Genomic and biotechnological insights on stress-linked polyphosphate production induced by chromium(III) in *Ochrobactrum anthropi* DE2010

Eduard Villagrasa $^1$ · Raquel Egea $^2$ · Neus Ferrer-Miralles $^{1,3,4}$ · Antonio Solé $^1$

Received: 18 November 2019 / Accepted: 21 June 2020 / Published online: 26 June 2020
© Springer Nature B.V. 2020

Abstract
The resistance of microorganisms to heavy metals in polluted environments is mediated by genetically determined mechanisms. One such mechanism includes the intracellular sequestration of heavy metals in polyphosphate (polyP) inclusions. In Cr(III) contaminated mediums, *Ochrobactrum anthropi* DE2010 is able to bind and sequester Cr(III) in polyP inclusions. In order to further study the relationship between Cr(III) tolerance and polyP production in *O. anthropi* DE2010, we carried out whole genomic sequencing, analysis of single nucleotide polymorphisms (SNPs), polyP chemical quantification, and determination of the relative abundance and morphometry of polyP inclusions. In the *O. anthropi* DE2010 genome, six polyP and pyrophosphate (PPi) metabolic genes were found. Furthermore, genomic analysis via SNPs calling revealed that *O. anthropi* ATCC49188 and DE2010 strains had average variations of 1.51% in their whole genome sequences and 1.35% variation associated with the principal polyP metabolic gene cluster. In addition, the accumulation of polyP in the DE2010 strain and number of polyP inclusions found were directly correlated with the concentration of Cr(III) in contaminated cultures. The results presented in this study may enhance the understanding of polyP production in response to Cr(III) toxicity in the *O. anthropi* DE2010 strain. This knowledge may facilitate the successful removal of Cr(III) from the natural environment.

Keywords Chromium(III) · Cytoplasmic inclusions · Genome sequencing · *Ochrobactrum anthropi* DE2010 · Polyphosphate production

Introduction
Chromium occurs in nature in bound forms in the earth’s crust (Jacobs and Testa 2005). Although it exists in several oxidation states, the most common and stable forms are the Cr(0), trivalent Cr(III), and hexavalent Cr(VI) species (Oliveira 2012). Human activities have harmed the natural environment, leading to large increases in the levels of toxic metals (e.g., revision in Ali et al. 2013; Masindi and Muedi 2018). Cr(III) is found in air, soil, and water after being released from industries that use chromium. This metal is also released into the environment from the burning of natural gas, oil, or coal (Wilbur et al. 2012). The permanence of its soluble forms that act as long term pollutants poses a serious threat, since they can be reoxidised to Cr(VI), which is carcinogenic (Chourey et al. 2006). For this reason, it is relevant to study the immobilisation of Cr(III) (Cheng et al. 2010; Millach et al. 2015). Cr(III) is considered less toxic than Cr(VI), but it can cause DNA damage and topoisomerase inhibition. Besides, it is involved in some human and
animal diseases with respiratory, reproductive, immunological, and development effects (Wilbur et al. 2012; Fatima and Rao 2018). Moreover, this metal has antibacterial and antifungal due to its oxidative damage-causing and biotoxic functions (Plaper et al. 2002; Paez et al. 2013). Microbial cells have adapted to the presence of heavy metal ions in their habitat by displaying specific resistance mechanisms. These mechanisms include cell surface bioabsorption, bioaccumulation outside or inside the cell, and biotransformation to less toxic forms (Chojnacka 2010; Hansda et al. 2016). One of the strategies to bioaccumulate heavy metals inside the cells involves capturing them within the intracellular inclusions of polyphosphate (polyP) (Kulakovskaya 2018a).

In a previous study, our research group isolated a strain from the Ebro Delta microbial mats (Tarragona, Spain), which was identified as Ochrobactrum anthropi DE2010 using the genotypic and phenotypic techniques (Villagrasa et al. 2019). O. anthropi DE2010 is a gram-negative, nonspore, rod shaped, marine, heterotrophic bacterium. In addition, O. anthropi DE2010 immobilises Cr(III) in cytoplasmic inclusions of polyP (Villagrasa et al. 2020). Under conditions of nutrient starvation and stress, such as the presence of heavy metals, some microorganisms can accumulate polyP via gene-regulated mechanisms (Baxter and Jensen 1980; Jensen et al. 1986; Kuroda et al. 2001; Narancic et al. 2012; Burgos et al. 2013; Millach et al. 2015). The potential for using heavy metal tolerant microorganisms in bioremediation prompted us to further characterise the response of O. anthropi DE2010 to Cr(III) exposure.

Inorganic polyPs are polymers of orthophosphate residues linked by phosphoanhydride P-O-P bonds (Albi and Serrano 2016). They are present in most organisms, including bacteria, archaea, and eukaryotes (Harold 1966; Kornberg et al. 1999; Rao et al. 2009). Metabolic and biological functions of polyP in bacteria and yeast are detailed elsewhere (Aschar-Sobbi et al. 2008; Oehmen et al. 2010; Rubio-Rincón et al. 2017). Inorganic polyP was initially considered a phosphate and energy storage molecule that seemed to be involved in diverse physiological and regulatory mechanisms in bacteria (Kornberg et al. 1999; Brown and Kornberg 2004; Rao et al. 2009). Among these is the bioaccumulation of heavy metals in intracytoplasmic inclusions (Gerber et al. 2016, Kulakovskaya 2018a). The main enzyme related to polyP biosynthesis is polyphosphate kinase 1 (PPK1, EC 2.7.4.1) (Akiyama et al. 1993; Rao and Kornberg 1996). However, a subsequent characterisation of the pathway indicated the involvement of two PPKs (PPK1 and PPK2) in the process. PPK1 is mainly involved in polyP synthesis by catalysing the reversible transfer of phosphate residues from ATP to polyP, and from polyP to ADP. On the other hand, PPK2 participates in the synthesis of polyP from GTP and is frequently associated with polyP degradation (Zang et al. 2002). Further, an exopolyphosphatase (PPX, EC 3.6.1.11) and its homologue exopolyphosphatase/guanosine pentaphosphate phosphohydrolase (PPX/GPPA, EC 3.6.1.40), hydrolyse polyP, liberate inorganic phosphate (Pi) and transform GDP into GTP (Akiyama et al. 1993). These PPK enzymes have been purified from Escherichia coli and their genes are found in several bacteria (Kornberg et al. 1999; Alvarez and Jerez 2004). Other enzymes involved in polyP metabolism include inorganic pyrophosphatases (PPases, EC 3.6.1.1), which are organised in two groups, namely, soluble (coding gene, ppa) and membrane embedded (coding gene, hppa). Soluble PPases (sPPases) are ubiquitous proteins with roles in the removal of the inorganic pyrophosphate (PPi) produced by anabolic reactions (Lahti et al. 1988). Membrane-bound, proton translocating, inorganic pyrophosphatases (H⁺-PPases) utilise PPi hydrolysis as the driving force for the movement of H⁺ across biological membranes (Rea and Poole 1993). Although some studies have proposed several roles for polyP in microbial metabolism, the mechanism by which PPi is transported from polyP inclusions remains unknown. Ruiz et al. (2001), however, found that PPI initiates polyP chain synthesis.

