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# "Use of acidophilic bacteria of the genus *Acidithiobacillus* to biosynthesize CdS fluorescent nanoparticles (quantum dots) with high tolerance to acidic pH"

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ABSTRACT

The use of bacterial cells to produce fluorescent semiconductor nanoparticles (quantum dots, QDs) represents a green alternative with promising economic potential. In the present work, we report for the first time the biosynthesis of CdS QDs by acidophilic bacteria of the *Acidithiobacillus* genus. CdS QDs were obtained by exposing *A. ferrooxidans*, *A. thiooxidans* and *A. caldus* cells to sublethal $Cd^{2+}$ concentrations in the presence of cysteine and glutathione. The fluorescence of cadmium-exposed cells moves from green to red with incubation time, a characteristic property of QDs associated with nanocrystals growth. Biosynthesized nanoparticles (NPs) display an absorption peak at 360 nm and a broad emission spectra between 450 and 650 nm when excited at 370 nm, both characteristic of CdS QDs. Average sizes of 6 and 10 nm were determined for green and red NPs, respectively. The importance of cysteine and glutathione on QDs biosynthesis in *Acidithiobacillus* was related with the generation of $H_2S$. Interestingly, QDs produced by acidophilic bacteria display high tolerance to acidic pH. Absorbance and fluorescence properties of QDs was not affected at pH 2.0, a condition that totally inhibits the fluorescence of QDs produced chemically or biosynthesized by mesophilic bacteria (stable until pH 4.5–5.0). Results presented here constitute the first report of the generation of QDs with improved properties by using extremophile microorganisms.


## 1. Introduction

The use of microorganisms to synthesize metal nanostructures has gained increased interest during the last years. The interaction of microorganisms with metal ions is the base of several applications, and the formation of different metallic nanostructures in bacterial cells exposed to gold, silver, lead, titanium and cadmium salts have been reported [1–4].

In particular, the biosynthesis of cadmium sulfide (CdS) nanoparticles has been extensively studied in different bacterial models because of their tremendous biotechnological applications [5,6]. Bacterial biosynthesis of CdS nanoparticles (NPs) offers several advantages over chemical methods since produced NPs display high stability, water solubility, biocompatibility, low costs and are environmentally friendly [5,6].

The formation of CdS NPs is related with the capacity of microorganisms to tolerate heavy metals, mainly by the participation of thiols and enzymatic activities that favor the conversion of metal ions into nanocrystals [5,7]. Previous examples of cadmium detoxification included the formation of CdS through the interaction of the metal with $S^{-2}$ on the surface of *Klebsiella pneumoniae* cells or inside engineered *Escherichia coli,* and the formation of peptide-capped intracellular CdS nanoparticle within *Schizosaccharomyces pombe* yeast cells [8–10].

During the last years, part of the research in Nanotechnology has been oriented to develop novel synthesis methods that allow the improvement of NPs properties and applications [11]. In this context, special interest exists in avoiding the sensitivity of NPs to some environmental conditions and compounds that disrupt the

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nanocrystal and affect the performance of QDs-based technologies. Among these conditions we can mention UV radiation, temperature and acidic pH.

Radiation affects the properties of NPs and their applications by causing photo corrosion in QDs Sensitized Solar Cells (QDSSCs) and UV photodetectors [12–14]. Also, QDs used in solar cells must tolerate significant changes in temperature, moving from high temperatures when exposed to light to very low temperatures during the night. Regarding pH, controlling pH-sensitivity of NPs favors their metabolic distribution and body secretion in biomedical applications [15]. In addition, intracellular trafficking studies require the use of highly florescent dyes that maintain their properties inside acidic intracellular compartments, such as lysosomes and vacuoles.

It has been reported that Cd and Cu-QDs are highly sensitive to pHs below 5,0 [16]. The exposition of CdS-capped CdSe QDs to acidic conditions degrades the QDs and quench their fluorescence, even in the presence of different coatings [17]. At acidic pH, Cd-QDs coated with mercaptopropionic acid or alkylated polycarboxylic acid rapidly aggregate and suffer acid-etching. Only those QDs stabilized by cationic charges at their surface (i.e. polyethyleneimine, PEI) can resist the acid-etching and maintain their spectroscopic properties [17]. In the same context, coating of CdSe/CdS/CdZn QDs with PEI protects the nanorystals from the exposure to gastric fluids (pH 1,0–2,0), allowing to mantain 60–70% of their fluorescence [18].

To date, NPs biosynthesis has not been described in acidophilic microorganisms. Acid-leaching solutions are characterized by high metal concentrations that are toxic to most life forms and have historically been considered 'sterile'. Nevertheless, there are numbers of microorganisms surviving in acid-leaching environments that present different mechanisms to tolerate acidic conditions and high concentrations of toxic metals [19]. Based on this, acidophilic bacteria from hydrometallurgical extraction processes represent an ideal system to study and understand the mechanism of biomineralization of NPs in acidic conditions. This would also allow to increase the capacity to extract metals such as copper and cadmium, but also could represent an alternative to generate high value metal NPs as a byproduct of mining operations. Estimations indicate that the global market of QDs will reach US$4704 million by 2020, at a Compound Annual Growth Rate (CAGR) of 63.61% from 2014 to 2020 [20].

In this work, three commercial strains belonging to the *Acidithiobacillus* genus were tested for their ability to produce CdS QDs. *A. ferrooxidans* (ATCC 23270), *A. thiooxidans* (ATCC 19703) and *A. caldus* strain ATCC 51756 were used to biosynthesize CdS QDs. Also, the stability of QDs to acidic environments was compared with those produced by other mesophilic bacteria or by chemical methods.

