Isolation of Toxin Producing Cyanobacteria from Aquatic Samples with *Lyngbya*

15

S. Nisha Nandhini, Kallingil Gopi Divya, and Nithaniyal Stalin

Abstract

Cyanobacteria are represented by morphologically diverse, ecologically important, phylogenetic Gram-negative prokaryotes. They constitute a phylogenetically coherent group of evolutionarily ancient phototrophic bacteria. The toxins produced by cyanobacteria are classified into endotoxins, hepatotoxins, lipopolysaccharides, and neurotoxins. Some species of *Lyngbya* can have Lyngbyatoxin-a and Aplysiatoxins, causing dermatitis with itching, burning, pain, rash, and blisters when in contact with human skin and could lead to loss of superficial layers of the skin by cell death. The effect of cyanobacterial intoxication includes epithelial cell damage in the digestive and respiratory tracts promoting tumour development. This chapter aims to elucidate the methodology for isolating the cyanotoxins from *Lyngbya*.

Keywords

Lyngbya · Lyngbyatoxin-a · Aplysiatoxins · Saxitoxins · Z8 medium · ASN-III medium

S. Nisha Nandhini
Department of Botany, St. Joseph's College (Autonomous), affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

K. G. Divya
Department of Pharmacognosy, Siddha Central Research Institute, Chennai, Tamil Nadu, India

N. Stalin (✉)
Botanical Survey of India, Central Regional Centre, Prayagraj, Uttar Pradesh, India

© The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2023

N. Thajuddin et al. (eds.), *Protocols for Cyanobacteria Sampling and Detection of Cyanotoxin*, https://doi.org/10.1007/978-981-99-4514-6_15

15.1 Introduction

Lyngyba is filamentous blue-green algae that flourish in freshwater. The unbranched filaments are covered by firm polysaccharide sheaths and are seen covering both water surfaces and benthic sediments (Speziale et al. 1988). The consumption of water contaminated with cyanobacteria will call gastroenteritis. Cyanobacteria can produce diverse toxins that may affect the central nervous system like anatoxin-a, anatoxin-a(s), and saxitoxins. How cyanotoxins have their toxic effects can vary among different groups. The principal toxins primarily affect liver, such microcystins, nodularin, and cylindrospermopsin (Otero and Silva 2022). The marine *Lyngbya majuscula* produces a several bioactive compounds including Dermatotoxins. Lyngbyatoxin-a a potent irritant and vesicant produce rashes and other skin reactions. Aplysiatoxins considered promoting tumour development. Similarly, *Lyngbya wollei* can produce a potent neurotoxin like saxitoxins. The toxic effects include damage to the digestive and respiratory tract's epithelial cells. The toxins produced by *Lyngbya* promote the development of a tumour and the development of fibropapillomatosis (Beasley 2020). Cylindrospermopsin is another important alkaloid cyanotoxin produced by *Lyngbya* consisting of tricyclic guanidine (Ohtani et al. 1992; Guzmán-Guillén et al. 2013). The structural variant 7-epicylindrospermopsin is highly toxic (Banker et al. 2001). Toxins released from the algal cells to the surroundings are accumulated in the organisms in the lower level of the food chain (Rücker et al. 2007; Kinnear 2010). Cyanobacterial intoxication requires accurately identifying the algal species in the contaminated water (Van der Merwe 2015). Toxicological analyses of biological specimens contracted with algal toxins are recommended. Since the toxicity of these cyanobacteria is strain specific, more than mere identification is needed to predict their hazardous level. Liquid chromatography and tandem mass spectrometry are the best methodologies adapted for the analysis (Puschner and Moore 2013).

15.2 Materials

- Z-8 medium
- Modified seawater BG-11 medium
- ASN-III medium
- Sterile agar
- Cycloheximide
- Germanium dioxide
- Cycloserine
- N-formimidoyl thienamycin monohydrate
- Distilled water
- Sterile containers
- Petri dishes
- Erlenmeyer flasks (125 mL, 500 mL, and 1 L)
- Culture vessels

- Dive knife
- Autoclave
- Incubator
- Cool white fluorescence tube

15.3 Methods

15.3.1 Sample Collection (Burja et al. 2002)

1. Collect the samples with a clean dive knife.
2. Place them in sterile sample containers and seal them underwater.
3. Ship to the laboratory within a day and process them within 1 week of collection.

15.3.2 Z-8 Medium

1. Wash the filaments with sterile Z-8 medium (Kotai 1972; Carmichael 1986).
2. Autoclave the sterile agar and wash it thrice over a period of 72 h with distilled water. Then, mix with the filter-sterilized nutrient solution.
3. Add 50 mg L^{-1} and 100 μg mL^{-1} of cycloheximide and germanium dioxide, respectively, to eliminate the growth of eukaryotes. Add cycloserine and N-formimidoyl thienamycin monohydrate (Imipenem) to prevent bacterial growth (Vaara et al. 1979).
4. Store the agar plates at 4 °C before use.
5. Cut 0.5 cm long segments and plate at the centre of Petri dishes containing 1% w/v agar and Z-8 medium. Prepare in triplicate.
6. Maintain the cultures in an incubator under 26 °C under 16/8 light and dark photoperiod.
7. Use cool white fluorescence tube with an irradiance of 22 μmol m^{-2} s^{-1} photon flux as a light source (Speziale and Dyck 1992).
8. Cut new trichomes that grow out of old ones away from the region of contamination. Transfer the trichome to another Petri dish containing the same medium. Repeat until unialgal culture is obtained.
9. Then, transfer the trichomes to 125 mL Erlenmeyer flasks containing 100 mL of Z-8 medium. Allow the filaments to adapt to the Z-8 medium for 2 weeks.
10. After 2 weeks, transfer to the 1-L Erlenmeyer flasks containing 800 mL of Z-8 medium.
11. Aerate the cultures at the rate of 43–71 Lh^{-1}.

