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Light scattering on PHA granules protects bacterial cells against the harmful effects of UV radiation

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Abstract
Numerous prokaryotes accumulate polyhydroxyalkanoates (PHA) in the form of intracellular granules. The primary function of PHA is the storage of carbon and energy. Nevertheless, there are numerous reports that the presence of PHA granules in microbial cells enhances their stress resistance and fitness when exposed to various stress factors. In this work, we studied the protective mechanism of PHA granules against UV irradiation employing *Cupriavidus necator* as a model bacterial strain. The PHA-accumulating wild type strain showed substantially higher UV radiation resistance than the PHA non-accumulating mutant. Furthermore, the differences in UV-Vis radiation interactions with both cell types were studied using various spectroscopic approaches (turbidimetry, absorption spectroscopy, and nephelometry). Our results clearly demonstrate that intracellular PHA granules efficiently scatter UV radiation, which provides a substantial UV-protective effect for bacterial cells and, moreover, decreases the intracellular level of reactive oxygen species in UV-challenged cells. The protective properties of the PHA granules are enhanced by the fact that granules specifically bind to DNA, which in turn provides shield-like protection of DNA as the most UV-sensitive molecule. To conclude, the UV-protective action of PHA granules adds considerable value to their primary storage function, which can be beneficial in numerous environments.

Keywords Polyhydroxyalkanoates · *Cupriavidus necator* · UV radiation · Turbidity · Integrating sphere · Nephelometry

Introduction

Bacteria are fascinating organisms due to their capability to cope with widely fluctuating environmental conditions such as changes in nutrient availability, temperature, pH value, or osmolarity. In addition, also radiation, which can be defined as energy manifested in the form of electromagnetic waves, can be considered an important stress factor and occurs in numerous ecological niches. UV radiation in sunlight is among the most common stressors and has many harmful impacts on living cells such as induction of oxidative pressure or the inducing of fatal changes to the molecular structure mainly of DNA, but also of RNA, lipids, and proteins (Gabani and Singh 2013). Generally, UV radiation is considered to be one of the most detrimental abiotic factors influencing microorganisms at both the community and single-cell level, thus severely affecting the diversity and dynamics of microbial communities. Moreover, it is expected that by the end of the twenty-first century, the intensity of UV radiation at the Earth's surface will increase by approximately 5–10% in temperate latitudes and by about 20% in high latitudes (Pérez et al. 2017). It can therefore be expected that the evolutionary significance of UV radiation and ability to face this stressor may even increase.

To cope with UV radiation, bacteria—and among them especially extremophiles inhabiting harsh environments exposed to harmful solar radiation—have evolved various strategies mainly based on efficient DNA repair mechanisms and active defense against UV-induced oxidative stress. Moreover,

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many pro- and eukaryotic microorganisms also rely on production of UV-protective metabolites such as pigments, mycosporine-like amino acids, scytonemin, ectoines, bacteriruberin, sphaerophorin, pannarin, or melanin (Koller et al. 2014; Singh and Gabani 2011).

Polyhydroxyalkanoates (PHAs) are polyesters accumulated by numerous prokaryotes in the form of intracellular granules (Tan et al. 2014). The number of granules can reach up to 10–15 granules per cell and their average diameter is about 200–400 nm, though individual values particularly depend upon the specific microorganism and the physiological state of the bacterial culture (Vadlja et al. 2016). The weight content of PHA in bacterial cells can reach up to 90% of cell dry weight, though bacteria regulate their diameters to confine the volumetric content of PHA granules at a level below 40 vol% (Mravec et al. 2016). The PHA polymer itself represents the hydrophobic core of the granules which is covered by numerous specific proteins with various functions. These proteins include PHA synthase, PHA depolymerases, regulatory proteins, and various PHA granule structural proteins which create a functional interface between the hydrophobic polymer and water-containing cytoplasm. These proteins are also responsible for intracellular localization of granules within bacterial cells. To emphasize their complexity and multifunctionality as de facto organelles, these granules are also referred to as "carbonosomes" (Jendrossek 2009).

The primary function of PHAs is the storage of carbon and energy. However, recent research has shown that the biological function of PHAs is much more complex and that the capability to accumulate PHA has many biochemical and biophysical consequences, enhancing the survival and fitness of bacterial cells when exposed to numerous stress factors including but not limited to high temperature (Pham 2004; Wu et al. 2011), low temperature (Tribelli and Lopez 2011), freezing (Obruca et al. 2016a; Pavez et al. 2009), or osmotic upshock (Obruca et al. 2017).

