3 Biotech (2018) 8:500
https://doi.org/10.1007/s13205-018-1522-6

SHORT REPORTS

Response to chromate challenge by marine *Staphylococcus* sp. NIOMR8 evaluated by differential protein expression

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Received: 13 September 2018 / Accepted: 17 November 2018 / Published online: 26 November 2018
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Abstract

Liquid Chromatography–Mass Spectrometry-Quadrupole Time of Flight (LC/MS QToF) protein profiling of marine-derived *Staphyloccous gallinarum* NIOMR8 was carried out to evaluate proteins conferring chromate (Cr^{6+}) resistance and possible metabolic pathways that were altered as a result. Expressional (up or down-regulation) responses to varying Cr^{6+} (0, 50, 100, 150, and 200 µg mL^{-1}) concentrations varied, with as many as 346 proteins identified. Most number of proteins—their numbers in parentheses—were up-regulated when grown in medium with 50 µg mL^{-1} (162) and, down-regulated in medium with 100 (281) or 200 µg mL^{-1} Cr^{6+} (280). Among these, eight proteins were commonly up-regulated, while 58 were commonly down-regulated across all conditions of Cr^{6+}. Expression of protein moieties in metabolic pathways like translation (38), transcription (14), replication (18) and repair (4), metabolism of carbohydrates (26), amino acids (27), nucleotides (17), and membrane transport (21) was evidenced. Up-regulation patterns suggest that reduction of molecular oxygen (5), DNA repair (4) and peptide misfolding (7) were the potential protective mechanisms employed to counter Cr^{6+} stress. Additionally, proteins associated with biofilm and cell wall biogenesis highlight their hypothetical involvement in toxicity tolerance. Results also indicate that at higher concentrations of Cr^{6+}, down-regulation of functional proteins impedes normal cellular functions.

Keywords *Staphyloccous gallinarum* · Marine · Chromate · LC/MS QToF

Introduction

Chromium salts and compounds are widely used in diverse industrial processes. Their release in wastewaters into natural ecosystems by improper treatment and/or disposal has converted chromium into a serious environmental contaminant. Of the commercially important trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) states, the former is less mobile, less water-soluble and much less toxic (Kamaludeen et al. 2003). Chromate (Cr^{6+}) being water-soluble and highly mobile rapidly enters the bacterial cytoplasm (Han et al. 2016). Chromate is intracellularly reduced to lower oxidation states [i.e., Cr^{3+} and Cr^{5+}]. The production of transiently highly reactive radical Cr(V) in redox reactions to generate reactive oxygen species leads to oxidative damage of DNA and of cellular components (Ackerley et al. 2006).This damage is responsible for the genotoxic effects of Cr^{6+} (Shi and Dalal 1990). Some bacteria can resist these toxic effects via protein function alterations (Mohapatra et al. 2017) although the processes involved in marine isolates are not widely examined. Proteomic methods can thus be used extensively to examine them and identify coping mechanisms involved at a genome level (Yung et al. 2014). Although bacterial Cr^{6+} reduction has been reported (Lloyd and Lovley 2001), prior proteomic studies focusing on bacterial isolates from non-contaminated environments is lacking. Thus the proteomic response in marine bacteria is worth investigating.

The protein profiling of a marine-derived *Staphylococcus gallinarum* NIOMR8 under Cr^{6+} exposure was carried out using a LC/MS QToF peptide fingerprinting (PMF) approach. Here we try to gain insight into the physiological response to chromium stress and the specific mechanisms of defense involved.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13205-018-1522-6) contains supplementary material, which is available to authorized users.

