Biological synthesis of fluorescent nanoparticles by cadmium and tellurite resistant Antarctic bacteria: exploring novel natural nanofactories

D. O. Plaza\textsuperscript{1,2}, C. Gallardo\textsuperscript{1}, Y. D. Straub\textsuperscript{1}, D. Bravo\textsuperscript{3} and J. M. Pérez-Donoso\textsuperscript{1*}

Abstract

Background: Fluorescent nanoparticles or quantum dots (QDs) have been intensely studied for basic and applied research due to their unique size-dependent properties. There is an increasing interest in developing ecofriendly methods to synthesize these nanoparticles since they improve biocompatibility and avoid the generation of toxic byproducts. The use of biological systems, particularly prokaryotes, has emerged as a promising alternative. Recent studies indicate that QDs biosynthesis is related to factors such as cellular redox status and antioxidant defenses. Based on this, the mixture of extreme conditions of Antarctica would allow the development of natural QDs producing bacteria.

Results: In this study we isolated and characterized cadmium and tellurite resistant Antarctic bacteria capable of synthesizing CdS and CdTe QDs when exposed to these oxidizing heavy metals. A time dependent change in fluorescence emission color, moving from green to red, was determined on bacterial cells exposed to metals. Biosynthesis was observed in cells grown at different temperatures and high metal concentrations. Electron microscopy analysis of treated cells revealed nanometric electron-dense elements and structures resembling membrane vesicles mostly associated to periplasmic space. Purified biosynthesized QDs displayed broad absorption and emission spectra characteristic of biogenic Cd nanoparticles.

Conclusions: Our work presents a novel and simple biological approach to produce QDs at room temperature by using heavy metal resistant Antarctic bacteria, highlighting the unique properties of these microorganisms as potent natural producers of nano-scale materials and promising candidates for bioremediation purposes.

Keywords: Fluorescent nanoparticles, Quantum dots, Green synthesis, Antarctica, Bacteria, Heavy metals

Background

Recently, the vastly explored field of nanotechnology has become a key area of technological development \cite{1}. In the last decade, fluorescent colloidal nanoparticles or quantum dots (QDs) have been intensively studied for both basic and applied research due to their unique size-dependent properties \cite{2}. These nanoparticles are crystalline arrangements sized 1–20 nm, composed by elements of groups II–IV, III–V or IV–VI (such as Cd, Te, Se, Zn, In, As), which form a core covered by an external layer or envelope \cite{3}. In comparison to organic fluorophores, QDs are brighter, more photostable, and display a narrower fluorescence emission spectra allowing multiplexing applications upon excitation by a single wavelength \cite{4}.

Currently, QDs are used on several biological, biomedical, optical and optoelectronic applications, ranging from biosensors to solar cells \cite{5,6}. QDs synthesis is one of the key challenges for applications development, since...
it determines the size, shape, and surface properties of nanoparticles [7]. Traditionally, the synthesis has been performed using both physical and chemical approaches [8], which are often expensive and harmful to the environment due to the use of high temperatures, inert atmospheres, and toxic reagents [9]. The development of greener (environmentally friendly) synthesis methods has been proposed as an alternative to solve these drawbacks, since they involve low costs, the absence of toxic chemicals, high performance, and produce nanomaterials with well-defined sizes and shapes [10].

The use of biological systems has emerged as a promising alternative to produce water-soluble nanoparticles because they are economic, eco-friendly, easily scaled up, free of toxic compounds, biocompatible and the synthesis is carried out under room conditions [11]. A wide range of organisms, including plants, worms, fungi, yeasts, and bacteria can synthesize QDs from different metal compounds [12, 13]. Prokaryotes possess outstanding additional advantages for QDs synthesis over eukaryotic systems such as faster growth rates and efficient strategies to overcome the inherent toxicity of heavy metals that constitute the core of these nanoparticles, which are very harmful to many organisms at low concentrations [14, 15]. These strategies likely include processes such as metal reduction and/or precipitation, generating non-toxic or less toxic metal nanoarrays [16, 17].

Glutathione (GSH) is a fundamental biological thiol involved in cell protection against different stresses, for example the stress produced by harmful metals [18]. This thiol is depleted by oxidative stress generators such as cadmium and tellurite ions [19, 20], which are usually used as substrates in QDs biosynthesis. A positive relationship between GSH content and the synthesis of nanocrystals has been reported in microbial QDs production [21, 22], highlighting the essential role of cellular antioxidant defenses for QDs generation.

