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ORIGINAL PAPER

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A simple mediatorless amperometric method using the cyanobacterium *Synechococcus leopoliensis* for the detection of phytotoxic pollutants

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Abstract The unicellular cyanobacterium *Synechoccocus leopoliensis* is used in a micro-electrochemical cell to generate photocurrents. The photocurrent is dependent on photosynthetic electron transport and is mediated by hydrogen peroxide formation following the reduction of oxygen on the acceptor side of photosystem I. This is the first known application of cyanobacteria in an electrochemical device where no artificial electroactive mediator is needed. The potential for the development of this micro-electrochemical cell for the detection of phytotoxic pollutants, such as herbicides and toxic metal cations, using the photosynthetic system of the cyanobacteria without interference from added electron acceptor is discussed.

Introduction

Water pollution constitutes an important environmental problem. There is a growing need for rapid and easy-to-operate bioassays to evaluate the sublethal toxicity of polluted water effluents (Blaise et al. 1997). Microscale bioassays can involve cellular or subcellular materials, such as tissue cultures or enzyme systems (Isomaa and Lilius 1995). Most require complicated manipulations and are time consuming.

Several pollutants found in contaminated effluents affect the photosynthetic electron transport chain. This is the case for several quinone-type herbicides such as phenol, urea, and triazine derivatives (Pfister et al. 1981). Nitrite and sulfite compounds also inhibit photosynthesis, as well as most heavy metals and other divalent toxic cations (Carpentier 2001). Detection of these chemicals is primordial because photosynthesis constitutes a vital process for plant species. These inhibitors directly affect the growth of phytoplankton, which is at the basis of the aquatic biomass. Thus, the use of photosynthetic materials in a phytotoxicity biotest constitutes a particularly interesting application. A major limitation of this approach is the relatively short active life-span of isolated photosynthetic membranes. In contrast, intact photosynthetic microorganisms are stable in their culture medium.

There have been some reports of the use of cyanobacteria for the electrochemical detection of herbicides and toxic divalent cations in polluted water. In those cases, the reducing equivalents produced by the electron transport chain were detected amperometrically, and a decrease of electric signal indicated the presence of inhibitors (Avramescu et al. 1999; Rawson et al. 1989; Rouillon et al. 1999). In the above application, artificial electron acceptors such as potassium ferricyanide or substituted benzoquinones were used as electroactive mediators between the cyanobacterial photosynthetic membranes and the working electrode (Avramescu et al. 1999; Rawson et al. 1989). A drawback of this approach is that the artificial acceptors can directly interact with divalent cations or other chemical pollutants with redox properties, or with the buffer used in the electrochemical media, and thus complicate the detection of phytotoxic compounds (Renganathan and Bose 1989, 1990).

A micro-electrochemical cell where isolated thylakoid membranes were used to generate photocurrent in the absence of electroactive mediators has been described in full (Mimeault and Carpentier 1988). It was demonstrated that this system uses the so-called Mehler reaction (Mehler and Brown 1952). In that case, dissolved oxygen is reduced at the acceptor side of photosystem I to form superoxide radicals. The latter spontaneously or enzymatically dismute into hydrogen peroxide that is degraded at a working platinum electrode kept at the appropriate potential (Agostiano et al. 1992; Goetze and Carpentier 1990)

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In this work we used the cyanobacterium *Synechococcus leopoliensis* in a simplified version of the previously developed micro-electrochemical cell. In this application, we take advantage of the endogenous production of hydrogen peroxide that is produced in a significant amount by the Mehler reaction in this type of cyanobacterium (Morales and De La Rosa 1989; Patterson and Myers 1973). The first application of intact cyanobacterium cells in a micro-electrochemical cell without added artificial electron acceptor is presented. The potential use of this system for a phytotoxicity bioassay is discussed.

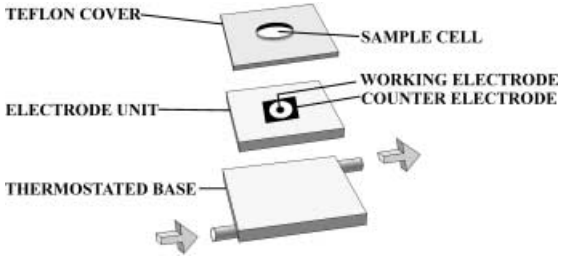

Fig. 1 Schematic representation of the micro-electrochemical cell used with cyanobacteria

Materials and methods

Culture of cyanobacteria

Synechococcus leopoliensis obtained from UTEX collection number 625 was grown in continuous cultures in Allen medium (Allen 1968). Cultures were grown at 25°C in a 16-h day and 8-h night cycle with gentle agitation using continuous bubbling with 2.5% CO_2. Cells were harvested by centrifugation (7 min at 2,500 *g*, room temperature) during mid-exponential growth and resuspended at the appropriate chlorophyll concentration in small volumes of 50 mM TES-NaOH (pH 7.8), 2 mM $MgCl_2$, and 1 mM NH_4Cl, which served as the electrochemical buffer. Chlorophyll was determined in methanol according to Porra et al. (1989).

