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Molecular taxonomic evaluation of *Anabaena* and *Nostoc* strains from the Mosonmagyaróvár Algal Culture Collection

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ABSTRACT

The taxonomy of genera *Anabaena* and *Nostoc* is very controversial. They are typically paraphyletic within phylogenetic trees and show similar morphological characters. The present study aimed to determine the taxonomic relationships among *Anabaena* and *Nostoc* strains of the Mosonmagyaróvár Algal Culture Collection (MACC) using 16S rRNA and *rbcLX* gene sequences. We concluded on the basis of the number of unsuccessful amplifications that more of the examined MACC *Nostoc* cultures are axenic than the *Anabaena* cultures. In agreement with previous studies we noticed that the applied phylogenetic algorithms gave congruent results in phylogenetic analyses. However, the genus *Nostoc* clearly was found not monophyletic in the present study and this finding differed from many of the previous studies. Molecular results contradicted the previous morphology-based classification of some MACC cyanobacteria strains, therefore polyphasic taxonomic approaches are required for the reliable identification of cyanobacterial species. Some strains seemed to be identical based on the alignment of 16S rRNA or *rbcLX* sequences.

1. Introduction

Cyanobacteria species represent an ancient lineage of Gram-negative photosynthetic prokaryotes. They are monophyletic but morphologically diverse. *Nostoc* and *Anabaena* cyanobacterial genera have been traditionally differentiated on the basis of morphological and life cycle characteristics. Identification of cyanobacteria strains in culture by a morphological based system usually leads to ambiguities. Loss of phenotypical attributes during serial inoculations has been observed in numerous microalgal cultures (Day et al., 2005; Lehtimäki et al., 2000; Gugger et al., 2002). According to Komárek and Anagnostidis (1989), the features of more than 50% of strains in collections do not correspond to the characteristics of the taxa to which they are assigned. Additionally, relatively few species grow under axenic culture conditions, which makes the identification even more difficult (Casamatta et al., 2005). To address the above challenges, it was essential to introduce a multidimensional classification system. Polyphasic taxonomy utilises all available data: (i) phenotypic information, such as chemotaxonomic features, morphology, staining behaviour, and culture characteristics, and (ii) genetic properties, such as G + C content, DDH value, and highly-conserved gene sequences. Numerous studies have demonstrated that genetic relationships sometimes conflict with the morphological classification (Lyra et al., 2001; Iteman et al., 2002). The data from the molecular taxonomic separation of *Anabaena* and *Nostoc* genus are also incongruent with the morphological analyses. Based on 16S rRNA gene sequence analysis, Svenning et al. (2005) divided the examined microalgal strains in four clades. Whereas clades II and III contained only *Nostoc* strains, clades I and IV included both *Nostoc* and other (e.g. *Anabaena*, *Aphanizomenon*, *Nodularia*) strains, thus suggesting paraphyletic origin. Within the genus *Anabaena* it is difficult to separate species and strains as they often disperse among other species, or even different genera, with a high similarity (Gugger et al., 2002; Lyra et al., 2001; Rajaniemi et al., 2005; Willame et al., 2006). Based on molecular markers, genus *Nostoc* forms a monophyletic group with high genetic diversity, and the different strains may represent individual species (Rajaniemi et al., 2005; Rasmussen and Svenning, 2001; Wilmotte and Herdman, 2001). However, Rajaniemi et al. (2005) also noted that in certain situations the opposite may be true. In these cases, the high similarities of the 16S rRNA sequence suggested that previously distinct morphospecies belong to a single species.

Although the application of 16S rRNA sequence for taxonomy is wide-spread, the low variability of this region does not allow discrimination among species or strains (Bosshard et al., 2006; Mignard and

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Flandrois, 2006). Sometimes this method was not able to differentiate between *Nostoc* and *Anabaena* strains (Giovannoni et al., 1988; Lyra et al., 2001). Conflicting results are often attributed to the alignment of short sequences or to differing rates of sequence evolution (Hoef-Emden et al., 2002). Therefore, it would be necessary to use full-length 16S rRNA gene sequences of about 1500 bp for a reliable phylogeny reconstruction (Nübel et al., 1997).

Consequently, application of multigene phylogeny is recommended. This approach has been used to study the evolution of various groups of algae (Hoef-Emden et al., 2002). In addition to the 16S rRNA, the RuBisCO large subunit gene sequence (*rbcLX*) has also been used as a phylogenetic marker in the taxonomy of these microorganisms.