## 2. Materials and methods

### 2.1. Bacterial strains and growth conditions

Three acidophilic bacterial strains were used in this study: *A. ferrooxidans* ATCC 23270, *A. thiooxidans* ATCC 19703 and *A. caldus* strain ATCC 51756. The culture medium used for bacterial growth was a basal medium adjusted to pH 2.3 [0.4 g/L $(NH_4)_2SO_4$, 0.4 g/L $MgSO4 \times 7H_2O$ and 0.056 g/L $KH_2PO_4$], supplemented with spherical prills of elemental sulfur ($S_8$) as an energy source for the growth of microorganisms [21,22]. Sulfur beads (1% w/v) previously colonized by the respectively *Acidithiobacillus* strain were used for bacterial growth. The liquid cultures of each strain were grown for 7 days in flasks with constant agitation (150 rpm) at their respective optimal growth temperature (28 °C for *A. ferrooxidans* and *A. thiooxidans*, and 40 °C for *A. caldus*) [21,22].

### 2.2. Cadmium resistance assays

MICs–$CdSO_4$ of each acidophilic strain were determined over a wide range of metal concentrations (50–500 mM). The MIC value for cadmium was determined as the concentration showing a 90% decrease ($p < 0.01$) in bacterial growth when compared with the control, measured by direct counting in a Petroff-Hauser chamber after 7 days incubation. A Petroff–Hausser chamber model Thoma was used (double grid, 0.01 mm depth and 0.0025 $mm^2$ area), being the limit of detection for the technique $6{,}25 \times 10^5$ bacteria/mL.

### 2.3. Biosynthesis of nanoparticles

Biosynthesis of CdS nanoparticles was evaluated in the three acidophilic strains according to the protocol previously described by Gallardo et al. [23] with some modifications. Briefly, cells were grown to stationary phase in basal media pH 2.3, sedimented by centrifugation and then resuspended in phosphate buffers (pH 3, 5 and 7) with the presence of cadmium (0.66 mM) and thiols (cysteine and glutathione 5 mM). To do this, bacterial strains were concentrated by centrifugation, resuspended in phosphate buffer to reach an $OD_{620nm}$ 1.3 (approx. $1 \times 10^{11}$ cells/mL), and exposed to cadmium (0.33–5 mM) and thiols for 24 h at the optimum growth temperature of each strain. Samples were then centrifuged 5 min at $10{,}000 \times g$ and the fluorescence of cell pellets and supernatants was evaluated after excitation at 360 nm using a UV-transilluminator. To evaluate the fluorescence of cell pellets over time, pellets of cells exposed to biosynthesis conditions for different times were washed twice with 50 mM Tris-HCl pH 7.4, centrifuged 5 min at $10.000 \times g$, and exposed to UV light as described above.

### 2.4. Purification of intracellular nanoparticles

50 mL of an *A. thiooxidans* culture producing QDs were concentrated by centrifugation at $10{,}000 \times g$ for 5 min and washed twice with 50 mM Tris-HCl pH 7.4. Cell lysis was performed by mechanical methods using glass beads for 3 cycles of homogenization in a Bead Beate homogenizer (MP-Biomedicals). Subsequently, the resulting solutions were centrifuged 10 min at 14,000 x *g* and the supernatant solutions were loaded on Sephadex columns G75 for gel filtration. Fluorescent fractions were concentrated and characterized.

### 2.5. Purification of extracellular nanoparticles

50 mL of an *Acidithiobacillus* cell culture producing QDs were concentrated by centrifugation at $10{,}000 \times g$ for 5 min. The resulting fluorescent supernatant solution was collected. To remove unreacted reagents the reaction mixture was filtered using 0.2 μm filters, centrifuged twice in the presence of ethanol and the fluorescent fraction was then centrifuged at $7{,}000 \times g$ using Amicon filters with a 3 kDa pore size (0.3–0.5 nm) to obtain a concentrated solution (200 μL). Purified NPs were used for characterization experiments.

### 2.6. Absorption and fluorescence spectroscopy

Absorbance and fluorescence spectra of purified nanoparticles were determined by using a fluorescence multiplate reader, Synergy H1M (Biotek). Fluorescence spectra were obtained after excitation at 360 nm.

### 2.7. Dynamic light scattering (DLS)

The size of NPs composing the purified fluorescent fractions was determined using a S90 instrument (Malvern Instruments Limited,

**Table 1**
Cadmium MICs for *Acidithiobacillus* strains.

| Strain | MIC (mM) | | | | | |
|---|---|---|---|---|---|---|
| | #Cd | *Cd | *As | *Cu | *Zn | *Ni |
| *A. ferrooxidans* ATCC 23270 | 400 | 500[a] | 84[a] | 800[a] | 1071[b] | 1000[c] |
| *A. thiooxidans* ATCC 19703 | – | | | | | |
| *A. caldus* ATCC 51756 | 200 | | | 250[d] | 75[d] | |

# Data obtained in this work.
* MICs previously reported in the following references: a. Harvey & Crundwell [25]; b. Kondratyeva et al. [26]; c. Dew et al. [24] and d. Aston et al. [27].

UK) with a refraction index of 2.6 and 4-optical sides disposable cuvettes.

### 2.8. Transmission electron microscopy (TEM)

A suspension of purified nanoparticles was deposited on a copper grid and analyzed with a Low Voltage Transmission Electron Microscope 5 (LVEM5) (Delong Instruments) operated at 5.1 kV. Based on the TEM image of NPs a size distribution histogram was performed using the ImageJ software.

