BLUE-GREEN TRAVELERS

Cultivation of Cyanobacteria Associated with Sea Turtles

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Introduction

Isolating cyanobacterial strains and determining their taxonomy is of foremost importance for establishing their ecological significance. Some cyanobacterial symbiotic relationships, such as those with marine invertebrates like corals and sponges, have garnered attention and are well explored. However, specific epibiotic cyanobacteria associated with marine vertebrates like sea turtles have remained notably unexplored using culture dependent and independent methods.
This study addresses this gap by focusing on the cultivation and taxonomic exploration of cyanobacterial strains associated with sea turtles.

Objective

The study aims to isolate and characterize cyanobacterial strains from the skin and carapace of sea turtles, shedding light on their taxonomic identity and their genetic potential of selected cyanotoxin production.

Methodology

Biofilm samples were collected using non-invasive methods from skin and carapace of loggerhred, hawksbill and leatherback sea turtles from rehabilitation centers in Croatia and South Africa. Strain isolation was based on micromanipulation and streaking. Growth medium BG-11 (Rippka et al., 1979) was used with addition 35 g of marine salts and cycloheximide at a final concentration of 500 µg mL-1 for removal of eukaryotic contaminants. Morphological characterization was conducted using a light microscope Olympus BX43 with SC100 camera. Molecular characterization involved obtaining 16S rRNA and ITS sequences using primers 27F and 23S30R (Taton et al., 2003). Additionally, strains were screened for genetic potential to produce selected cyanotoxins (microcystin, nodularin, and saxitoxin) by amplifing *mycE*, *nod* and *sxtA* genes.

Conclusion

The successful cultivation, taxonomic identification and deposit into BCCM/ULC Cyanobacteria Collection provide a foundation for future studies on these strains that could tell us more about the functional significance of these cyanobacteria within the sea turtle microbiome context.

References

Rippka, E., Deruelles, J., & Waterbury, N. B. (1979). Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria. Journal of General Microbiology, 111, 1–61.

Taton, A., Grubisic, S., Brambilla, E., De Wit, R., & Wilmotte, A. (2003). Cyanobacterial Diversity in Natural and Artificial Microbial Mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): A Morphological and Molecular Approach. Applied and Environmental Microbiology, 69(9), 5157–5169. https://doi.org/10.1128/AEM.69.9.5157-5169.2003

Results

- 10 isolated cyanobacterial strains
- morphological characterisation
- deposited into Public Collection BCCM/ULC of cyanobacterial strains (Liège, Belgium)
- isolated 16S-ITS sequences
- strain ULC772 (Cy015) had the genetic potential for microcystin production

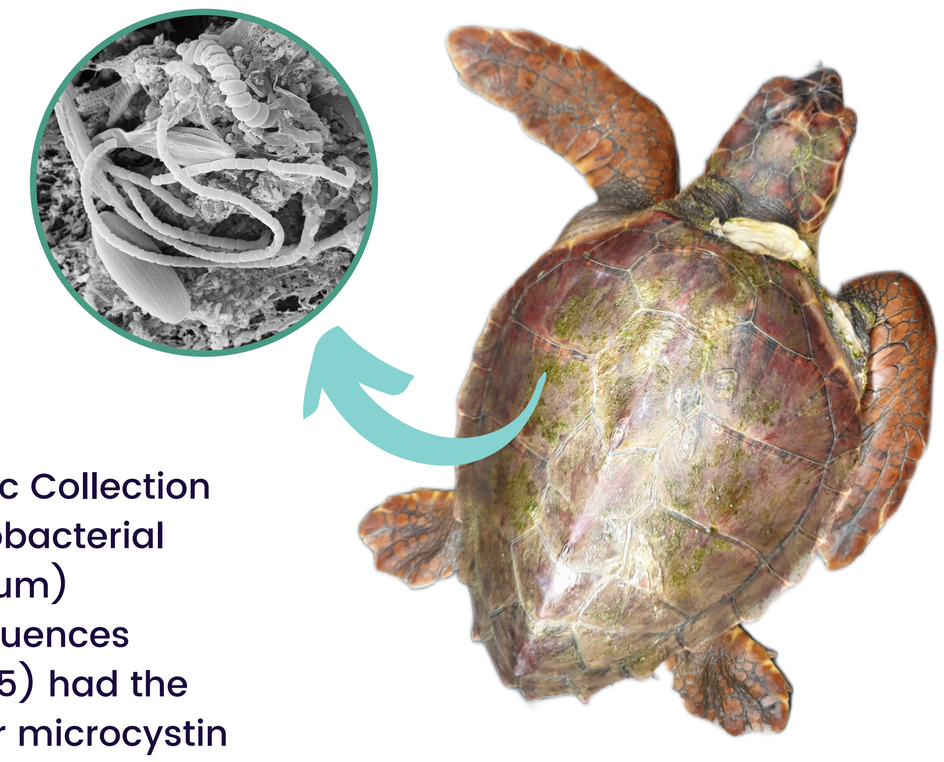

Figure 1. Loggerhead sea turtle with visible green biofilm on its carapace; upper left picture - scanning electron microscope image of biofilm sample from sea turtles containing cyanobacteria.

