Pigments from UV-resistant Antarctic bacteria as photosensitizers in Dye Sensitized Solar Cells

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Here we report the use of pigments produced by UV-resistant Antarctic bacteria as photosensitizers in Dye Sensitized Solar Cells (DSSCs). Pigments were obtained from red and yellow colored psychrotolerant bacteria isolated from soils of King George Island, Antarctica. Based on metabolic characteristics and 16S DNA sequence, pigmented bacteria were identified as Hymenobacter sp. (red) and Chryseobacterium sp. (yellow). Pigments produced by these microorganisms were extracted and classified as carotenoids based on their spectroscopic and structural characteristics, determined by UV–Vis spectrophotometry and infrared spectroscopy (FTIR), respectively.

With the purpose of develop green solar cells based on bacterial pigments, the photostability and capacity of these molecules as light harvesters in DSSCs were determined. Absorbance decay assays determined that bacterial carotenoids present high photostability. In addition, solar cells based on these photosensitizers exhibit an open circuit voltage ($V_{OC}$) of 435.0 [mV] and a short circuit current density ($I_{SC}$) of 0.2 [mA·cm$^{-2}$] for the red pigment, and a $V_{OC}$ of 548.8 [mV] and an $I_{SC}$ of 0.13 [mA·cm$^{-2}$] for the yellow pigment.

This work constitutes the first approximation of the use of pigments produced by non-photosynthetic bacteria as photosensitizers in DSSCs. Determined photochemical characteristics of bacterial pigments, summed to their easy obtention and low costs, validates its application as photosensitizers in next-generation biological solar cells.

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1. Introduction

During the last decades, research and development of photovoltaic technology has strongly increased in response to high world energy demand [1]. Solar energy is the most abundant renewable resource on our planet, however, its application as an alternative to the use of fossil fuels has a major drawback: the economic and environmental costs of their production [2]. As a way to solve this inconvenient, during the last decades researchers have worked on developing photovoltaic devices based on new materials or novel ways to make them interact. In this context, in 1991 Michael Graetzel developed a low cost and high efficiency solar cell known as Dye Sensitized Solar Cell (DSSC). This model is based on light-harvesting molecules (sensitizers) attached to a film of TiO$_2$ nanoparticles in presence of a redox electrolyte [3,4].

To date, there have been several types of photosensitizers for this kind of solar cell, such as organic dyes [5], ruthenium [6] and platinum complexes [7]. However, some of these materials display high toxicity and are very scarce in nature, revealing a hard, expensive and non-ecological fabrication process. In this context, natural dyes have been proposed as sensitizers because of their low obtention costs, easy manipulation and eco-friendly characteristics [8–10]. Different biological dyes such as anthocyanins [11–13], flavonoids [14] and carotenoids [15,16] have been used as photosensitizers in DSSCs. Also, pigments present in light-harvesting photosynthetic complexes from plants and bacteria have been used as sensitizers [17,18]. In the case of carotenoids, their biosynthesis has been reported in plants and some environmental microorganisms, but to date, only carotenoids extracted from plants have been used as sensitizers on DSSCs [19,20]. In this context, non-photosynthetic pigmented microorganisms constitute an efficient and still non-explored source of biological dyes for DSSCs.

Microorganisms inhabiting Antarctica have developed different defense mechanisms to survive the constant exposure to UV radiation. UV-mediated cellular stress produces an increase in reactive oxygen species (ROS), which damage DNA, lipids and proteins [21–23]. In general, microorganisms display mechanisms to counteract UV-damage such as improved DNA repair systems and antioxidant response, among others [24–27]. The synthesis of carotenoid pigments represents another antioxidant defense against oxidative stress that has been described in Antarctic bacteria [28–30].

Carotenoids are one of the most structurally diverse natural pigments described. Their structure is based on hydrocarbon chains with conjugated double bonds and a range of light absorption between 400 and 500 nm [31,32]. To date, carotenoid pigments used as sensitizers...
in DSSCs have been chemically synthesized or extracted from fruits and flowers [15,16], and the use of pigments extracted from bacterial cells has not been reported.

