Biodegradation and detoxification of bisphenol A by bacteria isolated from desert soils

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Received: 23 November 2018 / Accepted: 11 May 2019 / Published online: 22 May 2019
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Abstract

The endocrine-disrupting chemical bisphenol A (BPA) has attracted much attention because of its estrogenic activity and widespread environmental contamination. In this study, we investigated the BPA biodegradation abilities of various bacterial strains isolated from deserts and arid soils from southern Tunisia. Ten bacterial strains that belong to *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Klebsiella* sp. and *Pantoea* sp. showed high BPA removal potential in mineral salt medium (MSM) containing 1 mM BPA. BPA removal rates varied between strains and ranged from 36 to 97%. The strain G320 (*P. putida*) presented the highest BPA removal rate with 97% within 4 days at 30 °C. The half-life when increasing the BPA concentration to 3 mM was 2 days for strain G320, while total degradation was achieved within 8 days. BPA biodegradation products were identified by GC–MS, and their toxicity was assessed by an algal toxicity test. BPA detoxification was confirmed by evaluating the effect of its biodegradation metabolites on algal growth (dry weight), cells morphology and chlorophylls levels of *Tetraselmis* sp. strain V2. Results showed the interesting potential of desert soil's bacteria in BPA detoxification as well as the eventual use of the algal specie in toxicity assessment.

Keywords Bisphenol A · Biodegradation · Bacteria · Desert soils · Microalgae · Toxicity

Introduction

Bisphenol A [BPA; 2,2,-bis (hydroxyphenyl) propane; CAS Registry No. 80-05-7] is a man-made compound commonly used in the production of plastics, in particular, epoxy resins and polycarbonate with an annual production exceeding 5.0 million tons in 2010 (Huang et al. 2012). For many decades,

BPA has been intensively introduced into soils, sediments, ground and surface waters (Careghini et al. 2015). Industrial and municipal wastewaters are the most common sources of BPA contamination in aquatic environments (Stuart et al. 2011; Gorga et al. 2015; Azevedo et al. 2001). In addition to natural environments, human exposure to BPA for adults and children was confirmed (Lehmler et al. 2018). In fact, BPA has been detected in human serum, urine, tissue and blood of both occupationally and environmentally exposed individuals (Asadgol et al. 2014; Ribeiro et al. 2017). BPA has an acute toxicity toward aquatic organisms (Ebenezer and Ki 2012; Canesi et al. 2015; Ellen et al. 2009; Krishna et al. 2014; Zhenghua et al. 2008) and it has been confirmed to be a human endocrine-disrupting chemical (EDC) by the US Environmental Protection Agency (USEPA). The BPA estrogenic activity was first reported by Dodds and Lawson in (1936) and further supported by several studies (Krishnan et al. 1993; Olea et al. 1998; Segner et al. 2003). In fact, BPA influences growth, reproduction and biota development through interfering with the activities of several endocrine hormones, specific intracellular signaling pathways, and epigenetic regulators (Ziv-Gal et al. 2013; Wetherill

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13205-019-1756-y>) contains supplementary material, which is available to authorized users.

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et al. 2007; Fenichel et al. 2013; Booth et al. 2016). Recent data also suggested that human exposure to BPA causes hepatotoxic, immunotoxic, mutagenic and carcinogenic effects (Michalowicz 2014). Son et al. (2018) confirmed that BPA significantly affected human keratinocytes viability, induced apoptotic fraction and increased activation of DNA-damage marker protein. Moreover, human exposure to BPA may increase obesity, diabetes and heart diseases risks (Vom Saal et al. 2012; Shankar et al. 2012; Liu et al. 2013a). Thus, BPA removal from the natural environment is an increasing worldwide concern. Several BPA transforming or/and degrading organisms such as bacteria, fungi, algae and plants were effectively used to remove BPA from the environment (Michalowicz 2014; Zhang et al. 2013; Kamaraj et al. 2014; Mita et al. 2015; Vijayalakshmi et al. 2018). More and more BPA-degrading bacteria have been isolated from water, sediment, soil and wastewater treatment plants. They included both Gram-negative and Gram-positive strains (Das et al. 2018; Ebenezer and Ki 2016; Hou et al. 2014; Fouda 2015; Zhang et al. 2013). Different BPA degradation pathways have been proposed in the literature according to the intermediates detected during the degradation process for specific bacterial strains (Li et al. 2012; Zhang et al. 2013; Fischer et al. 2010; Vijayalakshmi et al. 2018). Until now, most BPA biodegradation studies were conducted by single-strain or bacterial consortia isolated from sediments or aquatic environments (Peng et al. 2015; Kamaraj et al. 2014; Eio et al. 2014; Yang et al. 2014; Li et al. 2012; Kang and Kondo 2002; Moussavi and Haddad 2019) but no studies were made for deserts and arid area's bacteria. The ability of desert microorganisms to cope with the harsh environmental conditions and to adopt complex survival strategies and special adaptation mechanisms to alleviate abiotic stresses, make them an interesting subject for study (Elleuche et al. 2015). In this work, we tried to gain a greater insight into BPA removal by studying the biodegradation ability of desert soil's bacteria toward BPA. *P. putida* G320 ability to degrade BPA was evaluated. The main BPA biodegradation metabolites were identified by GC-MS and their toxicity was evaluated through a micro-algal toxicity test on *Tetraselmis* sp. (growth, morphology and chlorophylls content). This study was conducted in order to better understand the biodegradation process of endocrine disruptors in nature and facilitating the evaluation of BPA metabolites toxicity.

