



CITOTOXICITY OF DIFFERENT SPECIES OF

THE GENUS GAMBIERDISCUS ISOLATED IN THE CANARY ISLANDS





Rossignoli A.E.*1, Tudó A.2, Cañete E.2, Diogène J.2, Figueroa R.I.1, Bravo I.1, Ramilo I.1, Blanco J.3, Riobó P.

¹Departmento de Microalgas Nocivas, Instituto Español de Oceanografía, Vigo, España.

² IRTA, Ctra. Poble Nou, km 5.5, Sant Carles de la Ràpita, España

³ Centro de Investigacións Mariñas (CIMA), Vilanova de Arousa, España

Departamento de Fisiología, Fotobiología y Pigmentos del Fitoplancton, IIM-CSIC, Vigo, España.

INTRODUCTION

Gambierdiscus genus includes species responsible for the ciquatera syndrome, an endemic food poisoning of some tropical and subtropical zones that is acquired by the consumption of fish contaminated with ciguatoxins, which have accumulated through food webs (Parsons et al., 2012). In recent years, the tropicalization caused by climate change is causing a possible distribution of *Gambierdiscus* towards higher latitudes and constitutes an emerging problem in the Canary Islands where more than 100 cases of ciguatera have been registered in the last decade (Rodríguez et al., 2017; Bravo et al., 2015).



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. Assays were taken at different time periods. Strains analyzed in the first and second periods are listed respectively in table 1 and 2

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 A) 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, 1.7um) column, Gradient as Yogi et al. 2014. Flow rate 0,4 µL/min. TSQ Quantum, Positive ion mode using SRM with Na+ adduct.











| | r: Santiago Frag | | | | | |
|----------|------------------|--|---|--|--|--|
| Strain | Specie | Origin | Ciguatoxin Concentration | | | |
| VGO1355 | G.silvae | La Gomera (Valle del Rey- Ch. Condesa) | 12.19 ± 0.62 fg/cell eq. | | | |
| VGO136(3 | G.silvae | La Gomera (Valle del Rey- Ch. Condesa) | (20.55 ± 2.67 fg/cell eq.) | | | |
| VGO1358 | G.silvae | La Gomera (Valle del Rey- Ch. Condesa) | 32.15 ± 0.34 fg/cell eq | | | |
| VGO1367 | G. caribaeus | La Gomera(San Sebastian-Pl La Cueva) | It is not possible to quantify (100% mortality without ouabain/veratridine) | | | |
| VGO1365 | G. caribaeus | La Gomera (Porto PI Santiago) | Negative (LOD <1.76 fg/cell eq) | | | |
| VGO1198 | G. australes | Las Américas, Tenerife | It is not possible to quantify (100% mortality without ouabain/veratridine) | | | |
| VGO1360 | G. australes | La Gomera (Valle Rey-Ch. Condesa) | It is not possible to quantify (100% mortality without ouabain/veratridine) | | | |
| VGO1356 | G. excentricus | La Gomera (Valle Rey-Ch. Condesa) | It is not possible to quantify (100% mortality without ouabain/veratridine) | | | |
| VGO1361 | G. excentricus | La Gomera (Valle Rey-Ch. Condesa) | It is not possible to quantify (100% mortality without ouabain/veratridine) | | | |
| VGO1197 | G.carolinianus | Alcalá, Tenerife | It is not possible to quantify (100% mortality without ouabain/veratridine) | | | |

| Table 1. Ciguatoxins concentration obtained in | the crude methanolic extract | (BEFORE CTXs:MTXs | partition) of 10 strains of Gambier | rdiscus by CBA-Neuro2A |
|--|------------------------------|-------------------|-------------------------------------|------------------------|
| | | | | |

| | Strain | Specie | Origin | CTXs:MTXs partition | Quantative data | Deat | th time MB | Α | MBA Observations | |
|-------|---------|----------------|------------------------|---------------------------------------|-----------------|-----------------|------------|-----------|---|---|
| | | | - 1.3 | , , , , , , , , , , , , , , , , , , , | CBA-Neuro2A | | | | | |
| 80 80 | VGO791 | G. excentricus | Pta. Hidalgo, Tenerife | MTXs | MTX + | 49min | 1h09min | 1h14min | Typical symptoms neurotoxic compound. | Ė |
| | VGO791 | | | CTXs | MTX + | 3h45min | 3h45min | 3h53min | Typical symptoms neurotoxic compound. | Ė |
| | VGO1198 | G. australes | Las Américas, Tenerife | MTXs | MTX + | 46min | 1h | 1h25min | Typical symptoms neurotoxic compound. | Ě |
| | VGO1198 | | | CTXs | MTX + | badly punctured | 1h04min | 1h11min | Very violent death of the two mice, jumping as in PSP | 龞 |
| | VGO1237 | G. caribaeus | La Estaca, El Hierro | MTXs | | Alive 48h | Alive 48h | Alive 48h | Inactivity and hypothermia after the injection and at least during the first 2 hours of continued observation. | i |
| | VGO1237 | | | CTXs | | Alive 48h | Alive 48h | Alive 48h | Inactivity and hypothermia after the injection and at least during the first 2 hours of continued observation. | |
| | VGO1167 | G. silvae | Pta. Hidalgo, Tenerife | MTXs | CTX+ | Alive 48h | Alive 48h | Alive 48h | Inactivity and hypothermia after the injection and at least during the first 2 hours of continued observation. | H |
| | VGO1167 | | | CTXs | CTX+ | Alive 48h | Alive 48h | Alive 48h | Inactivity and hypothermia after the injection and at least during the first 2 hours of continued observation. | |
| | VGO1180 | G. silvae | Pta. Hidalgo, Tenerife | MTXs | CTX+ | 4h53min | 5h34min | 5h34min | Lacrimation is observed in one of the mice. Strong tremors | E |
| 10101 | VGO1180 | | | CTXs | CTX+ | 44min | 1h32min | 1h19min | Lacrimation is observed in one of the mice. Strong tremors. Very violent death of the three mice, jumping as in PSP | Ė |

Table 2. Qualitative results of 5 strains of Gambierdiscus obtained AFTER dichloromethane (CTX fraction) and methanol (MTX fraction) partition by CBA-Neuro2A and Mouse Bioassay (MBA)

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, 54deoxyCTX1B or PCTX2 ciguatoxins.

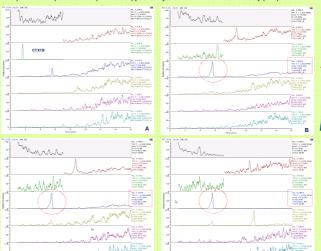
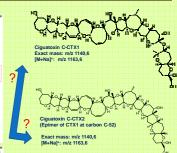


Figure 4. Chromatograms obtained by LC-MS/MS. A: CTX1B standard: B: G. silvae VGO 1355, C: G. silvae VGO 1358, D: G. silvae VGO 1363



CONCLUSIONS

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

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