The use of microorganisms to produce pigments or other natural products is an efficient process as consequence of the high yields of biomass that can be obtained and the simplicity to extract the natural dye. In this context, microbial biosynthesis processes constitute a cost effective and environmentally friendly alternative to produce biomolecules of technological interest [33–35].

In the present work, the use of bacterial pigments extracts as photo sensitizers in DSSCs is reported for the first time.

2. Materials and Methods

2.1. Isolation, Identification and Characterization of Antarctic Pigmented Bacteria

Soil samples were collected from King George Island (South Shetland Islands) during the 50th Antarctic Scientific Expedition (ECA 50) organized by the Chilean Antarctic Institute (INACH). For isolation of environmental bacteria, 100 mg of soil were suspended in 1 mL of sterile distilled water and vigorously stirred by vortexing. Samples were incubated at 28 °C for 1 h. After that, aliquots were seeded on R2A agar plates and incubated at the same temperature for two days. Environmental pigmented isolates were selected by morphology and coloration, and pure cultures were obtained by the streak plate method as reported before [36,37].

Selected isolates were identified by 16S rRNA sequencing. The genetic material for each bacterial isolate was extracted using DNeasy® Blood & Tissue Kit (Qiagen) according to manufacturer’s instructions. PCR amplification of the 16S rRNA gene was performed using the universal forward primer PA (5′-AGAGTTTGATCCTGGCTCAG-3′) and the universal reverse primer PH (5′-AAGGAGGTGATCCCGGCA-3′). The reaction mix consisted of 2 μL of extracted DNA as template, 1.25 μL of each primer, 12.5 μL of 2× GoTaq® Green Master Mix (Promega) and 8 μL of molecular grade water. The amplification steps include initial denaturation at 95 °C for 5 min, 42 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min 30 s, and a final extension at 72 °C for 10 min. Resulting amplicons were visualized by gel electrophoresis in agarose gels (1.5%) and sequenced (Macrogen Corporation, South Korea). Bacterial isolates were classified at the gender level by comparing obtained sequences (1398 bp for the yellow isolate and 1437 bp for the red isolate) with those present in the NCBI database using the BLAST algorithm.

Bacterial growth curves were determined by measuring the optical density at 600 nm (OD600) of bacterial cultures (100 mL R2A medium) grown at 28 °C with constant shaking.

To characterize the UV resistance of pigmented bacteria, bacterial isolates were grown in R2A medium at 28 °C with constant shaking until OD600 ~0.3 was reached. Then, cultures were diluted (serial dilutions of 1:10, 1:100, 1:1000, 1:10000 and 1:100000 were prepared) and 5 μL of each dilution were plated on R2A agar (in triplicates). Each plate was exposed to UV-C irradiation with a 15 W germicidal lamp (Model G15T8, 16 in. long, Sankyo Denki Co.) for 0, 5, 10 and 15 min and then incubated at 28 °C for 72 h. The same protocol was used for E. coli (BW25113) as control.

2.2. Extraction and Purification of Bacterial Pigments

Cultures of pigmented bacteria were grown in R2A medium at 28 °C for two days until saturation (OD600 ~0.8). Then, cells were centrifuged at 7,690 × g for 10 min, supernatants were discarded and collected cells were lyophilized (freeze-dried) for 24 h. Lyophilized cells were mixed with methanol for 10 min at room temperature and the methanol extract (colored supernatant) was separated from cells by centrifugation at 26,670 × g for 15 min at 4 °C (this also allows precipitating other biomolecules). This extraction process was repeated several times until the cells were completely bleached. All these steps were carried out under low light conditions to avoid pigment degradation.

