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Laboratory Exercises

A Simple Experiment to Show Photodynamic Inactivation of Bacteria on Surfaces*

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New suitable approaches were investigated to visualize the photodynamic inactivation (PDI) of bacteria immobilized on agar surfaces. The PDI capacities of a cationic photosensitizer (5,10,15,20-tetra(4-*N*,*N*,*N*-trimethylammoniumphenyl)porphyrin) and an anionic photosensitizer (5,10,15,20-tetra(4-sulfonatophenyl)-porphyrin) were analyzed on a typical Gram-negative bacterium *Escherichia coli* following two procedures. In Experiment I, the *E. coli* cells were grown as lawn on agar surface containing the sensitizers spread in a small area (10 nmol in ~0.6 cm^2). After irradiation with visible light (10 min, 90 milliwatts/cm^2), no cells were grown in the area containing the cationic porphyrin. In Experiment II, small colonies (~2-mm diameter) of *E. coli* on agar were treated with a solution of sensitizer (10 nmol) and irradiated with visible light for 3 h. Overnight incubation at 37 °C shows a growth delay of *E. coli* colonies treated with the cationic photosensitizer. In contrast, the anionic porphyrin did not produce appreciable photodamage. These experiments could be either used in an undergraduate project for natural science advance students or used for a postgraduate practical training course. This methodology illustrates the application of PDI to treat bacteria growing as localized foci of infection.

Keywords: Photodynamic inactivation, porphyrin, bacteria, sensitizers.

The emergence of antibiotic resistance among pathogenic bacteria has led to a major research effort to find alternative antibacterial therapies [1, 2]. A new promising approach to treat bacterial infections is called bacterial photodynamic inactivation (PDI, Scheme 1) [3, 4]. This is based on the administration of a photosensitizer, which is preferentially accumulated in the microbial cells. The subsequent irradiation with visible light, in the presence of oxygen, specifically produces cell damages that inactivate the microorganisms. Two oxidative mechanisms are considered to be principally implicated in the photodamage of cells. In the type I photochemical reaction, the photosensitizer interacts with a biomolecule to produce free radicals, whereas in the type II mechanism, singlet molecular oxygen, $^1O_2$, is produced as the main species responsible for cell inactivation [5, 6]. The reactive $^1O_2$ and other reactive oxygen species rapidly reacts with a variety of substrates including cholesterol, unsaturated fatty acids in lipid layers of membranes, amino acid residues such as cysteine, histidine, and tryptophan of protein structures, as well as nucleic acid bases of DNA, particularly guanine and thymine [3, 12]. These reactions induce damages in biomolecules, which conduce to a loose of appropriate biological functionality producing cell inactivation.

In general, Gram-positive bacteria are efficiently photoinactivated by a variety of porphyrins, whereas Gram-negative bacteria are usually resistant to the action of anionic or neutral agents [3]. The resistance of Gram-negative bacteria to the action of photoactivated sensitizers has been ascribed to the presence of highly organized outer membrane, which hinders the interaction of the photosensitizer with the cytoplasmic membrane and intercepts the photogenerated reactive species. Alternatively, cationic porphyrins have shown to photoinduce direct inactivation of Gram-negative bacteria without the presence of an additional permeabilization agent [7–9]. The positive charges on the photosensitizer molecule appear to promote a tight electrostatic interaction with negatively charged sites at the outer surface of the Gram-negative bacteria, increasing the efficiency of the photoinactivation processes [3].

There are two basic pathways, which have been proposed as mainly responsible for the lethal damage caused to bacteria by PDI.[1] The photodynamic activity can mainly produce changes in the cytoplasmic membrane and dam-

