



What makes *A. guillouiae* SFC 500-1A able to co-metabolize phenol and Cr (VI)? A proteomic approach

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ABSTRACT

Acinetobacter guillouiae SFC 500-1A is an environmental bacterium able to efficiently co-remediate phenol and Cr (VI). To further understand the molecular mechanisms triggered in this strain during the bioremediation process, variations in the proteomic profile after treatment with phenol and phenol plus Cr(VI) were evaluated.

The proteomic analysis revealed the induction of the β -ketoacid pathway for phenol oxidation and the assimilation of degradation products through TCA cycle and glyoxylate shunt. Phenol exposure increased the abundance of proteins associated to energetic processes and ATP synthesis, but it also triggered cellular stress. The lipid bilayer was suggested as a target of phenol toxicity, and changing fatty acids composition seemed to be the bacterial response to protect the membrane integrity. The involvement of two flavoproteins in Cr(VI) reduction to Cr(III) was also proposed. The results suggested the important role of chaperones, antioxidant response and SOS-induced proteins in the ability of the strain to mitigate the damage generated by phenol and Cr (VI).

This research contributes to elucidate the mechanisms involved in *A. guillouiae* SFC 500-1A tolerance and co-remediation of phenol and Cr(VI). Such information may result useful not only to improve its bioremediation efficiency but also to identify putative markers of resistance in environmental bacteria.

1. Introduction

Phenol and chromium are listed among the most dangerous environmental pollutants due to their toxicity and persistence [1,2]. They are usually discharged together in diverse industrial effluents insufficiently treated, such as those from leather tannery, wood treatment plants, car manufacturing and petroleum refineries, which causes the high concentrations detected in natural waters worldwide [3]. Therefore, the development of cost-effective and non-disruptive techniques for their remediation has become a global priority in the last years, and bioremediation could be an interesting option for such purpose [4].

In this sense, many bacterial strains have demonstrated to be useful for biodegradation of phenols and other monoaromatic compounds through different oxidation pathways. However, heavy metals are known to be powerful inhibitors of such activity [5]. Similarly, several microorganisms display good performance for enzymatic reduction of Cr(VI) to the less toxic species Cr(III) employing aliphatic compounds as

electron donors, but there is a limited number of strains capable of coupling aromatics degradation to Cr(VI) reduction [6–8]. Moreover, there is scarce information about physiological and molecular responses in bacteria during the simultaneous removal of these pollutants.

Acinetobacter guillouiae SFC 500-1A is an environmental isolate that efficiently co-remediates phenol and Cr(VI) in short time periods. Its ability to metabolize phenol through *ortho*-oxidation reactions was previously demonstrated. In addition, its enzymatic potential to reduce Cr(VI) to Cr(III) and immobilize it into the biomass was also studied [9]. Nevertheless, there are still many questions to be answered, such as the routes that drive the assimilation of phenol degradation intermediates, the main enzymes involved in Cr(VI) reduction, destination of contaminants and their derivatives, cellular damage caused by stress and antioxidant response, among others. In this sense, having an overview of protein profiles during exposure to these contaminants might help to elucidate the molecular networks involved in the bioremediation potential of this strain.

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In the present work, the proteomic variations of *A. guillouiae* SFC 500-1A in response to phenol and phenol plus Cr(VI) were evaluated by two dimensional electrophoresis coupled to mass spectrometry. The obtained results may help to understand the molecular mechanisms triggered by this strain and provide potential biomarkers for selecting new bacteria able to cope with these contaminants.

2. Materials and methods

2.1. Bacterial strain and culture conditions

A. guillouiae SFC 500-1A previously isolated from contaminated tannery sediments was used in this study [9,10].

For proteomic experiments, the strain was pre-grown during 20 h in TY broth [11] supplemented with phenol and Cr(VI), centrifuged (10,000 g, 4 °C, 15 min) and re-suspended in mineral medium plus 0.3% yeast extract (MMYE) [9] up to a cellular concentration of 2×10^9 CFU/ml. This bacterial suspension was employed to inoculate Erlenmeyer flasks (20% v/v) containing MMYE medium with and without pollutants and incubated at 28 ± 2 °C. Three conditions were tested by triplicate: medium MMYE (**control** condition), medium MMYE supplemented with phenol 300 mg/l (**phenol** condition) and with 300 mg/l phenol plus 10 mg/l Cr(VI) [**phenol + Cr(VI)** condition].