2.3. Optical and Structural Characterization of Pigments

Pigment extracts were characterized by UV–Visible spectrophotometry using a Synergy™ H1 Microplate Reader (BioTek Instrument Inc.). Absorbance spectrum between 300 and 700 nm (2 nm resolution) was measured. To determine the photostability of bacterial pigments, samples were normalized to 1.0 arbitrary unit of absorbance by dilution according to their maximum absorbance peak (450 and 478 nm for yellow and red pigment, respectively). Then, pigments were exposed to light (~70 mW·cm−2) and the decay of maximum absorbance peak of each sample was measured every 10 min during 1 h. To avoid solvent evaporation, the temperature of the experiment was controlled (16 °C). The experiment was performed in triplicate and a dark condition was used as control.

For the structural characterization, Fourier Transform Infrared Spectroscopy (FTIR) was performed. Samples were dried using a vacuum centrifugal concentrator (IR Concentrator Micro-Cerven NB-503CIR, N-Biotek Inc.) for 24 h and the powder was mixed with KBr to form a thin pellet. FT-IR spectra were performed in a Nicolet™ IS™FT-IR Spectrometer (Thermo Scientific Inc.) using a Smart iT™ Attenuated Total Reflectance (ATR) accessory provided with a single bounce Ge crystal. The scan frequency was 3700 to 700 cm−1.

2.4. Fabrication and Characterization of Dye Sensitized Solar Cells

DSSCs were produced following the protocol previously described by our group [38], with some modifications. To fabricate the electrodes, 50 × 50 × 2 mm size fluorine doped tin oxide coated glass (FTO glass) TEC15, with a surface resistivity of 13 [Ω/sq] and 85% transmittance was used. Conductive glasses were cleaned by successive sonication in absolute ethanol and deionized water for approximately 10 min to remove organic contaminants. The anode was prepared using a suspension of titanium (IV) oxide nanoparticles (TiO2 nanopowder from Sigma-Aldrich, ~21 nm particle size and anatase crystal structure) that was deposited on the glass through spin-coating at 2000 rpm for 10 s. The electrodes (TiO2 films) underwent a sintering process at 450 °C for 30 min.

Sensitization of TiO2 film was performed by direct adsorption. The electrode was treated twice with the pigment solution (in a concentration of 10 mg/mL) and incubated in darkness. The active area of the cells was 15 cm2. Moreover, the cathode or counter electrode was prepared from a solution of H2PtCl6·6H2O in isopropanol. 10 μL of the solution were dispensed on a FTO coated glass by spin-coating and heated 20 min at 400 °C.

Then, the photoanode and the counter electrode were assembled leaving a 127 μm space between them. Before sealing the cell, 100 μL of electrolyte was added. The electrolyte solution used was iodide/triiodide (1−/I−) prepared from KI (0.5 M), I2 (0.05 M) in ethylene glycol. Characterization of solar cells was performed in triplicate for each bacterial pigment using a solar simulator (SunLite™ Model 11002. Abet Technologies, Inc.) and an interface potentiostat-galvanostat (In- terface 1000. Gamry Instruments) to register the current-voltage curves and time-dependent photoresponse. Measurements were performed under constant conditions of temperature and irradiance at a one sun intensity as the light source (~100 mW·cm−2 and AM1.5).

3. Results and Discussion

3.1. Isolation and Characterization of Antarctic Pigmented Bacteria

Two pigmented bacteria were isolated from soil samples obtained from the King George Island in Antarctica. Isolates were obtained by
incubating in R2A media at 28 °C, a condition that favor the growth of bacteria present in soils [39]. From all isolates obtained, two pigmented bacteria were selected based on their color and growth characteristics (colonies were obtained after 48 h incubation at 28 °C). Yellow and red-pigmented bacteria were selected with the aim to evaluate two different pigments as photosensitizers in solar cells.

Red and yellow colored environmental isolates were designated as A9A5(R) and A9A5(A), respectively. Both Antarctic strains correspond to rod shape Gram-negative bacteria. Growths of both isolates were evaluated at 8, 18, 28 and 37 °C. No growth was observed at 8 and 37 °C. Despite isolates were able to grow at 18 °C, their optimal growth temperature was 28 °C (not shown). Based on this result, both isolates correspond to psychrotolerant bacteria. According to growth curves, A9A5(A) reaches the exponential phase of growth approximately 12 h before A9A5(R). However, both environmental isolates reach stationary phase after 48 h growth in R2A medium (Fig. 1).