### 2.9. pH-susceptibility of CdS QDs

Green and red cadmium sulfide QDs were prepared by different protocols and their stability to pH was evaluated. CdS QDs tested were: 1) QDs produced by *Acidithiobacillus*; 2) CdS QDs biosynthesized by *E. coli* BW25113 using the protocol described by Monrás et al. [5], and 3) Biomimetic CdS QDs prepared by a chemical method described by Pérez-Donoso et al. [24]. Purified QDs were diluted ten times in solutions adjusted at pH 1–3, 5 or 6, and incubated at room temperature for 40 min. Then, the emission spectrum of each QDs solution (exc. 360 nm) was determined.

## 3. Results and discussion

### 3.1. Acidithiobacillus *cadmium resistance*

The acidophilic strains used in this work naturally exhibit significant resistance to a great variety of metals such as Cu, Zn and Cr [25]. However, cadmium tolerance of sulfur-oxidizing strains has been poorly studied and there are significant variations in the resistance levels determined, probably as consequence of the use of different strains and/or experimental conditions such as culture media and $Cd^{2+}$ sources [26–29]. In addition, most QDs biosynthetic methods described to date involve the use of cadmium salts, such as $CdCl_2$, however, acidophiles have little tolerance to chloride ions. Chloride is known to inhibit both cell growth and $Fe^{2+}$ oxidation by enzyme systems in acidophilic iron oxidizers [30]. The growth rate of a mixed culture of acidophilic bacteria is reduced >50% in presence of NaCl (1,2 mM) as a consequence of the negative effect of chloride on sulfur oxidation [30]. Based on this, to determine $Cd^{+2}$ toxicity on *Acidithiobacillus* strains we used $CdSO_4$ as $Cd^{2+}$ source.

The minimum inhibitory concentration was determined (MIC) in three strains of the genus *Acidithiobacillus* grown in basal medium pH 2.3 (Table 1). High resistance levels were determined on the three strains evaluated, particularly when compared with the MIC presented by other microorganism such as *E. coli* (2 mM) that has been used for Cd-based QDs biosynthesis [9,31]. Cadmium resistance of the iron-oxidizing *A. ferrooxidans* (400 mM) was higher than in the sulfur-oxidizing bacterium *A. caldus* and *A. thiooxidans* (200 mM). These similarities in Cd-resistance in sulfur-oxidizing strains are in agreement with the strong similarities existing in metabolic and phenotypic characteristic between these two strains and could be evidence of a common Cd-resistance mechanism among these acidophiles [28,32]. The mechanism of cadmium toxicity and resistance in these strains is still unknown, and based on the information available it is not possible to associate the levels of Cd-resistance determined with specific cellular processes or targets, particularly with their capacity to oxidize sulfur or iron.

Several microorganisms, including bacteria and fungus, have been used for biosynthesizing cadmium-based QDs [5,23,33,34–36]. In most works reported to date biomolecules such as antioxidant thiols are the main precursors of the biosynthetic process, particularly GSH, CYS and $H_2S$ [5,23,34,36–40]. In the same way, it has been observed that the production of high concentrations of $H_2S$ by cysteine desulfhydrases enzymes in presence of CYS is responsible for the formation of CdS nanocrystals in *Rhodopseudomonas palustris* [37]. This year it was reported the in vitro production of CdS QDs by the enzyme cystathionine γ-lyase (smCSE) from *Stenotrophomonas maltophilia*, in a process involving $H_2S$ production in presence of CYS and GSH [7].

Recently, we reported the low temperature (15 °C) production of Cd-based QDs by Antarctic *Pseudomonas* sp. strains with increased tolerance to reactive oxygen species (ROS) and by Antarctic bacteria highly resistant to cadmium and tellurite [23,36]. In both cases the ability of these Antarctic isolates to biosynthesize CdS-QDs was linked to the generation of hydrogen sulfide (sulfur donor for CdS nanoparticle core formation) and their antioxidant defenses [23,36].

In this context, it has been reported that *Acidithiobacillus* genus produce sulfur-reduced compounds intracellularly (mainly sulfite and sulfide) and also that can storage polysulfur compounds in the form of R-Sn-R (R-, reduced thiol) [41–44]. Polysulfur compounds are involved in the generation of reduced species such as $H_2S$ and GSH [42], both of them associated with metal resistance and recently with the production of Cd-quantum dots [23,36].

Acidophilic sulfur-oxidizing bacteria produce very small quantities of $H_2S$ and most of it is rapidly consumed by the sulfide quinone oxidoreductase enzyme (SQR), or its used for amino acids generation [41,44]. Nevertheless, we determined that the addition of cysteine (5 mM) and glutathione (5 mM) to *Acidithiobacillus* cultures grown in basal medium pH 2.3 increase the production of hydrogen sulfide in culture headspaces (Supplementary Fig. S1 in the online version at DOI: http://dx.doi.org/10.1016/j.enzmictec.2016.09.005).

So, considering the high cadmium resistance determined in *Acidithiobacillus,* and the increase in $H_2S$ production in cultures amended with CYS and GSH, we decided to evaluate the capacity to biosynthesize CdS QDs by these bacterial strains.

### 3.2. Biosynthesis of CdS QDs by Acidophilic bacteria

Biosynthesis of CdS nanoparticles by *Acidithiobacillus* strains was carried out following the protocol previously described by Gallardo et al. [23] with some modifications as described in methods. QDs biosynthesis in *Acidithiobacillus* strains occurs in cells incubated at 28 °C for *A. thiooxidans*/*A. ferrooxidans* and 40 °C for *A. caldus* (Fig. 1). After 24 h exposure to biosynthetic conditions, bacterial pellets exhibit fluorescence when excited with UV light (360 nm), indicative of CdS QDs synthesis [5,23,35,36]. No variations in fluorescence colors and intensities were observed when pHs between 3.5 and 7 were used, however, no fluorescence was determined at pHs 2 and 3 (Supplementary Fig. S2 in the online version at DOI: http://dx.doi.org/10.1016/j.enzmictec.2016.09.005).