In the current study, we use genomic sequencing and the annotation of the environmentally isolated O. anthropi DE2010 to correlate polyP production and Cr(III) concentration with the following aims: (i) to detect the presence of polyP and PPI metabolic genes within the O. anthropi DE2010 genome; (ii) to apply an SNPs calling study between O. anthropi DE2010 and O. anthropi ATCC49188 to determine the overall differences in their genomic architectures and, in particular, polyP metabolic genes; (iii) to quantify the polyP in response to Cr(III); and (iv) to determine the relative abundance and morphometric characteristics of polyP cytoplasmic inclusions in Cr(III) contaminated cultures.

Materials and methods

Culture conditions, genome sequencing, assembly and annotation of O. anthropi DE2010

Ochrobactrum anthropi DE2010 was cultured on Luria–Bertani (LB) agar (tryptone (10.0 g/L), yeast extract (5.0 g/L), sodium chloride (10.0 g/L), and bacteriological agar (15.0 g/L) at pH 7.0 and 27 °C and preserved in cryoinstant vials (Thermo Fisher Scientific) at − 80 °C. Genomic DNA for whole genome sequencing (WGS) was extracted and isolated using the Puregene Core Kit A (Qiagen Sciences, Valencia, CA, USA) according to the manufacturer’s instructions. This genomic DNA was sequenced by Illumina MiSeq (https://www.illumina.com/systems/sequencing-platforms/miseq) which produced 19,362,809 paired-end reads with about 1160-fold coverage. The reads were filtered,
assembled, scaffolded, and validated using FastQC 0.11.3 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), SPADES 3.12.0 (Bankevich et al. 2012), and BLAST (https://blast.ncbi.nlm.nih.gov/BLAST), respectively. The genomic sequence was annotated using the Prokaryotic genome annotation pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/).

Identification of single-nucleotide polymorphisms (SNPs) and protein alignment

For this analysis, the *O. anthropi* ATCC49188 genome was used as the reference to call single-nucleotide polymorphisms (SNPs). Sequences with accession numbers NC_009667.1 and NC_009668.1 were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/genome/) (Sayers et al. 2019). Filtered reads were mapped to the *O. anthropi* ATCC49188 genome using the Bowtie 2.3.3.1 software package (https://bowtie-bio.sourceforge.net/bowtie2/index.shtml) (Langmead and Salzberg 2012). Results were processed with Samtools v1.9 (https://www.htslib.org/doc/samtools.html) (Li et al. 2009) and duplicated reads were removed using Picard (https://broadinstitute.github.io/picard/). Further, variant calling was performed using GATK v3.8 (https://software.broadinstitute.org/gatk/). Finally, the PPK and PPX protein sequences of the *O. anthropi* ATCC49188 and DE2010 strains were compared through a high-quality multiple sequence alignment created using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) (Sievers and Higgins 2018).

**Cr(III) stock solutions and *O. anthropi* DE2010 contaminated culture conditions**

For this study, a Cr(III) stock solution (50 mmol/L) was prepared by dissolving the 1.002 g of chromium nitrate salt (Sigma-Aldrich, Bellefonte, PA, USA) in 50 mL of double deionised water. The stock was sterilised by filtration through a 0.2 μm filter (Millipore, Merck Millipore). The Cr(III) stock was prepared just before use and its pH was adjusted at 6.5.

Cr(III) tested concentrations of 0.5, 2, 5, 7, and 10 mmol/L were obtained through the serial dilution of the 50 mmol/L stock solution. Uncontaminated (0 mmol/L) and contaminated cultures were prepared at the same conditions. To do so, 2 mL of a 24 h culture of *O. anthropi* DE2010 grown in LB (OD600 between 1.4 and 1.6, approximately 10¹⁰ cfu/mL) was inoculated into 18 mL of the LB liquid medium with the various tested Cr(III) concentrations (20 mL final volume) and further, its pH was adjusted at 6.5 to prevent metal precipitation. These cultures were used for all experiments and grown in an orbital shaking incubator (Infors HT Ecotron, Boston Laboratory) at 27 °C for 24 h.

**Cell lysis and polyphosphate quantification in Cr(III) contaminated cultures**

After being incubated for 24 h at 27 °C, all *O. anthropi* DE2010 cultures (non-contaminated and contaminated with 0.5, 2, 5, 7, and 10 mmol/L of the Cr(III) solution) were centrifuged at 5500xg for 15 min at 4 °C (Eppendorf 5804R refrigerated centrifuge) and the supernatants were discarded. Further, all the obtained pellets of bacteria cultures were resuspended in a 50 mmol/L Tris–HCl buffer (pH 7.0). All the suspensions of pellets were ultrasonicated with a SONY-REX (Bandelin, Berlin) system for 15 min in an ice bath, followed by centrifugation at 5500xg for 20 min at 4 °C to remove cell debris. Finally, the resultant supernatants were treated with a protease inhibitor cocktail tablet (Roche).