Moreover, there are reports stating that the presence of PHA granules in microbial cells also protects bacteria against UV radiation. For instance, a protective effect of PHA granules in bacterial cells against UV irradiation was observed in *Azospirillum brasilense* when PHA-rich (about 40 wt.% of PHA in cell dry weight) and PHA-poor cells (about 5 wt.% of PHA in cell dry weight) were compared (Tal and Okon 1985). The importance of PHA for the UV-radiation survival of *A. brasilence* was confirmed in following studies by Kadouri et al., who observed that the wild type was more resistant to numerous stressors, including UV radiation, than the PHA synthase deletion mutant incapable of accumulating PHA (Kadouri et al. 2003a). It was also more resistant than the PHA depolymerase deletion mutant, which was not capable of PHA hydrolysis (Kadouri et al. 2003b). Similarly, Zhao

et al. (2007) compared the stress resistance of the wild type of *Aeromonas hydrophila* and its PHA synthase negative mutant incapable of PHA synthesis. As the major outcome, the wild type was substantially more resistant to several stress factors, including UV irradiation. Furthermore, the UV-radiation protective capacity of PHA granules was also confirmed with genetically modified *Escherichia coli* which harbored genes enabling either PHA biosynthesis or both PHA biosynthesis and hydrolysis. Both transgenic strains were more resistant to UV radiation and other stress factors than the PHA non-producing wild type (Wang et al. 2009).

Nevertheless, despite the fact that numerous studies have reported that the presence of PHA granules in microbial cells provides protection against UV radiation; to our best knowledge, there are no studies exploring the potential mechanism of the protective action. Therefore, we experimentally confirmed the UV-protecting effect of PHA granules for *Cupriavidus necator*, a soil bacterium which is considered the most important model strain for PHA metabolism. Subsequently, various spectroscopic approaches were employed to shed light on the interaction of PHA granules in bacterial cells with UV radiation and to provide an explanation for their UV-protective mechanism.

Materials and methods

Microorganisms and cultivation

The PHA-producing strain *Cupriavidus necator* H16 (CCM 3726) was obtained from the Czech Collection of Microorganisms, Brno, Czech Republic, and its PHA non-producing mutant strain *Cupriavidus necator* PHB^{-4} (DSM-541) was purchased from the Leibnitz Institute DSMZ-German Collection of Microorganism and Cell Cultures, Braunschweig, Germany.

Cultivations were performed in Erlenmeyer flasks (volume 250 mL) containing 100 mL of mineral salt (MS) medium. The composition of the MS medium was 20 g fructose, 1 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, 11.1 g $Na_2HPO_4 \cdot 12\ H_2O$, 0.2 g $MgSO_4 \cdot 7\ H_2O$, 1 mL of microelement solution, and 1 L of distilled water; the microelement solution in turn was composed of 9.7 g $FeCl_3$, 7.8 g $CaCl_2$, 0.156 g $CuSO_4 \cdot 5\ H_2O$, 0.119 g $CoCl_2$, and 0.118 g $NiCl_2$ in 1 L of 0.1 M HCl. The flasks were inoculated with 5 mL of an overnight culture of a particular strain of *C. necator* grown in Nutrient Broth medium consisting of 10 g peptone, 10 g beef extract, and 5 g NaCl in 1 L of distilled water. The cells were cultivated for 72 h. The PHA content in microbial cells was determined by gas chromatography as described previously (Obruca et al. 2014).

UV challenge of bacterial strains C. necator H16 and C. necator PHB^{-4}

The suspension of bacterial cells cultivated for 72 h as described above was first diluted 30 times. Thereafter, the suspension of bacterial cells of *Cupriavidus necator* H16 was diluted to reach the cell density of the suspension of its mutant strain *Cupriavidus necator* PHB^{-4}. The solutions thus prepared were further diluted into approx. 10^8 CFU and 3 mL of the cell suspension was placed on a sterile Petri dish to form a thin layer (approx. 1 mm). After this, cells were exposed to UV radiation emitted by an UVA lamp (400–320 nm, height 25 cm) and samples were taken at regular intervals (15, 30, 45 min). From these samples, the number of viable cells was determined as CFU by plating of appropriately diluted cell suspensions on NB agars.

UV-Vis spectroscopy and nephelometry of bacterial cells

For UV-Vis spectroscopy characterization, the same cultivation suspension as described above was used. Firstly, both the suspension of bacterial cells of *C. necator* H16 and the suspension of its mutant bacterial strain were diluted step by step five times. The dilution process was repeated until the final solutions were diluted by a factor of 100. All solutions prepared this way were analyzed by UV-Vis absorption spectrophotometry (in a U-3900H, Hitachi) both in a regular transmission measurement mode and also in a spatially integrating mode (integration sphere attachment 60mmDIA for Hitachi U-3900H spectrophotometer). Simultaneously with the preparation of samples for UV-Vis spectroscopy, the number of viable cells was determined as described above.

