Bacterial death is an important event associated with carbon and nutrient cycles in aquatic ecosystems [1]. Although programmed cell death (PCD) is a regulated process largely known in eukaryotic organisms [2], it is still poorly understood in aquatic bacteria. In this study, we investigated the occurrence of PCD in aquatic bacteria collected from an impacted ecosystem (Batata Lake) located in Northern Brazilian Amazon. This ecosystem was drastically impacted by bauxite tailings and it is presently divided in an impacted area and a natural area. Water samples collected from the subsurface of this lake (n= 6) were studied by fluorescence microscopy using different probes for analysis of cell density (DAPI) and bacterial viability (LIVE/DEAD BacLight). DNA fragmentation was assessed by flow cytometry using Tunel assay, a marker for identifying PCD [2,3]. In parallel, samples were processed for transmission electron microscopy (TEM) [4] to evaluate bacteria alterations. A higher density of bacteria was found in the natural area of Batata Lake (p<0,05) than in the impacted area. Our cell viability results enabled direct visualization of live and dead bacteria and revealed a higher proportion of bacterial death in the impacted area compared to the natural area (p<0,01). DNA fragmentation analysis (TUNEL assay) showed that PCD is a phenomenon occurring in bacteria in this lake, with higher frequency in the impacted area (15,52%, p<0,05). TEM revealed typical ultrastructural changes indicative of apoptosis in bacteria from both areas, such as cell retraction, cytoplasmic condensation and non-disrupted degenerating cells. Quantitative EM analysis showed that 47,14% of aquatic bacteria in the impacted area exhibited signs of apoptosis. Altogether, our data demonstrate, for the first time, that PCD occurs in aquatic bacteria from tropical ecosystems and that this event may be an important mechanism for controlling bacterial communities in aquatic ecosystems.

References

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Fig. 1: Quantification of aquatic bacteria in a tropical ecosystem (Batata Lake, Brazil). (A) Bacteria density evaluated after staining with DAPI. (B) Live/dead bacteria were clearly observed as green (arrowhead) or red (arrow) structures. In (C), the percentage of live/dead cells are shown. (*) p< 0.001. Scale bar 10µm.

Fig. 2: Bacterial DNA fragmentation analysis by flow cytometry. (A, B) Representative histograms of DNA fragmentation in bacteria collected from impacted and natural areas and prepared for TUNEL assay. In (C), the percentages of cells exhibiting DNA fragmentation are shown. (*) p< 0.001.

Fig. 3: Transmission electron microscopy of aquatic bacteria reveals apoptosis-like alterations. (A) Bacterium with typical ultrastructure. In (B, C), bacteria show cytoplasmic condensation (arrows), grouped granules (G) and changes in electron-density. Two empty bacteria are indicated (*). Cellular debris are observed (C, arrowhead). Scale bar 150nm.