ASSESSMENT OF THE GROWTH AND TOXICITY OF DIFFERENT STRAINS OF *GAMBIERDISCUS* SPP.

Yefermin Darias-Dágfeel[1]; Francesco Pisapia[2]; Francesca Cucchi[2]; Juan Luis Gómez-Pinchetti[2]; Antera Martel[2]; Freddy Silva Sergent[1]; Andrés Sánchez Henao[1]; María José Ramos Sosa[1]; Daniel Padilla[1]; Natalia García-Álvarez[1] and Fernando Real[1]

[1]Institute of Animal Health and Food Safety (IUSA), University of Las Palmas de Gran Canaria (ULPGC), 35416-Arucas. Las Palmas, Spain.

[2]Spanish Bank of Algae, University of Las Palmas de Gran Canaria (ULPGC), Muelle de Taliarte, 35214 Telde, Spain.

INTRODUCTION

Dinoflagellates of the genus *Gambierdiscus* are ciguatoxins (CTXs) producers. CTXs bioaccumulate in the marine trophic chain and are responsible of ciguatera poisoning (CP) in humans. Ciguatera is a foodborne illness typically known as a tropical disease, but cases are being increasingly reported in the North-Eastern Atlantic. The identification of fish contaminated with CTXs has become more common in areas such as the Canary Islands. This study is part of the "CIGUARISK" Project and shows the comparative performance on cell growth and toxin production of two *Gambierdiscus* strains from the Atlantic region, *G. excentricus* IRTA-SMM-17-429 and *G. carolineanus* BEA 1923B, selected for fish feeding experiments and grown under laboratory-controlled conditions.

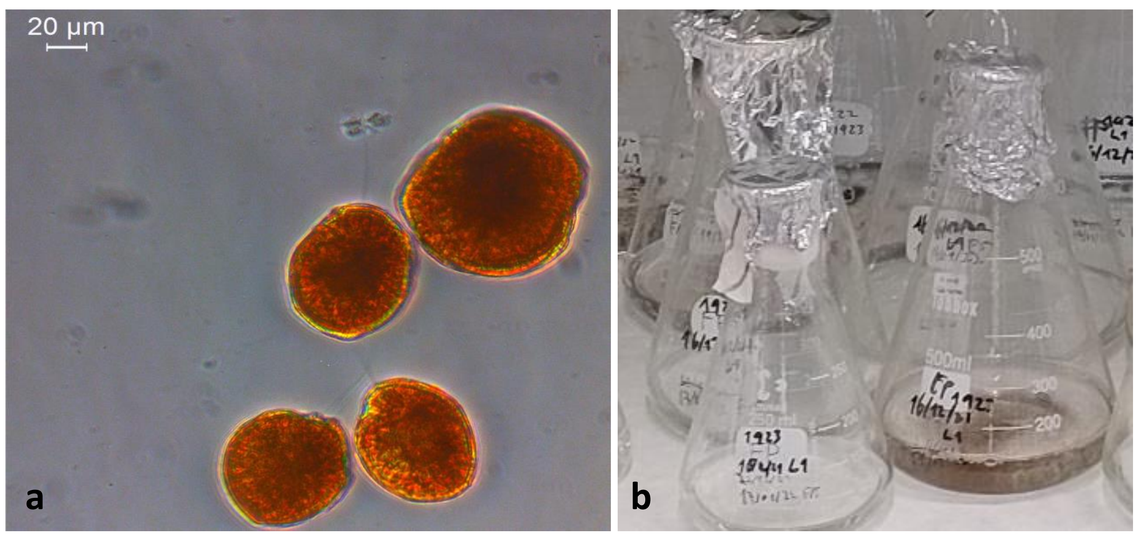

Figure 1.
(a) *Gambierdiscus excentricus* (IRTA-SMM-17-429) at the inverted microscope (20x).
(b) Cultures of the *Gambierdiscus carolineanus* strain (BEA1923B) under controlled conditions.

RESULTS

The results on the quantification of the CTX-like toxicity by *Gambierdiscus excentricus* (IRTA-SMM-17-429) extracts reached values of 27 fg CTX1B eq./cell (Fig. 4)