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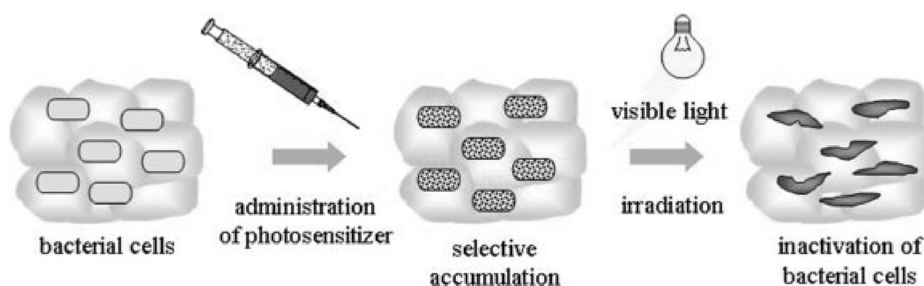
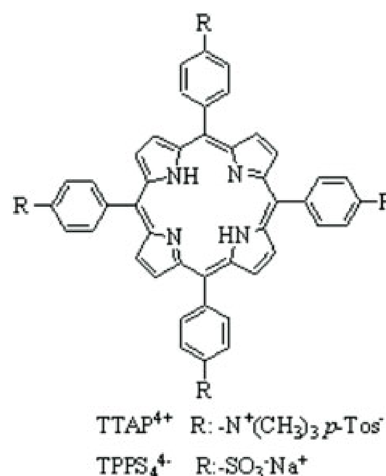
[1] The abbreviations used are: PDI, photodynamic inactivation; $TTAP^{4+}$, (5,10,15,20-tetra(4-*N*,*N*,*N*-trimethylammoniumphenyl)-porphyrin; $TPPS_4^{4-}$, (5,10,15,20-tetra(4-sulfonatophenyl)porphyrin; W, watt; mW, milliwatt; PBS, phosphate-buffered saline; CFU, colony-forming unit; TS, tryptic soy.



SCHEME 1. **Schematic representations of the bacterial PDI.**

age in the DNA [3]. The damage to cytoplasmic membrane can involve leakage of cellular contents or inactivation of membrane transport systems and enzymes. On the other hand, it is known that cationic porphyrins take part in complex formation with nucleic acids, inducing lesions upon photoactivation [10]. Actually, there are few studies on the affected subcellular sites and the nature of macromolecular damage to DNA, RNA, and proteins caused by PDI treatment of bacteria [11].

The purpose of this experiment is to demonstrate that PDI is a suitable approach to inactivate bacteria immobilized on a surface. Thus, two sensitizers, a cationic (5,10,15,20-tetra(4-*N*,*N*,*N*-trimethylammoniumphenyl)-porphyrin ($TTAP^{4+}$)) and an anionic (5,10,15,20-tetra(4-sulfonatophenyl)porphyrin ($TPPS_4^{4-}$)) photosensitizer (Scheme 2), were compared on a typical Gram-negative bacterium *Escherichia coli*. These studies show that only the cationic porphyrin is an effective photosensitizer to inactivate *E. coli* cells on surfaces.



SCHEME 2. **Chemical structure of porphyrins.**

This new approach to visualize the PDI of bacteria immobilized on agar surfaces could be an easy approach to test the photodynamic activity of several photosensitizers on the solid surface of one plate. This procedure has potential applications in photodisinfections of surfaces, particularly in hospital environments [2]. Also, this methodology could be used to visualize the treatment of microorganisms growing as localized foci of infection, on skin or on accessible areas to be irradiated with either artificial visible light or natural sunlight. The present experimental class might be either used in an undergraduate project for advanced students in the natural sciences or used for a postgraduate practical training course. For example, this procedure might be appropriate for a microbiology laboratory, which can be adapted in classes of bacteriology, in particular when the effects of antibiotic are discussed. In consequence, PDI can be introduced as an alternative approach to inactivate bacteria, which has the advantage over other therapies. PDI has selectivity not only because the photosensitizer can be targeted to localized bacterial infection but also because the irradiated light can be accurately delivered to the affected area. In addition to efficacy, PDI has shown other benefits [3]. First, the sensitizers used are highly selective; bacteria were killed at combinations of drug and light doses much lower than that needed for a similar effect on mammalian cells. Second, all investigated photosensitizers lack mutagenic activity, and the risk of selection of drug-resistant bacterial strains was not still reported [2–4, 12]. On the other hand, it could also be adopted into a postgraduate biochemistry or molecular biology course. In this way, an introduction about the mechanism of photoinactivation that occurs inside the bacterial cell, once the agent is introduced, should be given as explained above. In particular, it is an interesting training experiment for postgraduate photobiology students mainly interested in photooxidative mechanisms. Before attempting this experiment, the students should be informed about how photosensitizers work [5], given a short description of PDI application in solution including information about the binding of anionic and cationic sensitizers to cells [3, 12], and trained in microbiological handling of bacterial cultures.