Antarctica has recently emerged as an extraordinary source of microorganisms with exceptional antioxidant defenses. This challenging continent, considered the coldest and driest place on the planet, also presents other extreme conditions such as high levels of solar ultraviolet radiation, low nutrient availability, high salinity and long darkness periods, which increase the generation of oxidative stress. This unique mixture of stressful conditions limits the development of biological communities [23]. Despite this severe scenario, the presence of microorganisms is abundant [24, 25]. These organisms display specialized defense mechanisms against reactive oxygen species (ROS) that maintain their redox state under constant environmental stress [26, 27]. Some enzymatic and non-enzymatic adaptations described on Antarctic bacteria are: glutathione S-transferase [28], glutathione reductase [29], thioredoxin [30], catalases [31], superoxide dismutases [32], carotenoids [33] and oxide reductases [34]. Furthermore, the special abilities of some Antarctic bacteria to produce different nanostructures have been recently reported [29, 35, 36]. Thus, the extreme conditions of Antarctica would allow the development of bacteria with unparalleled capacity to cope with high concentrations of heavy metals and synthesize QDs naturally.

Herein, we report cadmium and tellurite resistant bacteria isolated from Antarctica with the ability to produce QDs at room temperature by using high concentrations of these oxidizing substrates. Different bacterial strains showed inherent capabilities for synthesizing fluorescent nanostructures with no additional reagents, representing a promising environmentally friendly methodology to produce valuable biological nanoparticles.

Results

Isolation and selection of cadmium and tellurite resistant bacteria

A total of 410 bacterial isolates were recovered from Antarctic samples using different culture media (see methods). Most of the isolates (92.3 %) were able to grow in LB medium, being selected for further assays. After, aiming to find bacteria for QDs biosynthesis at room temperature, those isolates able to grow at 15–28 °C (217) were chosen for assessing their metal resistance. Among these 217 isolates, a total of 35 resistant to CdCl₂ (200 mg/L), 44 to K₂TeO₄ (50 mg/L) and 16 to both toxic salts were obtained. The 16 isolates were then subjected to an initial biosynthesis test (exposure to CdCl₂ as previously described by Gallardo et al. [36]), to work only with QDs producing isolates. Finally, 12 resistant isolates displayed fluorescence within 24–48 h after cadmium exposure (Fig. 1a). Time dependent changes on the fluorescence were evaluated on one representative isolate (Fig. 1b). Changes in color emission across the incubation time (moving from green to red) were observed in bacterial pellets excited at 360 nm. This behavior is a key feature of QDs as has been reported previously [36]. Thus, these 12 isolates were selected for subsequent experiments.

Identification of metal-resistant bacteria

The results of 16S rRNA gene sequence analysis of the 12 metal-resistant bacteria revealed that they belong to genera Pseudomonas (eight isolates), Psychrobacter (three isolates) and Shewanella (one isolate), being renamed as Pse, Psy, and She, respectively. Some isolates evidenced low similarity scores to known species (Pse7 and Pse8), representing probably species not yet described (Fig. 2a). Phylogenetic analysis showed three clear clustering patterns in terms of genera and evidenced remarkable genetic distances among selected isolates (Fig. 2b).
Growth and metabolic characteristics of metal-resistant bacteria

Phenotypic characterization of the 12 resistant bacterial isolates evidenced differences among them (Table 1). The optimal growth temperature for the 12 isolates was 28 °C, nonetheless, isolates were able to grow at temperatures ranging from 10–28 °C. No growth was observed at 37 °C. All isolates were Gram-negative, mostly with homogeneous cream-colored colonies surrounded by abundant mucus. All selected isolates were resistant to Lincomycin and sensitive to Gentamycin and Kanamycin.

The metabolic profile reveals a high diversity among all selected isolates, particularly in terms of enzymatic activities, excepting β-galactosidase (negative for all isolates; Table 2). The predominant activities were nitrate
reductase and indole production (tryptophanase). In terms of substrate utilization, a transversal use of substrates was determined. All isolates were able to metabolize glucose, arabinose, gluconate, and malic acid.

Metal resistance levels of selected bacteria
MIC values for the 12 resistant bacteria ranged between 500–1400 and 62.5–1200 mg/L for CdCl₂ and K₂TeO₃, respectively (Table 3). Cadmium and tellurite resistant
bacteria presented elevated MIC values, which in some cases exceed seven and twenty times the concentrations used initially for selection, respectively. The strains that showed the highest cadmium resistance level were Pse1, Pse3 and the three Psychrobacter spp. (1400 mg/L), whereas for tellurite were Pse3 and Psy1 (1200 mg/L).

