



Polyphasic characterization of *Nostoc commune* (Cyanobacteria, Nostocaceae) isolated from rice growing agro-ecosystems of Dima Hasao district of Assam, North-East India

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Abstract

Two strains of *Nostoc commune* have been isolated from the soils of two different rice-growing agro-ecosystems, viz. flat and terrace paddy fields of Dima Hasao district of the state of Assam, North-East India. Phenotypic characterization was made for both the strains and their growth, pigments (chlorophyll *a*, total carotenoid content and phycobiliproteins) and biochemical properties (total carbohydrate and soluble proteins) were studied. Phylogenetic comparison was made utilizing 16S rRNA gene sequences. Both strains presented higher phycocyanin content than other biliprotein pigments. Total carotenoid content (TCC) was higher in the strain isolated from flat paddy field, while the isolate from terrace paddy field was richer in phycobiliproteins. 16S rRNA gene sequences of the isolated *N. commune* strains were compared with available sequences of other strains of *Nostoc* and *Anabaena* from various geographical locations. Gene sequences were clustered according to their geographical origin, which also reflected the disputed taxonomic position of the Nostocacean genera *Nostoc* and *Anabaena*.

Key words: Assam, cyanobacteria, morphology, *Nostoc commune*, polyphasic characterization, taxonomy

Introduction

Paddy cultivation in terraces and in the low lying flat valley lands are common land use practices in rice-growing countries of the world. Whereas flat paddy fields are very common, rice cultivation in terraces are restricted to the hilly parts of the world, including hilly terrains of Himalayan area (Sikkim, Himachal Pradesh and North-East India) in India. Such agro-ecosystems harbor diverse cyanophytes. Colonizing both terrestrial and aquatic habitats, *Nostoc* species-owing to their nitrogen fixing ability-constitute an important entity in rice fields (Dodds *et al.* 1995, Gao & Ai 2004). They are adaptive to a wide range of habitats due to their unique metabolic flexibility, with high dispersion capacities, large-sized populations and low extinction probabilities (Fenchel 2003).

Nostoc commune Vaucher ex Bornet & Flahault (1888: 203), known to be a highly drought-tolerant species (Takaichi *et al.* 2009), is one of the most dominant colonizers in the paddy fields of Dima Hasao district of Assam. This prokaryote is known to be a conspicuous cyanobacterial species in terrestrial microbial communities, with a worldwide distribution, particularly in nutrient-poor soils and on exposed limestone surfaces. Lipman (1941) mentioned the revival of *N. commune* from an 87-year-old herbarium specimen. Indeed, dried *N. commune* colonies can be successfully revived after more than one hundred years (Cameron 1962, Kviderova *et al.* 2011). *Nostoc commune* colonies which are partially hydrated are also known to have the potential to carry out photosynthesis, and are capable of fixing nitrogen in Arctic hydroterrestrial habitats as well (Novis *et al.* 2007, Kviderová *et al.* 2011).

Morphological diversity is known to have a strong correlation with biochemical parameters (Holton *et al.* 1968). These gram-negative prokaryotes exhibit a great morphological diversity along with wide spectra of physiological properties.