Materials and methods

Chemicals and reagents

BPA (99%) was purchased from Sigma Aldrich. Acetonitrile purchased from Suvchem was used for HPLC analysis was of analytical grade.

Soil samples collection

Soil samples were collected from three different locations in south Tunisia (Kebeli, Gabes and Tozeur) during September 2014. Each sample was collected from 1 to 10 cm in depth and stored in a sterile bottle for further analysis.

Media and culture conditions

The nutrient agar medium was used for nonselective isolation of different bacterial species from soil samples, it was prepared by dissolving 5 g NaCl, 5 g peptone, 2.5 g yeast extract, 10 g meat extract and 15 g agar in 1 L distilled water, pH was adjusted to 7. For BPA biodegradation study, Mineral Salt Medium (MSM) supplemented with 1 or 3 mM BPA was used as a working medium. The ingredients of MSM were as follows (g L⁻¹): K₂HPO₄ (0.3), KH₂PO₄ (0.3), KCl (0.1), NaCl (0.6), CaCl₂ (0.1), MgSO₄·7H₂O (0.1), NH₄Cl (0.1), trace element solution 1 mL (Daassi et al. 2013).

Isolation of bacteria

Desert soil samples (0.5 g) were separately suspended in 20 mL of nonselective nutrient broth medium (5 g L⁻¹ NaCl, 5 g L⁻¹ peptone, 2.5 g L⁻¹ yeast extract, 10 g L⁻¹ meat extract and 15 g L⁻¹ agar, pH was adjusted to 7). In order to increase bacterial diversity of the isolates, different culture conditions were conducted such as incubation temperature (37, 45 °C), NaCl (5, 20, 50 g L⁻¹) and glycerol (5, 15, 20%) concentrations. All cultures were incubated for 18 h at 150 rpm and then serial dilutions of the bacterial culture were plated separately onto solid media. After 24 h of incubation, individual colonies representing different morphological aspects were picked and streaked twice on new plates and incubated for 2 days then stored at 4 °C. Long period conservation of the strain was monitored in nutrient broth and glycerol (70–30%) at – 20 °C.

Screening of BPA-degrading bacteria

Growth on BPA as the sole carbon source was first tested by cultivating bacterial strains on MSM solid medium

containing 1 mM of BPA, plates were incubated at 37 °C for 3 days. Colonies that rose under these conditions were picked, checked for purity by microscopic examination and transferred to liquid media for conservation. Selected strains were inoculated in liquid MSM containing 1 mM of BPA supplemented with 1 g L⁻¹ yeast extract and incubated at 30 °C for 4 days at 150 rpm. Growth was evaluated by measuring the absorbance at 600 nm, and residual BPA concentrations were analyzed by HPLC.

HPLC analysis

After 4 days of incubation at 30 °C under shaking conditions, 1 mL of each culture was centrifuged for 10 min at 12,000 rpm and 4 °C. The resulting supernatant was filtered through a 0.22 µm filter to remove potential insoluble component. The filtrate was used for the quantification of the residual BPA concentration. HPLC analysis was conducted by a DIONEX UltiMate 3000 (Thermo Scientific) chromatograph. Compounds were separated on a C-18 column at room temperature. The mobile phase was water (A)/acetonitrile (B) with a total running time of 25 min and a flow rate of 1 mL min⁻¹. The mobile phase gradient was monitored as follow: solvent B (20%) for 5 min then increased to 70% over 10 min, maintained at 70% for 5 min and increased to 100% in 5 min until completion of the analysis. Compounds were detected using an UV/VIS detector at 278 nm.