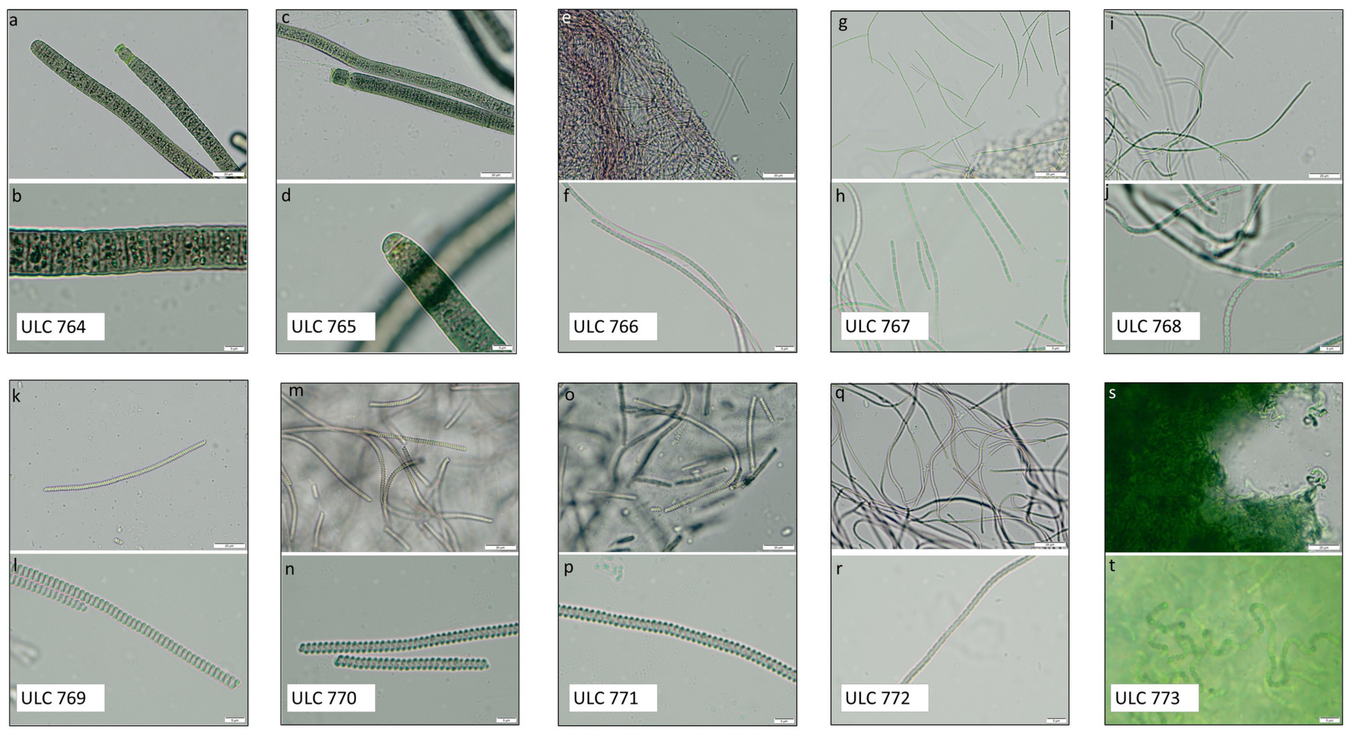

Figure 2. Microphotographs of cyanobacterial strains cultured and deposited in BCCM/ULC cyanobacteria collection; images a, c, e, g, i, k, m, o, q and s are taken under 40x magnification and have a scale bar of 20 µm, while b, d, f, h, j, l, n, p, r and t are taken under 100x magnification and have a scale bar of 5 µm.

Table 1. Information about identification, mearurements, sequencing results and PCR for cyanotoxins for strains deposited in BCCM/ULC Cyanobacteria Culture Collection; empty cells indicate that the PCR/sequencing was not performed for that strain.

ULC Number	Internal Code	Identification	Cell length (µm)	Cell width (µm)	16S-ITS sequence obtained	*mycE*	*nod*	*sxtA*
764	Cy002	*Lyngbya*	2.19	11.11		-	-	-
765	Cy004	*Lyngbya*	2.29	8.63		-	-	-
766	Cy006	*Pseudanabena*	1.46	1.04	+	-	-	
767	Cy007	*Leptolyngbya*	1.08	1.47	+	-	-	-
768	Cy009	*Leptolyngbya*	1.39	2.21	+	-	-	-
769	Cy011	*Spirulina*	3.08	1.13	+	-	-	
770	Cy012	*Spirulina*	3.48	1.19		-	-	
771	Cy013	*Spirulina*	3.31	1.10		-	-	
772	Cy015	*Leptolyngbya*	2.38	1.28	+	+	-	
773	Cy010.2	*Leptolyngbya*	1.33	1.32				

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