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Bacteria-based Sodium Alginate Formulation to Control Toxic *Microcystis* Blooms

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Abstract

Cyanobacterial bloom formation in freshwaters is a major socio-economic and health concern across the globe. Presently used chemical and physical control strategies are inefficient in complete removal of blooms and chemical application often lead to secondary pollution in water. Hence, the current study aimed to develop a bacteria-based formulation to control toxic bloom-forming *Microcystis aeruginosa* in freshwaters. Two bacterial strains, *Exiguobacterium acetylicum* and *Pseudomonas* previously isolated from Sri Lankan freshwaters and characterized for *M. aeruginosa* cell lysis and their microcystin (MC) toxin degradation were used in the study. Initially, suitability of bacterial strains to develop into solid formulations was evaluated through biofilm formation, antibiotic sensitivity and strain compatibility. Both strains formed biofilms on the surface of microtiter plates indicating their ability to attach and colonize on solid surfaces. The multiple antibiotic resistance indices for both strains were below the threshold risk level (0.2) against the seven tested classes of antibiotics. This result indicates that there is a low risk in introducing these bacterial strains to the natural environment. Further, plate assay showed that the two strains were compatible to stay together showing no antagonistic effect on the growth of each other. Having fulfilled all three criteria tested, the two strains were immobilized into beads (~5 mm) prepared from sodium alginate at 1:1 ratio of 1×10^8 cells/mL bacterial inoculum. Different weights (1.0, 2.0, 3.0 g) of bacteria-immobilized beads were enclosed in sachets made with Cambrella synthetic fabric. They were introduced to *M. aeruginosa* grown in BG-11 medium (OD=0.2, 730 nm) and kept at 26 °C. During incubation, growth stimulation of *M. aeruginosa* was visually observed in 1.0 and 2.0 g beads-containing cultures, whereas, gradual discoloration of colonies was observed with 3.0 g of beads. Microscopic observations also proved complete disintegration of *M. aeruginosa* colonies and lysis of cells in discolored cultures. After 15 days, *M. aeruginosa* cell lysis was estimated as a measure of chlorophyll degradation. The highest (8.4%) cell lysis was observed in cultures containing 3.0 g of beads. This indicates that bacteria cell lysis activity depends on the load of bacteria-immobilized beads. Degradation of MC toxin was estimated by enzyme-linked immunosorbent assay. The highest (57.6%) MC degradation was observed in cultures with 3.0 g of beads after 15 days of incubation. The efficiency of bacteria release from beads was tested by placing sachets in sterilized water. At 15 days, bacterial count in water was $x10^7$ CFU/mL, with all loadings of immobilized-bacteria indicating highly efficient bacteria release from the formulation. In conclusion, this study highlights that bacteria-based sodium alginate formulations can be made as a source of inoculum to control *M. aeruginosa* growth and MC degradation.

Keywords: Bacteria-based formulations, Cell lysis, *Microcystis* blooms, Microcystin

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