EXPERIMENTAL PROCEDURES

Photosensitizers—5,10,15,20-Tetra(4-*N*,*N*,*N*-trimethylammoniumphenyl)porphyrin ($TTAP^{4+}$) *p*-tosylate and (5,10,15,20-tetra(4-sulfonatophenyl)porphyrin ($TPPS_4^{4-}$) sodium salt from Aldrich were used without further purification. A stock solution of porphyrin (5×10^{-4} M) was prepared by dissolution in 1 ml of water. The concentration was checked by spectroscopy, taking into account the value of molar coefficient (ϵ); $TTAP^{4+}$ $\epsilon = 178\ 144$ M^{-1} cm^{-1} at 412 nm and $TPPS_4^{4-}$ $\epsilon = 163\ 000$ M^{-1} cm^{-1} at 413 nm in water [7].

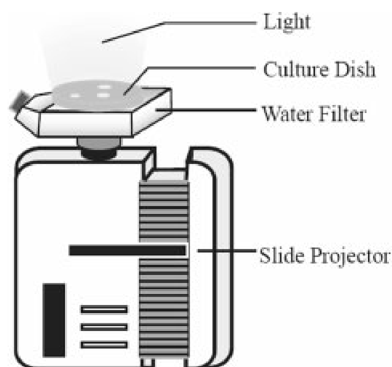
Irradiation System—The irradiation system is shown in Scheme 3. The light source used was a Novamat 130 AF slide projector equipped with a 150-W halogen lamp. The light was filtered through a 2.5-cm glass cuvette filled with water to absorb heat. A wavelength range between 350 and 800 nm was selected by optical filters. A radiometer (Radiometer Laser Mate-Q, Coherent) was used to determine light intensity. This device is calibrated and provides a measurement of the amount of light power incident on the detector. Thus, when the detector is localized in the treatment site instead of the culture, the light intensity is determined. In our equipment, it was 90 mW/cm^2.

Bacterial Strain and Preparation of Cultures—The *E. coli* strain recovered from clinical urogenital material was used as described previously [9]. The *E. coli* strain was grown aerobically at 37 °C in 30% w/v tryptic soy (TS) broth overnight. Aliquots (~40 μl) of this

culture were aseptically transferred to 4 ml of fresh medium (30% w/v TS broth) and incubated at 37 °C to the middle of the logarithmic phase (absorbance ~0.6 at 660 nm). Cells in the logarithmic phase of growth were harvested by centrifugation of broth cultures (3000 × *g* for 15 min) and resuspended in 4 ml of 10 mM phosphate-buffered saline (PBS, pH = 7.0). Then the cells were diluted 1/1000 in PBS, corresponding to ~10^6 colony-forming units (CFU)/ml, and subsequent dilutions were performed according with the experiment.

Experiment I—TS-agar plates (5-cm diameter) were spread with 20 μl (10 nmol) of porphyrin on an area of 0.6 cm^2 from a solution 5 × 10^{-4} M in water. The solvent was evaporated, and the plates were spread with the suspension of *E. coli* (~10^6 CFU/ml) in PBS. Then the plates were incubated at 37 °C for 20 min in the dark. After that, the plates were irradiated as described above for 10 min and incubated overnight at 37 °C in the dark. Plates with and without photosensitizers kept in the dark and plates without porphyrin and irradiated were used as controls. Each experiment was repeated separately three times.

Experiment II—Suspensions of *E. coli* (~10^2 CFU/ml) in PBS were spread on a 10-cm-diameter TS-agar dish and grown at 37 °C by 13 h. This procedure gives between 5 and 10 small colonies by plate. The sensitizers were added from a 5 × 10^{-4} M stock solution in water. The colonies were spread with porphyrin using 10 μl (10 nmol) of the stock solution. Before adding a new dose of sensitizer, the solvent of the drop was dried to avoid an increase in the area of treatment. At this time, the cultures were incubated for 10 min at 37 °C and irradiated for 3 h with visible light as described above. After irradiation, the plates were incubated for additional overnight at 37 °C. Controls with and without photosensitizers kept in the dark as well as illuminated controls without porphyrin were carried out. The variation in the area of *E. coli* colonies were estimated considering 100% to the increase in the area of the control. Each experiment was repeated separately three times. After the experiments, the cells were destroyed by treatment with sodium hypochlorite (10%) overnight and autoclaved.