This study focused on the *Nostoc* and *Anabaena* strains of the Mosonmagyaróvár Algal Culture Collection (MACC). The MACC collection has 580 strains isolated from soil samples and altogether 270 cyanobacteria and 500 eukaryotic microalgae strains. The strains serve as subjects to investigations related to plant hormone production; efficacy against plant pathogenic fungi; volatile organic compounds and lipid production used for biofuel production (Ördög et al., 2013; Stirk et al., 2013). MACC strains were previously classified based on the morphological attributes by the staff of the Centre for Ecological Research Balaton Limnological Institute (Hungarian Academy of Sciences). In this study, we characterised them by molecular taxonomic methods using both 16S rRNA and *rbcLX* gene sequences.

2. Materials and methods

2.1. Cultivation

Samples of 40 *Nostoc* and 40 *Anabaena* strains, obtained from the MACC were examined in this study. Stock cultures of the selected cyanobacterial strains were inoculated into 500 ml Erlenmeyer flasks containing 250 ml Zehnder-8 nutrient medium and incubated for a week in a culture apparatus described earlier by Ördög (1982). Afterwards, the culture suspensions were re-inoculated into new flasks to get an initial dry matter (DM) content of 10 mg/l. All culture suspensions were aerated with 20 l/h air, which was supplemented with 1.5% CO_2 during the light period. *Anabaena* and *Nostoc* strains were incubated for 5 and 7 days respectively in a light:dark cycle of 14:10 h, at an illumination of 130 μmol m^{-2} s^{-1} and at a temperature of 25 ± 2 °C. The culture suspensions were harvested in the logarithmic growth phase in 2 ml microcentrifuge tubes by centrifugation at 12,000*g* for 5 min at 4 °C. The supernatants were discarded, the pellets (0.3–0.5 mg DM/sample) frozen in liquid nitrogen, and stored at −80 °C before molecular investigations.

2.2. DNA extraction

Two microlitre cyanobacteria suspension was added to 100 μl 10% Chelex 100 solution from BioRad. The samples were incubated at 100 °C for 20 min followed by centrifugation at 12,000*g* for 1 min. The supernatant, which contained the DNA, was aliquoted and kept at −20 °C.

2.3. PCR amplification

Extracted DNA was amplified by PCR, separated by 1.5% (wt/vol) agarose gel electrophoresis, and visualised using ethidium bromide staining. 16S rRNA amplification was carried out in two steps resulting in two overlapping sequences. The first sequence was amplified by the 27F (Lane, 1991) universal and CYA781R (Nübel et al., 1997) cyanobacteria specific primers. Amplification of the second part of the 16S rRNA gene sequence was done by using the cyanobacteria specific CYA359F (Nübel et al., 1997) and universal 1492R (Lane, 1991) primer pair. The *rbcLX* gene sequences were amplified using the primer sequences CX-f and CW-r by Rudi et al. (1998). PCR amplifications were performed in a Veriti Thermal Cycler (Applied Biosystems).

The PCR cycling conditions were as follows: 98 °C for 5 min; 35 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min; 72 °C for 7 min, and a final cooling to 4 °C. Each PCR was carried out in 40 μl volume containing 0.5 μM of each primer, 0.25 mM dNTPs, 1.875 mM $MgCl_2$, 4 μl 10x Taq Buffer with KCl (Thermo Fisher Scientific Inc.) and 5 U of the mixture of *Taq* and *Pfu* polymerases (40:1) (Thermo Fisher Scientific Inc.). PCR products were excited by UV irradiation in a transilluminator, and well-separated bands were carefully excised from the gels using a sterile surgical scalpel. PCR products were purified with the QIAquick Gel Extraction Kit (Qiagen) and were sequenced by an external service (Macrogen Europe). Two biological (ie. from the harvesting of algal cells) and two technical replicates were used to determine the exact gene sequences.

2.4. Bioinformatic analysis of the amplified sequence

The obtained 16S rRNA and *rbcLX* sequences were deposited in GenBank, their accession numbers are listed in Table 1. Sequence similarity searches were done on the NCBI databases with a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Alignment, editing and phylogenetic tree constructions were carried out using the CLC Genomic Workbench software package, version 7.8.1 (CLCBIO, Aarhus, Denmark). Trees were created with CLC Genomic Workbench using the maximum-likelihood, UPGMA (Michener and Sokal, 1957) and Neighbour-joining (Saitou and Nei, 1987) algorithms. The significance was assessed using 500 bootstrap replicates. Average diversities and genetic distances (p-distance) were calculated using the Kimura two-parameter method (Kimura, 1980).

