REDUCED ANTIBIOTIC SUSCEPTIBILITY IN WASTEWATER *PLANKTOTHRIX* SP.: EVALUATION OF MINIMUM INHIBITORY CONCENTRATIONS BY A METHOD DESIGNED FOR CYANOBACTERIA - MOLECULAR IDENTIFICATION OF RESISTANCE GENES

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INTRODUCTION

Cyanobacteria are ubiquitous in aquatic ecosystems, being *Planktothrix spp*. one of the most common bloom-forming species in freshwaters worldwide [1]. In these environments, cyanobacteria are exposed to antibiotics and antibiotic resistant bacteria, but their role on water resistome was never investigated [2]. One limitation of testing antibiotic resistance in cyanobacteria is the lack of standardized methods and interpretative guidelines. Based on the standard broth microdilution method, we developed a procedure for testing antibiotic susceptibility in cyanobacteria [2]. This work aimed to test the applicability of that method in *Planktothrix mougeotti* from a wastewater treatment plant, in order to assess their susceptibility patterns and their putative contribution to the global pool of resistance determinants in freshwater.

MATERIAL AND METHODS

We investigated 8 strains of *Planktothrix mougeotti* previously isolated [3] from a wastewater treatment plant (WWTP) located in the north of Portugal (41º07'05.22''N, 8º34'015.40''W) and dimensioned for a population of 80,000 habitants. Antibiotic susceptibility was evaluated by a microdilution method previously adapted for cyanobacteria [2], against beta-lactams, aminoglycosides, quinolones, trimethoprim and tetracycline. Minimum inhibitory concentrations (MIC) were determined according to cyanobacterial cell density (optical density, OD_{450nm}) and microscopic examination of cultures integrity after 14 days of exposure to antibiotics. All strains were subjected to the search of antibiotic resistance genes and class 1, 2 and 3 integrons by PCR/sequencing.

References

[1] Catarina Churro, Elsa Dias, Valério E. 2012. Risk Assessment of Cyanobacteria and Cyanotoxins, the Particularities and Challenges of Planktothrix spp. Monitoring, InTech, DOI: 10.5772/37910.

[2] Dias, E., et al. 2015. Assessing the antibiotic susceptibility of freshwater cyanobacteria spp. Frontiers in Microbiology doi: 10.3389/fmicb.2015.00799.

[3] Martins J., Peixe L., Vasconcelos, V.M. 2011. Unraveling Cyanobacteria Ecology in Wastewater Treatment Plants (WWTP). Microb Ecol (2011) 62:241–256.

RESULTS

The tested *P. mougeotii* strains exhibited both a susceptible and a non-susceptible phenotype, depending on the antibiotic as exemplified in Figure 1, where green color denotes cyanobacterial growth. In this Figure it can observed that kanamycin inhibited cell growth above 0.2-0.8 mg/L, whereas trimethoprim did not had any effect on cyanobacteria. For each antibiotic, the susceptibility pattern did not differed considerably among the tested strains.

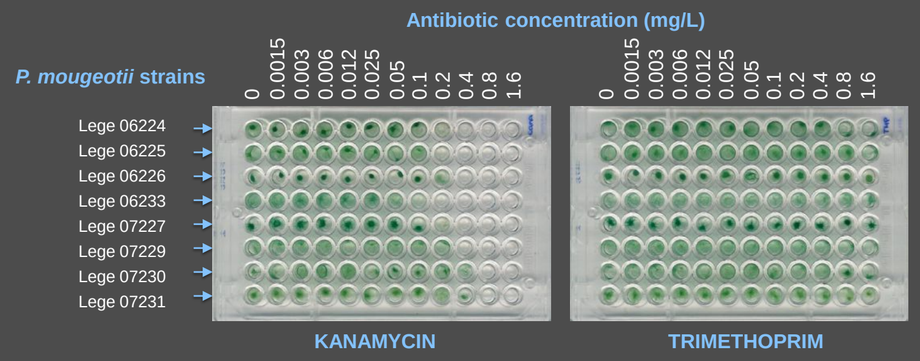

Figure 1. Microphotographs of microplates with cyanobacterial cultures exposed tor 14 days to kanamycin (A) and trimethoprim (B).