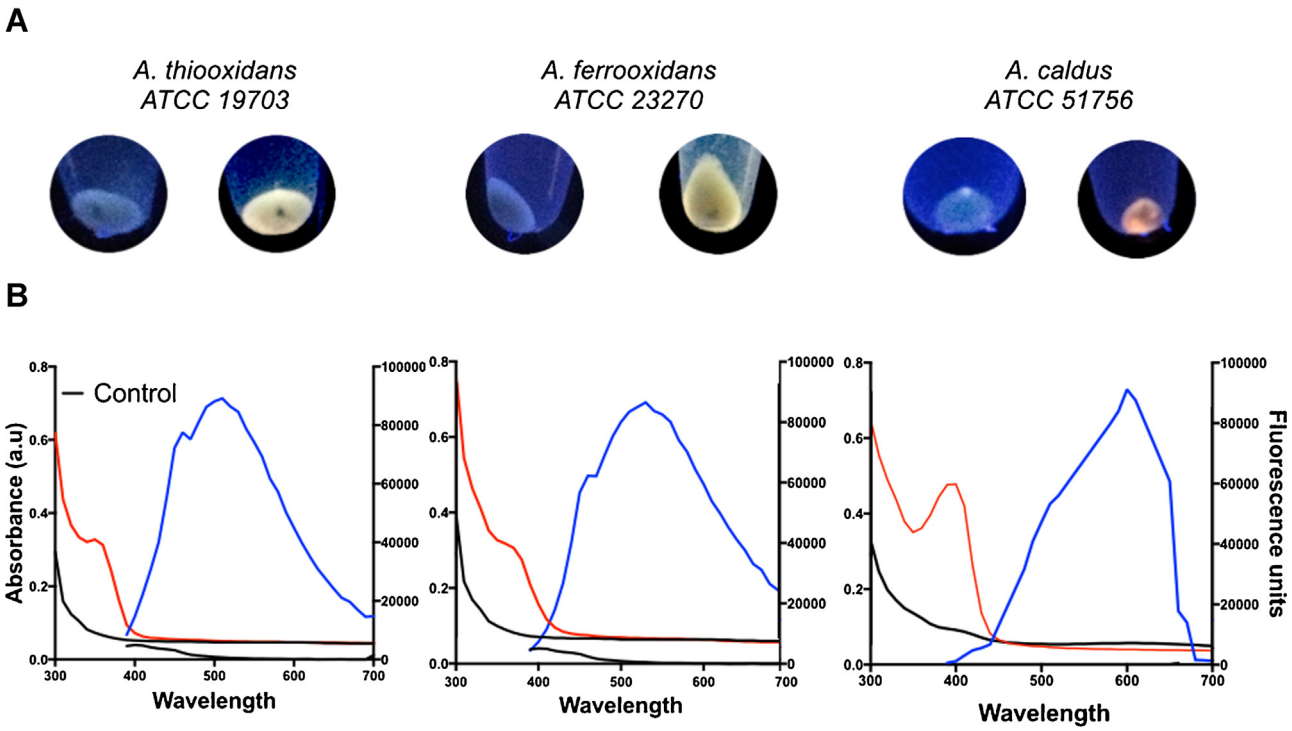


**Fig. 1.** Biosynthesis of CdS QDs by *Acidithiobacillus* strains. A) Acidophilic strains were exposed to biosynthesis conditions (cysteine 5 mM and cadmium 0,66 mM) for 24 h and the fluorescence of cell pellets exposed to UV was determined. Pellets of cells not exposed to QDs biosynthesis conditions are shown at the left. B) Spectroscopic properties of purified CdS nanoparticles produced by *Acidithiobacillus*. Absorption (red line) and emission spectra (blue line) (exc. 360 nm) of purified QDs were recorded. The black line on graphs correspond to the absorbance and emission spectra of controls obtained from cells not exposed to metals (only treated with cysteine).

Differences in the emission color of cell pellets were observed between strains, being *A. caldus* red fluorescent and *A. thiooxidans/A. ferrooxidans* green colored (Fig. 1A). These differences are most probably related with the high incubation temperature required for *A. caldus* that favors the production of bigger QDs. In addition, observed fluorescence in the three strains was consequence of the exposure to cadmium since in the negative control (no metal added) no emission was observed after UV-excitation (Fig. 1A).

The NPs present in fluorescent pellets produced by all biomining strains were purified and fluorescence/absorbance spectra of biosynthesized nanoparticles determined (Fig. 1B). The absorbance spectra of the three acidophilic strains shows one peak between 360 and 400 nm, that is in agreement with absorption properties of biological CdS nanoparticles synthesized in other microorganisms [23,35,36]. By the other hand, the emission profiles of purified samples after excitation at 360 nm display an emission peak between 470 and 550 nm for CdS-QDs obtained from green-yellow colored pellets and near 600 nm for those obtained from red pellets. Fluorescence spectra determined for QDs produced by *Acidithiobacillus* strains display the same characteristic than those described in other biosynthesized CdS QDs. Differences observed in emission spectra of purified QDs strongly suggests that cells are biosynthesizing QDs with different sizes and correlates with the color observed in cell pellets [23,36].

In order to evaluate changes in fluorescence color of cells over time, QDs biosynthesis of *A. caldus* ATCC 51756 was evaluated at different times of exposure to biosynthesis conditions. Fluorescence emission colors of cell suspensions changed at different exposure-times, moving from blue to red emission after 24 h incubation (Fig. 2A). This behaviour was observed in the three *Acidithiobacillus* strains evaluated (not shown) and is characteristic of QDs. Changes in fluorescence emission color are related with increasing nanocrystal size that changes the band gap and spectroscopic properties of nanoparticles [5,23,29,34,36].