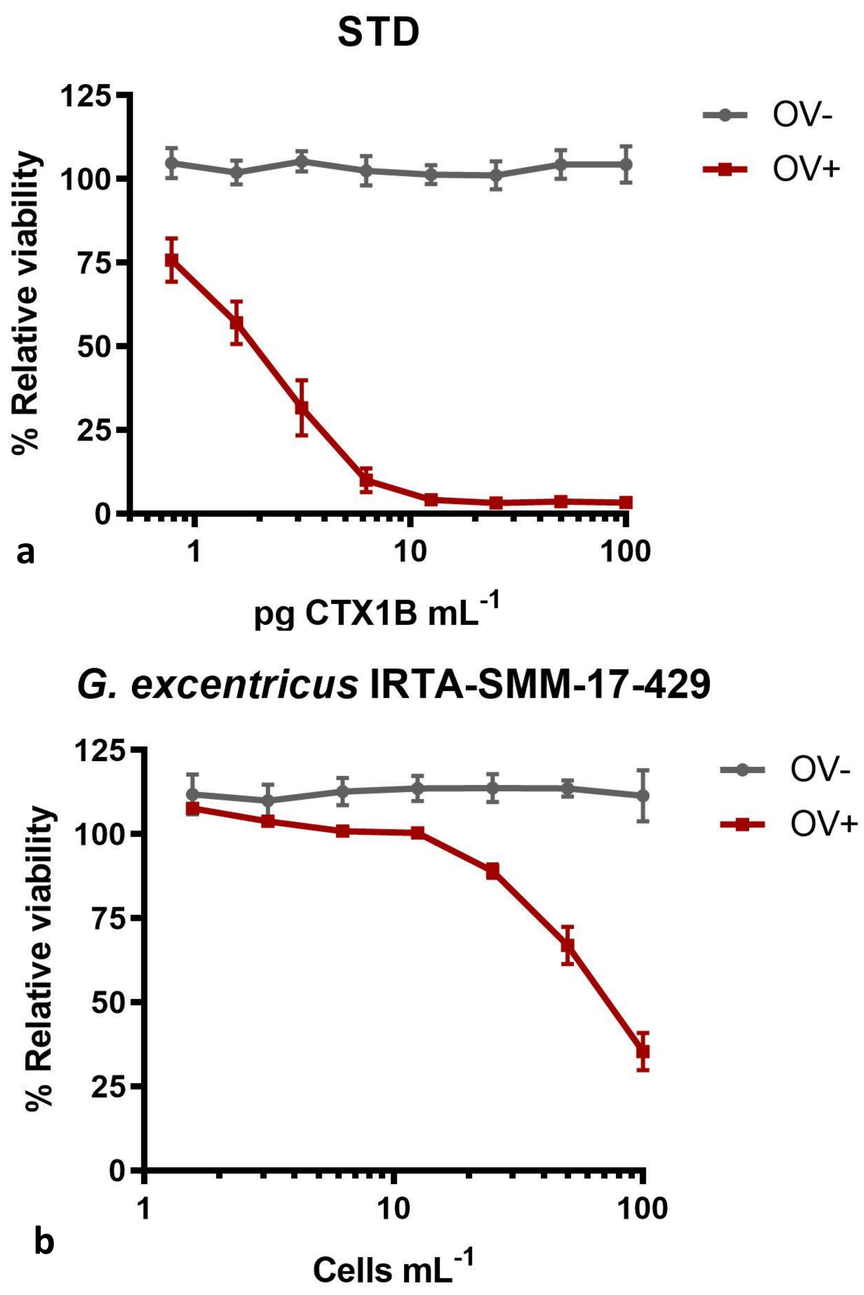

Figure 4.
Dose-response curves of N2a exposed to (a) CTX1B standard (0.78 to 100 pg mL-1) and (b) G. excentricus IRTA-SMM-17-429 extract (1.56 to 200 cells mL-1). G. excentricus caused cell mortality on the N2a cells in a dose-dependent manner in the presence of ouabaine (O) and veratridine (V), indicating the presence of ciguatoxins.

G. carolineanus did not show CTX-like toxicity with this assay (data not shown).

Acknowledgments: Financial support by CIGUARISK/CIGUAFOOD (**PID2019-108781RR-C21-PID2019 108781RR C22**), RASPA (**INTERREG MAC 2014-2020, MAC2/1.1a/305,**), and BIOALGRI (**PID2019-109476RB-C21**) is acknowledged. Yefermin Darias Dágfeel acknowledges support by **TESIS2021010048**.

References

Satake et al.(1993). The structure of CTX3C, a ciguatoxin congener isolated from cultured Gambierdiscus toxicus. Tetrahedron letters, 34(12), 1975-1978.

Pisapia et al.(2017). Toxicity screening of 13 Gambierdiscus strains using neuro-2a and erythrocyte lysis bioassays. Harmful Algae, 63, 173-183.

MATERIALS AND METHODS

Cultures flasks were kept in a chamber under controlled light (80 µmols photons m^{-2} s^{-1}) and temperature (24 ± 2°C) conditions, using L1 as culture medium (Fig. 1). Cells of *G. carolineanus* (BEA 1923B) and *G. excentricus* (IRTA-SMM-17-429) were harvested by centrifugation after a cultivation period of 60 days.