To determine the polyP content (PPK activity), each sample was analysed using methods described by Anschutz and Deborde (2016) that involve the reaction of molybdenum blue with soluble reactive phosphorus. Assays were performed in triplicate for each sample and results were obtained following the protocol described by Eixler et al. (2005) as well as by considering the previously described relationship between total and soluble cellular phosphorus.

**Transmission electron microscopy (TEM) coupled with (EDX) analysis and TEM imaging of *O. anthropi* DE2010 Cr(III) cultures**

To describe this stage of research in brief, 20 mL of cultures were incubated with Cr(III) (0, 0.5, 2, 5, 7, and 10 mmol/L). Cellular pellets were obtained by carrying out centrifugation at 5500xg for 15 min at 4 °C. Further, they were fixed for 2 h in the Millonig buffer (Millonig 1961), supplemented with 2.5% glutaraldehyde, and washed in the same buffer several times. Afterwards, cells were post-fixed in 1% OsO4 at 4 °C for 2 h. All the samples were then dehydrated in a graded series of acetone (30, 50, 70, 90, and 100%) and embedded in Spurr’s epoxy resin (Maldonado et al. 2010). consecutively, ultrathin Sects. (70 nm thickness) were obtained with a Leica EM UC7 ultramicrotome (Leica microsystems GmbH, Heidelberg, Germany). For TEM coupled with energy dispersive X-ray spectroscopy (EDX) analysis, the ultrathin sections were mounted on carbon-coated, 400-mesh titanium grids without contrast and examined with a JEOL-JEM 2010 TEM (Jeol, Tokyo, Japan). To determine the semiquantitative elemental composition of samples, EDX measurements were performed with an X-ray detector EDX spectrophotometer Link Isis-200 (Oxford Instruments, Bucks, England) and analysed with INCA 4.15 EDS software (Oxford Instruments, Bucks, England). For TEM imaging, the ultrathin sections were mounted on 200-mesh copper grids with contrast (uranile acetate and lead citrate) and examined under a JEOL-JEM 1400 TEM (Jeol, Tokyo,
Japan). The obtained TEM images of *O. anthropi* DE2010 non-contaminated and Cr(III) contaminated cultures were binarised using the image analysis software ImageJ 1.40 g (Wayne Rasband, NIH, USA). To perform this process, 100 cells from each case were analysed to quantify the number of electrondense inclusions and their diameters, areas, volumes, and circularities.

**Statistical analysis**

Statistical analyses were performed using ANOVA, Student's t test, and Tukey post-hoc test. Significant differences in ANOVA, Student's t test, and Tukey's test values were considered significant when \( p \leq 0.05 \). The results were expressed as the arithmetic mean for non-transformed data \( \pm \) the standard deviation (x \( \pm \) SD). The statistical analysis and graphical representations were obtained using SPSS software (version 20.0 for Windows 7) and Sigmaplot 12.0 software, respectively.

**Table 1** General features of *O. anthropi* DE2010 and genome information

| Item | Description or value |
|------|----------------------|
| Features of *O. anthropi* DE2010 (MIGS) | *Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Brucellaceae; Ochrobactrum; Ochrobactrum anthropi* |
| Classification | |
| Gram stain | Negative |
| Cell shape | Rod shaped and pleomorphic forms |
| Motility | Peritrichous flagellation |
| Sporulation | Non-sporulating |
| Temperature optimum | 27 °C |
| pH range | 5–9 |
| Salinity range | 0–70% NaCl |
| Relationship to oxygen | Strictly aerobic |
| Pathogenicity | Opportunistic human pathogen |
| Sample collection | 2010 |
| Geographic location | Spain: Tarragona |
| Latitude and Longitude | 40.33 N 0.35 E |
| Environment (biome and feature) | Marine soil and wetland (Ebro Delta) |
| Genome size (Mb) | 4.9 |
| GC content (%) | 56.52 |
| Total number of genes | 4683 |
| Coding sequence (CDS) | 4519 |
| rRNAs | 3 |
| tRNA | 48 |
| tmRNA | 1 |
| ncRNAs | 4 |
| Pseudo genes | 109 |

**Results**

**O. anthropi** DE2010 genome sequencing and gene detection of polyP and PPI metabolisms

This whole genome shotgun project has been deposited at INSDC (DDBJ/ENA/GenBank) under the accession number QMFN00000000. The version described in this paper is version QMFN01000000. All raw reads were deposited in the sequencing read archive (SRA) of NCBI with the accession number SRR7459269. The bioproject and biosample used in this study were also deposited at INSDC under the accession numbers PRJNA475095 and SAMN09379566, respectively.

The genomic assembly of *O. anthropi* DE2010 had a total length of 4.9 Mb, consisting of 26 contigs with an \( N_{50} \) length of 688,210 bp. Its GC content was 56.52% and it contained 4683 genes. Further, six genes related to polyP and PPI metabolism were found. The annotation of this genome revealed features that have been summarised in Table 1. The list of the identified genes is described in Table 2.
Comparison of SNPs and protein alignment between *O. anthropi* ATCC49188 and *O. anthropi* DE2010

The SNP calling of *O. anthropi* DE2010 against *O. anthropi* ATCC49188 revealed 72,465 SNPs (1.51% of the total genome length). From these variants, 2527 positions were polymorphic within the DE2010 strain. The *ppx* and *ppk* genes are located in the same operon (Keasling et al. 1993; Lee et al. 2006) and were found to be essential for polyP metabolism in bacteria. The SNP calling of *O. anthropi* ATCC49188 and DE2010 in this operon revealed a great degree of similarity with respect to *ppx* and *ppk1* sequences. Further, 51 variations (1.35%) via SNP analysis were found and studied in detail. All related data are shown in Figure S1 in the Supplementary material. The multiple alignments of identified proteins (*PPX* and *PPK*) revealed two mutations in PPX and one mutation in *ppk* (Text S1 and S2 [Supplementary material]). The identified mutations in the PPX protein corresponded to R286K and S465N, and were conservative and semi-conservative replacements, respectively. The catalytic domain of this enzyme is located in the region between residues 37 to 308 that includes the R286K conservative mutation, which may not affect protein function. In the case of the PPK amino acid sequence, the A36V semi-conservative mutation is not located in any of the identified catalytic domains of the enzymes and may not affect enzyme activity as well.

