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Effect of Bisphenol A on the extremophilic microalgal strain *Picocystis* sp. (Chlorophyta) and its high BPA removal ability

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ABSTRACT

Bisphenol A (BPA) effects and removal by an alkaliphilic chlorophyta, *Picocystis,* were assessed. BPA at low concentrations (0–25 mg L^{-1}) did not inhibit the *Picocystis* growth and photosynthesis during 5 days of exposure. At higher BPA concentrations (50 and 75 mg L^{-1}), the growth inhibition did not exceed 43%. The net photosynthetic activity was dramatically reduced at high BPA concentrations while, the PSII activity was less affected. The exposure to increasing BPA concentrations induced an oxidative stress in *Picocystis* cells, as evidenced by increased malondialdehyde content and the over-expression of antioxidant activities (ascorbate peroxydase, gluthation-S-transferase and catalase). *Picocystis* exhibited high BPA removal efficiency, reaching 72% and 40% at 25 and 75 mg L^{-1} BPA. BPA removal was ensured mainly by biodegradation/biotransformation processes. Based on these results, the extended tolerance and the high removal ability of *Picocystis* make her a promising specie for use in BPA bioremediation.

1. Introduction

Bisphenol A (BPA) is an organic compound extensively used for polycarbonate plastics and epoxy resins production (Michałowicz, 2014). The BPA production has been increased continuously during the last decade, to reach about 6.8 million tons per year (Jandegian et al., 2015). BPA has become a serious environmental problem since large mass of BPA-based product has been released into the aquatic environments whether by direct spills or by rainwater and drainage systems discharge. It was reported that BPA concentrations are approximately 150 µg L^{-1} in industrial wastewaters (Lee and Peart, 2000), 21 µg L^{-1} in rivers (Belfroid et al., 2002) and 17.2 µg L^{-1} in landfill leachates (Yamamoto et al., 2001). BPA can migrate through the food chain causing toxic and endocrine-disruptive effects on many aquatic organisms (Kang et al., 2007), even at concentrations less than 1 µg L^{-1} (Oehlmann et al., 2006). BPA is known to interfere with the endocrine system leading to adverse effects on the reproductive, neurological and immunological systems in both human and animals (Papazi and Kotzabasis, 2007).

Microalgae, as the primary producers and basis of the aquatic food chain, play a crucial role in the structure and functioning of most aquatic ecosystems and are the first targets to be affected by water pollutants. Microalgae are known by their ecological relevance (Arensberg et al., 1995) and sensitivity (Pavlic et al., 2006) to different pollutants, including BPA. Indeed, BPA can react with biologically active groups in algae causing the inhibition of many physiological process including cell division and photosynthesis (Ji et al., 2014; Zhang et al., 2014). Microalgae have also been reported to remove, accumulate and degrade several hazardous organic and inorganic pollutants, including BPA (Dosnon-Olette et al., 2010; Hirooka et al., 2005; Ji et al., 2013, 2014; Papazi and Kotzabasis, 2007; Petroutsos et al., 2007; Li et al., 2009; Lima et al., 2003).

Nowadays, increased attention is being paid to the isolation and characterization of tolerant algal strains able not only to tolerate and remove high concentrations of pollutants, but also to maintain sustainable growth under variable environmental conditions (Wang et al., 2016).

In this context, extremophilic microalgae seem to be suitable candidates. Indeed, such strains are already known to possess wider possibilities for physiological adaptation and genetic modifications that allow them to tolerate harsh environmental conditions and high anthropogenic pollution (Seckbach et al., 2007; Varshney et al., 2014). Those strains, maintained in continuous culture, may be useful, for the fast and sustainable purification of the aquatic environment.

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The goal of this work is to isolate tolerant algal species from wastewaters for bioremediation use. The present work represents the first one aiming to evaluate and compare, under laboratory culture conditions, the effects and the removal efficiency of BPA in an extremophilic microalgae strain *Picocystis* sp. isolated from alkaline, residual water, household sewage pond. Primarily, the effect of BPA on the growth, photosynthesis, lipid peroxidation and antioxidant activities will be investigated. Then, the ability of this strain to adsorb, accumulate, degrade/transform and remove BPA will be presented.

2. Materials and methods

2.1. Algal strains and culture conditions

Picocystis sp. was isolated from a household sewage "Essed valley" located in Center East of Tunisia (35°59′23″N, 10°30′10″E) at water pH 11. The purified strain was identified morphologically and further confined by 18S rRNA gene analysis. *Picocystis* sp. was cultivated in batch culture under sterile conditions in Zarrouk medium (Zarrouk, 1966). Experimental cultures were conducted at optimal growth conditions (30 °C, light intensity of 75 µmol photons m^{-2} s^{-1} and initial pH of 8.4) (Ben Ali et al., 2017).

Exponentially growing cultures of *Picocystis* were individually inoculated in 500 mL Erlenmeyer flasks containing 300 mL Zarrouk medium with an initial optical density of 0.07 at 680 nm. Cultures were exposed for five days to BPA at 0, 1, 10, 25, 50 and 75 mg L^{-1} initial concentrations. Stock solution of 1000 mg L^{-1} BPA was prepared in anhydrous ethanol. Ethanol concentration in the medium including the control groups did not exceed 0.5% (v/v), which showed nontoxic effects on the Chlorophyta strain. The testing solutions were diluted to the desired concentrations during the experiments.