SCHEME 3. **Visible light irradiation system.**

RESULTS AND DISCUSSION

Spectroscopy and Photodynamic Properties of Porphyrins—The absorption spectra of $TTAP^{4+}$ and $TPPS_4^{4-}$ porphyrins in water show the typical *Soret band* (~412 nm, ϵ ~1.7 × 10^5 M^{-1} cm^{-1}) and four less intense *Q bands* (~515, 550, 584, 640 nm, $\epsilon < 6 \times 10^3$ M^{-1} cm^{-1}), characteristic of free-base porphyrins (Fig. 1) [13]. This indicates that these porphyrins are excited by absorption of light in the visible region, mainly with blue light. Both sensitizers present a high efficiency of $^1O_2$ production, with quantum yields (Φ_Δ) of 0.77 for $TTAP^{4+}$ and 0.71 for $TPPS_4^{4-}$, in water [14, 15]. The Φ_Δ can be determined by using different methods [16]. One involves the direct observation of the luminescence at ~1270 nm produced by relaxation of $^1O_2$. Other possibilities are based on quantitative analysis of photooxidation reactions using specific molecular probes, such as 9,10-dimethylanthracene or diphenylisobenzofuran [17]. In both cases, the value of Φ_Δ for a new sensitizer is obtained by comparison with a standard. However, the values of Φ_Δ can significantly change in a different medium, diminishing when the sensitizer is partially aggregated [6]. Also, the biological microenvironment of the sensitizer can induce important modifications in the photophysics of the porphyrin established in solution [18].

PDI of E. coli Localized on a Surface—The experiments

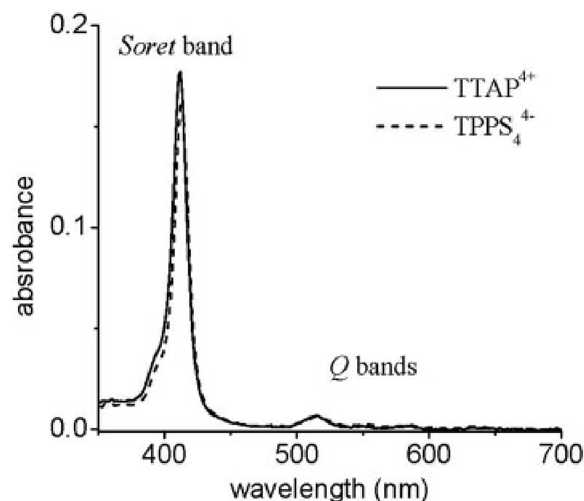
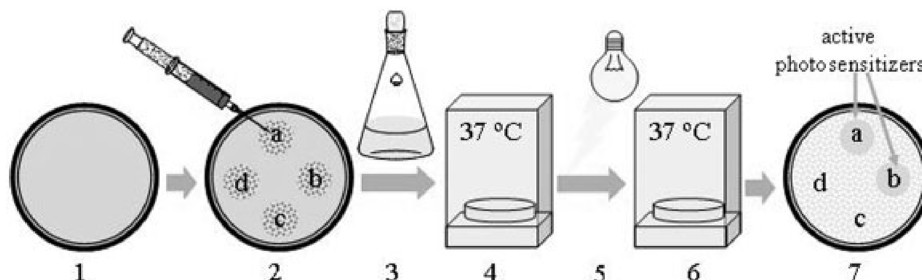


FIG. 1. **Absorption spectrum of porphyrins in water; [porphyrin] = 1 μM.**



SCHEME 4. **Experimental procedure to examine the photosensitizer activity on surface spread with bacterial cells.** *1*, agar plate with medium; *2*, homogenetic dispersion of the photosensitizers in a demarcate surface; *3*, spreading of bacterial cells, which allows obtaining a lawn of bacterium; *4*, short incubation time in the dark; *5*, irradiation with visible light; *6*, overnight incubation; *7*, growth of bacterial cells is not detected in the area treated with an efficient photosensitizer, as indicated by the *arrows*, whereas modification of the lawn is not observed for unsuccessful agents.