Relationship between polyP production and Cr(III) concentration in *O. anthropi* DE2010

Previous studies have noted that one gene, *ppk*, is mainly responsible for polyP production. For this study, the polyphosphate kinase (PPK) activity of cell extracts was tested using cells exposed to Cr(III) contamination in accordance with the evidence that has shown that polyP inclusions have a significant chelating effect on metal

| Table 2 Genes and encoded proteins for polyP and PPi metabolism in *O. anthropi* DE2010 |
|---------------------|---------------------|---------------------|
| Gene | GenBank accession number | Gene product | Activity |
| PolyP and PPi metabolisms | | | |
| *ppk1* | DNK03_06690 | Polyphosphate kinase 1 | Transfers the terminal phosphate residue of ATP to a growing chain of polyP in a reversible reaction |
| *ppx* | DNK03_06685 | Exopolyphosphatase | Mediates polyP degradation releasing orthophosphate from chain end |
| *hppa* | DNK03_06575 | K⁺-insensitive pyrophosphate-energized proton pump | Proton transmembrane pump that utilizes the energy of pyrophosphate hydrolysis as the driving force for proton movement |
| *ppx/pppa* | DNK03_08775 | Exopolyphosphatase/ pppGpp phosphohydrolase | Hydrolyses guanosine pentaphosphate (pppGpp) to guanosine tetraphosphate (ppGpp) |
| *ppk2* | DNK03_11830 | Polyphosphate kinase 2 | *ppk2*, at least in isolated form, seems to be designed for synthesis of GTP from polyP in contrast to *ppk1*, which strongly favors synthesis of polyP and exclusively from ATP |
| *ppa* | DNK03_19225 | Inorganic pyrophosphatase | Inorganic pyrophosphatase (PPase) catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate |

Fig. 1 Polyphosphate content (mg polyP/g dry weight) in the *O. anthropi* DE2010 cultures grown at increasing concentrations of Cr(III). Data from contaminated vs. non-contaminated samples were analysed using one-way ANOVA; values of **p < 0.005** were considered statistically significant. Data were expressed as mean ± SD (n = 3)
cations. The data collected indicated that under these stress conditions, *O. anthropi* DE2010 synthesised and accumulated polyP in a concentration-dependent manner (Figs. 1, S2 [Supplementary material]). A 23.08% change in the polyP concentration was achieved between 0 mmol/L (control) and 10 mmol/L of Cr(III). The statistical analysis showed that there was a significant difference between control and both 7 and 10 mmol/L Cr(III) samples (Fig. 1).

**Electron microscopy**

In a previous study, we demonstrated the colocalisation of Cr(III) with intracytoplasmic polyP inclusions using scanning transmission electron microscopy (STEM) coupled with EDX (Villagrasa et al. 2020). Nevertheless, the abundance of these inclusions in relation with the presence of chromium was not considered. In the present work, a semi-quantitative analysis regarding the relative abundances of P and Cr (in atomic %) in non-contaminated (control) and contaminated (10 mmol/L Cr(III)) samples was carried out using TEM–EDX (Fig. S3 and Table S1 [Supplementary material]). Comparing data from 1 and 3 EDX spectra, corresponding to polyP inclusions without and with Cr(III), respectively, an increment in the atomic percentage of Cr(III), from 0.1 to 3.59, and P, from 0.01 to 1.51, was detected. Moreover, the data from the EDX spectra 2 (grid) and 4 (cytoplasm in contaminated conditions) demonstrated that P and Cr were not present outside the cells and instead, were dispersed by the bacterial cytoplasm, indicating that the metal was only accumulated in the intracytoplasmic inclusions of polyP (Fig. S3 and Table S1 [Supplementary material]). At the same time, it was verified that the titanium grids used in these experiments only contained Ti (Fig. S3A and Table S1 [Supplementary material]).

In order to correlate the number of electrodense inclusions with Cr(III) concentrations in *O. anthropi* DE2010 cultures, a TEM study combined with an image analysis software (ImageJ) was carried out (Fig. 2 and Table 3). An increase in the presence of pleomorphic cellular forms and more destructured cytoplasm were observed as the metal concentration increased (Fig. 2). The circularity measurements indicated that polyP inclusions are circular. A six-fold increase in the number of inclusions along with different morphometric parameters assessed (diameter, area, and volume) between 0 mmol/L and 10 mmol/L samples were detected (Table 3). Statistically significant differences were obtained for comparisons between the 0 mM (control) and 10 mmol/L Cr(III) samples in terms of the diameter ($F = 903.41$) ($p < 0.05$), area ($F = 66.15$) ($p < 0.05$), and volume ($F = 5209.24$) ($p < 0.05$) results. Using the Tukey multiple comparisons post-hoc test, statistically significant differences ($p < 0.05$) were determined (Table 3).

These results suggest that the accumulation of polyP in cytoplasmatic inclusions may be one of the factors providing tolerance and resistance to *O. anthropi* DE2010 against Cr(III) via the formation of cation and polyP complexes.