Similarly, diluted bacterial suspensions of defined cell concentration were also investigated by means of nephelometry. As a simple nephelometer, we used a fluorometer (AMINCO-Bowman Series 2 luminescence spectrometer, Thermo Inc.) which was employed to detect scattered light at a fixed scattering angle of 90° to the incidental beam. Furthermore, this device offers the advantageous possibility of using different wavelengths ranging from 250 nm (ozone-free xenon lamps lowest wavelength) to approximately 850 nm (highest range of PMT detectors). To suppress detection of fluorescence or phosphorescence, which is common for biological samples at different excitation wavelengths (~ 340 nm for NADH, ~ 470 nm for flavonoids, etc.) a synchronous scan method was applied, where excitation and emission monochromators were set to the zero wavelength difference during scanning. In order to obtain more accurate results and also to protect the detector, slits were set to the minimum (1 nm bandpass). To compensate for the non-flat intensity profile of the excitation source (a 150-W xenon lamp), intensity was detected relative to the diode. The scan rate was set to 5 nm·s^{-1} and spectra were collected with a 1-nm resolution in the range 250–700 nm.

Analysis of intracellular ROS by flow cytometry

Determination of the intracellular level of reactive oxygen species (ROS) in bacterial cells of *C. necator* H16 and *C. necator* PHB^{-4} before and after 15 min exposition to UV irradiation (in details described above) was performed by flow cytometry employing CM-H2CFDA (Thermo Fisher Scientific), a fluorescent stain used as general oxidative stress indicator. Suspensions of cells with cell density of approx. 10^6 cells per mL were washed twice with PBS buffer; after that, 5 μL of CM-H2CFDA dissolved in HPLC-grade DMSO (the final concentration of the stain in 1 mL of the sample was 5 μM) was added to the suspensions, and the cells were then left to incubate in the dark at laboratory temperature for 10 min. After that, the fluorescence of stained as well as non-stained cells was immediately measured at single-cell level using the green fluorescence collecting channel (535 ± 35 nm) of the used flow cytometer (Apogee A50, ApogeeFlow Systems).

Results

UV exposure of C. necator cells

In the first experiment, cells of the PHA-accumulating strain *C. necator* H16 (the PHA content in microbial cells was 74% of cell dry weight as determined by gas chromatography) and its mutant strain which is not capable of accumulating PHA due to a mutation of the PHA synthase (Raberg et al. 2014) were exposed to a UV challenge. The viability of both bacterial strains was assessed during their exposition to UV irradiation in regular intervals; the results, expressed as the percentage of viable cells, are shown in Fig. 1. Generally, the PHA-containing culture demonstrated substantially higher resistance to UV radiation during the entire period of UV exposure, thus confirming the UV-protective effect of PHA granules which has been reported also for other microbial strains (Kadouri et al. 2003a, b; Tal and Okon 1985; Wang et al. 2009; Zhao et al. 2007). The decrease in viability of reference samples of both cultures, which were exposed to the same conditions but without being UV irradiated, was negligible (< 5%). Therefore, it can be stated that accumulation of PHA granules in cytoplasm represents a potent and generally observed strategy which protects bacterial cells from the harmful effects of UV irradiation.
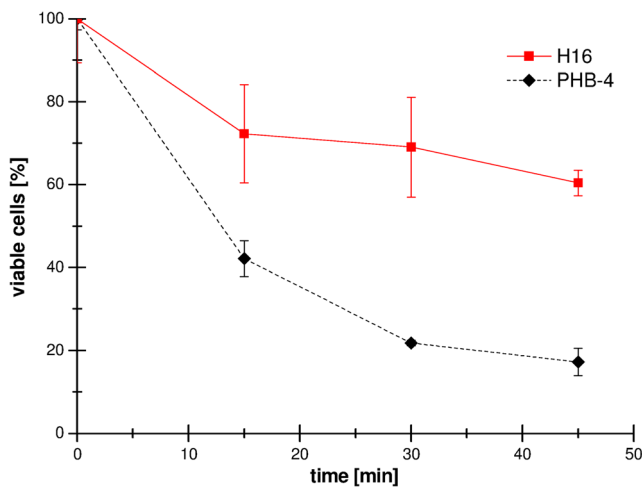
Fig. 1 Survival of both strains of *C. necator* during their exposition to UV irradiation

UV-Vis spectroscopy of bacterial cells

In order to understand how intracellular PHA granules interact with light, complex spectroscopic characterizations of the cell dispersions in the ultraviolet and the visible region were performed both for the PHA-accumulating strain (*C. necator* H16) and the non-accumulating mutant (*C. necator* PHB⁻⁴). Three different optical arrangements were used for this purpose.