Electrochemical measurements

A simplified version of a microelectrochemical cell previously described (Mimeault and Carpentier 1988) was used. It consisted of a two-electrode system where the working and counter electrodes were platinum flags and the counter electrode also acted as a reference electrode. The sample cell (80 µl) was composed of a 1-cm hole in a 1-mm-thick Teflon cover (Fig. 1) deposited on the surface of the concentric electrode system (working electrode in the center). The cell was maintained at 25°C using water circulation in a stainless steel base. Illumination (white light) was provided on the top of the cell by a fiber optic illuminator (Microview Canada, Thornhill, Ontario, Canada). A potential of 600 mV was imposed at the working electrode using a potentiostat (model 362, Princeton Applied Research, Princeton, N.J., USA) and the current was monitored on a chart recorder. There was no cover on the cell, thus the cyanobacterial samples could easily be replaced. The toxicity of some chemicals was evaluated by constructing a concentration-response curve. The concentration that inhibited 50% of the control photocurrent and 95% confidence limits were determined from the probit analysis of the data (Finney 1971).

Results

For the generation of photocurrent by the cyanobacterium *Synechococcus leopoliensis* in the micro-electrochemical cell, the potential of the working electrode was set at 600 mV and the system was equilibrated for 5 min before the light beam was turned on. The photocurrent started immediately upon illumination and reached a maximal value within a few minutes. The photocurrent is observed even in the absence of added electrochemical mediators, such as artificial electron acceptors, that could interfere with the effect of pollutants to be detected. This current originates from the efficient production

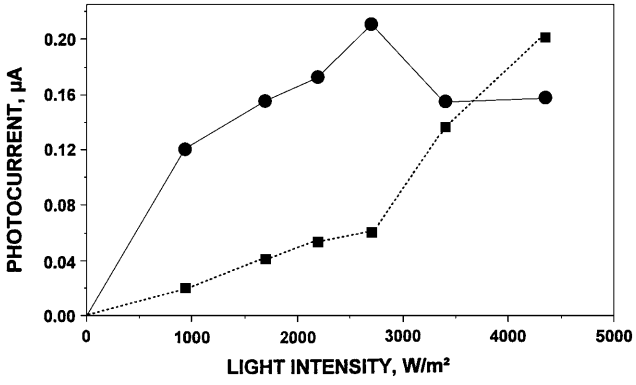

Fig. 2 Effect of incident light intensity on the photocurrent produced: ● in the presence of cyanobacteria at the chlorophyll concentration of 80 mg/l; ■ electrochemical buffer alone. The imposed potential was set at 600 mV

of hydrogen peroxide through the Mehler reaction in this cyanobacterium (Morales and De La Rosa 1989; Patterson and Myers 1973), which coincides with the known mechanism of photocurrent generation in this type of electrochemical cell (Agostiano et al. 1992; Goetze and Carpentier 1990). Such values are plotted for various incident light intensities in Fig. 2. A maximal photocurrent of around 0.21 µA can be obtained at the cell concentration used in Fig. 2 (chlorophyll concentration of 80 mg/l).

The photocurrents obtained with the cell filled only with the electrochemical buffer are also presented in Fig. 2. This photoeffect of the platinum electrode is kept below 0.05 µA if the light intensity does not exceed 2500 W/m^2. At higher light intensity, it became as high as the photocurrent obtained with the cyanobacterium cells. Even though in the presence of cyanobacterium the electrode must receive less direct light and this photoeffect should be minimized, it still limits the light intensity that can be used for proper measurement of the photosynthetic photocurrents at 2500 W/m^2.

As shown in Table 1, the cyanobacterial photocurrent can be inhibited by the herbicide diuron, a known photosynthetic inhibitor (Pfister et al. 1981). A diuron concentration of 15 ppm inhibited completely the photosynthetic photocurrent, leaving only the photocurrent due to the photoeffect at the electrode (result not shown). This in-

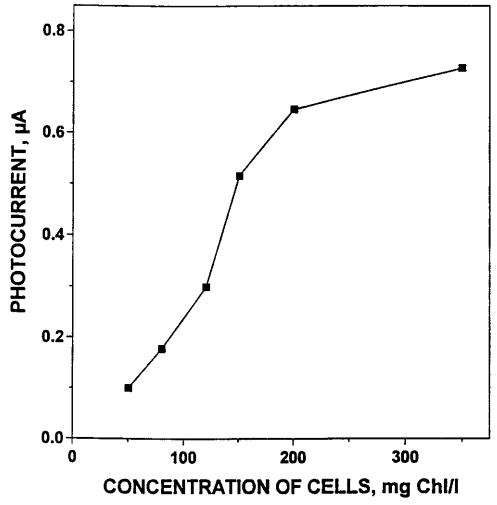

Fig. 3 The influence of the cell density in terms of chlorophyll concentration on the photocurrent produced. The light intensity was 2500 W/m^2; the imposed potential was set at 600 mV