P. mougeotti strains were particularly susceptible to streptomycin (MIC of 0.05 and 0.1 mg/L) (Table 1). They were also susceptible to ceftazidime, ceftriaxone, kanamycine and gentamicine, with MICs varying from 0.2 to 0.8 mg/L. None of the strains was susceptible to amoxicillin, tetracycline, norfloxacin, nalidixic acid and trimethoprim, up to 1.6 mg/L of antibiotic concentration.

Table 1. Minimun Inhibitory concentrations of antibiotics on *P. mougeotti*

P. mougeotii strains	MIC (mg/L)[a,b]									
	AMX	CAZ	CRO	KAN	GEN	STR	TET	NAL	NOR	TMP
Lege 06224	> 1.6	0.4	0.2	0.2	0.8	0.05	> 1.6	> 1.6	> 1.6	> 1.6
Lege 06225	> 1.6	0.8	0.4	0.2	0.8	0.1	> 1.6	> 1.6	> 1.6	> 1.6
Lege 06226	> 1.6	0.8	0.8	0.4	0.8	0.05	> 1.6	> 1.6	> 1.6	> 1.6
Lege 06233	> 1.6	0.4	0.2	0.2	0.4	0.05	1.6	> 1.6	> 1.6	> 1.6
Lege 07227	> 1.6	0.8	0.8	0.4	0.4	0.05	> 1.6	> 1.6	> 1.6	> 1.6
Lege 07229	> 1.6	0.8	0.4	0.4	0.8	0.1	> 1.6	> 1.6	> 1.6	> 1.6
Lege 07230	> 1.6	0.8	0.4	0.8	0.8	0.05	> 1.6	> 1.6	> 1.6	> 1.6
Lege 07231	> 1.6	0.4	0.4	0.4	0.8	0.1	> 1.6	> 1.6	> 1.6	> 1.6

[a]Median values of the three independent experiments.

[b]**AMX**, amoxicillin; **CAZ**, ceftazidime; **CRO**, ceftriaxone; **KAN**, kanamycin; **GEN**, gentamicine; **STR**, streptomycin; **NOR**, norfloxacine; **NAL**, nalidixic acid; **TET**, tetracycline; **TMP**, trimethoprim.

None of the strains exhibited any tested gene conferring resistance to AMX, TET, NAL, NOR and TMP. This suggests that cyanobacteria may be naturally non-susceptible to these antibiotics or that the PCR conditions requires further optimization for cyanobacteria.

Conversely, a pair of *strA-strB*-type genes were detected in 4 strains (Table 2). The mean of this finding should be further investigated considering that the STR susceptibility phenotype did not corresponded to STR resistance genotype. Additionally, a *sul*1-type gene and a class 1-type integrase (*intI*1) were also detected in some strains (Table 2).

Table 2. Antibiotic resistance genes in *P. mougeotti* strains

P. mougeotii strains	Antibiotic resistance genes[a]		
	strA-strB	*sul* 1	*intI*1
Lege 06224	-	-	-
Lege 06225	+	+	+
Lege 06226	+	+	+
Lege 06233	-	+	-
Lege 07227	+	-	-
Lege 07229	+	+	-
Lege 07230	-	+	+
Lege 07231	-	-	-

[a]Primers were those previously designed to amplify target sequences of antibiotic resistance genes from bacteria.

CONCLUSIONS

- The presence of antibiotic resistance genes and integrons in *Planktothrix mougeotti*, as well as their reduced susceptibility to some antibiotics, might be a result of their exposure to antibiotics and antibiotic resistance bacteria in the WWTP.
- The implementation of standard methodologies and guidelines for susceptibility testing in cyanobacteria is a fundamental step to characterize the role of cyanobacteria on environmental resistome.

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