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Materials and methods

Staphylococcus gallinarum NIOMR8 isolated from water samples collected along the Mandovi Estuary (Lat-15°30′ 19.206″ and Lon-73°50′ 9.5748″) was identified by 16S rRNA gene sequencing (NCBI Acc. No. KY673000, Pereira et al. 2017). Tolerating up to 400 µg mL^{-1} chromate, this strain was evaluated for its response to chromate stress. Cells were harvested, and pellets collected for protein extraction from cultures (mid-log phase) grown in seawater nutrient broth (HiMedia M002, India) supplemented with 0 (control without chromate), 50, 100, 150, and 200 µg mL^{-1} chromate, by centrifugation at 14,000 rpm for 10 min. All resulting pellets were first resuspended in 1 × phosphate buffer followed by 500 µL of urea thiourea buffer [7 M urea, 2 M thiourea, 20 mM Tris (pH 8), 4% CHAPS, 20 mM DTT, 0.8% IPG buffer (pH 3–10) (GE Healthcare, Sweden), 1 × protease inhibitor cocktail (Serva, Germany)]. The entire mixture was transferred to bead bashing tubes with zirconia beads and homogenized twice at 4.5 ms^{-1} for 40 s (FastPrep, MP Biomedicals, USA). Cell debris were separated from the supernatants by centrifugation and the protein content of the crude extracts (supernatant) quantified by Folin–Lowry method (Lowry et al. 1951).

Protein extracts (adjusted to uniform 100 µg) were methanol precipitated, vacuum dried (Eppendorf, Germany) and subjected to in-solution trypsin digestion (Pereira et al. 2018). The digests transferred to autosampler MS vials were analyzed by LC/MS QToF (6538 UHD accurate Mass QTOF LC/MS, Agilent Technologies, USA). The samples run as four technical replicates (A, B, C and D), were injected onto the Prot ID chip 150 II 300A C18 150 mm column. LC separations (in water and 90% acetonitrile) were carried out for 71 min using 0.1% formic acid as adduct and MS/MS was performed from 50 to 2000 *m/z* in positive ion mode. Spectral data acquired using Mass Hunter Data Acquisition software B.06.00 (Agilent Technologies, USA) was analyzed using Spectrum Mill MS Proteomics Workbench ver. B.04.01.141 (Agilent Technologies, USA) with the MS/MS search done against species-specific protein database.

Samples were grouped as technical replicates based on the chromate concentration administered, and the identified proteins were analyzed with Mass Profiler Professional ver. 13 (MPP, Agilent Technologies, USA). Differential significance of samples with respect to regulation of proteins was assessed with a fold change cut-off of 2.0 (applied at a *p* value of 0.2). Venn diagrams for proteins up- or down-regulated were generated by comparing proteins expressed in each condition (50, 100, 150, and 200 µg mL^{-1}) individually, versus control (0 µg mL^{-1}). All identified proteins were grouped based on functional classifications, as per KEGG (Kyoto Encyclopedia of Genes and Genomes) ver. 37 pathways.

Results and discussion

The objective of this study was to examine the proteomic responses of marine bacterial isolate *S. gallinarum* NIOMR8 to varying concentrations of Cr^{6+}.

Functional distribution of identified proteins

In all, 346 proteins corresponding to 12% of the predicted 2938 protein-coding ORF's within *S. gallinarum* proteome (Proteome ID: UP000032093) were identified across all five conditions (0, 50, 100, 150, and 200 µg mL^{-1}). Based on KEGG pathways and metabolic processes, they could be divided into 18 categories (Fig. 1). Most proteins—their numbers mentioned in parentheses—were involved in basic cellular upkeep such as translation (38), transcription (14), replication (18) and repair (4), metabolism of carbohydrate (26), amino acids (27) and nucleotides (17), as well as cellular processes (27) and membrane transport (21). A sizable fraction of proteins were found to group under proteins with enzymatic function (26). Proteins from metabolism of (i) cofactors and vitamins, (ii) lipids, (iii) glycans, (iv) terpenoids and polyketides; and (v) cell wall biogenesis were commonly grouped under other metabolic processes (34).

