Complete genome sequence of *Raoultella* sp. strain X13, a promising cell factory for the synthesis of CdS quantum dots

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Abstract

A novel cadmium-resistant bacterium, *Raoultella* sp. strain X13, recently isolated from heavy metal-contaminated soil, and this strain can synthesize CdS quantum dots using cadmium nitrate [Cd(NO₃)₂] and L-cysteine. Biomineralization of CdS by strain X13 can efficiently remove cadmium from aqueous solution. To illuminate the molecular mechanisms for the biosynthesis of CdS nanoparticle, the complete genome of *Raoultella* sp. strain X13 was sequenced. The whole genome sequence comprises a circular chromosome and a circular plasmid. Cysteine desulphydrase smCSE has been previously found to be associated with the synthesis of CdS quantum dots. Bioinformatics analysis indicated that the genome of *Raoultella* sp. strain X13 encodes five putative cysteine desulphydrases and all of them are located in the chromosome. The genome information may help us to determine the molecular mechanisms of the synthesis of CdS quantum dots and potentially enable us to engineer this microorganism for applications in biotechnology.

Keywords *Raoultella* sp. strain X13 · Genome sequence · CdS · Cysteine desulphydrase

Introduction

The genus *Raoultella* was first nominated by Drancourt et al. (2001). Members of *Raoultella* are closely related to the genus *Klebsiella* and can assimilate L-sorbose. Specific strains in this genus possess biotechnological potentials. For example, *Raoultella planticola* can degrade PAH (Ping et al. 2017), *Raoultella ornithinolytica* B6 produces 2,3-Butanediol (Kim et al. 2017) and *Raoultella* sp. SM1, a strain capable of precipitation of uranium by dissimilatory reduction, shows considerable potential in the use of remediation of heavy metal pollution (Sklodowska et al. 2018). Therefore, the genomic study of *Raoultella* is urgently needed. This

may facilitate the use of such type of biological resources. Either cadmium-resistant or detoxifying strains of *Raoultella* have not been described so far. In this study, we recently have isolated a cadmium-resistant and CdS-producing *Raoultella* sp. strain X13.

CdS quantum dots (QDs) have diverse applications in nanotechnology, such as in display technologies, in vivo or in vitro biomedical imaging/detection, solar cells, light-emitting diodes, photocatalysis, photoluminescence, infrared photodetectors, environmental sensors, and biological sensors (Klein et al. 1997; Mandal et al. 2004; Alivisatos et al. 2005; Medintz et al. 2005; Crouse and Crouse 2006; Nag et al. 2008; Yang et al. 2009; Ionov et al. 2010; Nozik et al. 2010). And the biosynthesis of CdS QDs using microorganisms could be a promising tool. To decipher the molecular mechanisms by which the bacterium synthesizes CdS QDs, and devise ways to utilize this bacterium in nanotechnology, we sequenced the whole genome of this bacterium X13.

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Materials and methods

Raoultella sp. X13, isolated from the cadmium contaminated soil, is an aerobic, Gram-positive bacterium that belongs to the family Enterobacteriaceae and produces CdS

Fig. 1 CdS QDs production by *Raoultella* sp. X13 and circular representation of the *Raoultella* sp. X13 genome. **a** UV–vis absorption spectra of the bacterium grown in M9 medium supplemented with cadmium nitrate [$\text{Cd}(\text{NO}_3)_2$, 1 mM] and L-cysteine (4 mM). **b** The emission spectra the bacterium grown in M9 medium supplemented with cadmium nitrate [$\text{Cd}(\text{NO}_3)_2$, 1 mM] and L-cysteine (4 mM) were recorded using a 380 nm excitation wavelength. **c** TEM micrograph shows the ultrathin section of the bacterial cells exposed for 2 days under the nanoparticle biosynthesis condition. **d** The cadmium removal by *Raoultella* sp. X13 in M9 medium supplemented with/without L-cysteine (4 mM) and photographs of the solutions inset. **e** Circular representation of the chromosome of *Raoultella* sp. X13 and **f** the plasmid GM host by *Raoultella* sp. X13. **e** From the outer- to the inner-most circle: the coding genes; COG; KEGG; GO; ncRNA. **f** The circular map for the plasmid GM was visualized in CGView. From the outside to the inside: the COG functional annotated taxonomic gene (arrows indicate the positive strand coding clockwise), the genome sequence position coordinates, the genome GC content, and the genome GC skew value distribution

nanoparticles in M9 medium supplemented with cadmium nitrate [$\text{Cd}(\text{NO}_3)_2$, 1 mM] and L-cysteine (4 mM). The size of CdS particles was analyzed by the transmission electron microscope (TEM). Genomic DNA of bacterial X13 was extracted using the SDS lysis method. The genome was sequenced by the Beijing Novogene Bioinformatics Technology Co., Ltd, using the single molecule real-time (SMRT) sequencing technology.