Residual phenol was measured hourly until the removal was around 50–75%. At this point growth and residual Cr(VI) concentration were also determined and cells were harvested and centrifuged (10,000 g, 4 °C, 15 min). Pellets were washed three times with 0.85% NaCl and kept at -20 °C.

Cr(VI) and phenol removal were evaluated by spectrophotometric methods, according to APHA-AWWA [12] and Wagner and Nicell [13], respectively.

2.2. Samples preparation for two dimensional electrophoresis (2DE)

Proteins extraction was carried out according Kim et al. [14] with modifications. For that, pellets were resuspended in Tris-HCl (20 mM; pH 8.0) and disrupted by ultrasonication. The obtained suspensions were treated with nucleases (final concentration: 50 µg/ml) and cell debris was removed by centrifugation (15,000 g, 30 min). Resulting supernatants were lyophilized and further rehydrated with buffer I [50 mM Tris-HCl; 0.3% w/v Sodium Dodecyl Sulphate (SDS); 0.2 M dithioerythritol (DTE)], heated (95 °C, 5 min) and then resuspended in IEF buffer composed of 7 M urea; 2 M thiourea; 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate hydrate (CHAPS); 1% DTE; 0.5% IPG buffer.

Protein concentration was determined using Bradford protein assay (BioRAD) and bovine serum albumin as standard.

2.3. 2DE

2DE was performed using the Immobiline polyacrylamide system. Isoelectric focusing (IEF) was carried out with pH 4–7 nonlinear, immobilized pH gradient strips (18 cm) employing Ettan™ IPGphor™ system (GE Healthcare, Uppsala, Sweden). Dry strips were rehydrated with 350 µL of IEF buffer containing 60 µg of proteins for 2D gel map construction, and 600 µg for protein identification through preparative gels. Electrical conditions were: 200 V for 7 h, from 200 V to 3500 V for 2 h, 3500 V for 2 h, from 3500 to 5000 V for 2 h, 5000 V for 3 h, from 5000 to 8000 V for 1 h, 8000 V for 3 h, 8000 V for a total of 80,000 V h.

After IEF, strips were equilibrated using two buffers, the first composed of 6 M urea, 2% w/v SDS, 2% w/v DTE, 30% v/v glycerol and 0.05 M Tris-HCl pH 6.8 for 12 min and the second of 6 M urea, 2% w/v SDS, 2.5% w/v iodoacetamide, 30% v/v glycerol, 0.05 M Tris-HCl pH 6.8 and a trace of bromophenol blue for a further 5 min. SDS-PAGE was carried out at 40 mA/gel constant current on 9–16% SDS polyacrylamide linear gradient gels at 9 °C. Analytical gels were stained

with ammoniacal silver nitrate [15] and digitized with a Molecular Dynamics 300S laser densitometer (4000 × 5000 pixels, 12 bits/pixel; Sunnyvale, CA, USA) for spot detection and protein map construction. For protein identification, preparative gels were attached to a glass surface using Bind-Silane (γ -methacryloxypropyltrimethoxysilane) (LKB-Produkter AB, Bromma, Sweden), stained with SYPRO Ruby (Bio-Rad Laboratories, Hercules, CA) and digitalized with a Typhoon 9400 laser densitometer (GE Healthcare) [16,17].

2.4. Image analysis and statistics

Two-dimensional image analysis was performed with Image Master Platinum 7.0 software (GE Healthcare). Spots were first detected and clarified by setting parameters such as filtering and smoothing. After that, gels belonging to each group were matched with intra-class reference gels known as *master gels* and then the three master reference gels were matched with each other. The algorithm of the software emphasized quantitative differences between gels, considering a spot to be differentially regulated when the mean relative percentage volume ratio ($\%V = V \text{ single spot} / V \text{ total spots}$) was ± 2 and satisfied the T-test ($p \leq 0.05$).

Statistical analysis was performed by ANOVA test followed by *post hoc* Tukey test.