Toxin extraction from *Gambierdiscus* samples. After harvested, cells were suspended in MeOH, disrupted using sonication (3 cycles of 10 min) and the supernatants collected after centrifugation at 4000 *g* for 5 min (4°C) (Fig. 2). Crude extracts were blow-dried under N_2 gas at 40 °C and the residue was resuspended in MeOH:H_2O (3:2, v/v). After the extraction step, separation of maitotoxins (MTXs) and CTXs was carried out by phases separation of DCM and MeOH according to Satake *et al.* (1993). The lipophilic CTXs were partitioned twice into the DCM soluble fraction (DSF) while the amphiphilic MTXs were partitioned into the aqueous methanol (aq. MeOH) soluble fraction (MSF) at the same proportion (Pisapia et al., 2017).

Figure 2. Laboratory toxin extraction process.

Neuroblastoma N2a assay. The N2a cytotoxicity assay is frequently used to estimate the presence of CTX-like toxicity in fish, shellfish or phytoplankton extracts. Ciguatoxins induce N2a cell mortality when cells are pretreated with ouabain (O) and veratridine (V), as they become sensitive to sodium channel activator toxins. The N2a cells were incubated for 24 h in 96-well plates. The CTX1B standard and *Gambierdiscus* extracts samples were added and incubated for 24 h. Cell viability was assessed after 16 h using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] and DMSO solutions (Fig. 3).

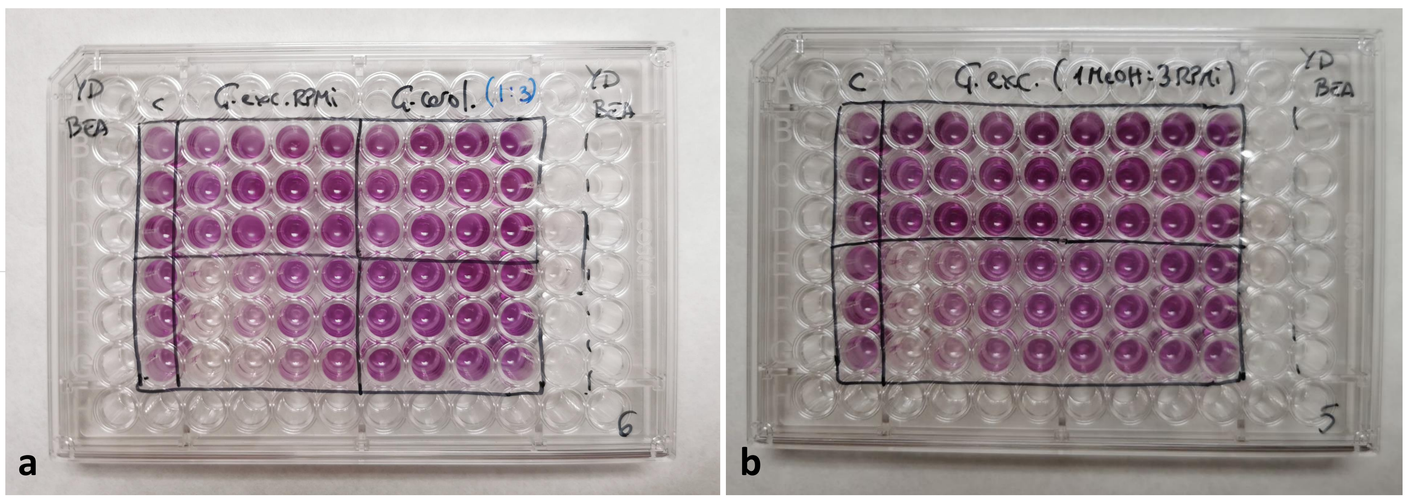

Figure 3.
(a) Microplate of N2a cytotoxicity (CBA-MTT) assay used for *G. excentricus* (IRTA-SMM-17-429) and *G. carolineanus* (BEA 1923B) analysis.
(b) Microplate of N2a cytotoxicity (CBA-MTT) assay used for *G. excentricus* (IRTA-SMM-17-429) quantification analysis.

CONCLUSIONS

- Under the same growth culture conditions *G. carolineanus* (BEA1923B) shows better growth capacity when compared to *G. excentricus* (IRTA-SMM-17-429).
- However, under such conditions *Gambierdiscus excentricus* (IRTA-SMM-17-429) showed CTX-like toxicity, while *G. carolineanus* (BEA 1923B) CTX activity was not detected.