3. Results

The Neighbour-joining, UPGMA and maximum likelihood algorithms were used in the phylogenetic reconstruction. Since the three methods gave congruent results for the major branching patterns of the trees, only the UPGMA tree (cladogram) with Kimura 80 distance measure are presented in the figures.

The sequencing of some samples failed: they resulted in too short reads or noisy peaks after multiple repeats, or the resulting sequences unequivocally proved to be of non-cyanobacterial origin. These cultures were excluded from the phylogenetic analyses.

3.1. Anabaena strains

Twelve different 16S rRNA sequences and 28 *rbcLX* sequences were identified.

3.1.1. Alignment

We found over 99% sequence similarity between strains MACC-177 and -146 (marked as group II/B in Fig. 1), strains MACC-103 and -206 (marked as group II/C in Fig. 1) and strains MACC-187 and -189 (marked as group II/D in Fig. 1) based on the 16S rRNA sequencing results. The *rbcLX* sequences were identical in strains MACC-113, -127, -128, -110, -255, -259, -798, -201, -797 and -57 (marked as group I in Fig. 2), strains MACC-103 and -206 (marked as group II/C in Fig. 2), strains MACC-269, -177, -146, -183, -174 and - 270 (marked as group II/B in Fig. 2) and strains MACC-247, -229, -133, -238, -134, -124 and -304 (marked as group II/A in Fig. 2).

3.1.2. Nucleotide BLAST

Morphological taxons, provenance and habitat information and the BLAST results of the amplified 16S rRNA sequences are shown in Fig. 1, next to the branches. Four strains belonged to the *Trichormus* species, five strains represented *Nostoc* species, and three strains resulted in uncertain classification based on the 16S rRNA sequences.

Table 1
GenBank accession numbers of 16S rRNA and *rbcLX* gene sequences used in the phylogenetic analyses.

Strain	Species name	GenBank accession number of 16S rRNA	GenBank accession number of *rbcLX*
MACC-57	*Anabaena variabilis*	MH702203	MH713634
MACC-103	*Anabaena constricta*	MH702204	MH713635
MACC-104	*Anabaena constricta*	–	MH713636
MACC-109	*Anabaena affinis*	–	MH713637
MACC-110	*Anabaena constricta*	MH702205	MH713638
MACC-113	*Anabaena constricta*	MH702206	MH713639
MACC-121	*Anabaena flos-aquae*	MH702207	MH713640
MACC-124	*Anabaena tenericaulis*	–	MH713641
MACC-127	*Anabaena variabilis*	–	MH713642
MACC-128	*Anabaena* sp.	–	MH713643
MACC-133	*Anabaena variabilis*	–	MH713644
MACC-134	*Anabaena* sp.	–	MH713645
MACC-136	*Anabaena miniata*	–	MH713646
MACC-146	*Anabaena constricta*	MH702208	MH713647
MACC-174	*Anabaena constricta*	–	MH713648
MACC-176	*Anabaena constricta*	–	MH713649
MACC-177	*Anabaena constricta*	MH702209	MH713650
MACC-183	*Anabaena* sp.	–	MH713651
MACC-186	*Anabaena* sp.	–	–
MACC-187	*Anabaena* sp.	MH702210	–
MACC-189	*Anabaena affinis*	MH702211	MH713652
MACC-201	*Anabaena oscillaroides*	MH702212	MH713653
MACC-206	*Anabaena constricta*	MH702213	MH713654
MACC-211	*Anabaena constricta*	MH702214	MH713655
MACC-221	*Anabaena constricta*	MH702215	MH713656
MACC-229	*Anabaena miniata*	–	MH713657
MACC-238	*Anabaena azollae*	–	MH713658
MACC-244	*Anabaena* sp.	MH702216	MH713659
MACC-247	*Anabaena* sp.	MH702217	MH713660
MACC-251	*Anabaena variabilis*	MH702218	MH713661
MACC-255	*Anabaena constricta*	–	MH713662
MACC-256	*Anabaena constricta*	–	MH713663
MACC-259	*Anabaena constricta*	–	MH713664
MACC-260	*Anabaena constricta*	MH702219	MH713665
MACC-269	*Anabaena constricta*	–	MH713666
MACC-270	*Anabaena constricta*	–	MH713667
MACC-304	*Anabaena sphaerica*	–	MH713668
MACC-307	*Anabaena variabilis*	–	MH713669
MACC-797	*Anabaena variabilis*	–	MH713670
MACC-798	*Anabaena hassalii*	–	MH713671
MACC-71	*Nostoc* sp.	MH702220	MH713672
MACC-112	*Nostoc commune*	MH702221	MH713673
MACC-125	*Nostoc* sp.	MH702222	MH713674
MACC-132	*Nostoc sphaericum*	MH702223	MH713675
MACC-139	*Nostoc* sp.	MH702224	MH713676
MACC-148	*Nostoc ellipsosporum*	MH702225	MH713677
MACC-150	*Nostoc* sp.	MH702226	MH713678
MACC-154	*Nostoc commune*	MH702227	MH713679
MACC-172	*Nostoc linckya*	–	MH713680
MACC-173	*Nostoc* sp.	–	–
MACC-175	*Nostoc muscorum*	–	MH713681
MACC-178	*Nostoc commune*	MH702228	MH713682
MACC-181	*Nostoc paludosum*	–	–
MACC-185	*Nostoc pruniforme*	–	MH713683
MACC-193	*Nostoc commune*	–	MH713684
MACC-198	*Nostoc punctiforme*	MH702229	MH713685
MACC-208	*Nostoc* sp.	MH702230	MH713686
MACC-210	*Nostoc punctiforme*	MH702231	MH713687
MACC-218	*Nostoc punctiforme*	MH702232	MH713688
MACC-231	*Nostoc* sp.	MH702233	MH713689
MACC-286	*Nostoc* sp.	MH702234	–
MACC-287	*Nostoc punctiforme*	MH702235	–
MACC-291	*Nostoc* sp.	–	MH713690
MACC-294	*Nostoc* sp.	MH702236	MH713691
MACC-420	*Nostoc muscorum*	–	MH713692
MACC-427	*Nostoc* sp.	–	–
MACC-461	*Nostoc* sp.	MH702237	MH713693
MACC-462	*Nostoc* sp.	MH702238	–
MACC-484	*Nostoc* sp.	–	–
MACC-498	*Nostoc* sp.	MH702239	MH713694
MACC-513	*Nostoc* sp.	MH702240	MH713695
MACC-605	*Nostoc* sp.	–	–
MACC-612	*Nostoc entophytum*	MH702241	–
MACC-627	*Nostoc* sp.	MH702242	MH713696
MACC-633	*Nostoc* sp.	MH702243	–
MACC-634	*Nostoc* sp.	–	MH713697
MACC-661	*Nostoc* sp.	MH702244	MH713698
MACC-668	*Nostoc* sp.	–	MH713699
MACC-683	*Nostoc* sp.	MH702245	MH713700
MACC-707	*Nostoc* sp.	–	MH713701