The identity of both isolates was determined by a 16S rRNA gene sequence analysis. The A9A5(R) isolate shared 85.85% similarity with Hymenobacter actinosclerus; which is a red pigmented, rod shaped and Gram-negative bacterium that was reported as a new species by Collins et al. [40]. The genus Hymenobacter was first described by Hirsch et al. (1998) to accommodate a group of red/pink-pigmented rods from Antarctic soils and sandstone [41]. Based on this, our bacteria was classified as Hymenobacter sp. A9A5(R).

The 16S rRNA gene analysis of A9A5(A) revealed that this isolate shared 95.90% sequence similarity to Chryseobacterium chaponense; a yellow pigmented, rod shaped Gram negative bacterium which was proposed as a new specie by Kämpfer et al. (2011) [42]. This bacterium was first described by Hirsch et al. (1994) to accommodate various species previously classified as Deinococcus. Chryseobacterium genus was created by Vandamme et al. (1994) to accommodate various species previously classified in the genus Flavobacterium [43]. Chryseobacterium strains produce translucent, shiny and with entire edges colonies. Chryseobacterium can also produce a bright yellow, non-diffusible and non-fluorescent flexirubin-type pigment [44]. All these phenotypes were also observed in our pigmented isolate. Based on this our bacteria was classified as Chryseobacterium sp. A9A5(A).

The tolerance to UV radiation of pigmented isolates was studied by exposing them to UV-C radiation for different times (Fig. 2). As expected for pigmented bacteria, Chryseobacterium sp. A9A5(A) and Hymenobacter sp. A9A5(R) were capable to grow after UV exposure (Fig. 2). Chryseobacterium sp. A9A5(A) grows gently after 5 min UV-exposure (Fig. 2, inset) but no growth was observed at longer times. Hymenobacter sp. A9A5(R) was able to grow even after 15 min UV-C exposure. As expected, the UV-sensitive bacterium E. coli was unable to grow after 5 min UV-exposure.

Obtained results indicate that both Antarctic isolates are resistant to UV, being Hymenobacter sp. A9A5(R) highly resistant to this stress. These results are in agreement with characteristics reported for both isolated species. H. actinosclerus has been obtained from irradiated pork and reported as radiation resistant [40]. This genus was found as dominant, in conjunction with Deinococcus, in samples obtained from solar panels [45]. It has also been proposed that these microorganisms tolerate excessive UV irradiation on the surface of the snow due to the protection provided by their own pigments [46]. In this context, members of this genus are known to synthesize red or pink carotenoids [47].

Some Antarctic environments are exposed to UV radiation for prolonged time periods, particularly near the polar circle where 24 h daylight exposures have been registered [48]. This generates a selection pressure that forces bacteria to develop protection mechanisms. Accordingly, a higher percentage of yellow, orange and red-pigmented bacteria have been determined on environments exposed to constant UV radiation [49]. Bacterial pigmentation is one mechanism that cells have developed to counteract the damage produced by UV-radiation and oxidative damage [30]. The antioxidant characteristic of bacterial pigments is related with the capacity of these biomolecules to interact with reactive oxygen species (ROS) inside cells, constituting a very effective mechanism to reduce oxidative damage [30].

3.2. UV–Vis Spectrophotometry and Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Bacterial Pigments

UV–Visible absorption spectra were determined to characterize the pigments extracts obtained from Antarctic bacterial isolates. Extracted pigments showed the characteristic absorption bands of carotenoids, between 400 and 550 nm (Fig. 3) [30,51].

Therefore, the measured spectra correspond to the absorption of chromophore groups present in the chemical structures of carotenones. β-carotene has a maximum absorption at 456 nm [52], similar to zeaxanthin [53]. According to this description, this last carotenoid matches with the extracted yellow pigment. In the case of the red pigment, absorption spectrum displays a slight shift to longer wavelengths when compared to β-carotene. Observed absorption maximum coincides with the characteristic absorption for lycopene [54] and canthaxanthin [55].

