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CHARACTERISATION OF HYDROGEN PEROXIDE-INDUCED PROGRAMMED CELL DEATH IN SELECTED MICROCYSTIN PRODUCING CYANOBACTERIA: CYTOTOXIC EFFECTS ON CELLULAR MICROCYSTIN CONTENT

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Cyanobacterial blooms can undergo programmed cell death (PCD) under natural and artificial stress conditions. This study aims to reveal the effects of PCD induced by H_2O_2 on cyanotoxin production, cell viability, and morphology of different cyanobacteria: *Microcystis* sp., *Fischerella* sp., *Nostoc* sp., *Pseudoanabaena* sp., and *Leptolyngbya* sp. Cyanobacteria (Initial cell concentration~$1x10^8$ Cells mL^{-1}) were grown as batch cultures in BG11 media. The cultures were grown in four different H_2O_2 concentrations:10 mg L^{-1},60 mg L^{-1},125 mg L^{-1},250 mg $L^{-1,}$ and four different exposure durations:3 hrs, 6 hrs, 9 hrs and 12 hrs. Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay was performed to analyse the cell viability by recording the absorbance at 595 nm using a microplate reader (FLUOstar Omega) and compared using OD_{750} values of controls. Microcystin (MC) was analysed using High-Performance Liquid Chromatography with reference to the MC standard (SIGMA ALDRICH 33578). Treatments were observed after 24 hrs using Olympus CKX41 inverted fluorescence microscope. The cultures significantly influenced cytotoxicity at different H_2O_2 concentration levels (p<0.05). The maximum cytotoxicity was observed within *Pseudoanabaena* sp. and *Leptolygbya* sp., reducing cell viability by 75.34% and 96.19%, respectively. *Microcystis* sp. and *Fischerella* sp. had the highest intracellular MCs content: 0.982 mg L^{-1} and 0.8519 mg L^{-1}, respectively. A positive correlation was found between average cell viability (%) and MC-LR content in cells (r=0.518, p>0.05), while a weak correlation was found between average cell viability (%) and total MC content in cells (r=0.329, p>0.05). A dose of 250 mg L^{-1} H_2O_2 at 24 hrs caused a decrease in green colour and chlorophyll red fluorescence intensity in cells compared to the controls. The Apoptosis conditions at higher H_2O_2 concentrations and extended incubation periods significantly affect cyanobacteria's cytotoxicity, morphology, and MC production and increase cyanotoxin production in cyanobacterial cells. Further investigation through molecular analysis is necessary to study cyanotoxin production in the context of apoptosis.

Key Words: Cell viability, Cyanotoxin, Microcystin-producing cyanobacteria, Programmed cell death.