerous studies (ROTHER & FAY 1979, CMIECH et al. 1984, HERDMAN 1987, LI et al. 1997, VAN DOK & HART 1997, HINDÁK 1999, BAKER & BELLIFEMINE 2000, KRAVCHUK et al. 2002, HORI et al. 2003, KARLSSON-ELFGREN & BRUNBERG 2004).

Besides this, growth characteristics of the genus *Anabaena* as a response to various conditions were studied (FOY et al. 1976, ROTHER & FAY 1979, KOHL et al. 1989, LEHTIMÄKI et al. 1997, GUPTA et al. 2002, MITROVIC et al. 2003, WESTWOOD & GANF 2004a, 2004b), buoyancy (KASHYAP et al. 1998, BROOKES et al. 1999, MITROVIC et al. 2001), competitive features (DE NOBEL (PIM) et al. 1997, DE NOBEL (PIM) et al. 1998, NALEWAJKO & MURPHY 2001) and other ecological aspects (REYNOLDS 1971, RAPALA & SIVONEN 1998, KRIVTSOV et al. 2001, HORI et al. 2002).

3. Taxonomic classification of the genus *Anabaena*

The genus *Anabaena* was established by Bory in 1822 (STULP & STAM 1985) and has been classified into the filamentous heterocystous cyanobacteria, subsection IV, family I (RIPPKA et al. 2001). Under the Botanical Code the genus *Anabaena* BORY ex BORN. et FLAH.. belongs to the order Nostocales (KOMÁREK & ANAGNOSTIDIS 1989). For many years, the descriptions of the 57 *Anabaena* species given by GEITLER (1932) were major points of reference for the classification of the genus. Later DROUET (1978) criticized this classification. In his opinion, only two *Anabaena* species exist (STULP & STAM 1985). From many species that were established in the past, only some are well known and accepted at present while others need a revision (KOMÁREK 1996).

3.1. Molecular and chemotaxonomic aspects

Molecular studies, comparing both the 16S rRNA sequences (RUDI et al. 1997, LYRA et al. 1997, LYRA et al. 2001, GUGGER et al. 2002b, ITEMAN et al.

2002, Rajaniemi et al. 2005) and for example some fatty acids or proteins (Lyra et al. 1997, Li et al. 1998, Gugger et al. 2002a), revealed a very close relationship between planktonic representatives of the genera *Anabaena* and *Aphanizomenon.* These conclusions suggest that the planktonic *Anabaena* and *Aphanizomenon* belong to the same genus, regardless of their morphological differences. Gugger et al. (2002b), however, point out that only a small number of *Aphanizomenon* strains have been investigated until now, which hinders taxonomic conclusions at the generic level. Hence, further research is necessary. Many other chemotaxonomic and molecular studies of the genera and their taxonomic consequences were published, for example Li et al. 1998, Fergusson & Saint 2000, Li & Watanabe 2001. Nevertheless, detailed overview would exceed the framework of this review.

3.2. Genus and species delimitation based on morphological features

The genus *Anabaena* is distinguished from the genus *Aphanizomenon* on the basis of botanical species *Anabaena oscillarioides* Bory and *Aphanizomenon flos-aquae* (L.) Ralfs. When the two species are compared, the genera are well distinguishable. However, both genera comprise a lot of ”transitional” species, in which the traditional diacritical features are not strictly delimited (Komárek & Anagnostidis 1989).

Fjerdingstad (1966) was among the first who warned of possible mistaking of *Aphanizomenon* for *Anabaena*. Komárek & Kováčik (1989) evaluated the morphological characteristics used for the differentiation of these genera, and the only reliable genus-specific feature appeared to be the structure of trichomes: the genus *Anabaena* has metameric trichomes whereas the trichome structure of the genus *Aphanizomenon* is subsymetric. However, this criterion can be rather problematic when most filaments in a population are too short to recognize trichome structure. Morphological and/or ecological evaluation and delimitation of both genera on generic (Anagnostidis et al. 1988, Komárek & Kováčik 1989, Hindák 2000) and species levels (Pankow 1965, Horecká & Komárek 1979, Baker 1981, Stulp 1982, Stulp & Stam 1982, Stulp 1983, Kováčik & Holečková 1984, Stulp & Stam 1984a, 1984b, Kohl et al. 1985a, 1985b, Stulp & Stam 1985, Komárková 1988, Li et al. 2000a, 2000b, Li & Carmichael 2003) have been the topics of numerous former and present studies.

Rippka et al. (1979) remarked that the morphological boundary between the genera *Anabaena* and *Nostoc* is not always clear as well. The distinction between these two genera has been traditionally based on the character of colony formation, but the gelatinous colonies typical of *Nostoc* spp. growing under natural conditions are rarely formed in cultures. But Rippka et al. (1979) reminds that the genus *Nostoc* differs from *Anabaena* by the developmental cycle and by the motility of trichomes. Molecular results show that these two genera are well separated genetically (RAJANIEMI et al. 2005).