Firstly, turbidity measurements were performed using a standard UV-Vis spectrometer, where the intensity of the transmitted light is measured and optical density is calculated for the particular wavelength of the incident light. As the wavelengths are altered to cover the whole UV-Vis region, spectra such as those shown in Fig. 2a are collected. It is evident that except for the shortest measured wavelengths (close to 200 nm), the PHA-accumulating strain shows a significantly higher optical density of the cell dispersion with comparable cell density compared to the non-accumulating strain. It should be emphasized that the optical density measured this way comprises two individual contributions. On the one hand, the intensity of the transmitted light is decreased via absorption of the specific wavelengths by the photoactive cell components. Absorption of the radiant energy then initiates diverse photophysical (e.g., light emission in the form of fluorescence) or photochemical processes, where the latter ones may often (mainly in the case of light in the UV spectral region) have harmful or even fatal effects on the cell fitness. The other contribution to the optical density is represented by the light scattered away from the direction of the incident beam. Unlike the light absorption, light scattering is rarely damaging. On the contrary, it can even have a protective "shielding" effect on the photo-labile cell components caused by attenuation of the local intensity of the incident light in the cell, which might reduce the level of cell damage (Paunescu
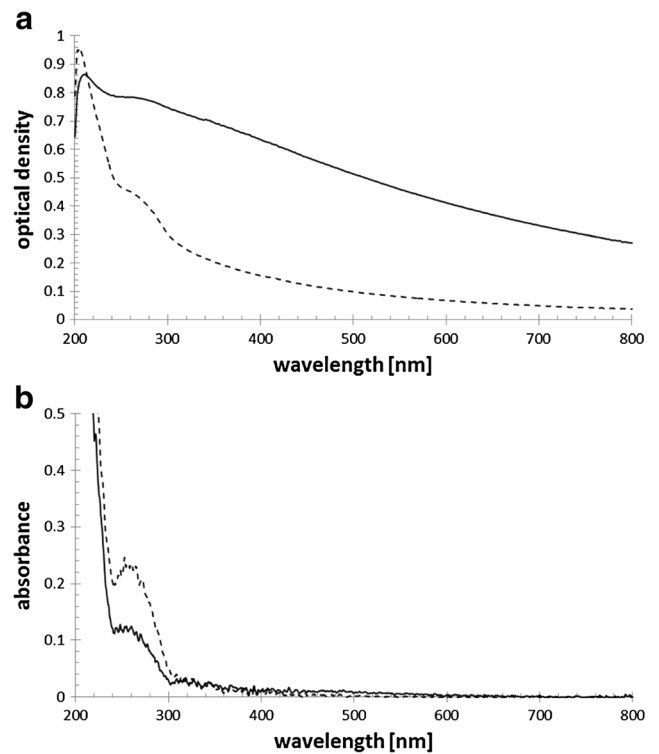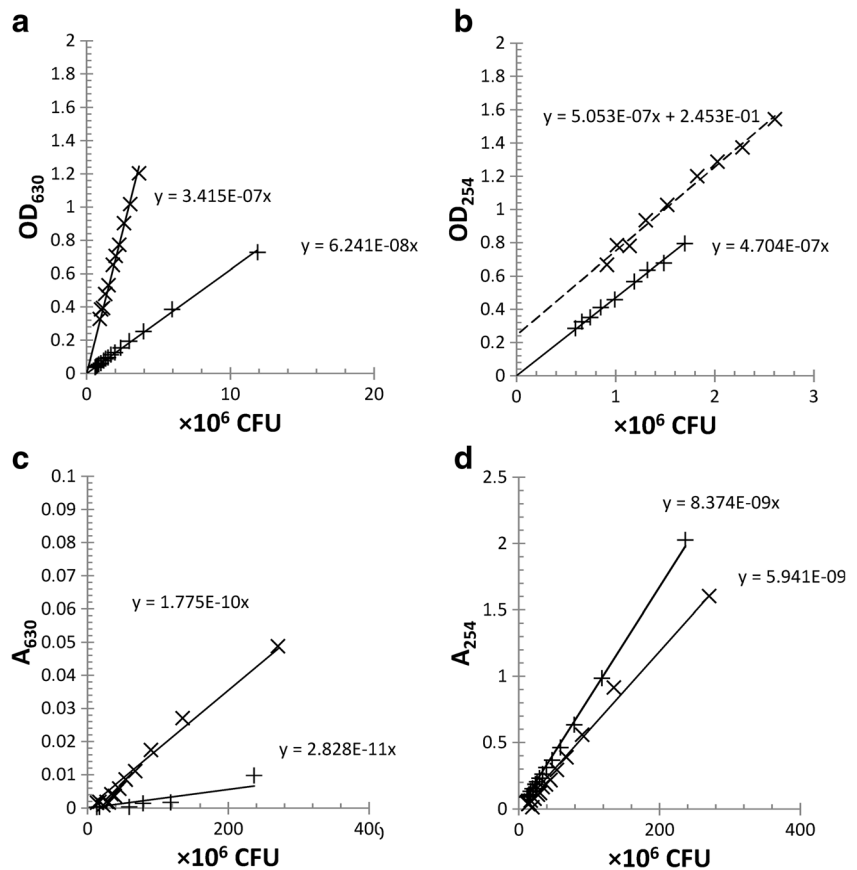