Two strains were *Scytonema crispum*, eight strains belong to *Trichormus variabilis*, one strain was *Nostoc calcicola*, four strains fell under the *Nostoc* genus, seven strains represented *Anabaena variabilis* and six strains resulted in uncertain classification based on the *rbcLX* sequences (Fig. 2).

3.1.3. Phylogenetic trees

The main topology of the trees was similar for the 16S rRNA and *rbcLX* genes. Three clusters and four subclusters within cluster II were formed in the 16S rRNA and the *rbcLX* tree. *Trichormus variabilis* strains were grouped in cluster I. *Nostoc* species were represented by cluster III and subcluster II/C and II/D. Some strains were grouped into subcluster II/B. According to the results of the *rbcLX* sequence analysis, we grouped two *Scytonema crispum* species in cluster I.

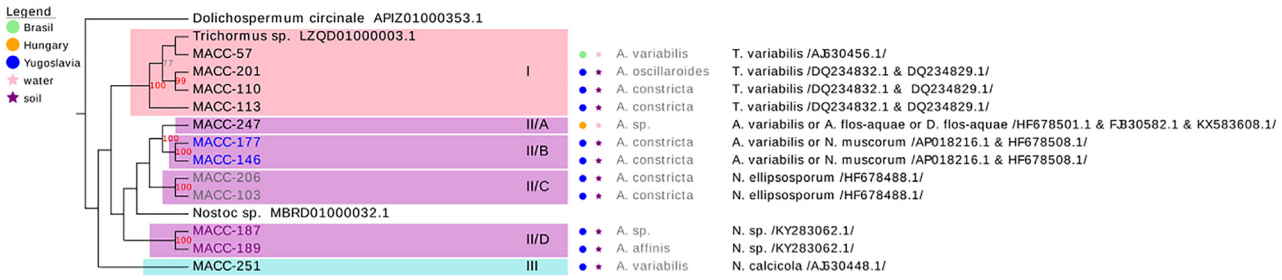

Fig. 1. Phylogenetic tree of the *Anabaena* isolates based on the nucleotide sequence of the 16S rRNA gene. All data obtained from the Genbank are indicated by accession numbers. Bootstrap values ≥70 are indicated at the branch nodes (70–79 values are grey, 80–89 values are blue and 90–100 values are red). Morphological classification is indicated on the right side of the tree with grey font colour. Most relevant BLAST results are on the right with black letters (A. = *Anabaena*, D. = *Dolichospermum*, N. = *Nostoc* and T. = *Trichormus*). Habitat information is labelled with circles and stars.