The chemical composition of bacterial pigment extracts was evaluated by FTIR (Fig. 4). FTIR spectra of both pigments show absorption peaks at 2850 and 2950 cm⁻¹. These bands are characteristic stretching

**Fig. 1.** Characterization of Antarctic isolates. a) Agar plate with pigmented A9A5(R) and A9A5(A) isolates and E. coli as non pigmented bacteria, b) growth curve for A9A5(R) and A9A5(A) strains, and c) 16S identification indicating most similar species.
vibrations of C—H bond, belonging to groups CH₂ and CH₃ (Fig. 4, signal B and C). Bending and scissoring of CH₂ and CH₃ groups between 1500 and 1350 cm⁻¹ (signal F) are also observed. In the fingerprint zone, typical bands between 1200 and 1300 cm⁻¹ corresponding to C—C bonds (signal G) were determined. Absorption at 1100–1150 cm⁻¹ corresponding to C—O stretching and bending vibrations was also observed (signal H).

The main difference between red and yellow pigment spectra corresponds to a characteristic absorption band for the vibration of the OH group, located at an approximate frequency of 3300 cm⁻¹ in the case of yellow pigment (signal A). This result suggests that the yellow pigment may correspond to lutein and/or zeaxanthin [56,57]. In the case of the red pigment, a strong signal near 1750 cm⁻¹ (signal D) with features of a carbonyl group (C==O) was also observed. The presence of this group could be consequence of the oxidation of lycopene, zeaxanthin and/or lutein. Oxidation occurs by incorporation of oxygen in any of the C=C double bounds (signal E) or by oxidation of the OH group in the chemical structure of carotenoid [58]. These oxidations result in the formation of carotenoids such as astaxanthin and canthaxanthin, all of them are red/orange keto-carotenoids that have been reported in microalgae, microorganisms and marine animals [59]. Canthaxanthin presents two carbonyl groups, a diketone [58]. Based on this, the red pigments most probably correspond to canthaxanthin.

In order to evaluate the purity of bacterial pigments, the extracts were runned on a chromatographic silica column. 9 and 4 fractions were obtained from red and yellow pigment extracts, respectively. We measured the absorbance spectrum of every fraction, and similar absorbance spectra were determined for each fraction obtained. All yellow and red pigments fractions display typical absorbance spectra of carotenoids (not shown). The main difference observed between fractions was the intensity of the signal, most probably reflecting differences in concentration.

Therefore, UV spectra and FTIR results suggests that the pigments from Antarctic bacteria belong to the family of carotenoids, specifically xanthophylls. The yellow pigment most probably corresponds to lutein, zeaxanthin or a mixture of both, and the red pigment mainly constituted by canthaxanthin (Fig. 5).
3.3. Photostability of Bacterial Pigments

Photostability is one of the most significant characteristics defining the potential application of photosensitizers in solar cells. In order to evaluate photostability of bacterial pigments, photodegradation was determined by measuring the absorbance decay at different times light exposure (Fig. 6).

The yellow pigment suffers a low and constant degradation in time, with the highest decay after 50 and 60 min (Fig. 6a). Moreover, the red pigment was much more photostable, keeping its maximum absorbance peak constant during all times evaluated (Fig. 6b). It has been reported that resistance to light degradation is due to the presence of structures with conjugated double bonds in carotenoid pigments, such as carbonyl and keto groups found in ionone rings [60]. Although it has been reported that natural carotenoids exhibit instability when exposed to light [31], photostable β-carotene-humic acid complexes have been chemically synthesized [61]. In contrast, natural pigments from Antarctic bacteria reported in our study proved to be quite stable to light under the evaluated conditions.

The presence of carotenoid pigments in some microorganisms is responsible for their survival in extreme environments [29,62]. In this context, obtained photostability results are in agreement with UV-resistance levels determined in Fig. 2, suggesting that high UV-resistance of Hymenobacter sp. A9A5(R) is consequence, at least on part, of the production of the red pigment canthaxanthin.