Comparing protein profiles of *Staphylococcus* sp. NIOMR8 subjected to chromate stress

Quantitative analysis of identified proteins revealed significant changes in protein expression due to Cr^{6+} exposure. As many as 162, 65, 84, and 66 proteins were up-regulated, respectively, when the isolate was grown in medium with 50, 100, 150, and 200 µg mL^{-1} Cr^{6+}, with the highest number in broth with 50 µg mL^{-1} Cr^{6+}. Among the 346, 8 proteins (~2%) were up-regulated in all tested Cr^{6+} concentrations (Fig. 2a). There was substantial down-regulation of proteins when grown in medium with 50, 100, 150, and 200 µg mL^{-1} Cr^{6+} (184, 281, 262 and 280 proteins, respectively) with many more down-regulated in medium with 100 or 200 µg mL^{-1} Cr^{6+}. Among these, 58 (~17%) were commonly suppressed in all concentrations of Cr^{6+} (Fig. 2b) possibly due to reduction/suppression of general cellular processes on exposure to heavy metals (Yung et al. 2014).

Chromate stress induced up-regulated proteins

The eight commonly up-regulated proteins along with their fold change (Table 1) predictably include proteins

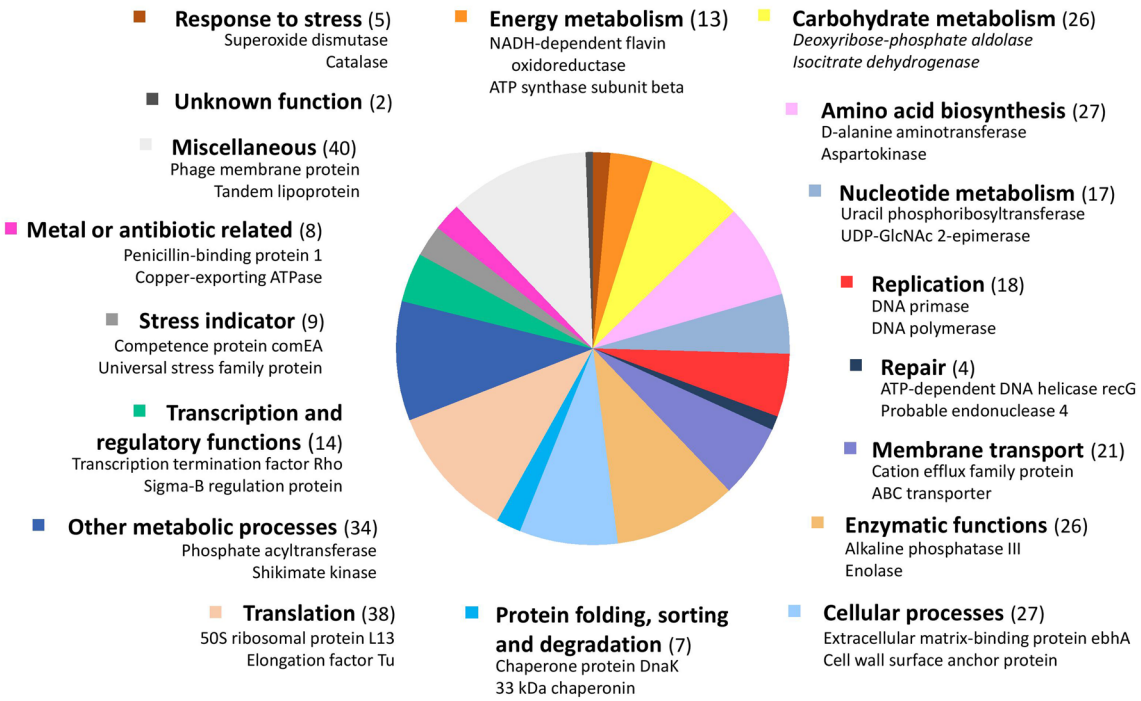

Fig. 1 Functional distribution of all proteins acquired for *Staphylococcus* sp. NIOMR8 under all conditions. Numbers in parentheses indicate number of proteins per category