Also, QDs present in green and red fluorescent cultures of *A. caldus* ATCC 51756 were purified and their spectroscopic characteristics determined. Green CdS QDs with an absorbance maximum at 350–400 nm were observed after 8 h incubation (Fig. 2B). Spectroscopic analysis revealed differences in absorbance spectra of aliquots obtained at different times. The absorbance peak at 370 nm become more defined at higher incubation times, a situation that most probably is consequence of an increase in the number of nanocrystals generated. In the case of fluorescence emission, a transition from cyan-green to orange-red was observed after 24 h exposure to biosynthesis conditions. Accordingly, emission spectra of purified QDs moves to longer wavelengths with exposure times (Fig. 2B). This phenomenon indicates a time-dependent increase on NPs size which is a characteristic of biosynthesized QDs [5,35,36]. Broad emission spectra were determined in purified NPs. This situation is commonly seen in biosynthesized NPs and most probably correspond to the generation of QDs with different sizes. Broad emission constitute a disadvantage when biological NPs are compared to those chemically produced. However, understanding the mechanisms of bacterial synthesis of NPs will contribute to improve the process and generate new protocols that allow the generation of NPs with narrow size distribution and well defined emission spectra.

To evaluate the size of NPs biologically produced by acidophilic bacteria, a DLS assay was performed on green and red QDs produced by *A. thiooxidans*. Obtained results confirmed the presence of nano-sized material in green and red QDs purifications, with average size dimensions of 6.9 and 10 nm, respectively (Fig. 3A and B). Both results are consistent with NPs sizes reported for this type of biological synthesis [30]. A polydispersity index of 0.64 and 0.85 were determined for green and red QDS, respectively. Accordingly, two main populations in the nanometric range were determined in both NPs purifications. The presence of populations near 100 and 400 nm in green and red NPs, respectively, could be consequence

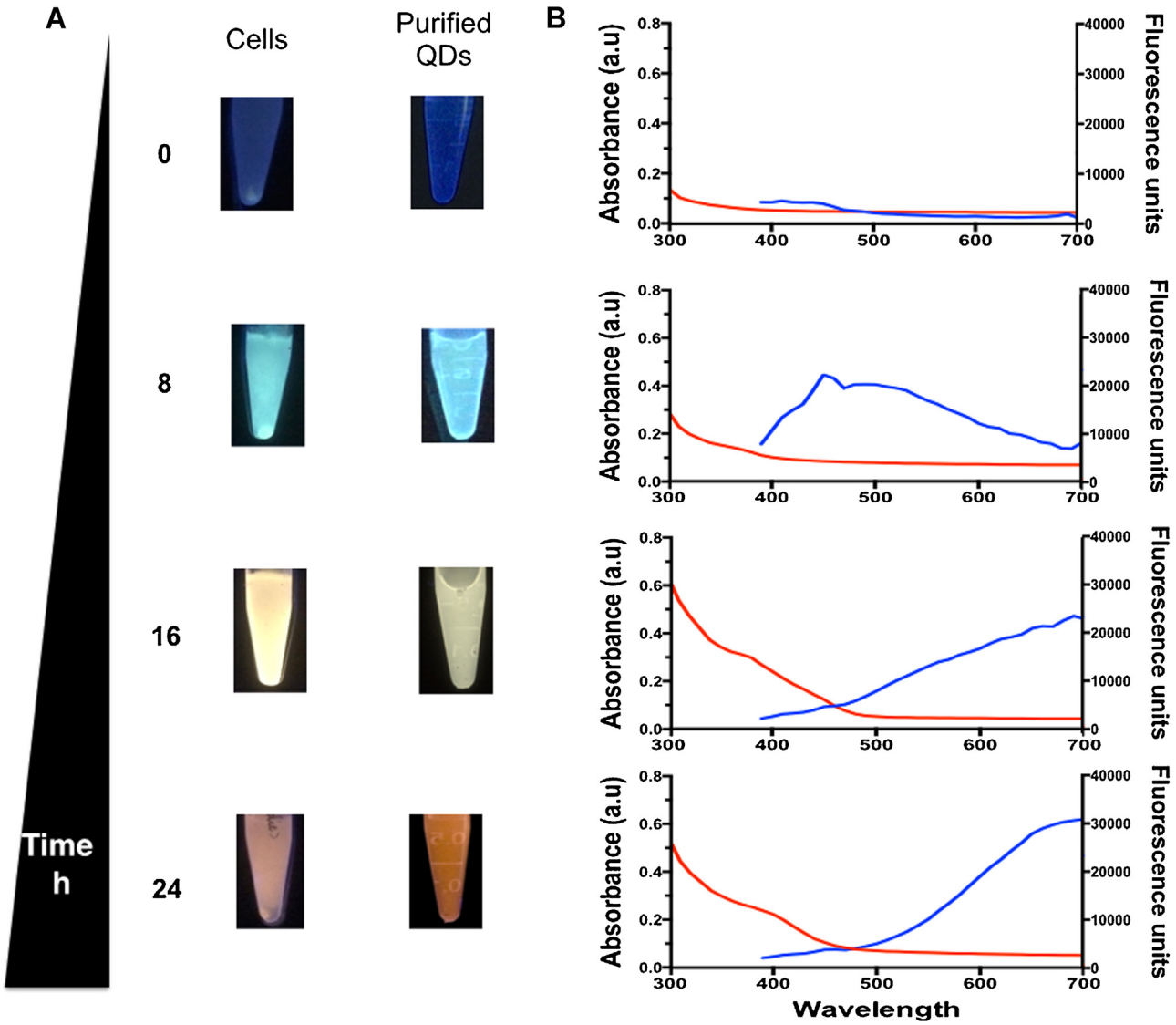


**Fig. 2.** Effect of incubation time on the spectroscopic properties of CdS QDs biosynthesized by *A. caldus* ATCC 51756. (A) Cells exposed for different times to biosynthesis conditions and the QDs corresponding purified fractions were exposed to UV light (360 nm). (B) QDs fluorescent fractions produced at different incubation times were purified and the absorption (red line) and emission spectra (exc. 370 nm, blue line) determined.