**Discussion**

Several reports have explored the capacity of some microorganisms to sequester heavy metals via the polyP metabolism (Orell et al. 2012, Acharya and Apte 2013, Andreeva et al. 2014, Kulakovskaya 2018a). In addition, our research group isolated three heterotrophic microorganisms from Ebro Delta microbial mats with the capacity to immobilise heavy metals, namely, *Paracoccus* sp. DE2007 (Diestra et al. 2007), *Micrococcus luteus* DE2008 (Maldonado et al. 2010), and *Ochrobactrum anthropi* DE2010 (Villagrasa et al. 2019). *Paracoccus* sp. DE2007 and *Micrococcus luteus* DE2008 can immobilise heavy metals in extracellular polymeric substances (EPS) (Baratelli et al. 2010; Maldonado et al. 2010; Puyen et al. 2012), whilst *O. anthropi* DE2010 is able to capture heavy metals

---

**Fig. 2** TEM images of *O. anthropi* grown in 0 mmol/L (a), 5 mmol/L (b), and 10 mmol/L (c) Cr(III) contaminated cultures. The arrows indicate intracytoplasmic electrodense inclusions. The scale bars represent 5 µm.
extra- and intra-cellularly in EPS and polyP inclusions, respectively (Villagrasa et al. 2020). Here, we reported the whole genome sequence of *O. anthropi* DE2010 and analysed the response of this strain to Cr(III) in contaminated cultures. The sequencing of the *O. anthropi* DE2010 genome revealed the presence of the key genes involved in polyP and PPI metabolism (Fig. 3), including *ppk* and *ppx* genes that comprise part of an operon, as expected. This configuration is maintained in the *O. anthropi* ATCC49188 genome, which was used as a reference in this study (Chain et al. 2011). Moreover, the genome of *O. anthropi* DE2010 contains a gene of the chromium/chromate efflux pump named *chrA* with the accession number DNK03_01860 (DDBJ/EMBL/GenBank). This gene is relevant in the sensitivity of *O. tritici* to transition metals (Almeida et al. 2020) and may have an important role in Cr(III) tolerance in *O. anthropi* DE2010.

The comparative genomics analysis between *O. anthropi* ATCC49188 and *O. anthropi* DE2010 revealed interesting findings regarding bacterial genome composition. Under selective or non-selective pressures, bacterial strains accumulate SNPs that lead to inter- and intra-strain diversity (Gohil et al. 2016). The present SNP study showed an average variability of < 1.6% between the analysed genomes (ATCC and DE2010 strains), which was slightly lower in the sequences of *ppx* and *ppk* genes, the most important polyP metabolic gene cluster. Aujoulat et al. (2014) studied genomic variations between different species of the same genus (*O. intermedium* and *O. ciceri*) and found higher percentages of polymorphic sites in different housekeeping genes such as *dnaK* (3.6%), *recA* (5.7%) and *rpoB* (7.4%). The low values obtained here in the *ppx* and *ppk* genes indicate that the polyP operon can be under selective pressure due to its evolutionary relevance wherein the genes enhance the capacity of *O. anthropi* DE2010 to survive toxic heavy metal contamination. Although several SNPs were located in the *ppx* and *ppk* genes, Clustal Omega results revealed that only a small fraction was present in the alignment of the corresponding protein sequences. In fact, the detected amino acid changes may not have profound influences on the activity of resultant enzymes, suggesting that polyP metabolism is preserved to cope with stress conditions such as the Cr(III) contamination assessed in this study (Text S1 and S2 [Supplementary material]).

### Table 3 Count and morphometric parameters of electrodense inclusions in *O. anthropi* DE2010 Cr(III) contaminated cultures

| Sample (mmol/L) | Number | Diameter (µm) | Area (µm²) | Volume (µm³) | Circularity (arbitrary units) |
|----------------|--------|---------------|------------|--------------|-----------------------------|
| 0              | 5      | 0.011 ± 2.45 × 10⁻⁴ | 3.80 × 10⁻⁴ ± 5.02 × 10⁻⁷ | 6.95 × 10⁻⁷ ± 1.88 × 10⁻¹¹ | 0.938 ± 0.006 |
| 0.5            | 6      | 0.013 ± 3.67 × 10⁻⁴ | 5.31 × 10⁻⁴ ± 1.13 × 10⁻⁶ | 1.14 × 10⁻⁶ ± 6.36 × 10⁻¹¹ | 0.950 ± 0.008 |
| 2              | 8      | 0.020 ± 0.003 ± 0.001 ± 1.11 × 10⁻⁴ ± 6.36 × 10⁻⁹ | 4.17 × 10⁻⁶ ± 6.36 × 10⁻⁸ | 0.945 ± 0.004 |
| 5              | 20     | 0.042 ± 0.008 ± 0.005 ± 8.04 × 10⁻³ ± 6.64 ± 6.36 × 10⁻⁶ | 3.86 × 10⁻³ ± 1.20 × 10⁻⁶ | 0.931 ± 0.010 |
| 7              | 24     | 0.049 ± 0.007 ± 0.007 ± 6.15 × 10⁻⁴ ± 6.64 ± 6.36 × 10⁻⁶ | 6.14 × 10⁻⁴ ± 8.08 × 10⁻⁶ | 0.945 ± 0.007 |
| 10             | 31     | 0.052 ± 0.009 ± 0.008 ± 0.001 ± 0.001 ± 6.36 ± 6.36 × 10⁻⁶ | 7.34 × 10⁻⁵ ± 1.71 × 10⁻⁶ | 0.941 ± 0.005 |

Data are expressed as mean ± standard deviation (SD)

---

**Table 3** Count and morphometric parameters of electrodense inclusions in *O. anthropi* DE2010 Cr(III) contaminated cultures

*ppk* and *ppx* genes that comprise part of an operon, as expected. This configuration is maintained in the *O. anthropi* ATCC49188 genome, which was used as a reference in this study (Chain et al. 2011). Moreover, the genome of *O. anthropi* DE2010 contains a gene of the chromium/chromate efflux pump named *chrA* with the accession number DNK03_01860 (DDBJ/EMBL/GenBank). This gene is relevant in the sensitivity of *O. tritici* to transition metals (Almeida et al. 2020) and may have an important role in Cr(III) tolerance in *O. anthropi* DE2010.