Identification of bacterial strains and phylogenetic tree

Using extracted genomic DNA of isolated strains, almost complete 16S rRNA gene was amplified through PCR using the bacterial domain universal primer sets fd1 and rp2 (Weisburg et al. 1991). The thermal cycling program was: initial denaturation of 95 °C for 5 min, followed by 35 cycles of 95 °C for 15 s, 54 °C for 15 s and 72 °C for 90 s, with a final elongation of 72 °C for 7 min. Appropriate positive and negative controls were run in parallel for each reaction to ascertain the absence of contamination or PCR failure. PCR products were then purified and sequenced directly using a BigDye197 Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and analyzed with an ABI-3130xl 48-capillary DNA sequencer (Applied Biosystems). Bacterial identification was conducted using blast analysis and species identity was ascertained when unambiguous high identity 98% was scored with only a single species.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 0.28458903 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is

drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The analysis involved 32 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 230 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 method (Tamura et al. 2013).

BPA degradation experiments

MSM containing 3 mM BPA added with 1 g L⁻¹ yeast extract was inoculated with 3% of a freshly prepared bacterial culture (OD₆₀₀ = 1.0) then incubated at 30 °C for 10 days. BPA removal was evaluated by measuring the daily residual BPA concentrations in the culture broth by HPLC. Bacterial growth was assessed by cell dry weight measurement in culture medium each 24 h. Samples (5 mL) were harvested, dried and weighed and the dry biomass was calculated. BPA biodegradation rate relating the eliminated amount of BPA per unit of dry weight biomass to time was also calculated. BPA containing cultures were conducted in duplicate and results are presented as mean values and the error bars described the standard deviation.

BPA degradation metabolites analysis

Extraction and derivatization

In order to elucidate the BPA detoxification potential of the selected bacterial strain, biodegradation intermediates were analyzed by GC-MS. Samples pH was adjusted to 2 with 6 M HCl solution and extracted with 2 volumes ethyl acetate. The extracted solution was dehydrated with anhydrous sodium sulfate and concentrated by rotator evaporation at 40 °C. 200 µL of concentrated extract was added with 45 µL of pyridine and 200 µL of N, Obis (trimethylsilyl) trifluoroacetamide (BSTFA) and incubated at 80 °C for 1 h.

GC-MS analysis

GC-MS analysis were performed by injecting the derivatized sample into a gas chromatograph equipped with a HP-5MS column (60 m*0.25 mm internal diameter; 0.25 µm film thickness) coupled to a quadrupole mass detector (GC-MS system 7980A-5975C, Agilent technologies). High-grade helium was used as the carrier gas with a flow rate of 1.2 mL min⁻¹. Injector and detector were set at

300 °C. The initial column temperature was maintained at 50 °C for 1.5 min, increased to 150 °C at a rate of 10 °C min⁻¹, then to 280 °C at 20 °C min⁻¹, and held at 280 °C for 6 min. Data were acquired in full scan and SIM modes. Each full scan mass spectrum, recorded in the *m/z* range 33–800 amu (atomic mass units), was searched in the Wiley-NIST2011 database for compounds identification.

BPA toxicity assays on microalgae

Algal culture conditions and median effective concentration EC₅₀ (96 h) determination

The marine microalgae *Tetraselmis* sp. strain V2 (Dammak et al. 2016) was used in this study. In order to evaluate to median effective concentration of BPA after 96 h of incubation EC₅₀ (96 h), algal cultures supplemented with different concentrations of BPA (0, 0.2, 1, 2.5 and 5 mg L⁻¹) were monitored. Cultures were inoculated in F/2 medium at pH 7.2 (Guillard, 1975) and incubated 96 h under a light intensity of 84 μmol photons m⁻² s⁻¹ at 25 °C with white fluorescent lamps (Compact Fluorescent Lamp, Superlight, Tunisia) with shaking manually four times per day. Algal cell density was recorded regularly by measuring the absorbance at 680 nm. Growth inhibition rates of cultures added with different concentrations of BPA were calculated according to the following equation (Šepić et al. 2003).

$$I = (\mu_c - \mu_t) * 100 / \mu_c \quad (1)$$

I (%): growth inhibition rate, μ_c : algal growth rate of the control, μ_t : algal growth rate at time *t*.

