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Electrochemical genosensors as a new approach on plant DNA detection and quantification for honey authentication

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INTRODUCTION

Honey is a natural sweet food with a rich nutritional composition and many health benefits (e.g., anti-inflammatory, antioxidant, and antimicrobial properties), often consumed as an alternative to processed sugars [1]. Nonetheless, a honey's nutritional composition differs based on the climate, soil, altitude, production method, and pollen source, consequently affecting its health benefits and market value [2]. So, the price of honey can fluctuate significantly depending on its botanical and geographical origins [3].

In this study, an electrochemical genosensor capable of detecting *Erica arborea* (the white heather) DNA with high sensibility and selectiveness was developed. Analyzing public database platforms, a 98 base-pair DNA-target probe capable of unequivocally detecting the pollen from *E. arborea* was selected and designed. The complementary probe to the DNA-target oligonucleotide sequence was then cut into a 28 base-pair thiolated DNA-capture probe and a 70 base-pair fluorescein isothiocyanate-labelled DNA-signaling probe. To increase the hybridization reaction, a self-assembled monolayer formed from mixing the DNA-capture probe with mercaptohexanol was employed.

The enzymatic amplification of the analytical signal was obtained by chronoamperometry using a POD/H_2O_2 system. A linear relationship between electrochemical intensity and DNA concentration was observed when DNA concentrations ranged from 0.03 to 2.00 nM.

The developed sensor was applied to the detection of the DNA from real *E. arborea* plant samples with promising results. This sensor will hopefully determine the geographic botanic origin of honeys and facilitate honey food safety and control.

MATERIAL & METHODS

Apparatus and electrodes

An iron mortar and pestle were utilized to extract the DNA from inside the heather plant and the amplification of the targeted DNA sequence carried out by conventional PCR with a MyCycler™ thermal cycler from Bio-Rad Laboratories. The electrochemical genosensors were assembled using screen-printed gold electrodes (SPGE) from Ωmetrohm (C223BT, DropSens, Switherland). The SPGE functioned as an electrochemical transducer. Moreover, an Autolab potentiostat (PGSTAT101, ΩMetrohm) equipped with the NOVA 1.11.0 research software was used to measure all the electrochemical signals. The chronoamperograms were measured with a -0.1 V potential during 60 s.

Oligonucleotides sequences and real DNA samples

Analyzing public database platforms, the synthetic probes were designed and bought from Eurogentec as a lyophilized salt. All the oligonucleotide solutions were stored at - 20ºC after being resuspended to 100 nM using Milli-Q ultrapure water. *E. arborea* samples (Figure 1) were obtained from the Botanical Garden of Porto, Portugal. Their genomic DNA was extracted by mechanically shredding the plants with liquid nitrogen in an iron mortar. *Castanea sativa* (the chestnut tree) was used as a negative control.

Figure 1. *Erica arborea* plant.

Electrochemical genosensor design

The sensor's methodology consisted of a sandwich hybridization between the 98-mer *E. arborea* target oligonucleotide and its cleaved complementary probes: the 28-mer DNA-capture probe and the 70-mer DNA-signaling sequence. The DNA-capture is initially attached to the SPGE, while the DNA-signaling probe, designed using a fluorescein isothiocyanate (FITC) to which anti-fluorescein antibodies labelled with horseradish peroxidase (POD) enzymes are attached, reacts with the 98-mer DNA-target probe before being applied to the SPGE (Figure 2).

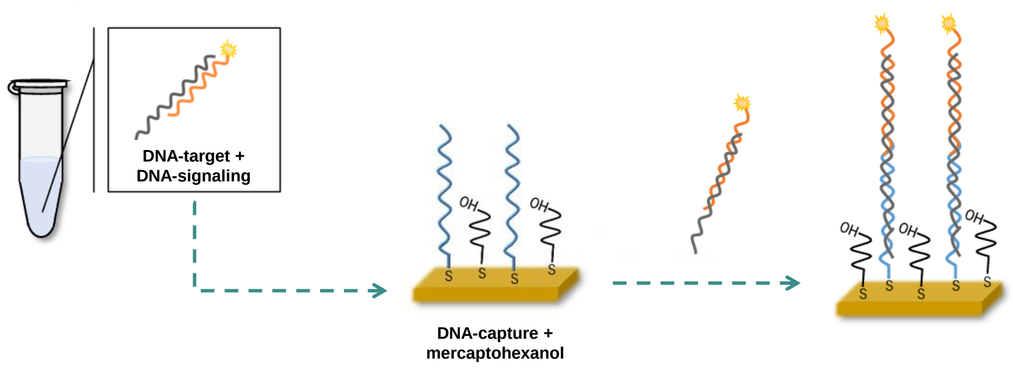

Figure 1. Sandwich hybridization reaction format. DNA-target in grey, DNA-signaling in orange, DNA-capture in blue, anti-fluorescein antibodies in yellow and mercaptohexanol in black.

RESULTS & DISCUSSION

Table 2. Selected analytical parameters levels used for the genosensor optimization.