References

- Agardh, C.A. (1824) *Systema Algarum*. Berling, Lund, 312 pp.
- Adhikary, S.P., Weckesser, J., Jürgens, U.J., Golecki, J.R. & Borowiak, D. (1986) Isolation and chemical characterization of the sheath from the cyanobacterium *Chroococcus minutus* SAG B.41.79. *Journal of General Microbiology* 132: 2595–2599.
- Ahluwalia, A.S., Rai, R.K. & Kumar, H.D. (1980) Chromatic adaptation and photoreversal in blue-green alga *Calothrix clavata* West. *Journal of Biosciences* 2: 63–68. <http://dx.doi.org/10.1007/BF02703135>.
- Becerra-Absalón, I., Rodarte, B., Osorio, K., Alba-Lois, L., Segal-Kischinevzky, C. & Montejano, G. (2013) A new species of *Brasilonema* (Scytonemataceae, Cyanoprokaryota) from Tolantogo, Hidalgo, Central Mexico. *Fottea* 13: 25–38.
- Bennet, A. & Bogorad, L. (1973) Complementary chromatic adaptation in a filamentous blue green alga. *The Journal of Cell Biology* 58: 419–435.
- Bornet, É. & Flahault, C. (1888) Revision des Nostocacées hétérocystées continues dans les principaux herbiers de France. *Annales des Sciences Naturelles, Botanique, Septième Série* 7: 177–262.
- Cameron, R.E. (1962) Species of *Nostoc* Vaucher occurring in the Sonoran Desert in Arizona. *Transactions of the American Microscopical Society* 81: 379–384.
- Chu, H.J. & Tsang, C.T. (1988) Research and utilization of cyanobacteria in China: a report. *Archiv für Hydrobiologie* 80: 1–4.
- Desikachary, T.V. (1959) *Cyanophyta*. Indian Council of Agricultural Research, New Delhi. 669 pp.
- Dodds, W.K., Gudder, D. A. & Mollenhauer, D. (1995) The ecology of *Nostoc*. *Journal of Phycology* 31: 2–18. <http://dx.doi.org/10.1111/j.0022-3646.1995.00002.x>
- Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791. <http://dx.doi.org/10.2307/2408678>.
- Fenchel, T. (2003) Biogeography for bacteria. *Science* 301: 925–926. <http://dx.doi.org/10.1126/science.1089242>
- Finkel, Z.V. (2001) Light absorption and size scaling of light-limited metabolism in marine diatoms. *Limnology and Oceanography* 46: 86–94.
- Gao, K. & Ai, H. (2004). Relationship of growth and photosynthesis with colony size in an edible cyanobacterium, ge-xian-mi *Nostoc* (Cyanophyceae). *Journal of phycology* 40: 523–526. <http://dx.doi.org/10.1111/j.1529-8817.2004.03155.x>
- Geitler, L. (1932) Cyanophyceae. *Rabenhorst's Kryptogamen Flora von Deutschland, Österreich und der Schweiz* 14: 1–1196.
- Haande, S., Rohrlack, T., Ballot, A., Røberg, K., Skulberg, R., Beck, M. & Wiedner, C. (2008) Genetic characterisation of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) isolates from Africa and Europe. *Harmful Algae* 7: 692–701. <http://dx.doi.org/10.1016/j.hal.2008.02.010>
- Hariot, P. (1891) Le genre *Polycoccus* Kützing. *Journal de Botanique* 5: 29–32.
- Herbert, D., Philipps, P.J. & Strange, R.E. (1971) Chemical analysis of microbial cells. In: Norris, J.R. & Ribbons, D.W. (eds.) *Methods in Microbiology. Vol. VB*. Academic Press, New York, pp. 209–344.
