

CITOTOXICITY OF DIFFERENT SPECIES OF THE GENUS GAMBIERDISCUS ISOLATED IN THE CANARY ISLANDS

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INTRODUCTION

Gambierdiscus genus includes species responsible for the ciguatera syndrome, an endemic food poisoning of some tropical and subtropical zones that is acquired by the consumption of fish contaminated with ciguatoxins...

Gambierdiscus cells cultures



Neuro2A cell based assay

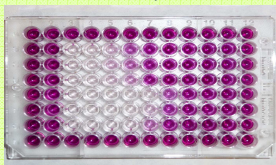


Figure 1. Gambierdiscus australes. Author: Santiago Fraga.

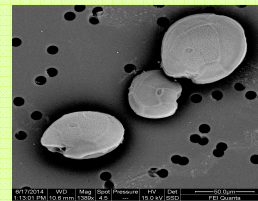


Figure 2. Gambierdiscus silvae. Author: Santiago Fraga



Figure 3. Sampling place

MATERIAL AND METHODS

Strains of five Gambierdiscus (G. australes, G. caribaeus, G. carolinianus G. excentricus and G. silvae) species obtained from the Canary Islands were cultured in order to estimate their toxicity.

Culture conditions: K medium without silicates, salinity 35, 25°C, 12:12h L:D

Toxins analysis: Caillaud et al., 2010.

Neuro-2a cell based assay (CBA-Neuro2A): Caillaud et al., 2012.

Mouse bioassay (MBA): Banner et al., 1960 and Yasumoto et al., 1984.

Solid phase extraction: Strata FL-PR Florisil (170 µm, 80 Å) cartridges 500 mg/6mL. Eluate with Acetone:methanol (7:3), dried under N2 gas and redissolved in methanol.

LC-MS/MS conditions: Acquity BEH C18 (2.1x100mm, 1.7µm) column, Gradient as Yogi et al. 2014. Flow rate 0.4 µL/min. TSQ Quantum, Positive ion mode using SRM with Na+ adduct.

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CBA-Neuro2A results before CTX:MTX partition (Table 1)

-Variations were observed in the toxicity of the different Gambierdiscus species.

-100% mortality before the addition of ouabain/veratridine was observed in strains with MTX.

- Dichloromethane: methanol partition is necessary in order to quantify strains with MTX.

-CTX could be quantified only in G. silvae strains.

Neuro2A and MBA results after CTX:MTX partition (Table 2)

-CTX and MTX were present in both fractions.

-Mice died more quickly and more violent when MTXs were present in the extracts.

-MTXs are the most toxic by biological assays but they do not accumulate in fish and are quickly eliminated by organisms.

-G. silvae, was the only species without MTXs. This specie only contains CTXs.

LC-MS/MS results (Figure 4)

-Toxicity observed by biological assays in the three G. silvae strains could be attribute to a compound with m/z [M+Na]+ 1163.6, the same m/z as C-CTX-1 and C-CTX2 ciguatoxins.

-Another peak present in some chromatograms with m/z [M+Na]+ of 1117.6, could be corresponding with 52-epi-54deoxyCTX1B, 54-deoxyCTX1B or PCTX2 ciguatoxins.

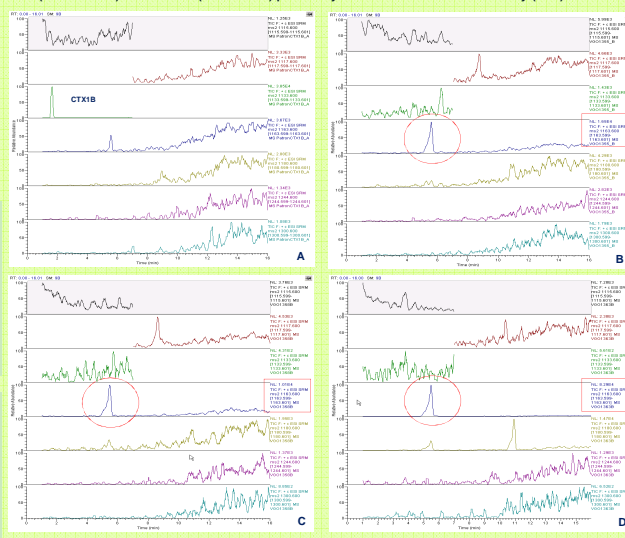
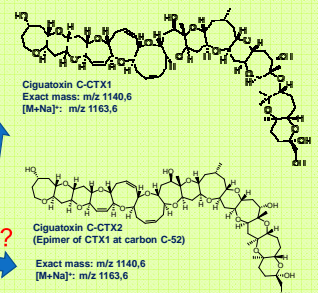


Figure 4. Chromatograms obtained by LC-MS/MS. A: CTX1B standard; B: G. silvae VGO 1355; C: G. silvae VGO 1358; D: G. silvae VGO 1363



CONCLUSIONS

- 1. G. silvae was just the species which showed only CTXs, therefore this microalgae is a very useful tool to improve MTX:CTX partition methodology.
2. Among different Gambierdiscus species here analyzed, G. silvae represents the highest potential risk of ciguatera in Canary Islands.
3. Identification of Gambierdiscus species present in seawater is crucial to determine the risk of ciguatera.

REFERENCES

Banner, A.H., Scheuer, P.J., Sasaji, S., Helfrich, P. and Alender C.B. (1960). The Annals of the New York Academy of Science, 90, 770-787.
Bravo, J., Cabrera, F., Ramirez, A. and Acosta, F. (2015). Journal of Aquaculture and Marine Biology, 3(1):00053.DOI:10.15406/jam.2015.03.00053

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