2.5. Protein identification by peptide mass fingerprinting

Differentially regulated spots were identified by peptide mass fingerprinting (PMF) using mass spectrometry (MS) as previously described [17]. Spots stained with Sypro ruby were mechanically excised with Ettan Spot Picker (GE Healthcare) and destained in 2.5 mM ammonium bicarbonate and 50% acetonitrile (ACN). After dehydration in ACN, the spots were rehydrated in trypsin solution and digested overnight at 37 °C. Each digested protein was spotted onto the MALDI target, dried, covered with a matrix solution of α -cyano-4-hydroxycinnamic acid in 50% v/v acetonitrile and 0.5% v/v trifluoroacetic acid, and allowed to dry again. Peptide masses were acquired by ultrafleXtreme™ MALDI-ToF/ToF (Bruker Corporation, Billerica, MA, United States). PMF search was performed using MASCOT software (Matrix Science Ltd., London, UK, <http://www.matrixscience.com>). The search parameters were set as follows: Swiss-Prot/TrEMBL and NCBI nr as databases, *Proteobacteria* as taxonomy, 100 ppm as mass tolerance, one missed cleavage site as acceptable, carbamidomethylation (iodoacetamide alkylation of cysteine) as fixed modification, and oxidation of methionine as a possible modification. The biological functions of the identified proteins were searched in the UniProt database (www.ebi.uniprot.org) and NCBI database (www.ncbi.nlm.nih.gov).

3. Results and discussion

3.1. Growth and removal of phenol and Cr(VI) by *A. guillouiae* SFC 500-1A

A. guillouiae SFC 500-1A was able to grow in the presence of phenol and phenol plus Cr(VI), and to remove them in a few cultivation hours. Table 1 shows how bacterial growth was stimulated by phenol but negatively affected by Cr(VI).

Phenol degradation above 50% was detected in both conditions after 7 h, while Cr(VI) removal was around 38% in the same time period. At this time, samples were collected and proteomic assays were carried out.

These results were in agreement with previous reports that indicated the ability of *A. guillouiae* SFC 500-1A to simultaneously degrade phenol and reduce Cr(VI) to Cr(III) through enzymatic mechanisms [9].

Table 1
Bacterial growth and contaminants removal.

| | Control | Phenol* | Phenol* + Cr(VI)** |
|-----------------------------|---|---|---|
| Initial cell count (CFU/ml) | $2.8 \times 10^8 (\pm 0.3 \times 10^8)$ | $3.1 \times 10^8 (\pm 0.2 \times 10^8)$ | $3.0 \times 10^8 (\pm 0.3 \times 10^8)$ |
| Final cell count (CFU/ml) | $1.1 \times 10^9 (\pm 0.8 \times 10^8)$ | $1.7 \times 10^9 (\pm 0.9 \times 10^8)$ | $8.4 \times 10^8 (\pm 0.6 \times 10^8)$ |
| Removed phenol (%) | – | 60.8 (± 2.3) | 54.2 (± 1.2) |
| Removed Cr(VI) (%) | – | – | 38 (± 3.9) |

* Initial concentration 300 mg/l.

** Initial concentration 10 mg/l.

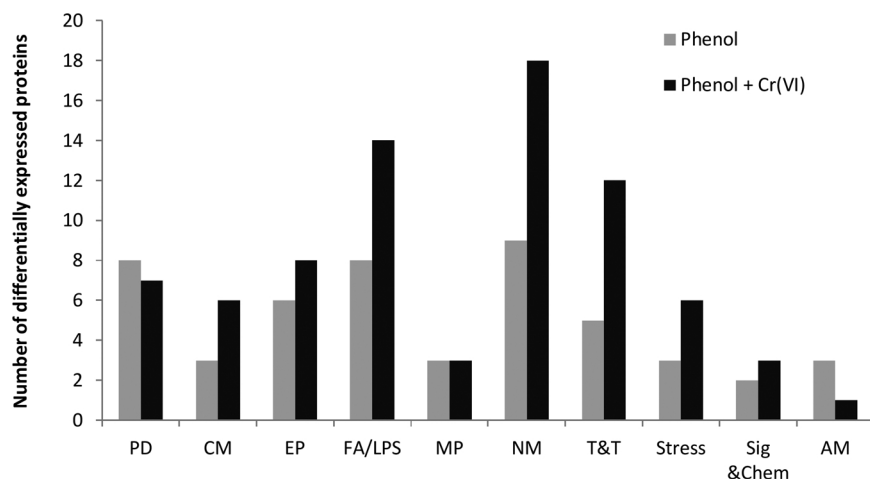


Fig. 1. Number of identified protein spots whose abundance varied following *A. guillouiae* sp. SFC 500-1A exposure to phenol and phenol plus Cr(VI). Categories: PD (Phenol degradation), CM (Carbon metabolism), EP (Energetic processes), FA/LPS (Fatty acids and LPS metabolism), MP (Membrane proteins and transporters), NM (Nitrogen metabolism), T&T (Transcription and translation), Stress (Stress processes and antioxidant response), Sig&Chem (Signaling, regulation and chemotaxis), AM (Metabolism of aromatic compounds).

