TOXIC DINOFLAGELLATES (*DINOPHYSIS* SPP.) DETECTION BY GENOSENSORS AND MOLECULAR BIOLOGY APPROACHES

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Over the years, the marine ecosystems integrity has been compromised, due to multiple factors that disrupt the natural balance of phytoplankton. Factors such as the unregulated runoff of agricultural and industrial wastes into the aquatic environment and higher surface temperatures [1] are believed to have transformed these ecosystems into favorable habitats for algae growth and proliferation. As a result, multiple species may produce harmful toxins that significantly affect the integrity of rivers, lakes, estuaries, and coastal areas [2]. Although these microorganisms are mostly harmless, certain species, namely belonging to dinoflagellates (e.g. *Dinophysis* spp.) produce toxins that pose a risk for human health. Therefore, the need for technological developments towards fast and precise detection of these toxin-producing microalgae is critical to prevent socioeconomical damages, as well as to assess the ecological status of marine ecosystems. The goal of this work was to develop analytical approaches based on electrochemical genosensors devices in order to create a low-cost platform able to detect two dinoflagellate species from the genus *Dinophysis*: *D. acuminata* and *D. acuta*, which are lipophilic toxin producers responsible for diarrhetic shellfish poisoning (DSP) in humans [3]. The design of this DNA-based sensor consists of three steps: i) Sensing phase: consisted by a mixed self-assembled monolayer composed by a linear DNA capture probe and mercaptohexanol onto disposable screen-printed gold electrodes surface; ii) Hybridization of complementary DNA sequence (DNA target) by using a sandwich format assay with enzymatic labels and iii) Electrochemical detection by chronoamperometry using an enzymatic scheme to amplify the electrochemical signal. The best analytical conditions used to study the relationship between electrochemical signal and DNA target concentration, to produce the best electrochemical genosensor device are described in Table 1. Molecular biology tools were used to validate the electrochemical genosensor.

Table 1: Optimized analytical variables in the development of the electrochemical genosensor.

Variables evaluated	Tested range	Selected value
DNA-capture probe concentration (µM)	0.25-1.00	0.25
MCH concentration (µM)	0.00-1.00	0.10
MCH incubation time (min)	15-60	7.5
Homogeneous hybridization incubation time (min)	15-60	30
Antibody concentration (µM)	0.5-2	2
Antibody incubation time (min)	15-60	60
Heterogeneous hybridization incubation time (min)	15-60	60
Homogeneous hybridization temperature (ºC)	25, 98	98

References

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