

**Isolation, identification and characterization of
Microcystin degrading bacteria for water treatment
solution**

By

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of

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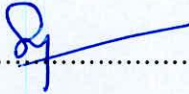
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DECLARATION OF STUDENT

I declare that the work presented in this thesis is my own, except where otherwise acknowledged, and has not been submitted in any form for another degree or qualification at any other academic institution. The work described in this thesis was carried out under the supervision of Prof.M.M.Pathmalal as the chief investigator while Prof.B.G.D.N.K. De Silva and Dr. S.D.M. Chinthaka were the co- supervisors.



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ABBREVIATIONS

Advanced Oxidation Processes	AOP
Cylindrospermopsin	CYN
deoxy-cylindrospermopsin	doCYN
Deoxy ribo nucleic acid	DNA
High Performance Liquid Chromatography	HPLC
Microcystin-LR	MC-LR
Microcystins	MCs
National water supplies and drainage board	NWSDB
Nodularins	NOD
Non-ribosomal Polypeptide	NRPS
Powdered activated carbons	PAC
Protein Phosphatases Types 1 A	PP1
Protein Phosphatases Types 2 A	PP2
Reverse osmosis	RO
Room temperature	RT
Slow sand filtration	SSF
Total nitrogen	TN
Total phosphate	TP

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Fathima Sumaiya Idroos

ABSTRACT

Microcystin –LR (MC-LR) is considered to be the most dominant type of MCs present in Sri Lankan water bodies. Hence, there is a significant interest in water treatment strategies that ensures the removal of MCs, with the exploitation of microbes, which is considered to be a possible environmental friendly approach. Detection of MC-LR concentration and isolation of potential MC-LR degraders were carried out in 25 water bodies of Sri Lanka from 1st September 2011 to 31st August 2014. The highest MC-LR concentration was recorded from Girandurukotte reservoir as $158.9 \pm 4.71 \mu\text{g ml}^{-1}$. A total of 386 bacterial strains were isolated from all water bodies and out of them four isolates namely, *Bacillus cereus* (12GK), *Stenotrophomonas maltophilia* (4B4 and 1 JAY) and *Rahnella aquatilis* (13UL) have shown an overall metabolism of MC-LR. This is the first report of MC degrading *R.aquatilis* belonging to class gammaproteobacteria.

B.cereus, the most efficient MC-LR degrading bacterium showed 100 % removal of MC-LR within eight days of incubation at 28⁰C where both *S. maltophilia* species and *R.aquatilis* showed 100 % removal of MC-LR within 10 days of incubation. Highest metabolism of MC-LR by all four bacterial strains was shown at 32⁰C. *B.cereus* showed 100 % removal of MC-LR, at the end of 6th day of incubation at 32⁰C. *S. maltophilia* and *R. aquatilis* required 8 days to show 100 % removal of MC-LR at 32⁰C. MC-LR degradation efficiency of each bacterial strain was optimized at different concentrations

of Phosphates (0.005 ppm to 0.05 ppm) and nitrates (0.1ppm to 2.5 ppm). MC-LR degradation rate of *B. cereus* and *R. aquatilis* increased from $0.43 \pm 0.05 \mu\text{g day}^{-1}$ to $0.94 \pm 0.15 \mu\text{g day}^{-1}$ and from $0.38 \pm 0.01 \mu\text{g day}^{-1}$ to $0.56 \pm 0.17 \mu\text{g day}^{-1}$, respectively when phosphate concentration was increased from 0.005 to 0.01ppm. Phosphate concentrations higher than 0.01ppm resulted a decrease in MC-LR degradation rate of *B. cereus* and *R. aquatilis*. *S. maltophilia* showed highest MC-LR degradation rate of $0.34 \pm 0.01 \mu\text{g day}^{-1}$ (4B4) and $0.38 \pm 0.002 \mu\text{g day}^{-1}$ (1 JAY) respectively when total phosphate concentration of the medium was increased up to 0.02 ppm and higher levels of phosphate showed a decrease in degradation of MC-LR. A rapid degradation of MC-LR was recorded by all four strains, with the increase of nitrate concentration in the medium from 0.1 to 0.4 ppm. MC-LR degradation rate for *B.cereus* increased from $1.76 \pm 0.05 \mu\text{g day}^{-1}$ to $3.98 \pm 0.15 \mu\text{g day}^{-1}$; In *S. maltophilia*, 4B4 and 1 JAY, MC-LR degradation rate increased from $1.98 \pm 0.17 \mu\text{g day}^{-1}$ to $3.55 \pm 0.18 \mu\text{g day}^{-1}$ and from $1.78 \pm 0.03 \mu\text{g day}^{-1}$ to $3.76 \pm 0.06 \mu\text{g day}^{-1}$ respectively, where as in *R. aquatilis* MC-LR degradation rate increased from $1.86 \pm 0.05 \mu\text{g day}^{-1}$ to $3.55 \pm 0.11 \mu\text{g day}^{-1}$. Nitrate concentrations higher than 0.4ppm reduced MC-LR degradation rates of all strains Moreover, all four bacterial strains showed abilities to degrade other MC variants (MC-LF, MCLW, MC-RR) and Nodularin (NOD). *B. cereus* acted as the predominant degrader of MC-LR and MC-LF showing a complete degradation of these toxin variants within eight and twelve days of incubation respectively. Both *S .maltophilia* strains (4B4 and 1JAY) showed a complete removal of MC- LR, MC-RR and MC – LF within 10, 12 and 14 days of incubation accordingly. However, *R. aquatilis* showed a complete degradation only for MC- LR within 10 days of incubation.

The molecular studies confirmed that all four bacterial strains harbor *mlrA*, *mlrB*, *mlrC* and *mlrD* genes in them. This confirms that these bacterial strains follow the traditional pathway of MC-LR degradation and break the toxic compound into non harmful products. Moreover, a sand filter was also developed incorporating the best degrader of MCs. Biofilm of the sand filter was developed by attaching bacteria into citric acid treated raw cotton. MC-LR elimination potential of sand filters was experimented using two full-scale sand filters: an experimental sand filter and a control sand filter. The control filter showed a 1% removal of MC-LR within three hours and 12% removal after four days. The experimental filter showed 3% removal of MC-LR within three hour of incubation and 90% removal of by the end of four days. Therefore, present study has provided a potential solution to treat Microcystin contaminated water by exploiting environmental bacteria.