The growth inhibition curve was plotted using the BPA concentration as the abscissa and the growth inhibition rate at 96 h as the ordinate. Microsoft Excel was used for the linear regression. Toxicity of BPA and its biodegradation products was evaluated at the calculated EC₅₀ (96 h). For this purpose, three algal cultures were prepared in duplicate as follows:

Culture (A): Control algal culture.

Culture (B): Algal culture containing 5 mg L⁻¹ BPA before bacterial inoculation.

Culture (C): Algal culture containing 5 mg L⁻¹ BPA after bacterial inoculation during 10 days. BPA biodegradation metabolites were added to the algal culture after centrifugation and filtration through a 0.22 μm filter to remove bacterial cell from the media. Cultures were incubated 15 days at the previously described conditions.

Assessment of BPA metabolites toxicity on algal growth, morphology and chlorophylls content

Algal cultures growth was estimated by the measurement of cell density and biomass (dry weight). For dry weight determination, samples (10 mL) were centrifuged and washed two times with deionized water. The cells pellets were dried at 105 °C to constant weight. Microscopic observations of cells morphology and motility were carried out at the end of the experiment by an inverted microscope (MOTIC microscopy AE2000, Germany). Pigments content of the algal biomass was measured spectrophotometrically after extraction in 100% ethanol. Chlorophyll a (Chl a), chlorophyll b (Chl b)

Fig. 1 BPA degradation percentages by selected BPA-degrading strains

Fig. 2 16S rRNA sequence-based phylogenetic tree showing the evolutionary relationships between newly isolated strains and their closest relatives. Bootstrap values over 95% are represented. The scale bar indicates 0.02 changes per nucleotide position

and total chlorophylls (Chl *t*) concentrations were calculated according to the following Eqs. (2, 3, and 4) of Lichtenthaler and Wellburn (1983) and Kumar et al. (2010).

$$\text{Chlorophyll a (mg L}^{-1}\text{)} = 15.6 * A_{666(\text{nm})} - 7.340 * A_{653(\text{nm})} \quad (2)$$

$$\text{Chlorophyll b (mg L}^{-1}\text{)} = 27.05 * A_{653(\text{nm})} - 11.21 * A_{666(\text{nm})} \quad (3)$$

$$\text{Total chlorophyll (mg L}^{-1}\text{)} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (4)$$

Results and discussion

Screening of BPA-degrading bacteria

A collection of 340 bacterial strains that were isolated from desert soils was tested for BPA assimilation. About 80% of tested strains were not able to assimilate BPA as sole carbon source, however, 10 strains showed a great ability to grow on 1 mM BPA. In liquid MSM, HPLC analysis of cultures supernatants demonstrated that strains G61, G56, G320, G280, G94, Tz33, G39, Gb30, Tz5 and Tz38 were able to assimilate BPA as sole carbon source, but there were differences in their efficiency. The BPA removal rates at 30 °C after 4 days ranged from 36 to 97% and the highest

Fig. 3 BPA degradation by G320 strain in MSM containing 3 mM BPA. (Continuous line): residual BPA (%); (dashed line): biomass dry weight (g L⁻¹) for strain G320

degradation levels were obtained with strains G320 and G280 (Fig. 1).

Molecular identification of bacterial strains

The 10 bacterial strains which were highly active in BPA degradation were identified by 16 S rRNA sequence analysis. As shown in Fig. 2, strains G58, G61 and G280 were closely related to *Klebsiella* sp., strain Gb30 to *P. aeruginosa*, strain G49 to *Pantoea* sp. strains Tz38, Tz33, Tz5 and G39 to *E. cloacae* and finally strain G320 to *P. putida*. All sequences had 99% or more homology compared to sequences in the nucleotide database NCBI (National Center for Biotechnology Information). This study confirms that the

genera *Pseudomonas*, *Enterobacter*, *Klebsiella*, and *Pantoea* encompass strains that have the ability to degrade BPA and reports for the first time that *E. cloacae* can use BPA as the sole carbon source.