Fig. 2 Example of typical UV-Vis spectra of *C. necator* H16 (full line) and *C. necator* PHB⁻⁴ (dashed line). a Turbidity measurement of dispersions with cell density 1×10^6 CFU. b Absorbance measurement of dispersions with cell density 1×10^6 CFU

et al. 2014). Therefore, turbidity measurements such as those described above do not provide a relevant explanation of a harmful effect of light on the cells with light because they provide no direct information about the relative involvement of the light absorption and light scattering, respectively, in the interaction of the cells and their constituent with light.

With respect to this, we further measured the spectra of cell dispersions using the same UV-Vis spectrometer equipped with an integrating sphere accessory, specially designed for the absorbance measurement of turbid samples. Examples of the collected spectra are shown in Fig. 2b. In these spectra, effective suppression of the light-scattering artifacts can be clearly seen. No significant light absorption was found in the Vis region, which confirms the assumption that in this wavelength region, the optical density of the sample can be interpreted solely as a consequence of light scattering. With respect to this finding, additional interesting outcomes can further be deduced from the previously described differences in the turbidity spectra of dispersions of PHA-accumulating and non-accumulating strains with the same cell density. Generally, a significant increase in light scattering in the case of the PHA-accumulating cells of the *C. necator* H16 strain is evidently caused by light scattering on the cell ultrastructure, namely on the PHA granules present in the cell cytoplasm.

Figure 3 presents results from both types of spectroscopic assays in a more quantitative way. Fig. 3a shows very good

Fig. 3 Summarized results from UV-Vis turbidimetry and absorbance assays of cell suspensions of PHA-accumulating *C. necator* H16 strain (×) and PHA non-accumulating *C. necator* PHB^{-4} strain (+), respectively. **a**, **b** Dependency of optical density at 630 and 254 nm on cell density. **c**, **d** Dependency of absorbance at 630 and 254 nm on cell density

a

$y = 3.415E\text{-}07x$

$y = 6.241E\text{-}08x$

OD_{630}

$\times 10^6$ CFU

b

$y = 5.053E\text{-}07x + 2.453E\text{-}01$

$y = 4.704E\text{-}07x$

OD_{254}

$\times 10^6$ CFU

c

$y = 1.775E\text{-}10x$

$y = 2.828E\text{-}11x$

A_{630}

$\times 10^6$ CFU

d

$y = 8.374E\text{-}09x$

$y = 5.941E\text{-}09$

A_{254}

$\times 10^6$ CFU

linearity of the dependency of optical density at 630 nm on the cell density for both strains. Evidently, light-scattering effects are cumulative regardless of a contribution of the light scattered by PHA granules. OD_{630} appears to represent a robust parameter suitable for quantification of cell density. Nevertheless, careful calibration is needed wherever intracellular light scatters occur, as is the case when PHA granules or other cell inclusions occur. However, it is evident from Fig. 3b that in the case of *C. necator* H16 strain, contributions of light scattering and light absorption in UV wavelength region are no longer cumulative (the dashed fitting line does not cross the origin of coordinates). Nevertheless, in this region, the crucial results are shown in Fig. 3d where a relative decrease of around 30% in the single cell absorption coefficient at 254 nm can be deduced. Finally, Fig. 3c is shown in order to illustrate the negligible residual apparent absorbance of the cell suspensions in the VIS region. This apparent absorbance represents an experimental artifact coming from the scattered light which does not reach the aperture of the integrating sphere.