**Biosynthesis of fluorescent nanoparticles**

Based on MICs and the fluorescence determined in Fig. 1, we selected Pse3, Pse8 and Psy1 isolates to assess their capacity to biosynthesize QDs under different temperatures and metal concentrations. Optimal conditions for nanoparticles bioproduction such as incubation temperature and metal concentrations were determined. The experiments were carried out by two treatments: exposing bacteria to (a) CdCl2 (10 mg/L) or (b) CdCl2 (10 mg/L) + K2TeO3 (0.5 mg/L). The effect of incubation temperature was investigated at 10, 15 and 28 °C during 96 h (Fig. 3). A QDs-characteristic change on fluorescence emission color, moving from green to red, was observed in bacterial pellets over time, with slight differences among strains. The fluorescence of cells exposed to CdCl2 increased with temperature, evidencing an optimal biosynthesis temperature of 28 °C for the three bacterial strains evaluated. Low fluorescence was observed in cells treated with CdCl2 + K2TeO3, particularly when compared to CdCl2 exposed cells. In addition, a black precipitate was observed, most probably corresponding to Te4+ reduction [37].

Selected bacteria were then exposed to high concentrations of CdCl2 and K2TeO3. The concentrations of both metals assayed for QDs biosynthesis were chosen based on MICs and bacterial growth curves of each metal-resistant isolate (data not shown). Four concentrations of CdCl2 (5, 10, 62.5 and 500 mg/L) and K2TeO3 (0.25, 0.5, 3, and 25 mg/L) were evaluated and bacterial cultures were incubated at 28 °C for 4 days (Fig. 4).

In agreement with results of Fig. 3, strains treated only with CdCl2 presented higher fluorescence than those exposed to both salts. The Pse3 strain exposed to CdCl2 presented fluorescence at three concentrations (5, 10, and 62.5 mg/L) (Fig. 4a), whereas Pse1 and Psy1 strains emitted fluorescence at all CdCl2 concentrations tested, including 500 mg/L (Fig. 4b, c). For the treatment with both salts, at any K2TeO3 concentration evaluated the fluorescence emitted from the three strains was strongly affected, and increased tellurite concentrations were positively correlated with increasing black precipitates (black spots in the pellets).

**Transmission electron microscopy**

To study the effects of biosynthetic conditions on cellular ultrastructure of the three resistant bacteria, transmission electron microscopy (TEM) was performed on cells exposed to CdCl2 (condition in which the highest fluorescence emission was obtained). The micrographs obtained

### Table 3 Cadmium and tellurite MICs of metal-resistant Antarctic bacteria

| Strains | Pse1 | Pse2 | Pse3 | Pse4 | Pse5 | Pse6 | Pse7 | Pse8 | Psy1 | Psy2 | Psy3 | She |
|---------|------|------|------|------|------|------|------|------|------|------|------|-----|
| CdCl2   | 1400 | 500  | 1400 | 500  | 500  | 1000 | 1000 | 1000 | 1400 | 1400 | 1400 | 500 |
| K2TeO3  | 400  | 125  | 1200 | 62.5 | 400  | 125  | 400  | 600  | 1200 | 500  | 125  | 125 |

Concentrations are expressed as mg/L.
from TEM evidenced different changes in cell morphology and ultrastructure of the three strains tested (Fig. 5). The major changes were observed in both, cell envelope and poles. In *Pseudomonas* strains exposed to cadmium (*Pse3* and *Pse1*), an increase of periplasmic space, particularly at the cell poles, was observed (arrows of Fig. 5a, b). Moreover, a high number of structures resembling outer membrane vesicles (OMV) at the cell periphery were detected. In addition, the presence of electron-dense nanostructures was observed at the bacterial endings, particularly in the periplasmic space of *Pse1* (Fig. 5b, arrows).

**Spectroscopic characterization of biosynthesized nanoparticles**

The optical properties of QDs produced by the three resistant strains were analyzed by determining the absorption and fluorescence spectra (Fig. 6). The inset of the figure shows the fluorescence of a representative solution after purification. Absorption spectra were similar for QDs produced by all strains displaying increased absorption at 400 nm as reported for biosynthesized CdS nanoparticles (not shown) [21, 36]. Regarding the emission spectra, wide peaks between 450–600 nm when excited at 365 nm were observed. All spectroscopic characteristics of purified QDs correspond to those previously reported for CdS biosynthesized nanoparticles [21, 36, 38, 39].