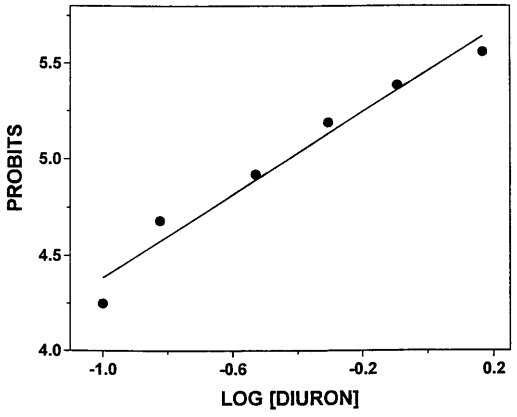

Fig. 4 Inhibition of the photosynthetic photocurrent in the presence of various concentrations of diuron represented on the probit scale. The chlorophyll concentration was 2500 mg/l, the light intensity was set at 250 W/m^2, and the working electrode was poised at 600 mV

Table 1 Determination of the concentration of various phytotoxic compounds that inhibits 50% of the photosynthetic photocurrent (IC_{50})[a, b]

Compound	IC_{50} ppm	95% confidence interval ppm	Correlation coefficientof the linear regression
Diuron	0.35	0.335–0.361	0.98
Atrazine	3.0	2.87–3.18	0.97
$CuCl_2$	8.1	7.9–8.2	0.97
$HgCl_2$	10.7	8.5–12.9	0.97
$Pb(NO_3)_2$	158	144–172	0.93

[a] Values obtained from probit analysis of the data
[b] Chlorophyll concentration=250 mg/l; light intensity=2500 W/m^2

hibitory action demonstrated the photosynthetic origin of the cyanobacterial photocurrent (Table 1).

Increasing the cell density in terms of chlorophyll concentration greatly increased the photocurrent produced (Fig. 3). A chlorophyll concentration of 250 mg/l provided almost the optimal photocurrent, which is about 0.7 µA. In that case the photosynthetic photocurrent is one order of magnitude stronger that the signal obtained with the buffer alone (electrode photoeffect). It is noteworthy that at low chlorophyll concentration (50 mg/l) the photocurrent approaches the value of the photocurrent with the buffer alone. Hence, in the determination of the inhibitory effect of pollutants, a constant value of 0.05 µA, taken as that current from the electrode photoeffect, was subtracted from the total photocurrents.

To illustrate the potential application of this type of cyanobacterium-based photoelectrochemical cell as a bioassay for phytotoxicity monitoring, we have analyzed the effect of various phytotoxic compounds on the photocurrent produced. The action of various concentrations of pollutants on the photocurrent was measured to obtain concentration-response curves. Those curves present a sigmoidal shape that can be linearized using the usual probit scale. A typical result is presented in Fig. 4 for diuron. Similar probit representations were obtained for the various products analyzed. The concentration that inhibits 50% of the photocurrent (IC_{50}) within the 95% confidence limits obtained from the probit analysis are presented in Table 1. Diuron and atrazine are well-known herbicides and inhibitors of photosynthetic electron transport; copper, mercury, and lead are toxic metals cations that are also inhibitory for the electron transport chain. Linear correlations (probit traces) were obtained with very good correlation factors in all cases (Table 1).

Discussion

The objective of this low-cost, easy-to-use, and rapid method should be to develop a general system for the monitoring of pollutants in highly contaminated agricultural or industrial effluents. The test can detect a large spectrum of toxic compounds that inhibit photosynthesis, and thus may be developed as a general-purpose test to localize the sites or effluents where a more-complete and specific test should be performed.

The values obtained for IC_{50} indicate that this proposed phytotoxicity bioassay is not sensitive enough to measure directly the lower concentrations expected in most water samples, such as drinking water, compared with the sophisticated and costly techniques using gas chromatography, high-performance liquid chromatography, atomic absorption, or mass spectrometry, which are more sensitive. However, it should be useful in some applications where the concentration of metal(s) is potentially high, for example in sewage sludge. We have already demonstrated the detection of the phytotoxic pollutants in sewage sludge using photosynthetic materials (Rouillon et al. 2000). The volume of samples required is very small (80 µl). Thus, for less-polluted ali-

quots, the pre-concentration of contaminated samples would be indicated to increase the sensitivity of the approach (Piletskaya et al. 1999). The sensitivity of cyanobacteria can be further increased using permeabilized cells (Papageorgiou et al 1988).

In contrast to isolated photosynthetic membranes, which completely lose their activity after 10 days of storage at 5°C (Carpentier 1999), cyanobacterial cells keep nearly 75% of their function for more than 60 days under similar storage conditions (Rouillon et al. 1999). Moreover, the life-span of the cyanobacterial cells can be further prolonged by entrapment (Papageorgiou et al. 1988) or immobilization (Avramescu et al. 1999; Rouillon et al. 1999) in various matrices. Finally, we should note that in this micro-electrochemical cell, whole-chain electron transport activity is measured without the addition of exogenous artificial electron donor or acceptor, which reveals this approach valuable for the study of algal photosynthesis. Hence, there are several avenues to further develop this biotest, which should support its potential future application.

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