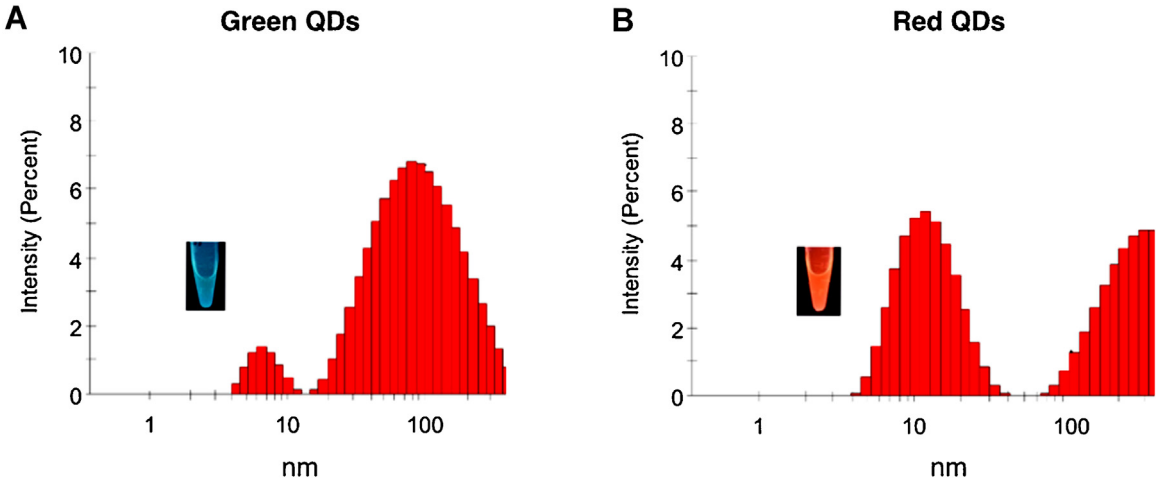


**Fig. 3.** Size determination of CdS QDs biosynthesized by *A. thiooxidans*. DLS analysis were performed to green (A) and red (B) fractions of purified nanoparticles.