- Holton, R.W., Blecker, H.H. & Stevens, T.S. (1968) Fatty acids in blue green algae: possible relation to phylogenetic position. *Science* 160: 545–547.
- Huang, Z., Liu, Y., Paulsen, B.S. & Klaveness, D. (1998) Studies on polysaccharides from three edible species of *Nostoc* (cyanobacteria) with different colony morphologies: comparison of monosaccharide compositions and viscosities of polysaccharides from field colonies and suspension cultures. *Journal of Phycology* 34: 962–968.
- Johnson, H.E., King, S.R., Banack, S.A., Webster, C., Callanaupa, W.J. & Cox, P.A. (2008) Cyanobacteria (*Nostoc commune*) used as a dietary item in the Peruvian highlands produce the neurotoxic amino acid BMAA. *Journal of Ethnopharmacology* 118: 159–165. <http://dx.doi.org/10.1016/j.jep.2008.04.008>
- Komárek, J. & Anagnostidis, K. (1989) Modern approach to the classification system of Cyanophytes. 4-Nostocales. *Algological Studies* 82: 247–345.
- Kützing, F.T. (1843) *Phycologia generalis*. F.A. Brockhaus, Leipzig, 458 pp.
- Kvídová, J., Elster, J. & Šimek, M. (2011) In situ response of *Nostoc commune* s.l. colonies to desiccation in Central Svalbard, Norwegian High Arctic. *Fottea* 11: 87–97.
- Lamprinou, V., Hernández-Mariné, M., Pachiadaki, M.G., Kormas, K.A., Economou-Amilli, A. & Pantazidou, A. (2013) New findings on the true-branched monotypic genus *Iphinoe* (cyanobacteria) from geographically isolated caves. *Fottea* 15: 15–23.
- Lipman, C.B. (1941) The successful revival of *Nostoc commune* from a herbarium specimen eighty-seven years old. *Bulletin of the Torrey Botanical Club* 68: 664–666.
- Lukešová, A. (1993) Soil algae in four secondary successional stages on abandoned fields. *Algological studies* 71: 81–102.
- Martinez, M.R., Evangelista, C.L. & Pantastio, J.B. (1977) *Nostoc commune* Vauch. as a potential fertilizer in rice-fish culture: a preliminary study. *Philippine Journal of Crop Science* 2: 252–255.
- Masters, J.C. and Brothers, D.J. (2002) Lack of congruence between morphological and molecular data in reconstructing the phylogeny of the galagonidae. *American Journal of Physical Anthropology* 117: 79–93. <http://dx.doi.org/10.1002/ajpa.10001>
- Moraes, C.C., Sala, L., Cerveira, G.P. & Kalil, S.J. (2011) C-phycoyanin extraction from *Spirulina platensis* wet biomass. *Brazilian Journal of Chemical Engineering* 28: 45–49.
- Neilan, B.A. (1995) Identification and phylogenetic analysis of toxigenic cyanobacteria by multiplex randomly amplified polymorphic DNA PCR. *Applied and Environmental Microbiology* 61: 2286–2291.
- Novis, P.M., Whitehead, D., Gregorich, E.G., Hunt, J.E., Sparrow, A.D., Hopkins, D.W., Elberling, B. & Greenfield, L.G. (2007) Annual carbon fixation in terrestrial populations of *Nostoc commune* (Cyanobacteria) from an Antarctic dry valley is driven by temperature. *Global Change Biology* 13: 1224–1237. <http://dx.doi.org/10.1111/j.1365-2486.2007.01354.x>