The comparative genomics analysis between *O. anthropi* ATCC49188 and *O. anthropi* DE2010 revealed interesting findings regarding bacterial genome composition. Under selective or non-selective pressures, bacterial strains accumulate SNPs that lead to inter- and intra-strain diversity (Gohil et al. 2016). The present SNP study showed an average variability of < 1.6% between the analysed genomes (ATCC and DE2010 strains), which was slightly lower in the sequences of *ppx* and *ppk* genes, the most important polyP metabolic gene cluster. Aujoulat et al. (2014) studied genomic variations between different species of the same genus (*O. intermedium* and *O. ciceri*) and found higher percentages of polymorphic sites in different housekeeping genes such as *dnaK* (3.6%), *recA* (5.7%) and *rpoB* (7.4%). The low values obtained here in the *ppx* and *ppk* genes indicate that the polyP operon can be under selective pressure due to its evolutionary relevance wherein the genes enhance the capacity of *O. anthropi* DE2010 to survive toxic heavy metal contamination. Although several SNPs were located in the *ppx* and *ppk* genes, Clustal Omega results revealed that only a small fraction was present in the alignment of the corresponding protein sequences. In fact, the detected amino acid changes may not have profound influences on the activity of resultant enzymes, suggesting that polyP metabolism is preserved to cope with stress conditions such as the Cr(III) contamination assessed in this study (Text S1 and S2 [Supplementary material]).
On the other hand, previous studies demonstrated the polyP production in response to numerous stress factors such as (i) nutrient starvation in the Paracoccus sp. strain (Lee and Park 2008); (ii) wastewater phosphorus removal by Chlorella sp., Lyngbya sp., and Anabaena sp. (Mukherjee et al. 2015); and (iii) heavy metal toxicity by bacteria, microalgae, or cyanobacteria, among others (Suzuki and Banfield, 2004; Millach et al. 2015; Kulakovskaya 2018a).

The results obtained in this study demonstrated that O. anthropi DE2010 is a significant candidate that has the potential to minimise Cr(III) toxicity by chelating the metal in polyP inclusions, producing a fourfold increase in polyP concentration and sixfold increase in polyP inclusion number, both in 10 mmol/L Cr(III) cultures with respect to control cultures (0 mmol/L). These results are in agreement with those obtained by Andreeva et al. (2014), which demonstrated that the concentration of polyP in C. humicola cells in cultures contaminated with other metals including Cd(II) and Mn(II) increased 3.9- and 3.4-fold, respectively, in comparison with non-contaminated controls. Moreover, the studies by Boswell et al. (1999), Choudhary and Sar (2011), and Acharya and Apte (2013) corroborated the results indicating that electrondense polyP inclusions were increased in heavy metal contaminated cultures using high-resolution electron microscopy techniques. Similar evidence was found by Kulakovskaya et al. (2018b) in yeast. Taken together, these results indicate that polyP production of O. anthropi DE2010 in Cr(III) contaminated cultures seems to be regulated in a concentration dependent manner.

In conclusion, our results demonstrate the genome sequence of O. anthropi DE2010 is a valuable source of information that can be used to analyse the metabolic response of the bacteria to Cr(III). In this study, heavy metal contamination of O. anthropi DE2010 cultures resulted in dose-dependent polyP accumulation; and an increment in the number of polyP inclusions was observed in contaminated cultures. According to the results obtained in this work, future investigations of processes and metabolic polyP pathways involved in Cr(III) removal in O. anthropi DE2010 are required and may facilitate the use of this bacteria in bioremediation efforts.

Acknowledgements We express our thanks for CIBER in Bioengineering, Biomaterials & Nanomedicine (CIBER-BBN) financed by Instituto Carlos III with assistance from European Regional Development. The authors also acknowledge ICTS “NANBIOSIS”, and, more specifically, the Protein Production Platform of CIBER-BBN at the UAB scPBioES scientific-technical service (https://www.nanbiosis.es/portal/1-modal-production-platform-ppp/) and to the UAB scientific-technical service SGB (https://sct.uab.cat/genomica-bioinformatica/es). We also appreciate the help and collaboration of Cristina Sosa, Estefania Solsona and Neus Bonet-Garcia and the valuable comments and suggestions of Prof. Isabel Esteve.

Funding This research was supported by the following Grants of Ministerio de Economía y Competitividad (CTQ2014-54553-C3-2-R and CGL2008-01891 to AS and RTA2012-00028-C02-02 to NM) and UAB postgraduate scholarship to EV.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in this publication.

References

Acharya C, Apte SK (2013) Novel surface associated polyphosphate bodies sequester uranium in the filamentous, marine cyanobacterium, Anabaena torulosa. Metallomics 5(12):1595–1598
Andreeva N, Ryazanova L, Dmitriev V, Kulakovskaya T, Kulaev I (2014) Cytoplasmic inorganic polyphosphate participates in the heavy metal tolerance of Cryptococcus humicola. Folia Microbiol 59(5):381–389. https://doi.org/10.1007/s12223-014-0310-x
Akiyama M, Crooke E, Kornberg A (1993) An exopolyphosphatase of Escherichia coli. The enzyme and its ppG in a polyphosphate operon. J Biol Chem 268(1):633–639
Albi T, Serrano A (2016) Inorganic polyphosphate in the microbial world. Emerging roles for a multifaceted biopolymer. World J Microbiol Biotechnol 32:27. https://doi.org/10.1007/s12223-015-1983-2
Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91(7):869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075
Almeida MC, Branco R, Morais PV (2020) Response to vanadate exposure in Ochrobactrum tritici strains. PLoS ONE 15(2):e0229359. https://doi.org/10.1371/journal.pone.0229359
Alvarez S, Jerez CA (2004) Copper ions stimulate polyphosphate degradation and phosphate efflux in Acidithiobacillus ferrooxidans. Appl Environ Microbiol 70(9):5177–5182. https://doi.org/10.1128/AEM.70.9.5177-5182.2004
Anschutz P, Deborde J (2016) Spectrophotometric determination of phosphate in matrices from sequential leaching of sediments. Limnol Oceanogr Methods 14:245–256. https://doi.org/10.1002/lom3.10085
Aschar-Sobbi R, AbramovAY, Diao C, Kargacin ME, Kargacin JG, French JR, Pavlov E (2008) High sensitivity, quantitative measurements of polyphosphate using a new DAPI-based approach. J Fluoresc 18:859–866. https://doi.org/10.1007/s10895-008-0315-4
Aujoulat F, Romano-Bertrand S, Masnou A, Marchandin H, Jumas-Bilak E (2014) Niches, population structure and genome reduction in Ochrobactrum intermedium: clues to technology-driven emergence of pathogens. PLoS ONE 9:1:e83376. https://doi.org/10.1371/journal.pone.0083376
Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19(5):455–477. https://doi.org/10.1089/cmb.2012.0021
Baratelli M, Maldonado J, Esteve I, Solé A, Diestra E (2010) Electron microscopy techniques and energy dispersive X-ray applied to determine the sorption of lead in Paracoccus sp. DE2007. In: Menendez-Vilas A (ed) Current research technology and education topics in applied microbiology and microbial biotechnology. Formatex Research Center, Badajoz, pp 1601–1608
Baxter M, Jensen TH (1980) Uptake of magnesium, strontium, barium, and manganese by Plectonema boryanum (Cyanophyceae)
with special reference to polyphosphate bodies. Protoplasma 104:81–89.