2.2. Growth inhibition test

Picocystis growth was followed by measuring the daily changes in $OD_{680\,nm}$ of both control and BPA-treated cultures during five days. Growth curves were obtained by plotting $OD_{680\,nm}$ values and incubation time. The areas under each growth curve were calculated using Origin 8.5 software (OriginLab, Northampton, USA). The growth inhibition percentage (PI%) was evaluated according to de Orte et al. (2013) by the following equation:

$$PI\% = [\frac{Control\ area - Treated\ area}{Control\ area}] \times 100$$

where, Control area and Treated area are the integrated areas under the growth curve of control and treated culture, respectively.

2.3. Photosynthetic activity

2.3.1. Net photosynthetic oxygen evolution (NPS)

Oxygen evolution was measured using a fiber-optic oxygen microsensor (Firesting O_2; Pyro-Science, Germany). For the measurement of O_2 evolution rate in the light, cell cultures were collected and placed in a flask containing a microsensor spot with red flash dye at the same culture conditions. Then, they were kept in darkness for 30 min to record the respiratory O_2 consumption rate in the dark. The concentration of dissolved O_2 expressed as nmol mL^{-1} was calculated from the percentage of air saturation by correcting for the temperature and salinity of the medium according to Quicker et al. (1981). The sum of O_2 evolution rate in the light and the respiratory O_2 consumption rate in the dark was considered as the net O_2 evolution rate and normalized to the chlorophyll *a* (Ch*a*) concentration for the corresponding culture (nmol O_2 min^{-1} μg^{-1} Ch*a*) under the assumption that the respiration rate was equal in light and in darkness. Ch*a* content was determined after extraction in 100% ethanol according to the equation established by Ritchie (2006).

2.3.2. Photochemical efficiency of PSII (Fv/Fm)

A sample of 2 mL was collected daily from each culture (control and BPA-treated culture) and diluted with fresh medium to a final optical density at 680 nm of 0.49. Before the measurement, each micro-algal sample was dark-adapted for 15 min at room temperature to complete re-oxidation of PSII reaction centers. The chlorophyll a fluorescence transients (OJIP) were induced by a fixed excitation wavelength at 650 nm and light intensity of 3000 µmol photons m^{-2} s^{-1}, and recorded from 10 µs up to 1 s using the Aquapen fluorometer (Photon Systems Instruments, Brno, Czech Republic). The OJIP-test parameters were determined from transient analysis according to Strasser et al. (2000) and the maximal PSII photochemical efficiency (Fv/Fm) was derived.

2.4. Lipid peroxidation and antioxidant activities

250 mL of each culture were centrifuged and collected; the cell pellets were mixed with 2 mL of 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1 mM Na_2 EDTA and 1 mM ascorbic acid and homogenized on ice bath by the Microson Ultrasonic cell disruptor at 200 W with total time of 1.5 min. The homogenate was then centrifuged at 10,000 × *g* for 10 min at 4 °C. The supernatant was considered as cell-free enzyme extract and was stored at 80 °C for lipid peroxidation, antioxidant activities and total proteins analyses.

The MDA content was quantified based on its chromogenic reaction with thiobarbituric acid as described by Heath and Packer (1968) and expressed as µmol MDA per mg protein.

Ascorbate peroxidase (APX) activity was assayed as described by Nakano and Asada (1981). Catalase (CAT) activity was determined according to the method of Claiborne (1985). Glutathione S-transferase (GST) activity was assayed as described in the method of Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.

Enzymatic activities of APX, CAT and GST were expressed as unit per mg protein. One unit is defined as the amount of enzymes that catalyze the conversion of one µmol of substrate per minute.

The protein contents of the samples were determined according to the Hartree method (Hartree, 1972), using bovine serum albumin as a standard.

2.5. Determination of BPA removal

At the fifth day, 150 mL of 0, 25, 50 and 75 mg L^{-1} BPA-treated cultures were centrifuged at 10,000 × *g* for 10 min. The supernatant (S1) was stored for the determination of the residual BPA in the medium. The cell pellets were washed three times with de-ionized water and then centrifuged. The supernatant (S2) was used for the determination of BPA adsorbed on the microalgae surface as described by Li et al. (2009). The pellets were than mixed with 3 mL of dichloromethane-methanol (1:2 v/v) and disrupted by sonication for 45 min (Ji et al., 2014). After centrifugation for 10 min at 4500 × *g*, the resulting supernatant (S3) was conserved to determine the accumulated BPA within the microalgal cells.

BPA was extracted from S1, S2 and S3 in 5 mL of dichloromethane. The extraction step was repeated three times to ensure total extraction of the BPA amount. The extract was evaporated to dryness under nitrogen and re-suspended in 2 mL of methanol.

The same extraction procedure was applied for media without algae cells exposed to the same culture conditions during five days and under different BPA concentrations, to determine the abiotic removal of BPA. All samples (S1, S2 and S3) were stored at 4 °C in the dark prior to HPLC analysis.

2.5.1. HPLC analysis

BPA quantification was carried out using Agilent series 1260 HPLC-DAD instrument (Agilent Technologies, Waldbronn, Germany) equipped by a Zorbax Eclipse XDB-C18 column, serial number