BPA degradation by *P. putida* G320 strain

Among the ten BPA-degrading isolates, *P. putida* G320 strain was selected for its BPA-degrading ability. HPLC analysis of the culture supernatant during incubation indicated that half of the initial BPA concentration was degraded within 2 days and all BPA was depleted after 8 days of incubation (Fig. 3). The growth-linked BPA biodegradation was evaluated and reached after 48 h of incubation 1.25 mM

Table 1 GC–MS identification of BPA biodegradation metabolites by *P. putida* G320 after 10 days' incubation

Chemical compounds	Structure	Retention time (min)
(a) Bisphenol A		25.45
(b) Methyl cinnamate		16.54
(c) Cyclohexylcarboxylic acid		13.84
(d) 2-Methyl, propanoic acid		7.69
(e) Propanoic acid		12.39
(f) Diglycolic acid		13.61
(g) β-Hydroxybutyric-acid		14.07
(h) Glycerol		14.84

BPA ($\text{g DW}_{\text{biomass}}^{-1}$). The BPA biodegradation rate after 24 h of incubation was $0.041 \text{ mM BPA (g DW}_{\text{biomass}} \text{ h)}^{-1}$. The sharp decrease in BPA concentration in the first days can be explained by the combination of adsorption and biodegradation mechanisms by the strain G320. Although there are many bacteria able of degrading BPA, those with high BPA biodegradation ability are rare (Michałowicz 2014). Better results were demonstrated by Moussavi and Haddad (2019) describing enhanced bacterial peroxidase-mediated BPA degradation in a batch bioreactor. BPA biodegradation rate was found to be $10.8 \text{ mg BPA (g}_{\text{biomass}} \text{ h)}^{-1}$ due to H_2O_2 infusion into the bioreactor. Oshiman et al. (2007) described that *S. bisphenolicum* AO1 was able of degrading 100 mg L^{-1} BPA only within 10 h. Similar results were demonstrated by Matsumura et al. (2015) who described that *Pseudomonas* sp. strains SU1 and SU4 are capable of mineralizing 115 mg L^{-1} BPA within 48 h. Kamaraj et al. (2014) reported that *Pseudomonas* sp. KU1 and KU2 and *Bacillus* sp. KU3 showed, respectively, $78 \pm 4\%$, $81 \pm 3\%$ and $74 \pm 2\%$ efficiency in degrading 1000 ppm BPA within 12 days. Vijayalakshmi et al. (2018) reported that *Pseudomonas aeruginosa* (PAB1) is able to degrade 16.30 mM BPA at optimal conditions (pH: 6.94, temperature: 32.61). For *P. putida* specie, strain G320 seems to be more performant in BPA biodegradation compared to KU5 strain described by Kang and Kondo (2002) which degraded about 1 mg L^{-1} BPA within 10 days.

BPA degradation metabolites analysis

BPA degradation by *P. putida* was assessed by GC–MS analysis of the culture supernatant extract. Library search has identified multiple products with shorter retention time than BPA (Table 1). Some of those compounds have been previously reported as BPA degradation products such as carboxylic or polycarboxylic acids and β -hydroxybutyric acid (Gassara et al. 2013; Daassi et al. 2016). These findings are confirmed by Husain et al. (2013) who reported the accumulation of acidic intermediates after biological and enzymatic BPA treatments. However, known BPA metabolites that are more toxic than BPA like *p*-hydroxybenzoic acid, *p*-hydroxyacetophenone, *p*-hydroxybenzaldehyde, hydroquinone, and phenol were not detected (Ike et al. 2002; Lu et al. 2013; Fischer et al. 2010; Eio et al. 2014; Vijayalakshmi et al. 2018). The only phenolic compound identified in this study was the methyl cinnamate. This metabolite was previously reported as a laccase BPA degradation metabolite (Daassi et al. 2016).

BPA toxicity assays on microalgae

Determination of the median effective concentration of BPA at 96 h

Algae are sensitive to a wide range of pollutants, in particular, endocrine-disrupting chemicals and are effective bioindicators in ecotoxicity assessments (Ebenezer and Ki 2012; Ebenezer and Ki 2016; Liu et al. 2013b). In this study, BPA concentration that inhibited the growth of *Tetraselmis* sp. strain V2 by $50\% \text{ EC}_{50}$ (96 h) was calculated to be 2.39 mg L^{-1} . The tested strain was very sensitive to BPA compared to *Cyclotella caspia*, *Cochlodinium polykrikoides* and *Chlorella vulgaris* which EC_{50} (96 h), EC_{50} (72 h) and EC_{50} (120 h) values were 7.96 mg L^{-1} , 68.15 mg L^{-1} and 39.8 mg L^{-1} , respectively (Li et al. 2008; Ebenezer and Ki 2012; Ji et al. 2014). The EC_{50} (96 h) of BPA for the freshwater algae (*Chlorella pyrenoidosa* and *Scenedesmus obliquus*) were 44.9 mg L^{-1} and 33.9 mg L^{-1} , respectively (Li et al. 2017). Although previous findings suggest that the marine algae genus *Tetraselmis* is more tolerant to toxic chemicals than other algae (Ebenezer and Ki 2013), V2 strain seems to be the most sensitive to BPA. This study leads to suggest that our specie may be relatively more efficient in metabolites toxicity assessment due to its high sensitivity.