Boswell CD, Dick RE, Macaskie LE (1999) The effect of heavy metals and other environmental conditions on the anaerobic phosphate metabolism of Acinetobacter johnsonii. Microbiology 145(7):1711–1720. https://doi.org/10.1099/13500872-145-7-1711

Brown MR, Kornberg A (2004) Inorganic polyphosphate in the origin and survival of species. Proc Natl Acad Sci USA 101:16085–16087. https://doi.org/10.1073/pnas.0406990101

Burgos A, Maldonado J, De los Ríos A, Solé A, Esteve I (2013) Effect of copper and lead on two consortia of phototrophic microorganisms and their capacity to sequester metals. Aquat Toxicol 140–141:324–336. https://doi.org/10.1016/j.aquatox.2013.06.022

Chain PSG, Lang DM, Comerci DJ, Malfatti SA, Vergez LM, Shin M, Ugalde RA, Garcia E, Tolmasy ME (2011) Genome of Ochrobactrum anthropi ATCC 49188T, a versatile opportunistic pathogen and symbiont of several eukaryotic hosts. J Bacteriol 193(16):4274–4275. https://doi.org/10.1128/JB.05335-11

Cheng Y, Yan F, Huang F, Chu W, Pan D, Chen Z, Zheng J, Yu M, Lin Z, Wu Z (2010) Bioremediation of Cr(VI) and immobilization as Cr(III) by Ochrobactrum anthropi. Environ Sci Technol 44(16):6537–6563. https://doi.org/10.1021/es100198v

Chojnacka K (2010) Biosorption and bioaccumulation—the prospects for practical applications. Environ Int 36:299–307

Choudhary S, Sar P (2011) Uranium biomining by a metal resistant Pseudomonas aeruginosa strain isolated from contaminated mine waste. J Hazard Mater 186(1):336–343. https://doi.org/10.1016/j.jhazmat.2010.11.004

Chourey K, Thompson MR, Morrell-Falvey J, Verberkmoes NC, Brown SD, Shah M, Zhou J, Doktycz M, Hettich RL, Thompson DK (2006) Global molecular and morphological effects of 24-hour chromium(VI) exposure on Shewanella oneidensis MR-1. Appl Environ Microbiol 72:6331–6344

Diestra E, Esteve I, Burnat M, Maldonado J, Solé A (2007) Isolation and characterization of a heterotrophic bacterium able to grow in different environmental stress conditions, including crude oil and heavy metals. In: Méndez-Vilas A (ed) Communicating current knowledge: background and history. In: Guertin J, Jacobs JA, Testa SM (eds) 2005 Global molecular and morphological effects of 24-hour chromium(VI) exposure on Shewanella oneidensis MR-1. World J Microbiol Biotechnol 34:139. https://doi.org/10.1007/s11274-018-2523-7

Kulakovskaya T, Ryazanova L, Zvonarev A, Khokhlova G, Vainshtein M (2018) The biosorption of cadmium and cobalt and iron ions by yeast Cryptococcus humilico at nitrogen starvation. Folia Microbiol 63:507–510. https://doi.org/10.1007/s11274-018-0583-6

Kuroda A, Nomura K, Ohtomo R, Kato J, Ikeda T, Takiguchi N, Ohtake H, Kornberg A (2001) Role of inorganic polyphosphate in promoting ribosomal protein degradation by the ion protease in E. coli. Science 27:705–708. https://doi.org/10.1126/science.1061315

Lahti R, Pitkäranta T, Valve E, Ilia I, Kuoko-Käläske E, Heinonen J (1988) Cloning and characterization of the gene encoding inorganic pyrophosphatase of Escherichia coli K-12. J Bacteriol 170(12):5901–5907

Langmead B, Salzberg S (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923

Lee HW, Park YK (2008) Characterizations of denitrifying polyphosphate-accumulating bacterium Paracoccus sp. strain YKP-9. J Microbiol Biotechnol 18(12):1958–1965. https://doi.org/10.4014/jmb.0800.162

Lee S, Lee Y, Lee Y, Choi Y (2006) Molecular characterization of polyphosphate (PolyP) operon from Serratia marcescens. J Basic Microbiol 46:108–115. https://doi.org/10.1002/jobm.200510038

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R (2009) 1000 Genome project data processing subgroup. The sequence alignment/map format and SAMtools. Bioinformatics 25(16):2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Maldonado J, Diestra E, Domènech AM, Villagrasa E, Puyó ZM, Esteve I, Solé A (2010) Isolation and identification of a bacterium with high tolerance to lead and copper from a marine microbial mat in Spain. Ann Microbiol 60(1):113–120. https://doi.org/10.1007/s12228-010-0019-2

Masindi V, Muedi KL (2018) Environmental contamination by heavy metals. Heavy Metals, Hosam El-Din M. Saleh and Refaat F. Aglan, IntechOpen https://doi.org/10.5772/intechopen.76082