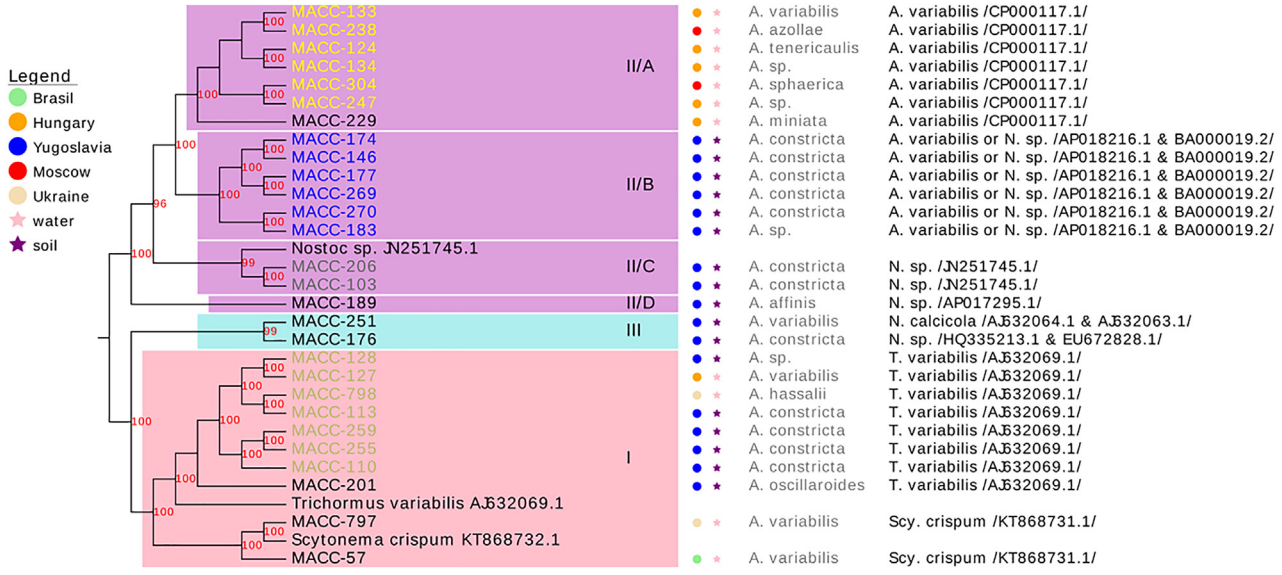

Fig. 2. Phylogenetic tree of the *Anabaena* isolates based on the nucleotide sequence of the *rbcLX* gene. All data obtained from the Genbank are indicated by accession numbers. Bootstrap values ≥70 are indicated at the branch nodes (70–79 values are labelled in grey, 80–89 values in blue and 90–100 values are labelled in red). Morphological classification is indicated on the right side of the tree with grey font colour. Most relevant BLAST results are on the right with black font colour (A. = *Anabaena*, N. = *Nostoc*, Scy. = *Scytonema* and T. = *Trichormus*). Habitat information is marked with circles and stars.