Results and discussion

As shown in Fig. 1a, b, the nanocrystals in the culture exhibited an absorption peak at $\lambda = 382$ nm and a fluorescence peak at $\lambda = 468$ nm (excitation at $\lambda = 380$ nm). The spectral characteristics of the culture are similar to those of the quantum confined CdS nanoparticles (band gap of the bulk CdS: absorption edge at $\lambda \approx 515$ nm). The transmission electron microscope (TEM) micrograph showed that the size of CdS particles was within the range for the CdS quantum dots (Fig. 1c). Compared with the control without Cys, when the bacterium was inoculated into the medium containing 4 mM L-Cys, the color of the culture was initially white then became yellow after 24 h (Fig. 1d, inset). The cadmium removal ratio by the bacterium X13 reached 100% in the medium containing Cys, but it was only 22.4% when the bacterium grew without Cys (Fig. 1d). These data indicated that the biosynthesis of CdS via the Cys lysis pathway facilitated the Cd removal in the aquatic environment.

After raw data filtering from the PacBio RS II sequencer, 99,497 long reads occupying 1,001,203,739 bps were generated and assembled de novo using an SMRT Link v5.0.1 assembler (Li et al. 2010). The tools used for genome component prediction were as follows: the coding gene was retrieved using the GeneMarkS program (Besemer et al. 2001) (<http://topaz.gatech.edu/GeneMark/>); transfer RNA

(tRNA) and ribosomal RNA (rRNA) were predicted as previously described (Lowe et al. 1997; Lagesen et al. 2007); open reading frames were predicted using the clusters of orthologous groups (COG) database (<http://www.ncbi.nlm.nih.gov/COG>), the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, and the non-redundant GenBank protein database; and the graphical circular map of the genome was constructed and visualized using Circos (Krzywinski et al. 2009).

The whole genome sequence of *Raoultella* sp. strain X13 was obtained with no gaps. The main features of the genome are summarized in Table 1. The circular chromosome comprises 5,404,711 bps, which correspond to 4375 protein-coding genes, 1 microsatellite sequence, 74 minisatellite sequences, 25 rRNA genes, and 85 tRNA genes with an average G + C content of 55.94% (Table 1; Fig. 1e). The plasmid, undetectable by agarose gel electrophoresis, possesses 43,768 bps with an average G + C content of 34.90%.

Gene ontology terms were assigned to X13 genes for functional categorization. On the chromosome, a total of 3749 genes were divided into 48 subcategories, falling within the 3 main categories of biological processes, cellular components, and molecular functions (Fig. S1). When searching the KEGG pathway database, a total of 3310 genes were assigned to 40 pathways (Fig. S2). Among these genes, 414, 388, and 289 genes were assigned to the membrane transport, carbohydrate metabolism, and amino acid metabolism subcategories, respectively. The COG database classified 4279 gene products into 24 classes based on the orthologous groups (Fig. S3). Carbohydrate transport and metabolism (614) was found to be the largest category, followed by amino acid transport and metabolism (544), and transcription (485).

Since strain X13 can synthesize CdS nanoparticles in the presence of cysteine and $\text{Cd}(\text{NO}_3)_2$, and reports have demonstrated that biosynthetic CdS nanoparticles depend on H_2S production from L-cysteine catalyzed by cysteine desulfhydrase (Wang et al. 2001; Edwards et al. 2013; Gallardo et al. 2014; Yang et al. 2015), we focused on the genes that potentially encode cysteine desulfhydrase. Cysteine desulfhydrase hydrolyzes L-cysteine to produce H_2S , NH_3 , and pyruvate (Cunningham et al. 1993; Wang et al. 2001). Until recently, only five active cysteine desulfhydrases have been characterized in *Fusobacterium nucleatum* (Fukamachi et al. 2002), *Streptococcus anginosus* (Yoshida et al. 2002), *Prevotella intermedia* (Yano et al. 2009), *Stenotrophomonas maltophilia* (Dunleavy et al. 2016), and *Treponema denticola* (Chu et al. 1997) (Table S1). Among them, only smCSE, obtained from *Stenotrophomonas maltophilia* was reported to biomineralize CdS nanocrystals in the lab (Dunleavy et al. 2016). We found that five open reading frames code for putative cysteine desulfhydrases (in chromosome) as shown in Table 1. Among them, CYS713, CYS737,

Table 1 General feature and specific genes of the X13 genome

Genome analysis		
General feature		
Items	Element and characteristics	Value
Chromosome	Size (bp)	5,404,711
	Gene number	5017
	GC content	55.94%
	rRNA	25
	tRNA	85
	Minisatellite DNA	70
	Microsatellite DNA	1
	Coding proteins	4375
Plasmid	Size (bp)	43,768
	Genes	51
	GC content	34.90%
Specific genes probably associated with CdS production		
Gene accession no	Predicted protein size (number of the amino acid residues)	Identity to smCSE
GM000713	396	29%
GM000737	382	61%
GM004373	397	25%
GM004422	463	NA
GM004651	317	41%

CYS4373, and CYS4651 showed 29%, 61%, 25%, and 41% identity with smCSE, respectively, based on the amino acid sequences. These putative enzyme activities and biomineralization potentials should be validated in the near future. The genome sequences of *Raoultella* sp. strain X13 may help us to determine the molecular mechanisms of the synthesis of CdS quantum dots and enable us to engineer this micro-organism for applications in biotechnology.

Nucleotide sequence accession number

The complete chromosome sequence and plasmid sequence of *Raoultella* sp. strain X13 have been submitted to NCBI GenBank database under accession numbers CP030874 and CP030875, respectively.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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