Curious is the unclearness of the difference between the genera *Anabaena* and *Pseudanabaena* in the past. Some phycologists have long expressed doubts about the validity of the genus *Pseudanabaena*, since the trichomes resemble those of *Anabaena* sp. without heterocytes (GEITLER 1932). In 1972, Bourrelly reported that *Pseudanabaena* spp. may exceptionally form heterocytes, and, therefore, considered the boundary between this genus and *Anabaena* imprecise (BOURRELLY 1972 cited by RIPPKA et al. (1979)). However, PRINGSHEIM (1968) had shown earlier that two strains of *Pseunabaena* were incapable of fixing nitrogen aerobically or of heterocyst formation under a wide variety of growth conditions. Also recent molecular studies confirm that *Pseudanabaena* is a separate and well defined genus (ISHIDA et al. 2001, KOMÁREK & KAŠTOVSKÝ 2003).

In accordance with different patterns of akinete development in relation to heterocyst position, KOMÁREK & ANAGNOSTIDIS (1989) suggested the division of former genus *Anabaena* into two well-defined genera, *Anabaena* and *Trichormus.*

The genus *Anabaena* is rather heterogenous, as within the genus we can distinguish two groups which differ in the planktonic or periphytic mode of life (and life form in solitary or clustered trichomes, or in mats, respectively) and particularly by the ability to produce gas vesicles (KOMÁREK & ANAGNOSTIDIS 1989). They are (1) the typical *Anabaena* species (subg. *Anabaena*, type species *A. oscillarioides* BORY ex BORN. et FLAH. 1886) creating filaments without gas vesicles unified into mats on the substrate, floating or living in soils and (2) the planktonic *Anabaena* species (subg. *Dolichospermum* (RALFS ex BORN. et FLAH.) KOM. et ANAGN. 1989, type species *A. flos-aquae* BRÉB. ex BORN. et FLAH. 1886) usually growing in solitary trichomes or in clusters of trichomes, obligatorily containing gas vesicles (aerotopes). Current molecular results indicate that benthic and planktonic *Anabaena* are not monophyletic. Planktonic *Anabaena* strains were intermixed in one conjoint cluster with *Aphanizomenon,* suggesting that they could be assigned to a single genus according to the genetic data (RAJANIEMI et al. 2005).

This review focuses on planktonic representatives of the genus *Anabaena*.

3.3. Investigation of natural morphology and morphological changes in relation to varying growth conditions and culturing

Cyanobacterial classification at generic and species level was originally created on the basis of morphological features.

An initial approach, but still frequently used, is the research of morphological diversity of *Anabaena* representatives in field samples (GEITLER 1932, PANKOW 1965, STARMACH 1966, HILL 1976a, 1976b, 1976c, HICKEL

1982, HICKEL 1985, CRONBERG & KOMÁRKOVÁ 1988, KOMÁRKOVÁ 1988, SANT'ANNA 1991, KOMÁRKOVÁ-LEGNEROVÁ & CRONBERG 1992, KOMÁRKOVÁ-LEGNEROVÁ & ELORANTA 1992, LI & WATANABE 1999, HINDÁK 2000, NAZ et al. 2004).

The first successful attempts to isolate planktonic cyanobacteria from natural samples and to culture monospecific strains were performed in the late 1950's and during the 1960's (EBERLY 1965). Detailed laboratory studies on morphology changes in cyanobacterial isolates were performed later (RIPPKA et al. 1979, ROTHER & FAY 1979, LI et al. 2000b).

The cultivation of cyanobacterial strains in artificial isolation from other species is a suitable method to use to reach various ecological and taxonomic conclusions. It makes it possible to predict definable conditions and to attribute a particular feature or response to them (FOGG et al. 1973). On the other hand, the utilization of cyanobacterial cultures for taxonomic purposes leads to several serious problems.

As mentioned above, a lot of cyanobacterial taxa have resisted isolation in culture until now (ZEHNDER 1985, CASTENHOLZ 1992). ZEHNDER (1985) presents that especially larger species, which have a comparatively low surface to volume ratio and thus their growth rate is usually lower, have not been successfully cultured yet because they are often overgrown by smaller species. Another painful trouble is that the strains of the same species differ one from another; that is to say they can vary greatly under a range of conditions and produce morphologically different developmental stages and morphological forms (ANAND 1988). This is the reason for numerous identification mistakes (KOMÁREK & ANAGNOSTIDIS 1989). Many cases of the designation of cyanobacetrial strains by different names in different culture collections (KOMÁREK 2002) or by different scientists (ANAND 1988) have been observed. This is why ANAND (1988) calls for precise studies of the variability of culture material combined with the complete knowledge of natural populations. To date, no one has ever published a comprehensive volume on "Cyanophytes in axenic cultures" (ZEHNDER 1985). Only Rippka and coworkers (RIPPKA et al. 1979) tried to evolve a better system for the classification of cyanobacteria by designating reference cultures.

The genus *Anabaena* is considered morphologically stable. STULP (1982) determined that incubation of the same strains under different light intensities and temperatures did not influence the use of morphological characters in distinguishing these as separate species. Some other studies are in agreement with Stulp's observations (RIPPKA et al. 1979, LI et al. 2000b; ZAPOMĚLOVÁ 2004). KOMÁREK (1996) also regards single species of *Anabaena* as morphologically constant: their typical forms repeatedly occur at different localities and times.