**Discussion**

The selection process of cadmium and tellurite resistant bacteria from Antarctica revealed an extensive number of isolates resistant to each salt. After the initial QDs biosynthesis test, 12 resistant isolates displayed the ability to generate these fluorescent nanoparticles under the conditions evaluated. The 16S rRNA gene analysis classified the 12 resistant isolates up to genus level. All belong to Gammaproteobacteria, a class that predominates in several Antarctic environments [40]. Based on optimal growth temperatures, the 12 selected isolates were classified as psychrotolerant bacteria [41]. Some records have uncovered a predominance of psychrotolerant bacteria over psychrophilic ones in habitats dominated by low temperatures [42].
Bacillus subtilis has been previously associated with a high production of silver nanoparticles in nitrate reductase, just the NADH-dependent enzyme subject of extensive controversy. Recently, it has been suggested that other enzymes or molecules may be involved in the QDs biosynthetic process described here.

Halococcus salifodinae [48]. Interestingly, not all resistant bacteria showed nitrate reductase activity, suggesting that other enzymes or molecules may be involved in the QDs biosynthetic process described here.

Regarding heavy metal resistance, the 12 resistant bacteria displayed nitrate reductase activity, suggesting that other enzymes or molecules may be involved in the QDs biosynthetic process described here.

Regarding heavy metal resistance, the 12 resistant bacteria showed MIC values ranging from 62.5–1200 mg/L. These results indicate the presence of highly resistant bacteria, particularly if compared with resistance levels reported for several eukaryotic and prokaryotic cells (MICs between 1–50 mg/L) [37]. It has been reported that Antarctic bacteria are able to cope and/or adapt to pollutants even in low human-impacted environments [49–51], illustrating their stunning attributes as promising candidates for bioremediation purposes.

In Antarctica, most trace elements have a natural origin, and the accumulation of noxious metals have been subject of extensive controversy. Recently, it has been advertised that an increase in cadmium levels is linked to human activities [52]. Additionally, some penguins possess ways of absorption, elimination and bioaccumulation of this harmful element, which would support its presence in the Antarctic ecosystem [53]. These precedents suggest that Antarctica is not free of moderate cadmium anthropogenic pollution [54]. In the case of tellurite, no reports showing its presence in Antarctic territory have been published. Despite this, Arenas et al. [55] isolated some tellurite-resistant bacteria, emphasizing their remarkable ability to cope with such a highly oxidative toxicant.

The synthesis of QDs can be easily detected by specific color changes during cell incubation, [56]. The intracellular fluorescence color shift observed in all resistant bacteria across time, from green to red, is a unique feature of these nanocrystals [3, 21, 36].

To date, the mechanisms involved in biological synthesis of QDs remain poorly understood. Chemical methods follow steps involving nucleation and crystals growth [57]. On the other hand, QDs biosynthesis appears to be more complex. In general, bacterial synthesis is achieved by a reduction step followed by metal precipitation [58]. Some studies suggest that some bacterial defense mechanisms against oxidative stress generated by toxic metals, as thiols, could direct the formation of QDs [21, 59]. In this thiol-oxidizing interaction, the oxidant is neutralized by conversion to a less toxic byproduct. During the cell-response to the stress generated by Hg, Ag, As, Pb and Cd, GSH forms spontaneously ion-conjugates, which irreversibly depletes intracellular metal concentration [59, 60]. For the biosynthesis of CdTe nanocrystals, Te²⁻ ions are required to form the nanoparticle core [58]. Nevertheless, in our results, an intracellular black precipitate evidenced a marked reduction of tellurite to Te⁰, as widely reported in Gram-negative tellurite resistant bacteria [37]. Accordingly, the low fluorescence observed in bacterial cells treated with the mixture of cadmium and tellurite salts, may be due to the formation of CdS nanocrystals instead of CdTe, probably as consequence of the lack of Te²⁻ ions and an accelerated precipitation to Te⁰. Lately, Monrás et al. [21] pointed out the vital importance of thiols, especially GSH, and the cellular redox state for CdS and CdTe production in Escherichia coli. These antecedents support the astonishing antioxidant defenses of resistant bacteria from Antarctic as enhanced QDs producers.