involved in translation (3), cell cycling (1), protein folding, sorting and degradation (1), membrane transport (1), transcription (1) and enzymatic function (1). Universal stress family protein, ATP-dependent DNA helicase recG, penicillin-binding protein 1, 33 kDa chaperonin, coenzyme A disulfide reductase, DNA double-strand break repair rad50 ATPase, competence protein comEA, L-lactate dehydrogenase were among the 118 found up-regulated only at 50 µg mL^{-1} Cr^{6+} (Fig. 2a). Capsular polysaccharide synthesis enzyme, anthranilate synthase component I, superoxide dismutase (SOD), universal stress protein family, ATP synthase subunit delta were among the 35 proteins that were up-regulated specifically at 100 µg mL^{-1} Cr^{6+}. Similarly, universal stress family domain-containing protein, bifunctional preprotein translocase subunit SecD/SecF, nitric oxide synthase oxygenase, cell wall-associated biofilm protein, cell wall surface anchor protein were among the 43 proteins that were up-regulated only at 150 µg mL^{-1} Cr^{6+}; and pyruvate dehydrogenase E1 component beta subunit, capsular polysaccharide biosynthesis protein Cap5N, biofilm-associated protein, OsmC-like protein were among the 32 proteins that were up-regulated only at 200 µg mL^{-1} Cr^{6+} (Supplementary data). Chaperone protein DnaK (DnaK) was up-regulated in 50 and 150 µg mL^{-1} Cr^{6+} and cell wall surface anchor family protein was up-regulated in only 50 and 100 µg mL^{-1} Cr^{6+}. Putative cell wall-associated protein/RHS repeat family and trigger factor were up-regulated in both 150 and 200 µg mL^{-1} Cr^{6+}.

Presence of proteins such as universal stress family protein, competence protein comEA, universal stress family domain-containing protein and OsmC-like protein indicate intracellular stress. Coenzyme A disulfide reductase and L-lactate dehydrogenase are reported with roles in cell redox homeostasis and oxidative stress response each by Boylan et al. (2006) and Richardson et al. (2008). SOD known to catalyze superoxide removal was specifically detected only in chromate-exposed cells by Thompson et al. (2010, *Pseudomonas putida* F1) and Ackerley et al. (2006, *Escherichia coli* K-12). DNA repair proteins, competence protein comEA, ATP-dependent DNA helicase recG (genome maintenance and SOS response) ATP-dependent nuclease subunit B and DNA double-strand break repair rad50 ATPase (double-stranded breaks via homologous recombination) repair DNA, damaged as a consequence of oxidative stress. Hu et al. (2005) similarly, reported the up-regulation of DNA repair proteins in chromate stressed *Caulobacter crescentus*. DnaK, 33 kDa chaperonin and 60 kDa chaperonin function in peptide folding and transport along with trigger factor and bifunctional preprotein translocase subunit SecD/SecF. These are known to prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides. Khan et al. (2015) also reported enhanced expression of protein folding machinery in *Escherichia coli* P_4 such as DnaK