In order to provide an experimental verification of the assumption that the difference between the optical density and absorbance of the cell suspensions is correctly assigned to the intensity of scattered light, we performed also a basic nephelometry assay of the same samples. Measurement was done with the synchronous scan method as was described above. We focused on monitoring the changes in scattered light intensity between the PHA-accumulating and non-accumulating strain suspension of the same cell density, where a high sensitivity of the fluorimeter photomultiplier and its strong light source provide an undisputed instrumental advantage. Figure 4 shows the ratio of the intensities of scattered light normalized per unit CFU for *C. necator* H16 and *C. necator* PHB^{-4}, respectively, vs. the wavelength. We use this means of data demonstration in order to suppress experimental artifacts coming from uneven light intensity emitted from the xenon lamp of the fluorimeter at different wavelengths (we assume that the results for both strains will be affected equally).

From the spectrum, it is evident that the nephelometry experiments confirmed the higher intensity of scattered light for the PHA-accumulating strain in the whole tested optical region. Furthermore, the relative efficiency of light scattering by the PHA producer as compared to the mutant strain increases significantly in the UV-region, where the light absorbance measurement revealed the most profound differences in intensity in the light absorption of the two strains. This finding was reproducible as far as similar results were found regardless of the particular suspension cell density. Therefore, it can be summarized that nephelometry confirmed the conclusions of the previous two spectroscopic assays.

Fig. 4 Ratio of the intensities of single-cell scattered light for *C. necator* H16 strain and PHA non-accumulating *C. necator* PHB^{-4} strain at 90° as determined by nephelometry
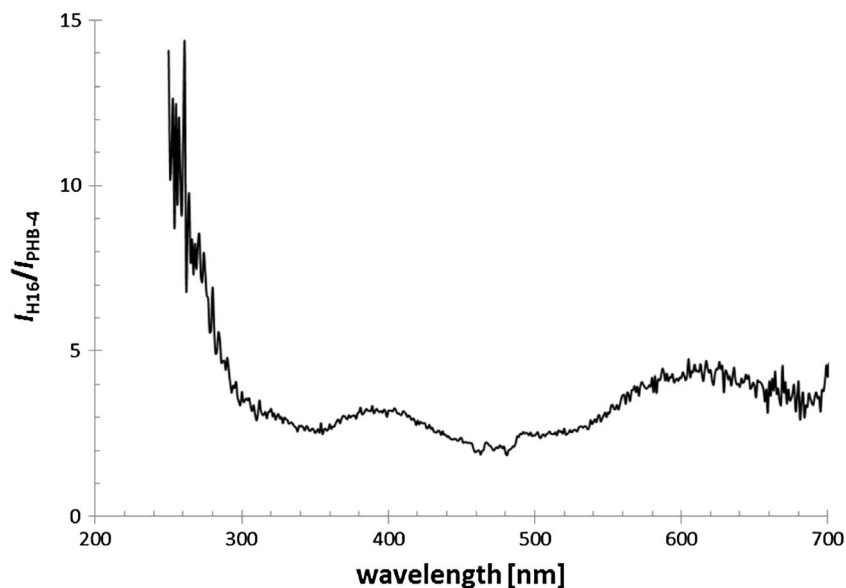

ROS analysis by flow cytometry

To investigate whether scattering of UV irradiation on PHA granules influences the intracellular level of ROS in UV-challenged cells, the amount of ROS before and after UV exposition was analyzed by flow cytometry employing fluorescent stress indicator CM-H2CFDA. Green fluorescence of this stain is activated by its reaction with ROS (Dong et al. 2015). Hence, at single-cell level, we analyzed intensity of green fluorescence of both bacterial cultures prior and after their exposition to UV irradiation in stained as well as non-stained bacterial cells; the results are demonstrated in Fig. 5. The mean value of intensity of green fluorescence of PHA granules containing bacterial was only slightly (approx. 10%) increased after exposition of the cells to UV irradiation. On the contrary, when the culture of cells not containing PHA granules was exposed to UV irradiation, green autofluorescence of

non-stained cells increased about 1.8-fold and, moreover, green fluorescence of CM-H2CFDA stained cells raised 3.6-fold. This clearly indicates that UV irradiation induces formation of substantially higher amount of ROS in PHA negative cells than in PHA-rich cells.