A fundamental parameter for bioproduction of QDs is the temperature. Most CdS nanocrystals microbial synthesis reported to date are carried out at 37 °C [15]. Some studies showing lower synthesis temperatures than 37 °C have used different bacterial strains such as Lactobacillus sp. [39], R. palustris [61], R. sphaeroides [46], Brevibacterium casei [62] and some Antarctic strains.
Nonetheless, heretofore no bacterial strain has biosynthesized CdS nanocrystals at as high concentrations of CdCl₂ as determined in the present study, evidencing the enormous potential of Antarctic bacteria like powerful QDs producers and bioremediation agents in simultaneous processes at room temperature. Furthermore, most biosynthesis protocols using different microorganisms and plant extracts use an external source of S, as Na₂S [63], whereas in the procedure described here just metal precursors are added (no other additional reagent is required). Hence, the fundamental advantages of using Antarctic bacteria over previous bacterial QDs methods rely on avoiding the use of any additional reagents, room conditions to carry out the synthesis process, and producing nanoparticles using higher metal concentrations. We foresee that Antarctic microorganisms will contribute to a new era in future industrial production of nanomaterials and in the implementation of bioremediation tools.

Changes on the cellular ultrastructure were observed for the three representative isolates under QDs biosynthesis conditions, indicating physiological adaptations, essentially at the cell poles, as previously described for silver nanoparticles [16] and QDs [21]. The nanometric structures present in the cells endings subjected to QDs biosynthesis conditions were similar to those previously observed in the biosynthesis of Ag NPs [36]. The presence of structures similar to OMV is also observed. Cells utilize OMV to deal with diverse environmental stresses and their presence in NPs biosynthesis has been reported [43, 64–67]. The formation of OMV in R. sphaeroides has been linked to the transport of CdS nanostructures [46]. In this context, the production of OMV could be stimulated during biosynthesis of QDs as a way to eliminate nanoparticles from cells (entrapped within vesicles).

Finally, the wide fluorescence emission spectra of purified nanoparticles might be consequence of a high size-dispersion of nanoparticles [15, 68]. This is a typical behavior of QDs biosynthesis most probably related with differences in the metabolic state of culture cells when interacting with metals. In addition, time depending changes in emission colors (moving from green to red) observed in cell pellets excited at 360 nm is a characteristic feature of biosynthesized QDs [36]. Altogether, size and spectroscopic characteristics displayed on NPs produced by Antarctic bacteria after metal-exposure confirm the generation of fluorescent nanoparticles.

Conclusions
The present study demonstrates the successful synthesis of fluorescent nanoparticles by highly cadmium and tellurite resistant bacteria isolated from Antarctica. This ecofriendly approach was carried out under room conditions and higher concentrations of heavy metals than those previously reported. Further research will be critical to understand the biochemical and molecular mechanisms underlying the nanoparticles biosynthesis in order to identify key components that could improve the technology.

Finally, our findings provide meaningful insights for the development of greener strategies for QDs production and highlight the unique properties of microorganisms from Antarctica as potent natural producers of nanoscale materials and promising candidates for bioremediation purposes.

Methods
Sampling
Samples were collected during the 48th Chilean Antarctic Expedition, sponsored by the Chilean Antarctic Institute (INACH) in January 2012. Environmental samples of seawater, soil, sediments and ice were obtained from King George, Greenwich, Livingston, Deception, and Southern Shetlands Islands. Samples were taken from each site and placed in sterile 50 mL Falcon tubes and cold stored until their use.

Isolation of bacteria
100 mg (solid) or 100 µL (liquid) of samples were suspended on 1 mL (final volume) of sterile distilled water and stirred by vortexing for 30 min. Then, 10 µL of each suspension was used to inoculate 990 µL of different growth media, including Luria–Bertani (LB, 1X and 0.1X), Saline LB (4 % NaCl), R2A [69], ATCC [70] and marine medium (1 g yeast extract, 10 g tryptone, 5 g peptone in 1 L of sterilized sea water), incubated at 4–37 °C for 24–48 h. Pure bacterial cultures were obtained after successive transfers of single colonies to the corresponding medium and then stored by freezing at −80 °C in liquid medium with 30 % (v/v) glycerol.

Selection of cadmium and tellurite resistant bacteria
Bacterial isolates were screened by plating on LB agar supplemented with 200 mg/L CdCl₂ and 50 mg/L K₂TeO₃ (Sigma-Aldrich). Bacterial growth was evaluated after 24–48 h, and bacteria able to grow in both culture media were separated from the rest and designed as resistant isolates.