in vitro are separated in two laboratory periods, which can be performed separately. These require that the instructor or stockroom prepare the overnight culture and the sterilized materials before the laboratory period. The results shown below represent typical student data. The capacity of these porphyrins to bind to bacterial cells of *E. coli* was previously studied in PBS suspension [19]. Porphyrin binding to bacterial cells can be determined by fluorescence analysis. This procedure involves the incubation of the culture with a determined amount of photosensitizer in the dark for a particular time. After centrifugation, the cell pellet is resuspended in aqueous sodium dodecyl sulfate, which breaks the cells. The concentration of sensitizer in the supernatant is measured by spectrofluorometry by comparison with a calibration curve obtained with standard solutions of the sensitizer. When *E. coli* cultures are treated with 1 μM sensitizer, the $TTAP^{4+}$ porphyrin is rapidly (<5 min) bound to bacterial cells, reaching a binding value of ~0.4 nmol/10^6 cells. Under similar conditions, the binding of $TPPS_4^{4-}$ porphyrin to *E. coli* cells is about an order of magnitude lower than that of $TTAP^{4+}$. Photosensitized inactivation of *E. coli* cells treated with 1 μM sensitizer for 30 min at 37 °C in PBS suspension indicates that $TTAP^{4+}$ produce an ~4-log decrease (~99.984%) of cell inactivation after a light fluence of 108 J/cm^2. Under these conditions, no inactivation effect was found for cultures treated with $TPPS_4^{4-}$. This result is expected due to its low binding to *E. coli* cells, indicating that this anionic porphyrin is an unsuccessful sensitizer for Gram-negative bacteria. Taking into account these results in PBS solution, the photodynamic activity of these photosensitizers was evaluated in *E. coli* cells immobilized on TS-agar. This approach can be used to inactivate bacteria growing *in vivo* as localized foci of infection, on skin, or on accessible mucous membranes [20, 21]. Also, photodynamic treatment has been proposed as a new possibility for protecting foods from microbial spoilage [22].

Experiment I, PDI of E. coli Cells Growing as Lawn on Agar Surface Impregnated with the Sensitizer—In this experiment (Scheme 4), the cells were grown as lawn on TS-agar surface containing the sensitizer in a small area. Firstly, 10 nmol of different porphyrins were homogenously distributed in ~0.6 cm^2 on TS-agar. The solvent was evaporated, and the plates were spread with a suspension of *E. coli*, which allows obtaining a lawn of bacteria. The cultures were kept in the dark for 20 min at 37 °C. During this period, the binding of sensitizer to *E. coli* cells can take place. Afterward, the plates were irradiated with visible light for 10 min and incubated overnight at 37 °C in the dark. Characteristic results are shown in Fig. 2. As can be observed, growth of *E. coli* cells was not detected in the area treated with tetracationic $TTAP^{4+}$ porphyrin (Fig. 2*A*). In contrast, modification of the lawn was not observed for controls treated with porphyrins and kept in the dark (Fig. 2*B*). Thus, the photodynamic inactivation was mainly observed for plates treated with $TTAP^{4+}$.

Experiment II, PDI of E. coli Cells Growing in Colonies on Agar Surface—The procedure of these experiments is summarized in Scheme 5. To start with the experiment, appropriate dilutions of *E. coli* suspensions in PBS were spread on TS-agar plates to obtain about 10 separated colonies. The cultures were incubated for ~13 h at 37 °C to form colonies of ~2-mm diameter. The colonies were treated with 10 nmol of porphyrin, which was homogeneously distributed on the colony from a stock solution in water. The cultures were kept in the dark for 10 min at 37 °C. During this period, the binding of sensitizer to *E. coli* cells can take place. The plates were then illuminated with visible light for 3 h. Afterward, the plates were incubated overnight at 37 °C in the dark.

Characteristic results for porphyrins are shown in Fig. 3. As can be observed, growth delay of *E. coli* colonies on TS-agar was clearly evidenced for colonies treated with $TTAP^{4+}$ porphyrin with respect to control without sensitizer. A comparable increase in the area size was also obtained for a control containing porphyrin but without