Discussion

Solar UV radiation on Earth can be considered an important stress factor which influences numerous living systems. Therefore, the influence of UV radiation on whole ecosystems has been studied for various aquatic environments (Häder et al. 2007), or high-altitude regions (Farías et al. 2009; Pérez et al. 2017). Moreover, UV-radiation resistance is also an important topic for astrobiology (Khodadad et al. 2017). The harmful effect of UV radiation is complex and it includes

Fig. 5 Mean intensity of green fluorescence of stained and non-stained cells of *C. necator* H16 and *C. necator* PHB^{-4} prior and after UV exposition as determined by flow cytometry
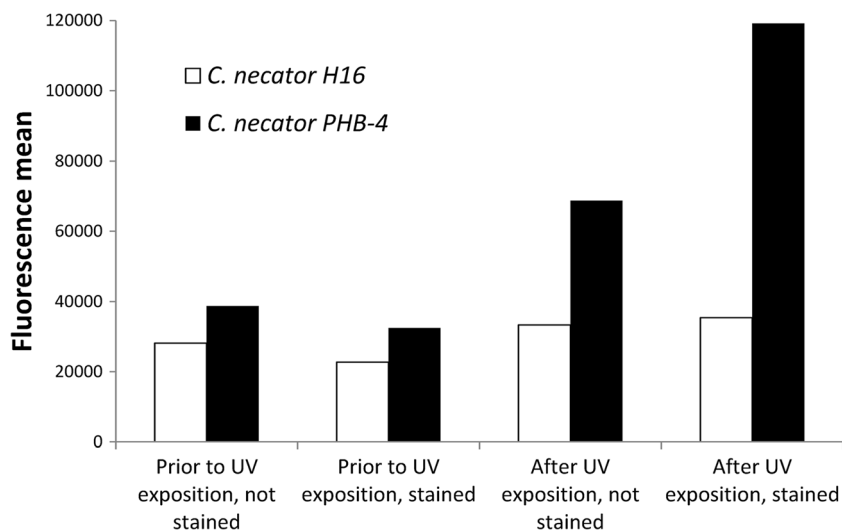
numerous cell-damaging mechanisms. First and foremost, UV radiation is known for its mutagenic potential because DNA directly absorbs UVB radiation and this radiation induces numerous grave changes in DNA structure, among which the formation of dimeric pyrimidines, photo-adducts, and DNA–protein cross-links are considered the most important (Ravanat et al. 2001). Furthermore, despite the fact that DNA does not absorb UVA radiation, UVA can be absorbed by endogenous photosensitizers which may damage DNA throughout subsequent reactions (Ravanat et al. 2001). Apart from DNA, also other photosensitive biomolecules such as RNA, proteins, or lipids can be damaged by direct or indirect absorption of UV radiation, though changes in these molecules' structure do not have such fatal consequences as is the case for DNA due to their quick turnover. In addition, UV radiation induces the formation of reactive oxygen species (ROS) which may further damage crucial cell components such as DNA, RNA, proteins, and lipids (Kim et al. 2015).

There are numerous reports that the capability to accumulate PHA enhances the stress resistance of bacteria against various stressors (Ayub et al. 2009; Kadouri et al. 2003a, b; Obruca et al. 2016a, b, 2017; Tal and Okon 1985; Wang et al. 2009; Zhao et al. 2007). This can be considered an important "added value" to their primary biological function—storage of carbon, energy, and also reduction power. Generally, it has been observed that the presence of PHA granules influences the overall biophysical properties of bacterial cells, which further increases stress survival when they are exposed to various stress factors. For instance, the presence of PHA granules enhanced the rate of water transport out of the cells during freezing, which subsequently protected bacterial cells from formation of intracellular ice; this substantially contributes to PHA's cryo-protective effect (Obruca et al. 2016a). Moreover, PHA polymers in native intracellular granules represent a unique amorphous form of matter which resembles "supercooled" liquid in its properties (Bonthrone et al. 1992). The liquid-like properties of PHA granules seem to play a crucial role in the protective mechanism of PHA against osmotic upshock, since the presence of PHA granules turned out to reduce the level of plasmolysis in challenged cells and, moreover, according to the results of transmission electron microscopy analysis, PHA granules were even capable of stabilizing membranes of bacterial cells by closing the holes in the cytoplasmic membrane (Obruca et al. 2017). Therefore, even the simple presence of PHA granules in cytoplasm can be beneficial for bacterial cells when exposed to stress conditions.

Moreover, the enhancement of the UV resistance of PHA-accumulating bacteria which was reported in this work (Fig. 1), as well as by other authors (Kadouri et al. 2003a; Tal and Okon 1985; Wang et al. 2009; Zhao et al. 2007), is most likely primarily based on the biophysical consequence of the presence of PHA granules in cells. According to our results, PHA granules do not considerably absorb UV radiation but they are