Optimal growth temperature
Resistant bacterial isolates were grown in LB medium until OD₆₀₀ = 0.2 was reached. Serial dilutions were performed in 96-well microplates and 5 µL of each dilution were used to inoculate LB plates. The incubation temperatures assayed were 4, 10, 15, 28 and 37 °C and bacterial growth was checked after 24 h inoculation.
**Identification of resistant bacteria**

Metal-resistant bacterial isolates were taxonomically classified by 16S rRNA gene sequencing analysis. For gene amplification a Colony PCR kit (Bionix Red, Bioline) was used. A single colony of each isolate was heated at 95 °C for 10 min to disrupt the cells. PCR reaction was performed using universal primers U515 F [71] and U1492 R [72] as follows: pre-heating 5 min at 95 °C, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing 30 s at 55 °C and extension 30 s at 72 °C, plus a final extension at 72 °C for 10 min. PCR products were evaluated by agarose gel (1 %) electrophoresis (stained with GelRed) and sequenced by Macrogen (Korea). Presence of chimeric sequences were discarded by DECIPHER tool [73]. The sequences were analyzed using tools from Ribosomal Database Project (RDP) (http://www.rdp.cme.msu.edu) [74]. Sequences were aligned using MUSCLE method [75] and grouped according to their closest neighbors in phylogenetic trees by neighbor-joining method. Distances were determined using the Maximum Likelihood by software MEGA v.6.0.6 [76]. The reproduction of each branch was performed by 1000 bootstrap analyses.

**Growth and metabolic characteristics of resistant bacteria**

Individual characteristics of selected bacterial colonies were evaluated using LB medium at 28 °C. Cell characterization was performed by Gram staining and by observation of morphology using a light microscope. Growth of resistant bacteria under different antibiotics was tested by Kirby-Bauer method [77] using 10 commercial strips (Valtek) for 24 h.

Metabolic characterization consisted in assaying substrates utilization and acid production from various sugars using API 20NE strips (BioMérieux Inc. Durham, NC). The results were obtained after 24–48 h inoculation. All assays were carried out according to the manufacturer indications.

**Minimal inhibitory concentration**

MIC values for cadmium and tellurite were determined by the method previously described by Pérez et al. [37]. Briefly, 10,000 mg/L of CdCl₂ and K₂TeO₃ stock solutions were placed in 300 µL of LB medium. Then, serial dilutions were performed in 96-well microplates and inoculated with 5 µL of bacterial cultures grown previously until an OD₆₀₀ ~0.5 was reached. Then, cells were collected and resuspended in two vials with phosphate-buffer saline (PBS) 50 mM pH 7.4, one containing CdCl₂ (10 mg/L) and other CdCl₂ (10 mg/L) + K₂TeO₃ (0.5 mg/L). Both treatments were incubated at 28 °C by 5 days. Each 12 h tubes were centrifuged at 12,000 rpm for 5 min, and QDs production (fluorescence of cellular pellets) was evaluated by UV light (365 nm) exposition using a transiluminator.

**Transmission electron microscopy**

Two strains were selected and treated with CdCl₂ (10 g/mL; as detailed above), and grown under QDs biosynthesis conditions for 36 h. Fluorescent cells were collected by centrifugation (10,000 rpm for 5 min), fixed using glutaraldehyde 2.5 % in cacodilate buffer 0.1 M pH 7.2 during 6 h at room temperature and washed with the same buffer for 18 h at 4 °C. Samples were fixed with aqueous osmium tetroxide (1 %) and uranile acetate (1 %). Finally, samples were dehydrated and infiltrated with an epoxy resin overnight. Ultrathin Sects. (60–70 nm thick) were obtained using an ultramicrotome (Sorval MT-5000) and placed in copper grids (Formvar carbon 300 mesh, grid hole size of 63 μm). Electron microscopy images were collected using a Phillips Tecnai 12 BioTwin microscope at 80 kV.

**Purification of biosynthesized nanoparticles**

Cell disruption was performed by adding 1 N sodium hydroxide (NaOH) to fluorescent bacterial pellets and incubating at 90 °C for 5 min, allowing QDs to stay in solution. Then, tubes were centrifuged at 10,000 g for 10 min in order to discard cellular debris. Aqueous phase (with the nanoparticles) was obtained after a separation of fluorescent fraction with chloroform (2:1) at room temperature. The resultant fraction was further purified by chromatography using a Sephadex G-75 column equilibrated and eluted with PBS buffer 50 mM. All obtained fractions were exposed to UV light (365 nm) to evaluate fluorescence, and then were concentrated using Amicon (10 kDa) tubes by centrifugation at 7000 rpm for 30 min.

**Spectroscopic characterization of biosynthesized nanoparticles**

Absorbance and fluorescence spectra of purified nanoparticles were determined by using a multiplate reader Synergy H1 M (Biotek) at room temperature. Emission spectra were obtained after excitation at 365 nm and recorded in the range of 400–700 nm.