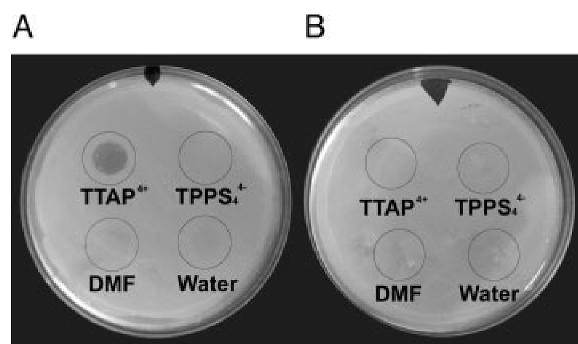
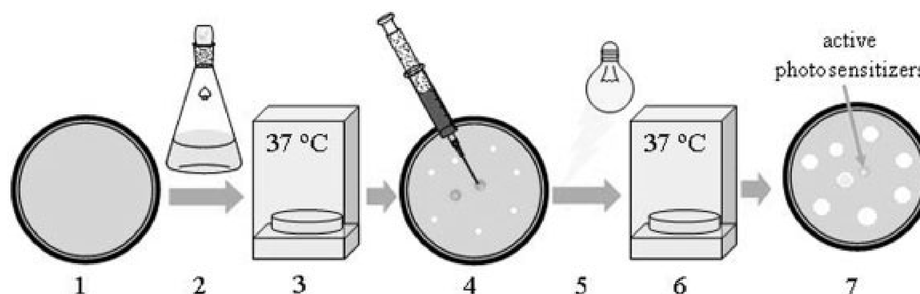


FIG. 2. **PDI of *E. coli* cells on TS-agar, irradiated with visible light for 10 min (90 mW/cm^2) (*A*) and kept in the dark (*B*).** The *dark circles* indicate the area where 10 nmol of sensitizer was spread from a solution 5×10^{-4} M in water.



SCHEME 5. **Experimental procedure to examine the photosensitizer activity on small colonies.** *1*, agar plate with medium; *2*, spreading of bacterial cells, which allows forming ~10 colonies; *3*, incubation for ~13 h at 37 °C to obtain small colonies (~2-mm diameter); *4*, homogenetic dispersion of the photosensitizers on the colonies; *5*, irradiation with visible light; *6*, overnight incubation; *7*, growth delay is observed in colonies treated with an efficient photosensitizer, as indicated by the *arrows*, whereas a similar colony size is found for unsuccessful agents in comparison with the untreated controls.

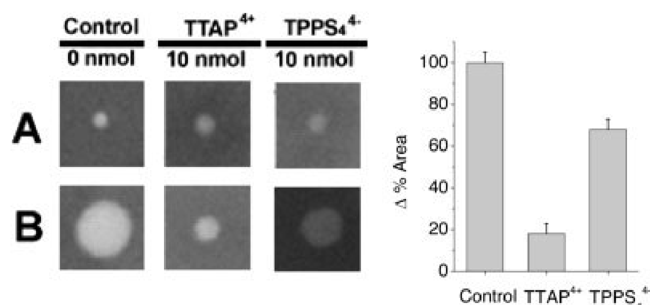


FIG. 3. **Growth delay of *E. coli* colonies on TS-agar treated with 10 nmol of $TTAP^{4+}$ and $TPPS^{4-}$ porphyrins and irradiated with visible light (90 mW/cm^2) for 3 h.** *A*, colonies immediately after PDI treatment; *B*, colonies incubated overnight at 37 °C. Sensitizer was spread on the colony from a stock solution (5×10^{-4} M) in water, and the culture was incubated for 10 min at 37 °C before irradiation. *Scale bars*, 2 mm.

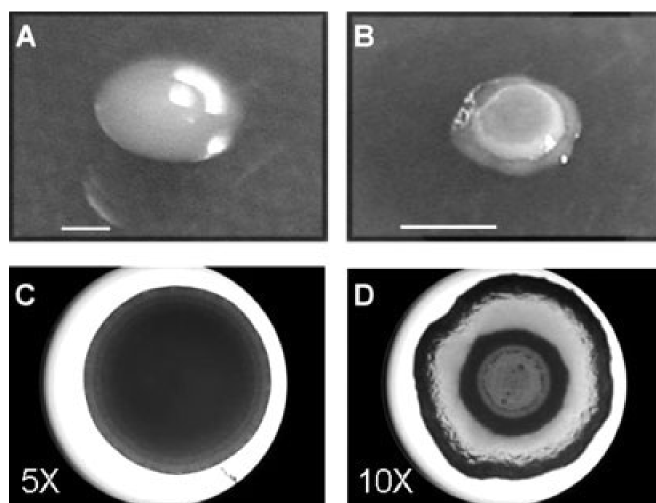


FIG. 4. **Colony morphology of *E. coli* cells.** *A*, colony control without sensitizer; *B*, colony treated with $TTAP^{4+}$, irradiated with visible light for 3 h and grown overnight for two days at 37 °C; *C* and *D*, microscopic visualization of colonies *A* and *B*, respectively. *Scale bars*, 2 mm.

irradiation. Thus, the growth delay obtained after irradiation of the cultures treated with the porphyrin is due to the photosensitization effect of the agent produced by visible light. The variations in the area of *E. coli* colonies were estimated considering 100% to the increase in the area of the control. Thus, for the colonies treated with $TTAP^{4+}$ porphyrin, the change in the area size was very small ($<$18%) in comparison with colonies containing the anionic porphyrin ($\sim$68%).