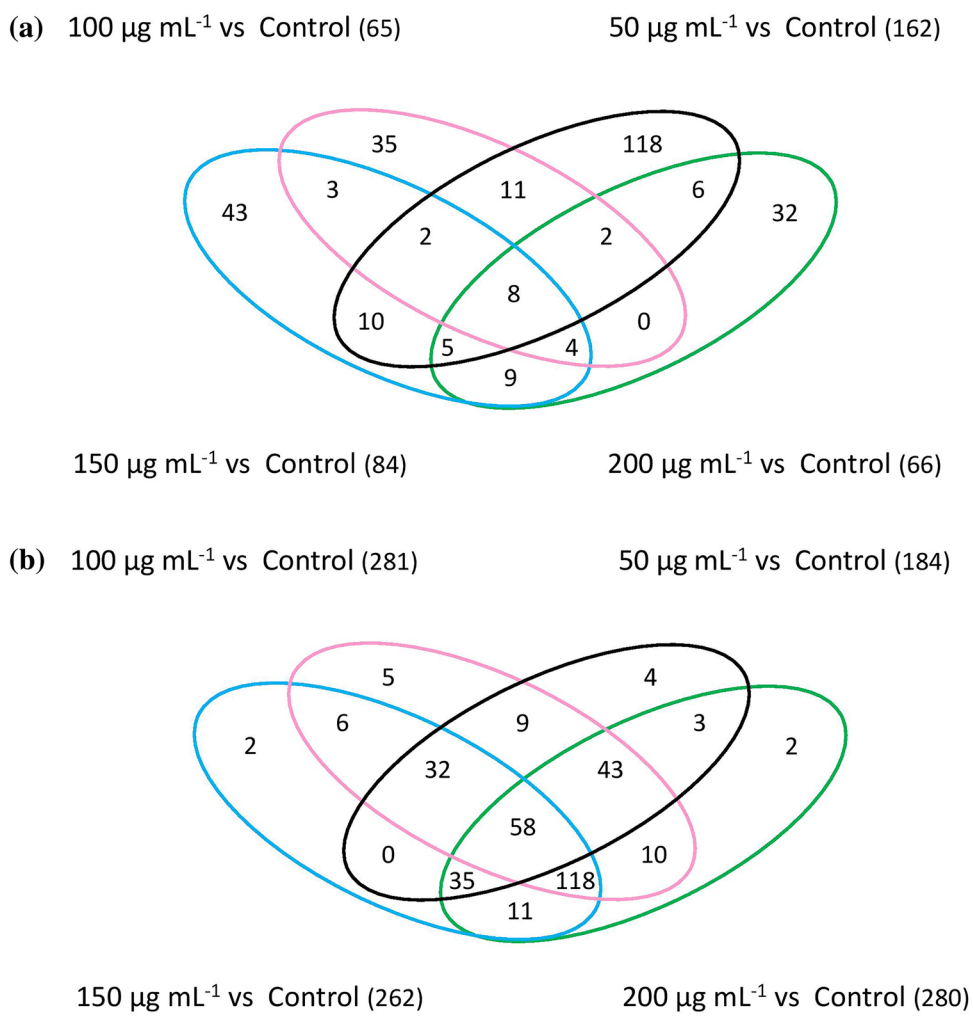

Fig. 2 Venn diagrams of proteins **a** up-regulated and **b** down-regulated in response to different chromate conditions. Each condition lists (entities) the number of proteins for each condition (50, 100, 150 or 250 µg mL^{-1}) against the control (0 µg mL^{-1})

under metal induced stress, suggesting that these proteins rectify misfolded proteins generated as a direct result of oxidative stress. Capsular polysaccharide synthesis enzyme, cell wall surface anchor protein, capsular polysaccharide biosynthesis protein Cap5N, putative cell wall-associated protein/RHS repeat family, cell wall-associated biofilm protein and biofilm-associated protein all have annotated functions in cell wall and envelope biogenesis. Thompson et al. (2010) similarly reported expression of such proteins on chromate exposure highlighting possible biosorption and metal ion entrapment (Lameiras et al. 2008). Proteins expressed in this study thus suggest potential chromate resistance mechanisms employed by *Staphylococcus* sp. NIOMR8 summarized in Fig. 3.

Chromate stress induced down-regulated proteins

Most proteins commonly down-regulated in all conditions (58) were part of metabolic processes such as carbohydrate (7), nucleotide (3), amino acid (3) and energy metabolism (3), translation (7), transcription (6), replication (4) and other cellular processes (25). Branched-chain amino acid transport system II carrier protein, 5′-nucleotidase, 50S ribosomal protein L30 and 50S ribosomal protein L11 were down-regulated only at 50 µg mL^{-1} Cr^{6+}. Histidine kinase, elongation factor tu (Fragment), clumping factor B, 30S ribosomal protein S9 and 2-oxoisovalerate dehydrogenase were down-regulated only at 100 µg mL^{-1} Cr^{6+}. Similarly, branched-chain alpha-keto acid dehydrogenase subunit E2 and pathogenicity island protein; and ribosome maturation factor RimM and cell division protein FtsZ were uniquely down-regulated at 150 and 200 µg mL^{-1} Cr^{6+}, respectively (Supplementary data).