**Authors’ contributions**

All authors contributed extensively to the present work. DOP participated in the experimental design, performed most of the experiments, analyzed the data and wrote the manuscript. CG participated in bacteria isolation from
Antarctica samples, PCR experiments and helped with developing methods. YDS participated in resistant bacteria selection, MIC determination and discussed the results. DB assisted with project organization and participated in the design of the study. JMP as the corresponding author, conceived the study, participated in experimental design, contributed to data analysis, and coordinated the manuscript writing. All authors have critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

Author details
1 BioNanotechnology and Microbiology Laboratory, Center for Bioinformatics and Integrative Biology (CBIB), Facultad de Ciencias Biológicas, Universidad Andres Bello, República # 239, Santiago, Chile. 2 Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Sergio Livingstone Pohlhammer # 1007, Santiago, Chile. 3 Laboratorio de Microbiología Oral, Facultad de Odontología, Universidad de Chile, Sergio Livingstone Pohlhammer # 943, Santiago, Chile.

Acknowledgements
This work was supported by Fondecyt 1151255 (J. M. P.), 1111:00076 (D.B.), Anillo ACT 1111 and 1107 (D. B. and J. M. P.), and INACH Grant MG-19-11 (J. M. P. and D. B.). D. O. Plaza gratefully acknowledges support from Programa de Formación de Capital Humano Avanzado de CONICYT for graduate fellowship and INACH Grant MG-01-13. The authors are also grateful for the valuable contribution of N. Bruna, F. Vega, G. Ullona and F. Venegas. Finally, we thank Alejandro Munizaga and staff from the Catholic University of Chile for their support on Electron Microscopy facilities.

Competing interests
The authors declare that they have no competing interests.

Received: 27 January 2016 Accepted: 27 April 2016
Published online: 06 May 2016

References
1. Adams FC, Barbante C. Nanoscience, nanotechnology and spectrometry. Spectrochim Acta Part B. 2013;86:3–13.
2. Kovalenko MV, Mann L, Cabot A, Hens Z, Talapin DV, Kagan CR, Klimov XVI, Rogach AL, Rees P, Milliron DJ, Guyot-Sionnont P, Konstantatos G, Parak WJ, Hyeon T, Korgel BA, Murray CB, Hess W. Prospects of nanoscience with nanocrystals. ACS Nano. 2015;2:1012–57.
3. Kershaw SV, Susha AS, Rogach AL. Narrow bandgap colloidal metal chalcogenide quantum dots: synthetic methods, heterostructures, assemblies, electronic and infrared optical properties. Chem Soc Rev. 2013;42:3033–87.
4. Liu T, Liu B, Zhang H, Wang Y. The fluorescence bioassay platform on quantum dots nanoparticles. J Fluoresc. 2005;15:729–33.
5. Selinsky RS, Doring FA, Faber MS, Wight JC, Jin S, Sarah R. Quantum dot nanoscale heterostructures for solar energy conversion. Chem Soc Rev. 2013;42:2963–85.
6. He X, Ma N. An overview of recent advances in quantum dots for biomedical applications. Colloids Surf B Biointerfaces. 2014;124:118–31.
7. Hines DA, Kamat PV. Quantum dot surface chemistry: ligand effects and electron transfer reactions. J Phys Chem C. 2013;117:14418–26.
8. Biju V, Toth T, Anas A, Sujith A, Ishikawa M. Semiconductor quantum dots and metal nanoparticles: synthesis, optical properties, and biological applications. Anal Bioanal Chem. 2008;391:2496–95.
9. Karakoti AS, Shukla R, Shanker R, Singh S. Surface functionalization of quantum dots for biological applications. Adv Colloid Interface Sci. 2014;215:28–45.
10. Kharissova OV, Dias HV, Kharisov BI, Olvera B, Jiménez VM. The greener synthesis of nanoparticles. Trends Biotechnol. 2013;31:240–8.
11. Mohanpuria P, Rana NK. Biosynthesis of nanoparticles: technological concepts and future applications. J Nanopart Res. 2008;10:507–17.
12. Thakkar KN, Mhatre SS, Parikh RY. Biological synthesis of metallic nanoparticles. Nanomedicine. 2010;6:257–62.
13. Dutan N, Marcato PD, Durán M. Mechanistic aspects in the biogenic synthesis of extracellular metal nanoparticles by peptides, bacteria, fungi, and plants. Appl Microbiol Biotechnol. 2011;90:1609–24.
fluorescent semiconductor nanoparticles (CdS) by oxidative stress resistant Antarctic bacteria. J Biotechnol. 2014;187:108–15.