The only morphological feature that is known to have a wide variation in one and the same species is the spirality of trichomes (KOMÁREK &

ANAGNOSTIDIS 1989). It is unclear to what degree the coiled types can produce straight morphotypes, particularly in culture (KOMÁREK 2002). This statement is supported by several observations of monospecific *Anabaena* isolates. HICKEL (1982) noticed straightening of originally tightly coiled trichomes of *Anabaena spiroides* var. *minima* f. *compacta* (currently classified as *A. compacta* (NYGAARD) HICKEL 1985) after three years of cultivation. On the other hand, some authors designated the spirality of *A. compacta* as very stable (WILLAME et al. 2004, RAJANIEMI et al. 2005). BOOKER & WALSBY (1979) reported on helical and straight forms of *Anabaena*, which they used for further study of sinking velocities and ecological consequences. Also, ZAPOMĚLOVÁ (2004) observed trichome straightening in two strains of *Anabaena* after three years of cultivation (*Anabaena crassa* (LEMMERMANN) KOMÁRKOVÁ-LEGNEROVÁ et CRONBERG 1992 and *A. spiroides* KLEBAHN 1895).

The instability of trichome coiling or concurrent existence of straight and coiled forms is also known in other genera of cyanobacteria: *Spirulina* (BAI 1985), *Nodularia* (KOMÁREK & ANAGNOSTIDIS 1989) and *Cylindrospermopsis* (SAKER et al. 1999, KOMÁREK & KOMÁRKOVÁ 2003, SHAFIK et al. 2003, CHONUDOMKUL et al. 2004).

In spite of the problems mentioned above, the exploration of monospecific cyanobacterial strains in laboratory conditions is still a favourite method for various studies. This applies to the genus *Anabaena,* too. Many papers based on the investigation of cyanobacterial cultures have been published focusing on the morphology of the genus *Anabaena* (STULP 1982, STULP & STAM 1982, STULP 1983, STULP & STAM 1984a, STULP & STAM 1984b, STULP & STAM 1985, LI et al. 2000b, RAJANIEMI et al. 2005), its akinete development (LI et al. 1997, VAN DOK & HART 1997, HORI et al. 2003), molecular features (BELTRAN & NEILAN 2000, FERGUSSON & SAINT 2000, LI & WATANABE 2001, LYRA et al. 2001, GUGGER et al. 2002b, RAJANIEMI et al. 2005) and their consequences for taxonomic classification of cyanobacteria.

Nowadays, research into the molecular features of cyanobacteria is more popular than the investigation of morphology. Reports oriented toward morphological changes of *Anabaena* strains in relation to certain growth conditions are of the earlier date of publication – usually around 1980 or before. RIPPKA et al. (1979) cited similar works concerning various other cyanobacterial genera and the situation seems to be analogous: all were published before the year 1980.

As remarked in Chapter 1., so-called the polyphasic approach is currently asserted in the classification of cyanobacteria. The first to combine morphological and molecular methods in the classification of the genus *Anabaena* were probably Stulp and Stam (STULP 1983, STULP & STAM 1984a, STULP & STAM 1985). The most important contemporary publications of the polyphasic approach applied to the genus are those of GUGGER et al. (2002b) and RAJANIEMI et al. (2005).

4. Summary

The precise knowledge of cyanobacterial morphology, its variability and possible morphological changes of one and the same strain under various conditions is necessary not only for taxonomic revisions of cyanobacterial classification based on the polyphasic approach but also for the routine determination. In the view of contemporary taxonomic approaches, understanding the behaviour of cyanobacteria in cultures is no less important than the knowledge of the morphological variability of natural populations.

Some imperfections can be seen in the research of morphological variability of the genus *Anabaena.* Most studies focusing on morphological changes as a response to growth conditions are not very recent – they were published around 1980 or earlier. Internet databases of scientific publications do not usually contain papers and abstracts published before the 1980's and, hence, the availability of such information is not optimal. Furthermore, earlier studies involved several strains only. Investigations of many more strains are necessary to generalise results not only for strains but also for whole species (morphospecies).

All works that have been published to date usually involve morphological variability either of natural populations or of cyanobacterial strains in cultures. As mentioned above, cultivation conditions can strongly influence the morphology of some strains. Therefore, further studies combining both approaches (observation of natural morphology and morphological changes in cultures) are necessary for reliable species delimitation on the basis of morphological features.

Another problem is that there is no study ranging over the whole morphological scale of the genus *Anabaena* and investigating the possible transient morphology of some natural populations or strains. The existing studies deal only with the strains that are typical representatives of single *Anabaena* species. Molecular results have confirmed that the genera *Anabaena* and *Aphanizomenon* are very closely related and could be assigned to a single genus. Numerous morphological observations support this finding but no comprehensive study exists.

Further examination of morphological variability within the genus *Anabaena* in relation to growth conditions and in comparison to representatives of the genus *Aphanizomenon* is required.

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