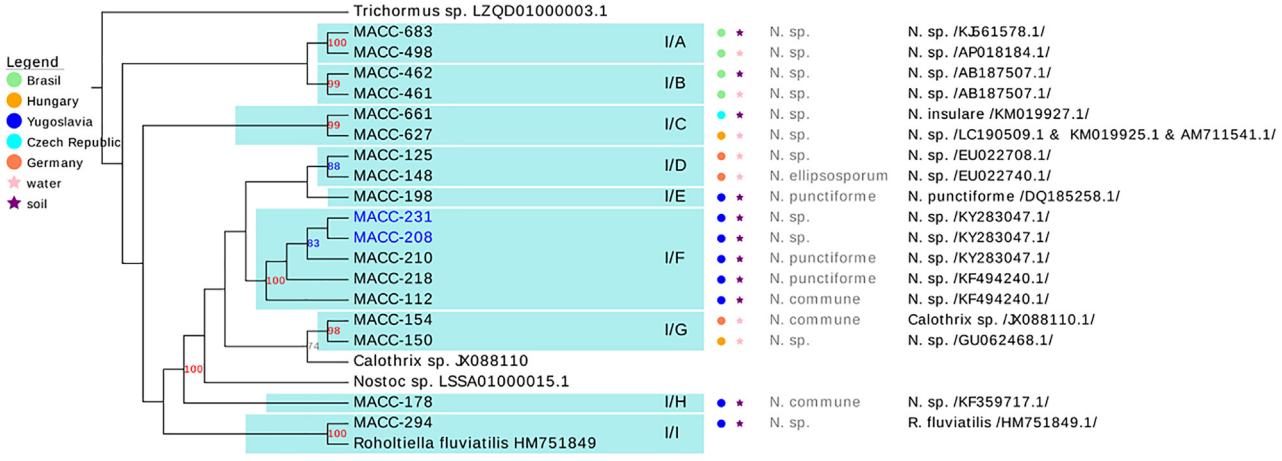

Fig. 3. Phylogenetic tree of the *Nostoc* isolates based on the nucleotide sequence of the 16S rRNA gene. All data obtained from the Genbank are indicated by accession numbers. Bootstrap values ≥70 are indicated at the branch nodes (70–79 values are labelled in grey, 80–89 values are in blue and 90–100 values are in red). Morphological classification is indicated on the right side of the tree with grey letters. Most relevant BLAST results are on the right with black letters (N. = *Nostoc* and R. = *Roholtiella*). Habitat information is marked with circles and stars.

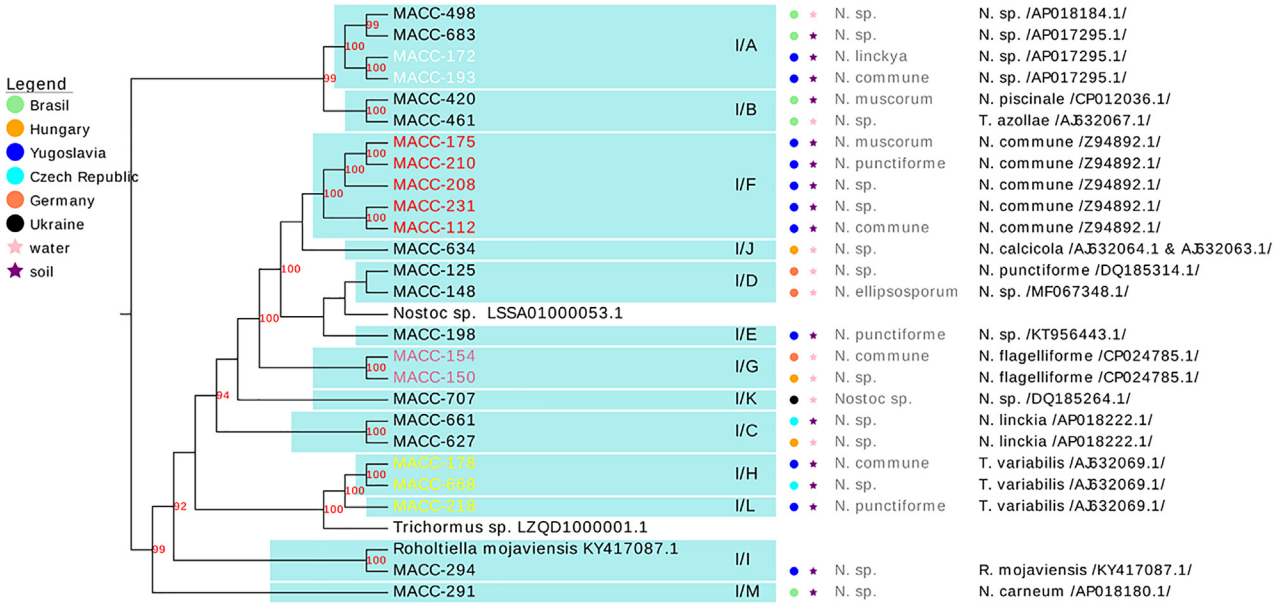

Fig. 4. Phylogenetic tree of the *Nostoc* isolates based on the nucleotide sequence of the *rbcLX* gene. All data obtained from the Genbank are indicated by accession numbers. Bootstrap values ≥70 are indicated at the branch nodes (70–79 values are labelled in grey, 80–89 values in blue and 90–100 values are highlighted in red). Morphological classification is indicated on the right side of the tree with grey font colour. Most relevant BLAST results are indicated on the right with black font colour (N. = *Nostoc*, R. = *Roholtiella* and T. = *Trichormus*). Habitat information is marked with circles and stars.