3.2. Proteome variations associated with “phenol” and “phenol plus Cr(VI)” treatments

Phenol and phenol plus Cr(VI) caused statistically significant changes in the relative abundance of 87 identified protein spots, which were classified into 10 functional groups (Fig. 1). Not surprisingly, most of them are involved in cellular metabolism of carbon, nitrogen and fatty acids (CM, NM, FA/LPS, respectively), phenol degradation (PD), and energetic processes (EP). The concentration of membrane proteins (MP) and proteins involved in signaling and chemotaxis also varied with the addition of pollutants.

Some enzymes related to the metabolism of aromatic compounds other than phenol (AM) were identified when the strain was exposed to phenol. The addition of Cr(VI) induced qualitative and quantitative proteome variations related to NM and CM, stress, fatty acids and lipopolysaccharides (LPS) metabolism (FA/LPS), as well as transcription and translation processes (T&T).

Master gels for the three analyzed conditions are presented in Fig. 2.

3.3. Proteomic variations in *A. guillouiae* SFC 500-1A exposed to phenol

3.3.1. Phenol degradation, carbon assimilation and energetic processes

Phenol-exposition caused an increase in the relative abundance of proteins related to phenol degradation, such as phenol hydroxylase, catechol 1,2-dioxygenase, muconate cycloisomerase, muconolactone isomerase and 3-oxoadipate CoA-transferase (Table 2.1). Phenol hydroxylase catalyzes the initial reaction in phenol biotransformation to catechol, which is then cleaved by catechol 1,2-dioxygenase to cis, cis-muconate. These results suggest that phenol degradation in this strain occurs through the β -ketoacid pathway, since catechol 1,2-dioxygenase, together with muconate cycloisomerase and muconolactone isomerase have been described as key enzymes for the degradation of aromatic compounds to β -ketoacid. Furthermore, 3-oxoadipate CoA-transferase is essential for the degradation products to reach the TCA cycle [18,19].

In addition, a decrease in the intracellular concentration of two key

enzymes of the TCA cycle (2-oxoglutarate dehydrogenase and succinyl-CoA synthetase) was observed while the relative abundance of the isocitrate lyase was increased (Table 2.2). Isocitrate lyase is involved in the bypass of TCA cycle to glyoxylate cycle, an anaplerotic pathway to synthesize cellular precursors [20]. Such bypass is not usual in bacteria that carry out phenol degradation through β -ketoacid formation, but it has been described in phenol-degrading bacteria through meta-fission pathway [21,22]. A similar behavior was reported in *Acinetobacter* sp. DW-1 grown on a mixture of phenol and acetate and in *A. oleivorans* DR1 during hexadecane degradation [19,23]. Given that glyoxylate shunt constitutes a shortcut for providing high biomass yield avoiding the CO_2 -releasing steps [24], it is likely that *A. guillouiae* SFC 500-1A needs to synthesize cellular precursors at a rapid rate during phenol detoxification.

The high abundance of an electron transfer flavoprotein and the catalytic subunit (β) of the ATP synthase protein suggest an active energetic metabolism when this strain is grown in the presence of phenol (Table 2.3). Furthermore, the induction of enzymes associated to B vitamins synthesis (pyridoxine 5'-phosphate oxidase and 6,7-dimethyl-8-ribityllumazine synthase) would indicate the need of cofactors for redox reactions during phenol oxidation and carbohydrates catabolism [25].

3.3.2. Metabolism of fatty acids

It is known that one of the main cellular targets of phenol toxicity is the phospholipid bilayer. For instance, phenol increases membrane fluidity, changes protein-lipid ratio and destabilizes its functioning. As a way to alleviate such effect, some bacterial cells are able to reorganize the fatty acids composition of their membrane in order to increase its rigidity [26].

In this context, the exposure of *A. guillouiae* SFC 500-1A to phenol caused an increase in the abundance of proteins associated to fatty acids biosynthesis and degradation (Table 2.4.1). For example, acyl-CoA synthetase is involved in fatty acid activation for the further β -oxidation, while acetyl-CoA carboxylase catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, a precursor of fatty acids synthesis.

