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ORIGINAL PAPER

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

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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

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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, non-spore, 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 (Ascher-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 reference 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 (OD_{600} between 1.4 and 1.6, approximately 10^{10} 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 centrifugated at 5500×*g* 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 SONOREX (Bandelin, Berlin) system for 15 min in an ice bath, followed by centrifugation at 5500×*g* for 20 min at 4 °C to remove cell debris. Finally, the resultant supernatants were treated with a protease inhibitor cocktail tablet (Roche).

To determinate 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 5500×*g* 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% OsO_4 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 UCG 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 2011 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 electrodense 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 ± the standard deviation (x ± SD). The statistical analysis and graphical representations were obtained using SPSS software (version 20.0 for Windows 7) and Sigmaplot 12.0 software, respectively.

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.

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

Item	Description or value
Features of *O. anthropi* DE2010 (MIGS)	
Classification	*Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Brucellaceae; Ochrobactrum; Ochrobactrum anthropi*
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 features	
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

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/gppa	DNK03_08775	Exopolyphosphatase/pppGpp phosphohydrolase	Hydrolyses guanosine pentaphosphate (pppGpp) to guanosine tetraphosphate (ppGpp)
ppk2	DNK03_11830	Polyphosphate kinase 2	*ppk*2, 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

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

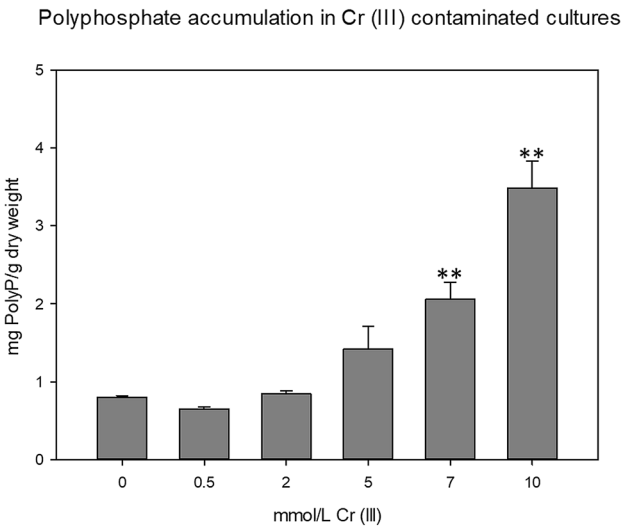

Fig. 1 Polyphosphate content (mg polyP/g dry weight) in the *O. anthropi* DE2010 cultures grown at increasing concentrations of Cr(III). Data from contaminated vs. non-contaminated samples were analysed using one-way ANOVA; values of $^{**}p < 0.005$ were considered statistically significant. Data were 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

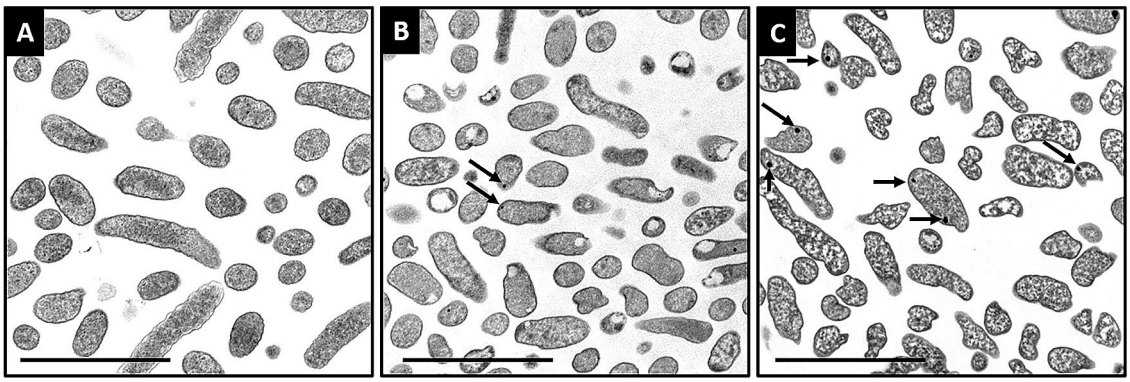

Fig. 2 TEM images of *O. anthropi* grown in 0 mmol/L (**a**), 5 mmol/L (**b**), and 10 mmol/L (**c**) Cr(III) contaminated cultures. The arrows indicate intracytoplasmic electrodense inclusions. The scale bars represent 5 µm