Conclusion

Protein profiling of *Staphylococcus* sp. NIOMR8 by LC/MS QToF analysis revealed differential expression of proteins under varying chromate stress. Up-regulation of proteins active in oxidative stress-response, reduction of molecular oxygen, DNA repair, and in peptide transport

Table 1 Proteins commonly up-regulated in *Staphylococcus* sp. NIOMR8 across varying chromate conditions and their fold change values

Gene	Annotation	Fold change per chromate concentration (μg mL^{-1}) in comparison to the control				Pathway involved (KEGG)	Function	Swiss-Prot ID
		50	100	150	200			
HMPREF1208_00854	DNA-binding protein HU	0.412	0.744	1.962	0.286	Cell cycle	DNA condensation	K8N651
groL	60 kDa chaperonin	8.274	8.553	13.480	4.055	Protein folding, sorting and degradation	Stress response	H7FGQ4
rpsS	30S ribosomal protein S19	4.238	4.483	14.490	8.771	Translation	Ribosomal protein	H7FG26
	Int activator	12.894	4.352	18.091	17.865	Transcription	Regulation of transcription	C8CH09
rplO	50S ribosomal protein L15	0.151	1.320	1.271	0.138	Translation	Ribosomal protein	C2LX53
ptsG	Glucose-specific phosphotransferase enzyme IIA component	13.643	4.535	4.745	4.859	Membrane transport	Phosphotransferase system (PTS)	K8N899
rplW	50S ribosomal protein L23	4.335	4.331	9.040	4.348	Translation	Ribosomal protein	A0A064C7K6
C273_07362	Carboxyl-terminal protease	10.109	8.837	12.249	0.082		Membrane component with peptidase activity	K9AY65

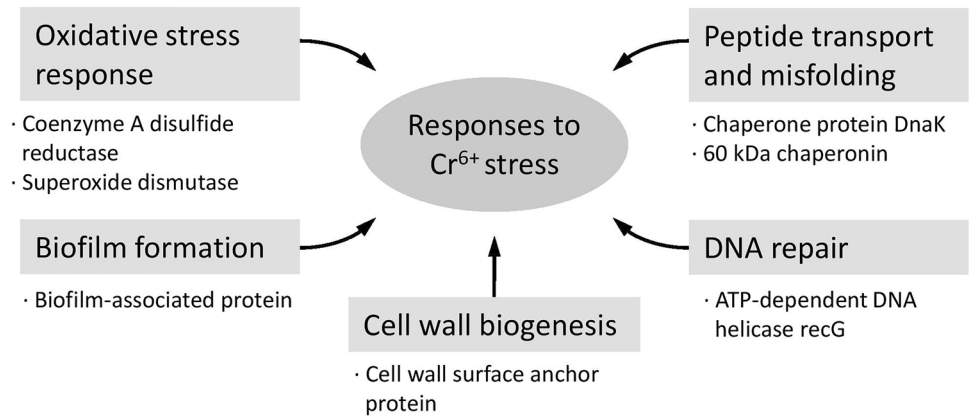

Fig. 3 Potential resistance mechanisms of marine-derived *Staphylococcus* sp. NIOMR8 to chromate. Proteins expressed in this study are listed below

(and folding) possibly promotes tolerance to induced stress. Proteins common to all conditions suggest that, some cellular functions are impeded by down-regulation of related proteins. However, the overall chromate tolerance machinery is intact enabling its endurance of Cr^{6+} stress. In this regard, examining the response of this strain to varying Cr^{6+} stress, to infer possible tolerance strategies employed by marine-derived bacteria is useful in recognizing heavy metal detoxification strategies.

Acknowledgements The authors thank the Director, CSIR-NIO for his support and providing all facilities to carry out this study. The authors acknowledge the funding support from CSIR project PSC0206. The first author acknowledges research fellowship by the University Grant Commission (UGC) (Sanction letter No: 18-12/2011(ii) EU-V). This is NIO's contribution No. 6316.

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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