KARST SCIENCE DAYS
SYMPOSIUM PROCEEDINGS
1 (2023), 5–6
https://doi.org/000000000000

Cold-adapted phototrophic microorganisms from Scărişoara Ice Cave: Suitable models for developing electrochemical biosensors for herbicides

Robert Ruginescu*[1], Alina Vasilescu[2], Roberta Banciu[2], Cristina Purcărea[1]

1 . Department of Microbiology, Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independenţei, 060031 Bucharest, Romania
2 . International Centre of Biodynamics, 1B Intrarea Portocalelor, 060101 Bucharest, Romania

The excessive use of herbicides has led to their accumulation in the environment, resulting in severe ecological and health consequences. Thus, the development of efficient on-site methods to detect herbicides is becoming increasingly important. Electrochemical biosensors that rely on the inhibition of photosynthesis of microorganisms by herbicides have emerged as an attractive alternative to conventional analytical techniques due to their high level of specificity, sensitivity, speed, and relatively low cost (Tucci *et al.*, 2019). Phototrophic microorganisms adapted to cold environments may serve as ideal models for developing biosensors that can operate effectively in low-temperature environments, such as seawater. In this context, our study aimed to explore the potential of microorganisms isolated from Scărişoara Ice Cave for developing cold-active electrochemical biosensors for herbicides.

In order to isolate phototrophic microorganisms, ice samples previously collected from the top of an ice block located in a sun-exposed site (Hillebrand-Voiculescu *et al.*, 2014) were inoculated in BG-11 growth medium (Stanier *et al.*, 1971) and incubated at 15 °C for 50 days under constant illumination. Green-pigmented colonies were purified by re-streaking. The phototrophic strain LT4 was identified among the obtained isolates by sequencing the plastidial 16S rRNA gene, which showed 100% identity (1319 bp) with the Antarctic microalga *Coccomyxa subellipsoidea* C-169. Cells and subcellular components, including thylakoids and PS II complexes, were isolated from the LT4 strain using previously described methods (Vavilin , 2011). These components were then either tested in solution or immobilized on screen printed electrodes as part of the development of herbicide electrochemical biosensors.

The photosynthetic activity of whole cells, thylakoids and PS II from *Coccomyxa subellipsoidea* TL4 was evaluated via amperometry measurements. The experimental system included a portable potentiostat, an electrochemical cell containing a screen-printed carbon nanotubes electrode (type DRP110D CNT, Metrohm Dropsens, Spain) and a white light lamp and adjustable light intensity for controlled lighting and a laptop with appropriate software for data acquisition and interpretation. The measurements were performed by applying a potential of 0.65 V in the electrochemical cell containing biological material (whole cells, thylakoids or PS II) in 20 mM phosphate buffer pH 7.1 with 0.15 mM NaCl, 5 mM $MgCl_2$ and 0.1 mM to 0.5 mM 2,6, diclorobenzoquinone (DCBQ) - as electrochemical mediator. The tests were done by applying 3 consecutive cycles of 1 minute light / 15 minutes dark and the intensity of the photocurrent generated in the electrochemical cell was expressed as average of the 3 measurements. The measurements were repeated with fresh biological material in the presence of diuron (0.26 µM-2.57 µM). The inhibition degree of the photosynthetic activity due to diuron was calculated from the decrease in photocurrent intensity (I_1), compared to the value recorded in the absence of the herbicide (I_0): Inhibition (%) = $(I_0 - I_1)/I_0 \times 100$.

The amperometry tests have emphasized that the photosynthetic activity of thylakoids is inhib-

*Corresponding author. *E-mail address*: robert.ruginescu@ibiol.ro (R. Ruginescu).

ited by 40.2±7.3% in the presence of 60 µg kg^{-1} diuron. By comparison, the same concentration of herbicide induced only 10.2±5.3% inhibition of the photosynthetic activity of the whole cells.

Stability tests have shown that the best results after 6 days at −20 °C were obtained for the samples that contained 2 M sucrose as additive. In these conditions the photosynthetic activity of thylakoids decreased to 55.6% of the initial value and the inhibition caused by 60 µg kg^{-1} diuron was 14.9±2.5%. Consequently, a more extensive study is needed to identify efficient additives to preserve the photosynthetic activity and functionality (i.e., sensitivity to diuron) of thylakoids kept at −20 °C. PS II preparations had low chlorophyll concentration (0.1 mg/mL), low photosynthetic activity and lack of sensitivity to 60 µg kg^{-1}. This leads to the conclusion that the stability of PS II must be improved and, related to this, the protocol for obtaining the PS II must be revisited. For the electrochemical sensing of photosynthesis-inhibiting herbicides, thylakoids appear as the most promising in terms of combined stability and sensitivity to diuron.

Acknowledgments. The authors acknowledge the financial support from the Romanian Executive Agency for Higher Education, Research, Development and Innovation Funding (UEFISCDI) through ERANET-MARTERA-MOBILTOX project, contract 224/04.01.2021.

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