The morphological changes of colonies were observed during the photodynamic experiments (Fig. 4). Colonies under PDI treatment and after 18 h of incubation in the dark at 37 °C show the appearance of Fig. 4*B*, which is characterized by a cellular growing from the base. In contrast, controls are characterized by convex appearance and glossy colonies (Fig. 4*A*). The variation in the colony morphology is schematically represented in Fig. 4*C* and 4*D*. As can be observed, the colony is homogeneously covered with sensitizer, and after irradiation, some levels of cells of the cover are inactivated. Viable cells remain after this PDI procedure in the colony core. Subsequent incubation overnight produces a side development of viable cells. A second growth overnight expands the new viable cells from the base of the colony, producing the colony crater form observed in Fig. 4*B*.

CONCLUSIONS

These laboratory experiments have been designed to observe the PDI produced by a cationic porphyrin on a typical Gram-negative bacterium *E. coli*, immobilized on surfaces. The trials are mainly proposed for either an undergraduate project for advanced students in the natural sciences or a postgraduate practical training course. In both experiments, the students must have training in microbiological handling of bacterial cultures. Also, the laboratory instructor must have the flasks with the culture and all the materials involved in the studies with cells sterilized beforehand.

The trials can be performed together in one laboratory period or in different laboratory periods taking into account the following considerations. (*a*) The necessary time to carry out the first laboratory period (Experiment I) is about 2 h, and the cultures must be observed the next day ($\sim$18–24 h after experiment) after overnight incubation; (*b*) the second laboratory period (Experiment II) requires about 4 h, and the colonies' changes must also be observed the next day; and (*c*) in consequence, it is possible to perform both experiments together, starting with Experiment II; during the irradiation time (3 h), Experiment I can be carried out.

In our experience, this laboratory class experiment was used as a training exercise included in a postgraduate curse for Ph.D. students, Focus in Biology. Our students did have experience in cultures because they were graduate students in microbiology. Before attempting this experiment, they were informed in classes about photosensitizers, photodynamic effect, and their applications in PDI of microorganisms. In the experiments, the students did have a comprehensible appreciation for how photodynamic effects can be used to inactivate bacteria. Also, the results were used to visualize the different behaviors observed for a cationic and an anionic porphyrin, which were interpreted considering that the positive charges on the photosensitizer molecule appear to promote a tight electrostatic interaction with negatively charged sites at the outer surface of the Gram-negative bacteria. This interaction produces an increase in the efficiency of the photoinactivation processes. During the experiment, the students were supervised by the instructor, and finally, they were evaluated by a research-style laboratory report that includes a discussion according to the observed results. Thus, this practical application of porphyrins to inactive bacteria allows an interesting learning of these topics and the basic knowledge of PDI processes in biological systems.

REFERENCES

[1] P. W. Taylor, P. D. Stapleton, J. P. Luzio (2002) New ways to treat bacterial infections, *Drug Discov. Today*, **7**, 1086–1091.
[2] M. Wainwright (1998) Photodynamic antimicrobial chemotherapy (PACT), *J. Antimicrob. Chemother.* **42**, 13–28.
[3] M. R. Hamblin, T. Hasan (2004) Photodynamic therapy: A new antimicrobial approach to infectious disease? *Photochem. Photobiol. Sci.* **3**, 436–450.
[4] G. Jori, S. B. Brown (2004) Photosensitized inactivation of microor-

