Chemotaxonomic identification of cyanobacteria by GC-MS FAME profile analysis

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Introduction

Cyanobacteria, known for their ability to synthesize toxic metabolites (cyanotoxins), can become dominant in water bodies with high concentrations of nitrogen and phosphorus, growing up excessively and forming visible blooms. These potentially lethal toxins, which might be present in water supplies, are increasingly a cause for concern worldwide. Therefore, the cyanobacteria species identification is an essential step in order to minimize possible health hazards related to cyanotoxins. Here we describe preliminar results of cyanobacteria fatty acids composition assessment (as fatty acid methyl esters – FAME), performed by GC-MS, of 10 toxigenic and nontoxigenic cyanobacteria strains. The aim of this study is to evaluate if there is a relationship among cyanobacteria fatty acids profiles, and evaluate the feasibility of developing databases that may be used for a rapid identification of cyanobacteria in the routine lab. Bacterial, yeast and mycobacterial identification systems, based on fatty acids profiles, are already commercially-available.

Results and Discussion

The sampling processing, which includes extraction and methylation (to obtain FAME) of freeze-dried cyanobacteria samples, was performed with the following strains: *Microcystis aeruginosa* (LTPNA02: nontoxigenic; LTPNA08 and ITEP28: microcystins producers); *Cylindrospermopsis raciborskii* (ITEP30: non-toxigenic; ITEP18: saxitoxins producer; CYP011K: cylindrospermopsin producer); *Sphaerospermopsis torques-reginae* (ITEP24, ITEP25 and ITEP26: anatoxin-a(s) producers); and *Pseudoanabaena* sp. (LTPNA06: nontoxigenic). Strains were cultured under the same temperature, light intensity, pH and nutrients content conditions. Profile analysis (determination of each selected FAME as percent of total FAME) were performed by GC-MS, and the results grouped for each species, as demonstrated in table 1.

Despite the FAME profile seemed to be characteristic of the studied species (with no significant differences between toxigenic and nontoxigenic strains), further analysis with a larger number of cultures from around the world (to avoid potential geographic bias) has to be performed to confirm this relationship and delineate each group.

Table1. FAME profile analysis of cyanobacteria species studied.

Fatty acid	*M. aeruginosa*	*C. raciborskii*	*S. torques-reginae*	*Pseudo-anabaena*
12:0	-	-	-	8,6
14:0	0,3-0,8	-	-	13,0
16:0	27-39,7	33-44,8	24,6-28,7	23,9
18:0	3,8-6,4	4,3-9,8	7,5-9,5	3,1
14:1	-	1,7-3,9	0-3,78	16,6
16:1	1,3-3,3	3,6-11,2	10,7-14,4	26,1
18:1n9	4,7-9,1	3,0-4,1	1,0-2,3	7,9
18:1n7	1,9-5,3	1,0-15,2	3,9-21,7	
16:2n4	-	-	0-1,1	0,7
18:2c	14,2-18,7	2,9-5,7	5,4-12,3	-
18:3n6	13,2-21,5	-	-	-
18:3n3	6,2-9,6	19,8-35,7	19,7-36,5	-
18:4n3	5,6-8,9	0-5,07		-
20:4n6	0,7-1,4	-	-	-
22:3	0,7-1,2	-	-	-

Furthermore, besides the comparison of the FAME profile of the unknown strain to a stored database using a covariance matrix, principal component analysis and a pattern recognition software should be applied. While the covariance matrix takes into account the mole-for-mole relationship of the conversion of one fatty acid to another (which might occur with temperature shifts or age differences), the pattern recognition software is necessary to allow calculations of ratios between fatty acid amounts, to be able to discriminate subtle differences between biovars or subspecies. The fully automated gas chromatographic analytical system commercialized for bacteria identification based on their unique fatty acid profiles already uses these computational tools.

Conclusions

It might be inferred that a cyanobacterial FAME library may be constructed as a promising tool for cyanobacteria species identification based on diferences and similarities in these chemicals markers.

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