3.2. Nostoc strains

Eighteen strains gave the expected 16S rRNA sequences, and 25 resulted in correct *rbcLX* sequences.

3.2.1. Alignment

Only MACC-231 and MACC-208 strains seemed to be identical based on the alignment of 16S rRNA sequences (Fig. 3). *rbcLX* alignment resulted in more conformity. Sequences were identical within the group I/B (strains MACC-420 and -461), the group I/C (strains MACC-661 and -627), the group I/F (strains MACC-112, -231, -208, -210 and -175), the group I/G (strains MACC-150 and -154), and the combined group of I/H and I/L (strains MACC-668, -178 and -218). In group I/A, only strains MACC-193 and -172 had identical 16S rRNA sequences (Fig. 4).

3.2.2. Nucleotide BLAST

Sixteen strains fell under the *Nostoc* genus, one of them was *Nostoc insulare*, one was *Nostoc punctiforme*. One strain belonged to *Roholtiella fluviatilis*, another one to *Chalothrix* species for the BLAST of 16S rRNA sequence (Fig. 3).

rbcLX sequences of 20 strains represented *Nostoc* species. Five of them were identified as *Nostoc commune*, two as *N. flagelliforme*, two as *N. linckia*. Furthermore, we identified one *N. calcicola*, one *N. punctiforme*, one *N. carneum* and one *N. piscinale* strain, too. Three strains belong to the *Trichormus variabilis* and one the *Trichormus azollae* taxa. Besides, there was a *Roholtiella mojaviensis* strain (Fig. 4).

3.2.3. Phylogenetic trees

The overall topology of the trees of the *Nostoc* strains was slightly different for the 16S rRNA and the *rbcLX* genes. One cluster and nine sub-clusters within cluster I were represented in the 16S rRNA tree. All of sub-clusters present in the 16S rRNA tree could also be recognised, in the *rbcLX* tree. Four sub-clusters (I/J, I/K, I/L, I/M) were only identified in the *rbcLX* tree. The isolates MACC-154, MACC-178, MACC-218 and MACC-461 were classified into different genera based on the two gene sequences. The sub-cluster I/F of the 16S rRNA tree was separated into two polyphyletic groups based on the *rbcLX* gene sequences (I/F and I/L). The *Trichormus variabilis* strains were contained by the clusters I/H and I/L in the *rbcLX* analysis and were absent in 16S rRNA tree. *Roholtiella* genus was represented by the sub-cluster I/I. *Nostoc species* were classified into the sub-clusters I/A-I/H in the 16S rRNA tree and the sub-clusters I/A-I/G in the *rbcLX* tree. Only one strain from *Calothrix* genus appeared in the sub-cluster I/G in the 16S rRNA tree and one strain from *Trichormus azollae* species appeared in the sub-cluster I/B in the *rbcLX* tree.

4. Discussion

4.1. Full-length 16S rRNA amplification

In the present study mixtures of universal and cyanobacteria specific primers were used to investigate a nearly full-length 16S rRNA gene sequence, avoiding the uncertainty and inaccuracy caused by the short sequences. In case of some non-axenic cultures, the universal components of primer pairs resulted in amplicon mixtures, thereby interfering with the further sequence analysis.

4.2. Comparison of BLAST results with morphological identification

Prior to this examination, there were no molecular taxonomic studies carried out using MACC strains. The classification of strains was done exclusively by morphological characteristics although numerous studies have demonstrated that morphological determination in itself is not always sufficient (Giovannoni et al., 1988; Wilmotte et al., 1994). Furthermore, morphological determinations of strains were not up to date, and in some cases there has been a change in official names since AlgaeBase (Guiry and Guiry, 2018). The most significant difference was the re-classification of *Anabaena variabilis* and *Anabaena azollae* taxa into *Trichormus variabilis* and *Trichormus azollae*.

4.2.1. Anabaena strains

The 16S rRNA-based taxonomical analysis of *Anabaena* strains provided a clear species-level match with the morphological-based assay in case of two strains (MACC-57 and MACC-251). Of these, only the result of the MACC-251 was corroborated by the result obtained from the *rbcLX* sequence. Further four strains exhibited strong similarity with

Trichormus variabilis sequences. The 16S rRNA sequences of five strains were more closely related to *Nostoc* species. MACC-247 strain was likely to be *A. variabilis* according to the *rbcLX* results.