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

Sample	Number	Diameter (μm)	Area (μm^2)	Volume (μm^3)	Circularity (arbitrary units)
0 mmol/L	5	$0.011 \pm 2.45 \times 10^{-4}$	$3.80 \times 10^{-4} \pm 5.02 \times 10^{-7}$	$6.95 \times 10^{-7} \pm 1.88 \times 10^{-11}$	0.938 ± 0.006
0.5 mmol/L	6	$0.013 \pm 3.67 \times 10^{-4}$	$5.31 \times 10^{-4} \pm 1.13 \times 10^{-6}$	$1.14 \times 10^{-6} \pm 6.36 \times 10^{-11}$	0.950 ± 0.008
2 mmol/L	8	$0.020 \pm 0.003^{**+}$	$0.001 \pm 1.11 \times 10^{-4**+}$	$4.17 \times 10^{-6} \pm 6.36 \times 10^{-8**+}$	0.945 ± 0.004
5 mmol/L	20	$0.042 \pm 0.008^{**++\$\$}$	$0.005 \pm 8.04 \times 10^{-3**++\$\$}$	$3.86 \times 10^{-5} \pm 1.20 \times 10^{-6**++\$\$}$	0.931 ± 0.010
7 mmol/L	24	$0.049 \pm 0.007^{**++\$\$\&\&}$	$0.007 \pm 6.15 \times 10^{-4**++\$\$\&\&}$	$6.14 \times 10^{-5} \pm 8.08 \times 10^{-7**++\$\$\&\&}$	0.945 ± 0.007
10 mmol/L	31	$0.052 \pm 0.009^{**++\$\$\&\&\wedge}$	$0.008 \pm 0.001^{**++\$\$\&\&\wedge}$	$7.34 \times 10^{-5} \pm 1.71 \times 10^{-6**++\$\$\&\&\wedge}$	0.941 ± 0.005

Data are expressed as mean ± standard deviation (SD)

$^{**}p < 0.01$: 0.5, 2, 5, 7, and 10 mmol/L treatments vs. control (0 mmol/L)

$^{+}p < 0.05$; $^{++}p < 0.01$: 2, 5, 7, and 10 mmol/L treatments vs. 0.5 mmol/L treatment

$^{\$\$}p < 0.01$: 5, 7, and 10 mmol/L treatments vs. 2 mmol/L treatment

$^{\&\&}p < 0.01$: 7, and 10 mmol/L treatments vs. 5 mmol/L treatment

$^{\wedge}p < 0.05$: 7 treatment mmol/L vs. 10 mmol/L treatment

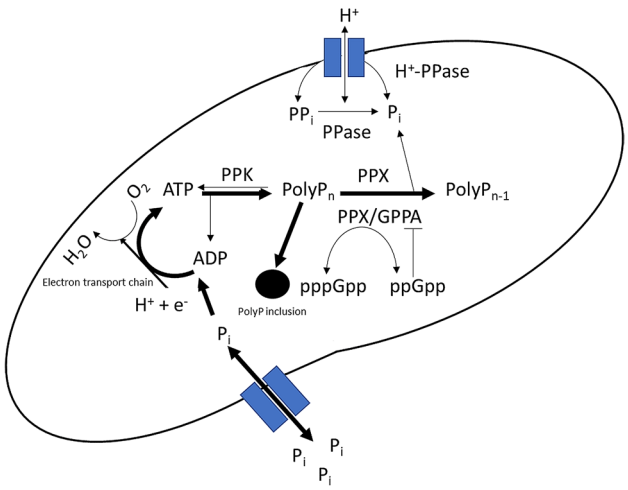

Fig. 3 Graphic representation of proposed metabolic pathways for polyP and PPi metabolism within *O. anthropi* DE2010. Abbreviations: Inorganic phosphate (P_i), inorganic pyrophosphate (PP_i), K^+-insensitive pyrophosphate-energized proton pump (H^+PPase), inorganic pyrophosphatase (PPase), guanosine pentaphosphate (pppGpp), guanosine tetraphosphate (ppGpp), polyphosphate kinase (PPK), exopolyphosphatase (PPX), and exopolyphosphatase/pppGpp phosphohydrolase (PPX/GPPA)

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 *chr*A 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 numbers, 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 electrodense 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.

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Compliance with ethical standards

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

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