Toxicity of BPA on algal growth

Microalgae are considered as an effective bioindicator in ecotoxicity assessment due to their sensitivity to a wide range of micropollutants such as EDCs and heavy metals (Šepić et al. 2003; Ebenezer and Ki 2012; Liu et al. 2013b).

Fig. 4 Effect of BPA on algal growth. A Control algal culture; B algal culture supplemented with $182 \mu\text{L}$ of MSM containing 3 mM BPA before bacterial inoculation; C algal culture supplemented with $182 \mu\text{L}$ of MSM supplemented with 3 mM BPA after incubation for 15 days with strain G320

Fig. 5 Effect of BPA on algal morphology. (1): Control algal culture; (2–4): algal culture supplemented with bacterial degraded BPA; (5–16): algal culture supplemented with untreated BPA

The assessment of BPA detoxification by strain G320 was performed using the marine algal strain *Tetraselmis* sp. strain V2. The algal growth in cultures A, B and C was measured by cell density (data not shown) and biomass dry weight determination. Based on the data presented in Fig. 4, it can be concluded that the inoculation of strain G320 in MSM medium decreased the initial toxicity of BPA. In fact, a considerable decrease in algal biomass was measured in culture (B) which contains 5 mg L^{-1} of untreated BPA; however, biomass in culture (C) was better than that of the control. The obtained results suggested that strain G320 efficiently detoxified BPA and maintained algal growth.

Microscopic observations of cultures A, B and C were conducted to elucidate the possible damages caused by

BPA and its biodegradation metabolites on algal cells morphology. Morphologically normal *Tetraselmis* sp. cells observed in control culture were filled with chloroplasts, had an oval shape, green in color and motile due to its four flagella (Fig. 5. 1). It was clearly observed in Fig. 5 that BPA metabolites generated by *P. putida* degradation in culture (C) were less toxic than the parent material, thus, they did not significantly affect the algal cells morphology (Fig. 5. 3, 4). In fact, slight change in the algal cells form was noticed in this case. Cells division proceeds normally (Fig. 5. 2) but algal motility was affected and majority of cells lost flagella. In culture (B), containing untreated BPA, algal cells were strongly damaged, they have lost motility and several cells deformations were

Fig. 6 Chlorophyll a, chlorophyll b and total chlorophyll contents in cultures A, B and C

described (Fig. 5. 5–16). Cells with circular or elongated form (Fig. 5. 5, 6) were observed under BPA stress. Some cells showed separation of the cell wall from the cytoplasm (Fig. 5. 8, 9), while cytoplasm disorganization, volume enlargement, as well as inclusions within cells increased as shown in Fig. 5. (7–12) and some cells were disrupted (Fig. 5. 13). Moreover, abnormal cell division and dead cells were observed (Fig. 5. 14–16). The toxic effects of untreated BPA such as shape changes, volume enlargement, increased cytoplasmic inclusion content, division abnormalities, decreased chlorophyll content and cell growth inhibition were previously described by Li et al. (2008) and Ebenezer and Ki (2012) as effects of *Cyclotella caspia* BPA and nonylphenol (NP) exposure. In the other hand, The Chla, Chlb and total Chl contents in micro-algal biomass showed a similar trend as cell density. That is, they strongly decreased in culture B but decreased slightly in culture C (Fig. 6). These results suggest that BPA metabolites toxicity could be lower than that BPA. Chlorophylls concentration reflects the photosynthetic ability and physiological status of algal cells.

Conclusion

In this works, BPA-degrading ability of bacteria isolated from desert soils from southern Tunisia was evaluated. Several strains possessed a very high BPA biodegradation potential. Strain G320 exhibited important BPA biodegradation ability suggesting the importance of desert soil bacteria in the natural treatment of environmental pollutants. Algal toxicity test suggests that *P. putida* G320 strain can be considered as an efficient isolate for BPA detoxification

and that the studied algae *Tetrraselmis* sp. can be selected as a model marine specie for toxicity assessment.

Acknowledgements This work was supported by the Ministry of Higher Education and Scientific Research of Tunisia.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication OR.

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