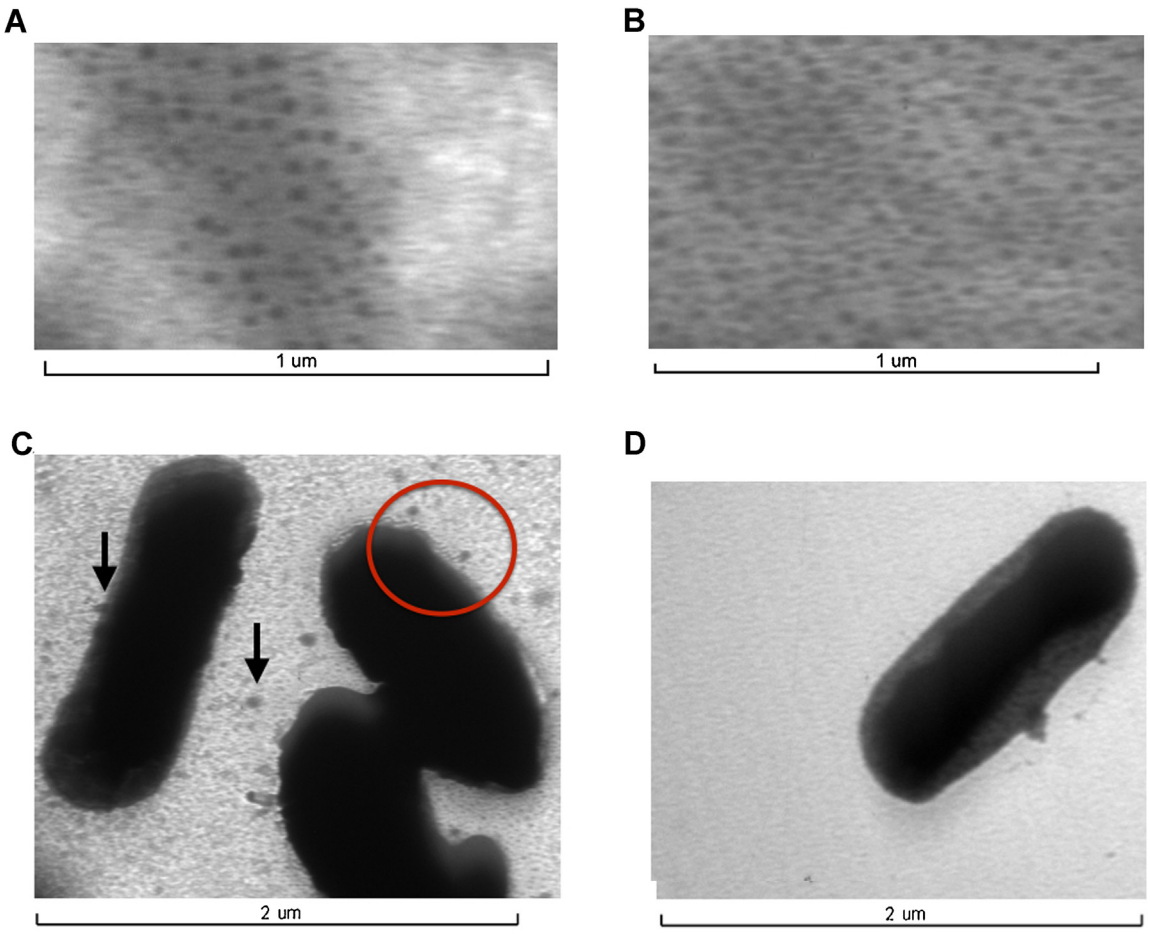


**Fig. 4.** TEM image of purified NPs and *A. thiooxidans* cells synthesizing NPs. A) The TEM image shows nanocrystals purified from pellets (A) or from cell supernatants (B) (see methods for details). C) *A. thiooxidans* cells synthesizing NPs. Electrondense extracellular material with nanometric dimensions is indicated by an arrow and the circle shows extracellular nanostructures probably interacting with components of the cell envelope. D) *A. thiooxidans* cells unexposed to biosynthesis conditions were used as control.

of aggregation. Experiments to determine the presence and importance of biological molecules on aggregation and properties of NPs are currently been performed in our laboratory.

Also, purified NPs produced inside and outside cells were evaluated by TEM. As observed, a uniform population of nanosized material with sizes below 40 nm was present in the fluorescent purifications obtained from cells (obtained from pellets or supernatants) (Fig. 4A and B). These results are in agreement with presented DLS studies and confirm the generation of nanostructures with sizes expected for QDs. As mentioned before, the presence of NPs of higher size has been reported in biological samples, and are probably consequence of an organic layer incorporated during the intracellular synthesis of NPs [5,33].

TEM analysis of cells synthesizing QDs was used to evaluate if the biosynthetic condition affect the cell morphology of *A. thiooxidans*, and also to determine the cellular location of NPs (Fig. 4C and D). Cells biosynthesizing QDs display the typical morphology of *A. thiooxidans* (compare with the control) a result that indicates that biosynthesis do not affect cellular morphology. Surprisingly no stress signals such as filamentation, degradation or polar localization of electron dense material was observed. Previous reports have indicated these signals in different bacterial cells producing QDs, particularly the accumulation of electron dense nanomaterial at the cell poles and the formation of structures similar to vesicles [5,30,31]. This observation is probably consequence of the high Cd-resistance determined in *Acidithiobacillus* strains. The presence of extracellular electron dense granules (arrow, Fig. 4C) was observed in cells producing QDs. This electron dense material was purified and the presence of nanomaterial with similar characteristics to that obtained from intracellular purifications was observed (compare Fig. 4A and B).

### 3.3. CdS QDs biosynthesized by A. thiooxidans are tolerant to acidic pH

The progress and future prospects of QDs applications are limited in part by their instability to some environmental conditions such as light exposure (photosensibility), solvents, osmolality, temperature and pH. Currently there is an increasing demand for controlling the properties of nanoparticles in order to increase their potential applications. For instance, it is known that the performance of QDs based technologies is affected by the constant exposure to photons in the visible and infrared spectra, and by UV overexposure [36].

In this context, the search of green, simple and controllable alternatives to synthesize NPs with novel properties is a topic of great interest [25,26]. Based on this, we evaluated the stability and fluorescence of QDs produced by *A. thiooxidans* to acidic pH. We compared the pH tolerance of green and red QDs produced by chemical methods, biosynthesized by the mesophilic bacteria *E. coli* and those produced by *A. thiooxidans* (Fig. 5). pH sensitivity was determined by measuring the fluorescence spectra of NPs and evaluating the fluorescence quenching produced by pH.

Obtained results indicate that QDs produced by *A. thiooxidans* display increased tolerance to acidic pH, maintaining their fluorescence properties at extremely acidic conditions such as pH 2.0.