37. Pérez JM, Calderón I, Arenas FA, Fuenteras DE, Pradesana GA, Fuenteras EL, Sandoval JM, Castro ME, Ales A, Vázquez CC. Bacterial toxicity of potassium tellurite: unveiling an ancient enigma. PLoS One. 2007;2:e211.

38. Dameron CT, Reese RN, Mehra RK. Biosynthesis of cadmium sulphide quantum semiconductor crystallites. Nature. 1989;338:596–7.

39. Prasad K, Jha AK. Biosynthesis of CdS nanoparticles: an improved green and rapid procedure. J Colloid Interface Sci. 2010;342:68–72.

40. Yu Y, Li H, Zeng Y, Chen B. Phylogenetic diversity of culturable bacteria from Antarctic sandy intertidal sediments. Polar Biol. 2010;33:669–75.

41. De Maayer P, Anderson D, Cary C, Cowan DA. Some like it cold: understanding the survival strategies of psychrophiles. EMBO Rep. 2014;15:508–17.

42. Shivaji S, Begum Z, Soma S, Nageswara S, Thamban M, Krishnan KP, Singh SM, Srinivas TNR. Antarctic ice core samples: culturable bacterial diversity. Res Microbiol. 2013;164:70–82.

43. Frias A, Manresa A, De Oliveira E, López-iglesias C, Mercade E. Membrane vesicles: a common feature in the extracellular matrix of cold-adapted Antarctic bacteria. Microbiol. 2010;59:475–86.

44. Safiuddin N, Wong CW, Yasumira AAN. Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. J Chem. 2009;6:61–70.

45. Kalimuthu K, Babu RS, Venkataraman D, Bilal M, Gurunathan S. Biosynthesis of silver nanocrystals by Bacillus licheniformis. Colloid Surf B. 2008;68:150–3.

46. Bai H, Yang B, Chai C, Yang G, Jia W, Yi Z. Green synthesis of silver nanoparticles using Rhodobacter Sphaeroides. World J Microbiol Biotechnol. 2011;27:273–8.

47. El-Shanshoury AER, Elsilk SE, Ateya PS, Ebeid EM. Synthesis of lead nanoparticles using culture supernatant of bacteria with microwave irradiation. J Microbiol. 2012;62:1803–10.

48. Srivastava P, Braganca JM, Kowshik M. In vivo synthesis of selenium nanoparticles from Antarctic shallow sediments towards heavy metals, antibiotics and antibiotic-resistant in psychrotrophic bacterial from Antarctic marine waters. Ecotoxicology. 2006;15:379–84.

49. Aronson RB, Thatje S, McClintock JB, Hughes KA. Anthropogenic impacts of Antarctic bacteria. J Biotechnol. 2009;27:265–72.

50. Lorenz N, Hintemann T, Kramarewa T, Katayama A, Yasuta T, Marschner MA, Srinivas TN. Antarctic ice core samples: culturable bacterial diversity. Res Microbiol. 2013;164:70–82.

51. El-Shanshoury AER, Elsilk SE, Ateya PS, Ebeid EM. Synthesis of lead nanoparticles using culture supernatant of bacteria with microwave irradiation. J Microbiol. 2012;62:1803–10.

52. Alipieva E, Zlatov AS, Polisichuk VA, Brukhovetskyi AP, Grigoriev DE, Federation R. Influence of quantum dots size dispersion on the fluorescence spectrum. Laser Phys Appl. 2013;87:701–7.

53. Reasoner DJ, Geldreich EE. A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol. 1985;49:1–7.

54. Chiong M, Barra R, González E, Vázquez C. Resistance of Thermus spp. to potassium tellurite. Appl Environ Microbiol. 1988;54:610–2.

55. Reysenbach AL, Pace NR. Reliable amplification of hyperthermophilic archaeal 16S rRNA genes by PCR. In: Robb FT, Place AR, editors. Thermophiles. New York: Cold Spring Harbor Press; 1995. p. 101–6.

56. Suzuki MT, Giovannianni SJ. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. Appl Environ Microbiol. 1996;62:25–30.

57. Wright ES, Yilmaz LS, Noguera DR. DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. Appl Environ Microbiol. 2012;78:717–25.

58. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, Brown CT, Porras-Alfaro A, Kuske CR, Tiedje JM. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 2014;42:633–42.

59. Edgar RC, Drive RM, Valley M. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32:1792–7.

60. Tamura K, Stecher G, Peterson D, Filipski A, Kuma S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30:7725–9.

61. Bauer AW, Kirby WM, Sherris JC, Turck M. A standard for antibiotic susceptibility testing. J Clin Pathol. 1966;19:453–6.