Based on the properties of bacterial carotenoid pigments, we evaluated their use in sensitized solar cells. A schematic representation of the solar cell used to evaluate the bacterial pigment extracts is shown in Fig. 7.

When pigments absorb light, they inject electrons from their excited levels to the conduction band of the TiO₂ nanoparticles film. The

![Chemical structures of carotenoids belonging to the xanthophyll family.](image_url)

Fig. 5. Chemical structures of carotenoids belonging to the xanthophyll family.

![Photostability of bacterial pigments. Pho](image_url)

Fig. 6. Photostability of bacterial pigments. Photodegradation of red and yellow pigments extracted from UV-resistant Antarctic bacteria was determined at their respective maximum absorbance peaks (450 and 478 nm for yellow and red pigment, respectively).
recirculation of the redox electrolyte in its oxidized-reduced state allows recharging the electrons lost by the oxidized dye while serving as a pathway for electron transfer between the two electrodes [3,4]. Thus, when light shines on the solar cell, the device directly converts sunlight into electricity and the current and voltage data can be recorded in an external circuit.

As shown in Fig. 8, the photovoltaic device constructed with bacterial pigments present the expected behavior for solar cells, evidenced by the characteristic shape on current-voltage curves and time-dependent photoresponse [Fig. 8, inset] [63]. Under darkness, no photovoltage response can be observed at the studied time interval, due to the negligible concentration of charge carriers in the valence band (holes) and to the presence of a Schottky barrier, indicative of the diode character of the sensitized TiO$_2$/electrolyte interface. Under illumination, the dynamic response of this interface in intermittent status indicates that processes such as photogeneration, diffusion and recombination are occurring [64,65]. Photovoltaic parameters derived from current-voltage curves are summarized in Table 1.

The solar cell sensitized with the yellow pigment presents an open circuit voltage ($V_{oc}$) of 548.79 [mV], a short circuit current density ($I_{sc}$) of 0.13 [mA·cm$^{-2}$] and an efficiency of 0.0323%. On the other hand, the solar cell with the red pigment showed a $V_{oc}$ of 435.02 [mV], a $I_{sc}$ of 0.2 [mA·cm$^{-2}$] and an efficiency of 0.0332%.

Sensitized solar cells with carotenoid pigments extracted from gardenia fruits and Asian flowers have reported efficiencies between 0.16 and 0.56% [15,16]. One disadvantage of using a natural dye as sensitizer is the aggregation of the biomolecule in the TiO$_2$ film. This phenomenon blocks the flow of electrons between the electrodes reducing the
photovoltaic parameters [66]. DSSCs constructed with bacterial pigments extracts display this behavior, a result that could explain the photovoltaic parameters obtained.

In terms of current and efficiency, parameters obtained sensitizing with the red pigment are similar than those obtained using the yellow pigment (Table 1). It has been reported that variations in current and voltage can be attributed to structural and optical differences that can facilitate the interaction with TiO₂ and/or improve the energy absorption [67–69]. In fact, among natural dyes, carotenoid sensitized solar cells have lower photovoltaic parameters due to the lack of chemical groups that favor the interaction with the semiconductor [16,70].

4. Conclusions

In this work, we reported for the first time the use of pigments produced by UV-resistant Antarctic bacteria as photosensitizers in solar cells.

The bacteria were isolated from Antarctic soil samples and identified as Hymenobacter sp. (red strain) and Chryseobacterium sp. (yellow strain). Both environmental isolates showed resistance to UV radiation. Extracted bacterial pigments are photosensible and belong to the carotenoid family, specifically xanthophylls. Finally, we demonstrated that bacterial carotenoids can be used as photosensitizers in DSSCs. Despite photovoltaic parameters of DSSCs based on bacterial pigments are still being studied, the results constitute a first step for the development of greener solar cells using these bacterial molecules. Future research should be focused on the search and isolation of microorganisms that produce new pigments with characteristics that favor its use in sensitized solar cells, such as higher performance as light harvesters or better interaction with the TiO₂ film.

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References