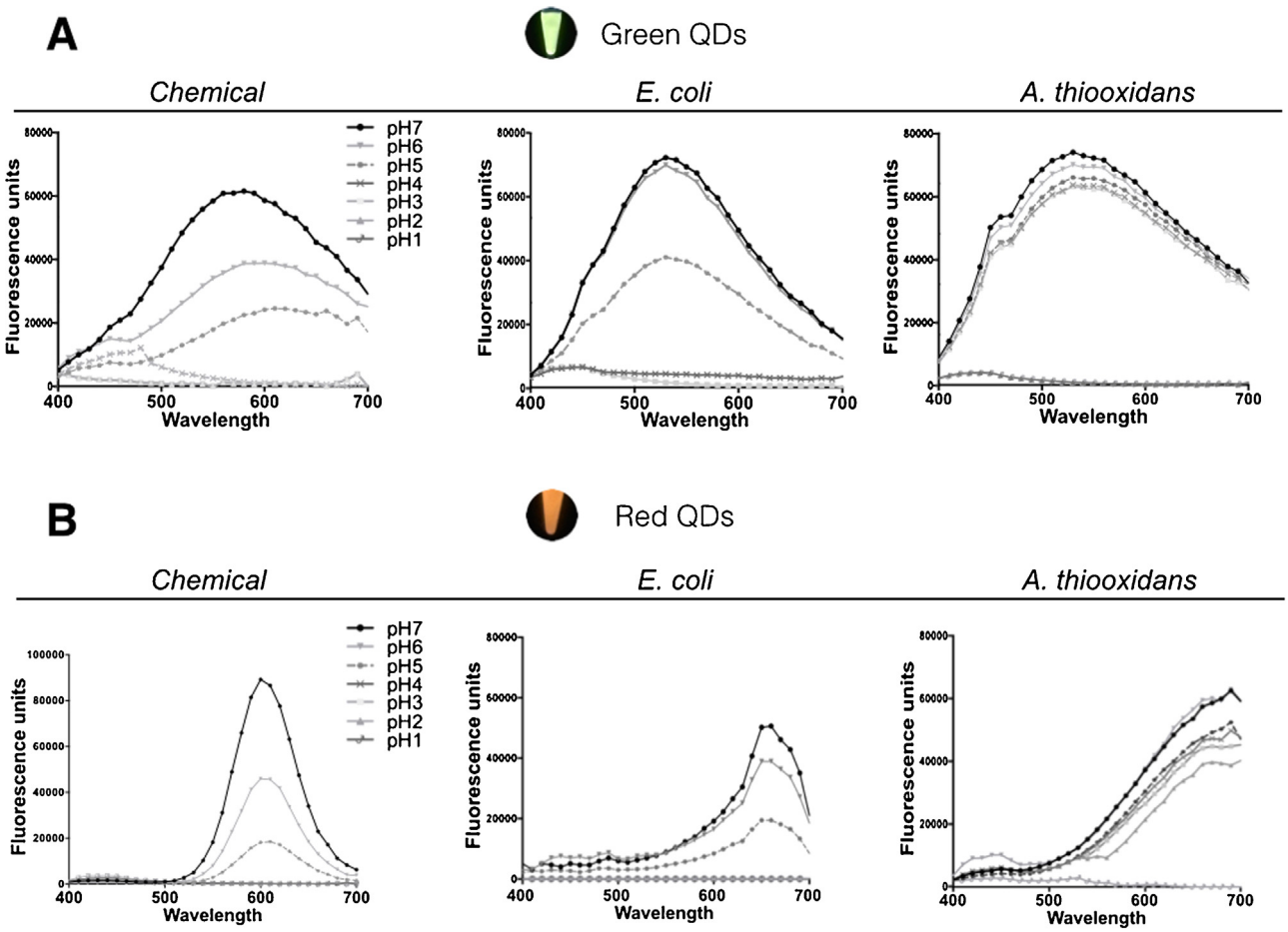


**Fig. 5.** pH tolerance of CdS QDs produced by *A. thiooxidans*, *E. coli* and chemically synthesized. The effect of pH on fluorescence emission spectra of red and green QDs was evaluated after 40 min exposure to different pHs.

Significant decreases in fluorescence spectra of chemical and *E. coli* produced QDs were observed at pH 5.0, which is in aggrement with previous reports indicating that exposure of CdS/CdSe QDs to acidic pH degradates the QDs and quench their fluorescence [17,18]., Interestingly, biological QDs (even those produced by *E. coli*) display higher tolerance to acidic conditions than those chemically produced (see spectra obtained at pH 6 and 5), a result that validates the properties of NPs produced by bacteria.

The increased tolerance to pH observed in QDs produced by *A. thiooxidans* is probably consequence of the presence of bacterial biomolecules that stabilize and/or protect the nanostructure. Interestingly, it has ben reported that the pH of leaching solutions has no significant impact in the absortion and fluorescence spectra of chemically produced *N*-acetyl Cysteine-coated CdTe/CdS QDs until 7 days incubation [45]. In addition the presence of cationic surface coating biomolecules could prevent that free protons reach the QDs surface, protecting the nanocrystal from acid etching as has been previously reported for chemical QDs coated with PEI [17,18]. To date, most antecedents about biomolecules that protect biosynthesized NPs report the presence of biological thiols such as GSH that improves aqueous solubility and biocompatibility of NPs [5,12].

## 4. Conclusion

Nowadays, there is a growing necessity to develop new, cost-effective, more efficient and environmentally friendly synthesis methods to produce NPs with improved tolerance to extreme conditions. In this general context, natural processes for synthesizing nanoscale materials represent a new and unexplored area of research. The use of extremophile microorganisms and their metabolism for the production of nanostructures could be an option to overcome these problems.

The present work is the first report describing the biosynthesis of CdS QDs by biomining microorganisms. In addition, QDs biosynthesized by acidophilic bacteria display increased tolerance to extreme pH, a property that has not been described in biological synthesis.

Obtained results indicate that sulfide generation enhances CdS QDs biosynthesis, probably by acting as sulfur donor for CdS nanoparticle core formation. Based on the similarity of the biosynthesis process with those described in mesophilic bacteria, excepting the pH, we believe that other unidentified cellular moieties (different from $H_2S$) like proteins, thiols or small biomolecules, must be involved in NPs formation and acid tolerance in *Acidithiobacillus*. Future research will involve the identification of proteins or biomolecules present in acid tolerant NPs in order to understand the biosynthetic process and use these biomolecules to improve the properties of NPs composed by other metals or produced by chemical methods.

The generation of nanoparticles by the mining industry could allow the creation of a new, sustainable and revolutionary business and research area related with NPs production. In the mining industry ferrous and sulfur-oxidizing bacterial cells are constantly exposed to metal contaminants such as cadmium. Accordingly, the use of these microorganisms to obtain metals from mining solutions and produce high value nanoparticles, will contribute to

increase the rentability of mining operations but most important will contribute to avoid its ecological effects.

Finally, the reported method is the first protocol for QDs biosynthesis in acidophilic bacteria currently used by the mining industry and has great potential in bioremediation of acid mine drainage and bioleaching of copper sulfide minerals. Also, for the first time is reported the generation of QDs with improved properties by using extremophile microorganisms. In our opinion, obtained results have significant implications in Bionanotechnology since open a totally new area of basic and applied research related with the use of extremophile bacteria in the biosynthesis of NPs with special properties and also with the study of the biomolecules improving the properties of the nanostructures produced.

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