For the *rbcLX* sequences, three strains matches with the morphological classification at the species level (MACC-127, -133, -251). However, we could not confirm this match using the 16S rRNA data of MACC-127 and -133 strains. The BLAST results of further 13 strains showed a close relationship with the *Trichormus* (or *Anabaena variabilis*) taxon, at least at the genus level, confirming the prior morphological classifications. However, five strains showed a closer relationship with *Nostoc* taxa. Two strains grouped to *Scytonema crispum* based on the *rbcLX* sequences.

The information about the natural habitat of the strains (Figs. 1 and 2) was consistent with the general environment information available in AlgaeBase at species level to the *Anabaena* strains.

The strains with uncertain classification and *Anabaena variabilis* strains (cluster II/A and II/B) sharply separated from *Trichormus variabilis* strains (cluster I) in both trees. This fact and the close clustering with *Nostoc* strains suggest that they belong to a *Nostoc* or an authentic (not *Trichormus variabilis*) *Anabaena* species.

4.2.2. Nostoc strains

BLAST analysis of the morphological *Nostoc* strains resulted mostly in *Nostoc* hits. However, the results of the two gene sequences were controversial in some cases. Also, the habitat information of the strains (Figs. 1 and 2) was not always consistent with the general environment information available in AlgaeBase at a species level.

4.3. Strains with identical genotype

4.3.1. Anabaena strains

Some strains were found with identical genotype for the examined gene sequences. MACC-103 and -206 *Anabaena* strains were identical to both gene sequences. Morphological identification and information about their origin supported this finding. MACC-146 and -177 had identical sequences for the examined genes and they could be the same according to morphology and origin information too. The present BLAST analysis results suggest that these strains belong to the same species, which is concordant with the previous morphological results. The rest of the conformity was not verified by both genes and just partially supported by morphological and origin data. Strains with identical genotype may belong to the same species. This is not confuted by the morphological classification of the strains of II/D clade and members of II/B. At the same time, morphological identification contradicts with the genotype results of yellow strains in II/A clade.

4.3.2. Nostoc strains

MACC-208 and -231 *Nostoc* strains were identical according to both gene sequences. The morphology confirmed this result only at the genus level, but they were collected from similar habitats. There were some strains identical to the previous ones, but morphology and 16S rRNA sequences did not support this. Only MACC-210 showed strong similarity (99,93%) with their 16S rRNA sequences. The identity of *rbcLX* sequences of MACC-150 and -154 was not confirmed by 16S rRNA sequences, morphology or origin. Although they originated from similar habitats, neither morphology nor the 16S rRNA sequences (which were missing) confirmed the concordance of MACC-172 and MACC-193 strains. The situation was comparable to MACC-178, -218 and -668 strains, except that in this case the origin only partially met. The morphological differences used in the original classification contradict many of the molecular difference detected in the present study.

4.4. Tree topologies

The tree building algorithms we used gave congruent results for the major branching patterns as can be seen in many other studies (Rajaniemi et al., 2005; Willame et al., 2006). The genera *Anabaena* and *Nostoc* seemed to be paraphyletic in the obtained topologies. This confirmed the observation of many other authors (Gugger et al., 2002; Iteman et al., 2002; Lyra et al., 2001; Rajaniemi et al., 2005; Svenning et al., 2005; Tamas et al., 2000; Willame et al., 2006). *Nostoc* strains were intermixed within the main clusters and not monophyletic as previously described by many authors (Rajaniemi et al., 2005; Rasmussen and Svenning, 2001; Wilmotte and Herdman, 2001).

5. Conclusion

We managed to complement the existing morphology-based taxonomical system of MACC with a new, molecular taxonomy results. In some cases, we got contradictious results. Regarding the morphological classification, it should be updated by also considering the nomenclature changes. In particular for *Nostoc* strains, *16S rRNA*-based BLAST results show fewer contradictions with the habitat information, so the 16S rRNA sequences seem to be more reliable.

It is challenging to produce perfectly axenic cyanobacteria cultures. In the absence of certain symbionts, the cyanobacteria cells became unviable which led to sequencing difficulties. We could conclude from the inaccuracy of the sequencing that more of the examined MACC *Nostoc* cultures are axenic than the *Anabaena (Trichormus)* cultures. We noticed that the three phylogenetic algorithms (Neighbour-joining, UPGMA, maximum likelihood) resulted in congruent outcomes.

The alignment of gene sequences revealed that there are some strains which seem to be identical. These strains could be suitable for similar biotechnological applications. Examination of similarities between the biological activities of these strains should also give interesting results.

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