15.3.3 Modified Seawater BG-11 Medium (Burja et al. 2002)

1. Subculture the samples in 500 mL Erlenmeyer flasks containing 200 mL of modified seawater BG-11 medium (Rossi et al. 1997).

2. Let the cultures to achieve equilibrium with their new environment at 20 °C and then inoculate in large-scale culture vessels.
3. Maintain the cultures under a 12-h light/dark regime (Lux 250) and at a temperature of 20 °C.
4. Aerate with 0.2 mm filtered air and subculture every 2 months.

15.3.4 ASN-III Medium (Rippka et al. 1979)

1. Culture the filaments in ASN-III medium and subculture repeatedly to obtain axenic culture.
2. Maintain the cultures under 25 ± 1 °C and 7.5 W/m^2 of temperature and light intensity, respectively.
3. Use cool-white, fluorescent tubes as the light source with a photoperiod of 14: 10 h.

15.4 Observation (Burja et al. 2002)

1. Use light microscopy to study the external morphology of *Lyngbya*. Autofluorescence images of filamentous *Lyngbya* visualized at the emission range of 400–500 nm under a fluorescence microscopy.

Acknowledgement S.N. acknowledge the Director, Botanical Survey of India, Kolkata; N.N. acknowledges the Department of Botany, St. Joseph's College (Autonomous), Tiruchirappalli, for infrastructural support. G.K. acknowledges the Department of Pharmacognosy, Siddha Central Research Institute, Chennai.

References

Banker R, Carmeli S, Werman M, Teltsch B, Porat R, Sukenik A (2001) Uracil moiety is required for toxicity of the cyanobacterial hepatotoxin cylindrospermopsin. J Toxicol Environ Health A 62(4):281–288

Beasley VR (2020) Harmful algal blooms (Phycotoxins)

Burja AM, Abou-Mansour E, Banaigs B, Payri C, Burgess JG, Wright PC (2002) Culture of the marine cyanobacterium, Lyngbya majuscula (Oscillatoriaceae), for bioprocess intensified production of cyclic and linear lipopeptides. J Microbiol Methods 48(2–3):207–219

Carmichael WW (1986) Isolation, culture and toxicity testing of toxic freshwater cyanobacteria (blue-green algae). In: Shilov V (ed) Fundamental research in homogenous catalysis, vol 3, pp 1249–1262

Guzmán-Guillén R, Prieto AI, Vasconcelos VM, Cameán AM (2013) Cyanobacterium producing cylindrospermopsin cause oxidative stress at environmentally relevant concentrations in sub-chronically exposed tilapia (Oreochromis niloticus). Chemosphere 90(3):1184–1194

Kinnear S (2010) Cylindrospermopsin: a decade of progress on bioaccumulation research. Mar Drugs 8(3):542–564

Kotai J (1972) Instructions for preparation of modified nutrient solution Z8 for algae. Norwegian Institute for Water Research Oslo 11(69):5

Ohtani H, Callahan RJ, Khaw BA, Fishman AJ, Wilkinson RA, Strauss HW (1992) Comparison of technetium-99m-glucarate and thallium-201 for the identification of acute myocardial infarction in rats. J Nucl Med 33(11):1988–1993

Otero P, Silva M (2022) The role of toxins: impact on human health and aquatic environments. In: The pharmacological potential of cyanobacteria, pp 173–199

Puschner B, Moore C (2013) Chapter 43—cyanobacteria. In: Small animal toxicology, pp 533–540

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology 111(1):1–61

Rossi JV, Roberts MA, Yoo HD, Gerwick WH (1997) Pilot scale culture of the marine cyanobacterium Lyngbya majuscula for its pharmaceutically-useful natural metabolite curacin A. J Appl Phycol 9(3):195–204

Rücker J, Stüken A, Nixdorf B, Fastner J, Chorus I, Wiedner C (2007) Concentrations of particulate and dissolved cylindrospermopsin in 21 Aphanizomenon-dominated temperate lakes. Toxicon 50(6):800–809

Speziale BJ, Dyck LA (1992) Lyngbya infestations: comparative taxonomy of Lyngbya wollei comb. nov. (cyanobacteria). J Phycol 28(5):693–706

Speziale BJ, Turner EG, Dyck L (1988) Giant Lyngbya. Aquatics 10(2):4–11

Vaara T, Vaara M, Niemelä S (1979) Two improved methods for obtaining axenic cultures of cyanobacteria. Appl Environ Microbiol 38(5):1011–1014

Van der Merwe D (2015) Chapter 31—cyanobacterial (blue-green algae) toxins. In: Handbook of toxicology of chemical warfare agents, pp 421–429