capable of efficient scattering of UV radiation as was indicated in the present study by the comparison of turbidity (Fig. 2a) and absorbance measurement (Fig. 2b) of the cells of PHA-accumulating C. necator and its PHA negative mutant. Furthermore, the fact that PHA granules efficiently scatter UV radiation was also confirmed by nephelometry measurement (Fig. 4). Because no considerable changes in cell dimensions were found for both strains in our previous work (Mravec et al. 2016), the significant increase in the light scattering of the C. necator H16 strain can be ascribed to the fraction of light scattered on the cell ultrastructure, namely on the PHA granules in the cell cytoplasm. This finding is in fact not surprising; a similar observation of an increase of the single cell light turbidity as a result of light scattering on inclusion bodies has previously been reported, e.g., for E. coli W3110 (Hwang and Feldberg 1990). Nevertheless, to the best of our knowledge, our results represent the first convincing experimental confirmation that intracellular PHA granules serve as effective in situ light-scatterers. Furthermore, according to the results presented in Fig. 2b, it is evident that UV radiation is absorbed by the bacterial cells quite effectively. The absorption band centered around 254 nm can be assigned to nucleic acid, especially to DNA. Nevertheless, from the comparison of the absorption spectra of cell suspensions with the same cell density, it can be seen that UV-radiation absorption in this wavelength region is considerably suppressed in the case of the PHA-accumulating strain. Bearing in mind that there is no significant difference in the cellular content of DNA for the two strains, this result supports the assumption of "shielding" effects of PHA granules resulting from their great light-scattering ability. Moreover, it can be stated that, apart from protecting DNA as the most sensitive molecule, scattering of UV irradiation on PHA granules also reduces level of intracellular ROS (see Fig. 5) generated by UV radiation. This new finding very likely substantially contributes to complex UV-protective function of PHA granules.

It should be pointed out that in natural producers, PHA granules are not randomly distributed in bacterial cells, but they are specifically attached to DNA. In C. necator, the attachment is performed via the protein PhaM which simultaneously binds to DNA and the PHA associated-protein PhaP5 (Wahl et al. 2012). Similarly, in Pseudomonas putida, the binding of PHA granules to DNA is enabled by the protein PhaF. This protein serves as a transcriptional regulator of PHA metabolism but it is also responsible for proper segregation of granules during cell division and ensures, under balanced conditions, equal distribution of granules between daughter cells. PhaF directs the PHA granules to the center of the cells, forming a characteristic needle array in the close vicinity of DNA (Galan et al. 2011). This might substantially contribute to a UV-protective effect since PHA granules represent a "shield" attached to the nucleoid which scatters UVB radiation away from the most sensitive molecule–DNA. Here, it

has to be emphasized that very recent findings by Karmann et al. (2017) show that, under carbon-limited conditions, the distribution of granules to daughter cells in statu nascendi occurs in an asymmetric way; the culture segregates into a PHA-rich and a PHA-poor subculture, thus displaying a "bistable behavior." Future investigations might provide insights if the PHA-rich subculture is definitely better protected when challenged by UV irradiation.

PHA metabolism reveals a cyclic nature, the so called PHA cycle, since in microbial cells the polymer is simultaneously synthesized and degraded (Kadouri et al. 2005). According to the results of Kadouri et al. (2003b), also the capability of intracellular PHA degradation is an important factor enhancing the UV-protective effect of PHA, since a PHA depolymerase deletion mutant strain of *Azospirillum brasilense* incapable of PHA degradation was shown to be more sensitive to UV irradiation than the wild type strain. The explanation can be that, due to the cyclic nature of PHA metabolism and activity of PHA depolymerase, a substantial amount of PHA monomers is present in bacterial cells. For instance, the intracellular concentration of 3-hydroxybutyrate (3HB) in the wild type strain of *C. necator* is 16.5-fold higher than in its PHA non-accumulating mutant. This is important since 3HB constitutes a potent chemical chaperone capable of preventing a model enzyme, lipase, against denaturation caused by high temperature or oxidative damage (Obruca et al. 2016b). Therefore, it is likely that the complete PHA cycle might in this way also prevent bacterial cells against oxidative pressure generated by UV radiation. Moreover, Ayub et al. (2009) suggested that PHA metabolism is essential for the maintenance of the redox state in *Pseudomonas* sp. 14-3 during oxidative pressure induced by exposure of bacterial cells to low temperatures.

In summary, the presence of PHA granules in bacterial cells has numerous biophysical and metabolic consequences, which alter the stress survival capacity of bacterial cells during their exposition to various stress factors. Their UV-protective action might be explained by their efficient UV-radiation scattering properties with high scattering efficiency in the wavelengths close to the DNA absorption maxima. Furthermore, presence of PHA granules in bacterial cells also protects them from ROS generated by UV irradiation since scattering of UV radiation on granules decreases levels of generated ROS and, moreover, PHA metabolism also provides efficient protection against oxidative stress induced by UV irradiation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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