- Nübel, U., Garcia-Pichel, F. & Muyzer, G. (1997) PCR primers to amplify 16S rRNA genes from cyanobacteria. *Applied and Environmental Microbiology* 63: 3327–3332.
- Oksanen, I., Lohtander, K., Sivonen, K. & Rikkinen, J. (2004) Repeat type distribution in trnL intron does not correspond with species phylogeny: comparison of the genetic markers 16S rRNA and trnL intron in heterocystous cyanobacteria. *International Journal of Systematic and Evolutionary Microbiology*, 54: 765–772.
<http://dx.doi.org/10.1099/ijs.0.02928-0>
- Parsons, T.R. & Strickland, J.D.H. (1965) Determination of phytoplankton pigments. *Journal of the Fisheries Research Board of Canada* 18: 117–127.
- Parsons, T.R., Maita, Y. & Lalli, C.M. (1984) *A manual of chemical and biological methods for seawater analysis*. Pergamon press, Oxford, 73 pp.
- Pisani, D., Benton, M.J. & Wilkinson, M. (2007) Congruence of morphological and molecular Phylogenies. *Acta Biotheoretica* 55: 269–281.
<http://dx.doi.org/10.1007/s10441-007-9015-8>
- Planet, P.J. (2006) Tree disagreement: measuring and testing incongruence in phylogenies. *Journal of Biomedical Informatics* 39: 86–102.
<http://dx.doi.org/10.1016/j.jbi.2005.08.008>
- Prakash, O., Verma, M., Sharma, P., Kumar, M., Kumari, K., Singh, A., Kumari, H., Jit, S., Gupta, S.K., Khanna, M. & Lal, R. (2007) Polyphasic approach of bacterial classification. An overview of recent advances. *Indian Journal of Microbiology* 47: 98–108.
<http://dx.doi.org/10.1007/s12088-007-0022-x>
- Rajaniemi, P., Hrouzek, P., Kaštovská, K., Willame, R., Rantala, A., Hoffman, L., Komárek, J. & Sivonen, K. (2005) Phylogenetic and morphological evaluation of the genera *Anabaena*, *Aphanizomenon*, *Trichormus*, and *Nostoc* (Nostocales, cyanobacteria). *International Journal of Systematic and Evolutionary Microbiology* 55: 11–26.
<http://dx.doi.org/10.1099/ijs.0.63276-0>
- Ramírez, M., Hernández-Mariné, M., Mateo, P., Berrendero, E. & Roldán, M. (2011) Polyphasic approach and adaptative strategies of *Nostoc cf. commune* (Nostocales, Nostocaceae) growing on Mayan monuments. *Fottea* 11: 73–86.
- Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. & Stanier, R.Y. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* 111: 1–61.
<http://dx.doi.org/10.1099/00221287-111-1-1>
- Rudi, K., Skulberg, O.M. & Jakobsen, K.S. (1998) Evolution of cyanobacteria by exchange of genetic material among phylogenetically related strains. *Journal of Bacteriology* 180: 3453–3461.
- Saitou, N. & Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425.
- Schagerl, M. & Müller, B. (2006) Acclimation of chlorophyll *a* and carotenoid levels to different irradiances in four freshwater cyanobacteria. *Journal of Plant Physiology* 163: 709–716.
<http://dx.doi.org/10.1016/j.jplph.2005.09.015>
- Silveira, S.T., Burkert, J.F.M., Costa, J.A.V., Burkert, C.A.V. & Kalil, S.J. (2007) Optimization of phycocyanin extraction from *Spirulina platensis* using factorial design. *Bioresource Technology* 98: 1629–1634.
<http://dx.doi.org/10.1016/j.biortech.2006.05.050>
- Singh, S.M., Singh, P. & Thajuddin, N. (2008) Biodiversity and distribution of Cyanobacteria at Dronning Mod Land, East Antarctica. *Acta Botanica Malacitana* 33: 17–28.
- Smoker, J.A. & Barnum, S.R. (1988) Rapid small – scale DNA isolation from filamentous cyanobacteria. *FEMS Microbiology letters* 56: 119–122.
<http://dx.doi.org/10.1111/j.1574-6968.1988.tb03161.x>
- Spiro, R.G. (1966) Analysis of sugars found in glycoproteins. *Methods in Enzymology* 8: 3–26.
- Svenning, M.M., Eriksson, T. & Rasmussen, U. (2005) Phylogeny of symbiotic cyanobacteria within the genus *Nostoc* based on 16S rDNA analyses. *Archives of Microbiology* 183: 19–26.
<http://dx.doi.org/10.1007/s00203-004-0740-y>
- Takaichi, S., Maoka, T. & Mochimaru, M. (2009) Unique carotenoids in the terrestrial Cyanobacterium *Nostoc commune* NIES-24:2-Hydroxymyxol 2'-Fucoside, Nostoxanthin and Canthaxanthin. *Current Microbiology* 59: 413–419.
<http://dx.doi.org/10.1007/s00284-009-9453-4>
- Tamaru, Y., Takani, Y., Yoshida, T. & Sakamoto, T. (2005) Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium *Nostoc commune*. *Applied and Environmental Microbiology* 71: 7327–7333.
<http://dx.doi.org/10.1128/AEM.71.11.7327-7333.2005>
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28:2731–2739.
<http://dx.doi.org/10.1093/molbev/msr121>
- Thajuddin, N. & Muralitharan, G. (2008) Application of PCR based fingerprinting in the phylogeny of marine cyanobacteria. *Indian Hydrobiology* 11: 25–41.
- Vaucher, J.P.E. (1803) *Histoire des Conferves d'eau douce. Contenant leurs différents modes de reproduction, et la description de leurs principales espèces, suivie de l'histoire des trémelles et des ulves d'eau douce*. J.J. Paschoud, Geneva, 285 pp.
- Weisberg, W.G., Barns, S.M., Pelletier, D.A. & Lane, D.J. (1991) 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* 173: 697–703.
- Whitton, B.A. & Potts, M. (2000) Introduction to the cyanobacteria. In: Whitton, B.A. & Potts, M. (eds.) *The Ecology of Cyanobacteria*. Kluwer Academic Press, Dordrecht, pp. 1–11.
- Wright, D., Prickett, T., Helm, R.F. & Potts, M. (2001) Form species *Nostoc commune* (Cyanobacteria). *International Journal of Systematic and Evolutionary Microbiology* 51: 1839–1852.
<http://dx.doi.org/10.1099/00207713-51-5-1839>