ganisms, *Photochem. Photobiol. Sci.* **5**, 403–405.
[5] M. Ochsner (1997) Photophysical and photobiological processes in photodynamic therapy of tumours, *J. Photochem. Photobiol. B Biol.* **39**, 1–18.
[6] M. C. DeRosa, R. J. Crutchley (2002) Photosensitized singlet oxygen and its applications, *Coordin. Chem. Rev.* **233–234**, 351–371.
[7] M. Merchat, G. Spikes, G. Bertoloni, G. Jori (1996) Studies on the mechanism of bacteria photosensitization by meso-substituted cationic porphyrins, *J. Photochem. Photobiol. B Biol.* **35**, 149–157.
[8] Y. Nitzan, A. Balzam-Sudakevitz, H. Ashkenazi (1998) Eradication of *Acinetobacter baumanni* by photosensitized agents *in vitro*, *J. Photochem. Photobiol. B Biol.* **42**, 211–218.
[9] D. Lazzeri, M. Rovera, L. Pascual, E. N. Durantini (2004) Photodynamic studies and photoinactivation of *Escherichia coli* using meso-substituted cationic derivatives with asymmetric charge distribution, *Photochem. Photobiol.* **80**, 286–293.
[10] H. Li, O. S. Fedorova, A. N. Grachev, W. R. Trumble, G. A. Bohach, L. Czuchajowski (1997) A series of meso-tris(*N*-methyl-pyridiniumyl)-(4-alkylamidophenyl) porphyrins: Synthesis, interaction with DNA and antibacterial activity, *Biochim. Biophys. Acta* **1354**, 252–260.
[11] M. Salmon-Divon, Y. Nitzan, Z. Malik (2004) Mechanistic aspect of *Escherichia coli* photodynamic inactivation by cationic tetra-meso(*N*-methylpyridyl)porphine, *Photochem. Photobiol. Sci.* **3**, 423–429.
[12] E. N. Durantini (2006) Photodynamic inactivation of bacteria, *Curr. Bioact. Comp.* **2**, 127–142.
[13] K. M. Smith (1975) *Porphyrins and Metalloporphyrins: General Features of the Structure and Chemistry of Porphyrin Compounds*, pp 20–27, Elsevier, Amsterdam.
[14] J. B. Verlag, A. Gaudemer, I. Kraljic (1984) Water soluble porphyrins and metallo-porphyrins as photosensitizers in aerated aqueous solutions. I. Detection and determination of quantum yield of formation of singlet oxygen, *Nouv. J. Chim.* **8**, 401–406.
[15] V. Gottfried, D. Peled, J. W. Winkelman, S. Kimel (1988) Photosensitizers in organized media: Singlet oxygen production and spectral properties, *Photochem. Photobiol.* **48**, 157–163.
[16] F. Wilkinson, J. G. Brummer (1981) Rate constants for the decay and reactions of the lowest electronically excited singlet state of molecular oxygen in solution, *J. Phys. Chem. Ref. Data* **10**, 809–899.
[17] R. W. Redmond, J. N. Gamlin (1999) A compilation of singlet yields from biologically relevant molecules, *Photochem. Photobiol.* **70**, 391–475.
[18] B. M. Aveline, R. W. Redmond (1999) Can cellular phototoxicity be accurately predicted on the basic of sensitizer photophysics? *Photochem. Photobiol.* **69**, 306–316.
[19] D. A. Caminos, M. B. Spesia, E. N. Durantini (2006) Photodynamic inactivation of *Escherichia coli* by novel meso-substituted porphyrins by 4-(3-*N*,*N*,*N*-trimethylammoniumpropoxy)phenyl and 4-(trifluoromethyl)phenyl groups, *Photochem. Photobiol. Sci.* **5**, 56–65.
[20] A. Orenstein, D. Klein, J. Kopolovic, E. Winkler, Z. Malik, N. Keller, Y. Nitzan (1998) The use of porphyrins for eradication of *Staphylococcus aureus* in burn wound infections, *FEMS Immunol. Med. Microbiol.* **19**, 307–314.
[21] S. Wood, B. Nattress, J. Kirkham, R. Shore, S. Brookes, J. Griffiths, C. Robinson (1999) An *in vitro* study of the use of photodynamic therapy for the treatment of natural oral plaque biofilms formed *in vivo*, *J. Photochem. Photobiol. B Biol.* **50**, 1–7.
[22] M. Kreitner, K.-H. Wagner, G. Alth, R. Ebermann, H. Foiby, I. Elmadfa (2001) Haematoporphyrin- and sodium chlorophyllin-induced phototoxicity towards bacteria and yeasts-a new approach for safe foods, *Food Control* **12**, 529–533.