Variables	Tested range	Selected value
DNA-capture concentration (µM)	0.25–10.00	1.00
MCH spacer concentration (µM)	0.00–1.00	0.50
MCH spacer incubation time (min)	5–30	5
Homogeneous hybridization incubation time (min)	15–60	30
Temperature (°C)	25-98	25
DNA-signaling concentration probe (µM)	0.13–0.50	0.50
Heterogeneous hybridization incubation time (min)	30–120	60
Antibody concentration (U/mL)	0.50–3.00	2.00
Antibody incubation time (min)	15–45	30

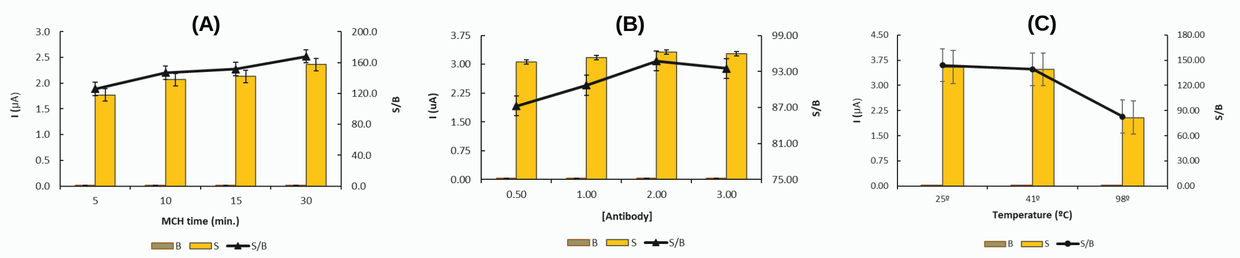

Figure 3. Chronoamperometric measurements registered when studying the effect of the (A) the mercaptohexanol (MCH) incubation time, (B) hybridization temperature and (C) antibody concentration. Blank (B) values represented in brown, signal (S) in light yellow and the corresponding S/B ratio in black.

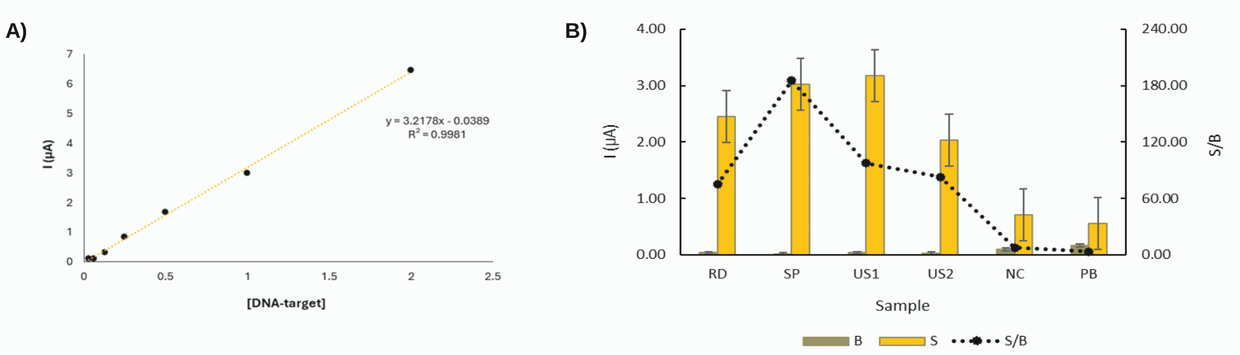

Figure 4. Results from the **(A)** calibration curve corresponding to the synthetic DNA-target concentrations ranging from 0.03 to 2.00 nM and **(B)** electrochemical signals detected from the complementary *Erica arborea* (RD, US1 and US2) and the non-complementary (NC and PB) amplified DNA to the synthetic DNA target (SP). Current intensity values of the blank assays (B) represented in brown, signal (S) in orange and the corresponding S/B ratio in black.

- All optimization helped enhance the electrochemical signal (Figure 3A–3C);
- A linear relationship (R^2 = 0.9981) between the intensity current and the synthetic target concentration was obtained in the 0.03–2.00 nM range, with a slope and intercept value of 3.22 ± 0.03 (µA/nM) and 0.04 ± 0.01 (µA), respectively (Figure 4A);
- The highest S/B value (S/B = 185.00) was obtained with the synthetic DNA-target probe at 1 nM. Nevertheless, the highest current intensity was observed for the *E. arborea* DNA sequence with 1.30 nM (US1), followed by the synthetic DNA-target probe (SP) with 1 nM (Figure 4B).
- All responses were within the calibration curve error range;
- The current responses from the non-complementary NC and PB samples exhibited the lowest current intensities (Figure 4B).

CONCLUSIONS & FUTURE WORK

- An electrochemical genosensor capable of identifying the DNA of E*rica arborea* plant, was developed with high sensitivity and selectivity.
- The sensor's high sensitivity and selectivity was achieved by designing a self-assembled monolayer composed of the thiolated-DNA-capture probe and MCH spacer and due to the sandwich format assay design, respectably.
- The developed genosensor was optimized for the detection and quantification of *E. arborea* plant samples with heather-specific synthetic DNA probes.
- This genosensor was able to detect with great selectivity both the synthetic and genomic DNA of the *E. arborea* at different concentrations.
- In the near future, real honey and pollen DNA will be applied to the developed sensor.

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