Millach L, Solé A, Esteve I, Solé A (2010) Isolation and identification of a bacterium with high tolerance to lead and copper from a marine microbial mat in Spain. Ann Microbiol 60(1):113–120. https://doi.org/10.1007/s12228-010-0019-2

Mukherjee C, Chowdhury R, Ray K (2015) Phosphorus recycling from phosphoorganic pyrophosphatase-accumulating bacterium with high tolerance to lead and copper from a marine microbial mat in Spain. Ann Microbiol 60(1):113–120. https://doi.org/10.1007/s12228-010-0019-2

Mukherjee C, Chowdhury R, Ray K (2015) Phosphorus recycling from phosphoorganic pyrophosphatase-accumulating bacterium with high tolerance to lead and copper from a marine microbial mat in Spain. Ann Microbiol 60(1):113–120. https://doi.org/10.1007/s12228-010-0019-2

Narancic T, Djokic L, Kenny ST, O’Connor KE, Radulovic V, Nikodinovic-Runic J, Vasiljevic B (2012) Metabolic versatility of Gram-positive microbial isolates from contaminated river sediments. J
Hazard Mater 215–216:243–251. https://doi.org/10.1016/j.jhazmat.2012.02.059

Oehmen A, Carvalho G, Lopez-Vazquez CM, van Loosdrecht MCM, Reis MAM (2010) Incorporating microbial ecology into the metabolic modelling of polyphosphate accumulating organisms and glycogen accumulating organisms. Water Res 44(17):4992–5004. https://doi.org/10.1016/j.watres.2010.06.071

Oliveira H (2012) Chromium as an environmental pollutant: insights on induced plant toxicity. J Bot. https://doi.org/10.1155/2012/375843

Orell A, Navarro CA, Rivero M, Aguilar JS, Jerez CA (2012) Inorganic polyphosphates in extremophiles and their possible functions. Extremophiles 16:573. https://doi.org/10.1007/s00792-012-0457-9

Páez PL, Bazán CM, Bongiovanni ME, Toneatto J, Albesa I, Becerra MC, Argüello GA (2013) Oxidative stress and antimicrobial activity of chromium(III) and ruthenium(II) complexes on Staphylococcus aureus and Escherichia coli. BioMed Res Int. https://doi.org/10.1155/2013/906912

Plaper A, Jenko-Brinovec S, Premzl A, Kos J, Raspor P (2002) Genotoxicity of trivalent chromium in bacterial cells. Possible effects on DNA topology. Chem Res Toxicol 15:943–949. https://doi.org/10.1021/tr010096q

Puyen ZM, Villagrasa E, Maldonado J, Diestro E, Esteve I, Solé A (2012) Biosorption of lead and copper by heavy-metal tolerant Micrococcus luteus DE2008. Bioreour Technol 126:233–237. https://doi.org/10.1016/j.biortech.2012.09.036

Rao NN, Kornberg A (1996) Inorganic polyphosphate supports resistance and survival of stationary-phase Escherichia coli. J Bacteriol 178:1394–1400. https://doi.org/10.1128/jb.178.5.1394-1400.1996

Rao NN, Gomez-Garcia MR, Kornberg A (2009) Inorganic polyphosphate: essential for growth and survival. Annu Rev Biochem 78:60. https://doi.org/10.1146/annurev.biochem.78.083007.093039

Rea PA, Poole RJ (1993) Vacuolar H+-translocating pyrophosphatase. Annu Rev Plant Physiol Plant Mol Biol 44:157–180. https://doi.org/10.1042/1042-0221446

Rubio-Rincón FJ, Lopez-Vazquez CM, Welles L, van Loosdrecht MCM, Brdjanovic D (2017) Cooperation between Candidatus Competibacter and Candidatus Accumulibacter clade I, in denitrification and phosphate removal processes. Water Res 120:156–164. https://doi.org/10.1016/j.watres.2017.05.001

Ruiz FA, Rodrigues CO, Docampo R (2001) Rapid changes in polyphosphate content within acidocalcisomes in response to cell growth, differentiation, and environmental stress in Trypanosoma cruzi. J Biol Chem 276:26114–26121. https://doi.org/10.1074/jbc.M102402200

Sayers EW, Agarwala R, Bolton EE, Brister JR, Canese K, Clark K, Connor R, Fiorini N, Funk K, Heffron T, Holmes JB, Kim S, Kimchi A, Kitts PA, Lathrop S, Lu Z, Madden TL, Marchler-Bauer A, Panch P, Schneider VA, Schoch CL, Pruitt KD, Ostell J (2019) Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 47:23–28. https://doi.org/10.1093/nar/gky1069

Sievers F, Higgins DG (2018) Clustal Omega for making accurate alignments of many protein sciences. Protein Sci 27:135–145. https://doi.org/10.1002/pro.3290

Suzuki Y, Banfield JF (2004) Resistance to, and accumulation of, uranium by bacteria from uranium-contaminated site. Geomicrobiol J 21:113–121

Villagrasa E, Ferrer-Miralles N, Millach L, Obiol A, Creus J, Esteve I, Sole A (2019) Morphological responses to nitrogen stress deficiency of a new heterotrophic isolated strain of Ebro Delta microbial mats. Protoplasma 256:105–116. https://doi.org/10.1007/s00709-018-1263-8

Villagrasa E, Ballesteros B, Obiol A, Millach L, Esteve I, Sole A (2020) Multi-approach analysis to assess the chromium(III) immobilization by Ochrobactrum anthropi DE2010. Chemosphere 238:124663. https://doi.org/10.1016/j.chemosphere.2019.124663

Wilbur S, Abadin H, Fay M, Yu D, Tencza B, Ingerman L, Klotzbach J, James S (2012) Toxicological profile for chromium. Agency for Toxic Substances and Disease Registry (US), Atlanta

Zhang H, Ishige K, Kornberg A (2002) A polyphosphate kinase (PPK2) widely conserved in bacteria. Proc Natl Acad Sci USA 99:16678–16683. https://doi.org/10.1073/pnas.26265199

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.