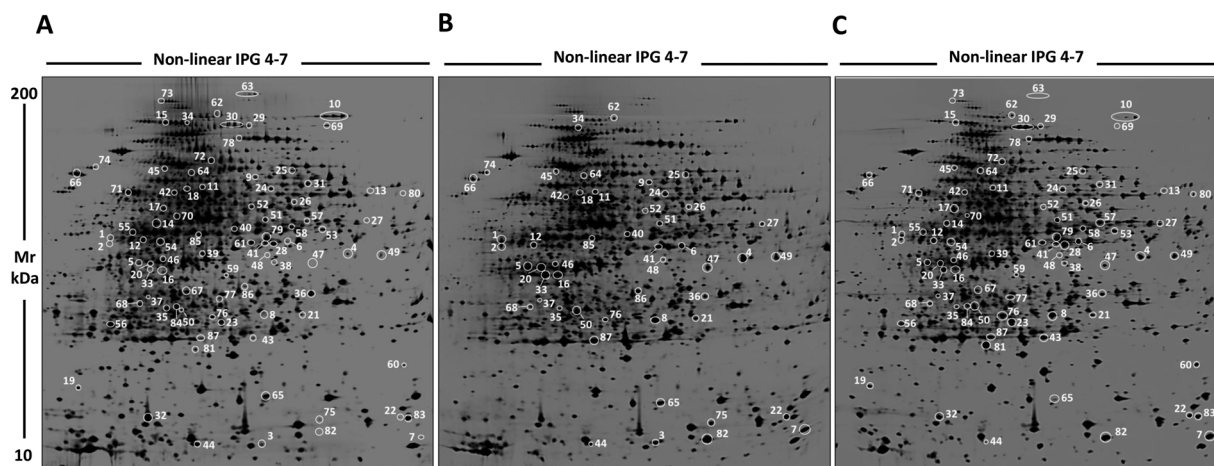


Fig. 2. Representative 2DE gels showing the proteome profile of *A. guillouiae* sp. SFC 500-1A in (A) control condition and in the presence of (B) phenol and (C) phenol plus Cr(VI). The identified protein spots whose relative abundance varied between the control condition and each treatment are indicated with numbers (corresponding to those reported in Table 2).

Moreover, methylisocitrate lyase (MICL) was among the most strongly expressed enzymes in the presence of phenol (Table 2.4.2). MICL and 2-methylisocitrate dehydratase belong to the methylcitrate cycle, which is crucial for the clearance of toxic propionyl-CoA formed during β -oxidation of odd-chain and branched-chain fatty acids [27]. These results suggest a possible membrane restructuring in *A. guillouiae* SFC 500-1A exposed to phenol, reducing the levels of unsaturated branched-chain fatty acids that destabilize the lipid bilayer. Adjusting the saturation degree of fatty acids has played a major role in the homeostasis of cytoplasmic membrane viscosity in other *Acinetobacter* strains exposed to toxic substrates [28,29].

Phenol led to a decrease in relative concentration of succinyl-CoA:3-ketoacid-coenzyme A transferase (SCOT) and 3-hydroxybutyrate dehydrogenase (HBDH), enzymes involved in degradation of polyhydroxyalkanoates (PHA), but there was no evidence about induction of PHA synthesis (Table 2.4.3). A similar behavior has been observed in other bacteria under stress situations. Apparently, the release of acetoacetyl-CoA from PHA granules increases oxidative stress due to generation of NADH and FADH₂, which are utilized by the electron transport chain. Therefore, the inhibition of PHA depolymerization would operate as bacterial antioxidant response [30]. This finding is important from a biotechnological perspective considering that *A. guillouiae* SFC 500-1A could be a PHA producer, although this potential does not appear to be stimulated by phenol.

3.3.3. Membrane proteins and transporters

The content of the outer membrane protein Omp38 and the porin OprB was increased up to 2.7-fold after phenol exposure (Table 2.5).

Omp38 is an alternative name used to refer to OmpA, a family of monomeric proteins located in the outer membrane with a wide range of functions [31]. Among them, it is to be highlighted the emulsification of aromatic compounds, which increases their bioavailability and allow their incorporation into bacterial cells [32]. Therefore, this outer protein may be involved in phenol uptake by the strain SFC 500-1A, as it was already observed in other *Acinetobacter* strains [33].

OprB is an outer membrane porin with high affinity for glucose that is also able to non-specifically bind other compounds with hydroxyl groups [34]. In some pseudomonads, for example, OprB was involved in phenol uptake and its disruption altered the microbial behavior in presence of the contaminant [35,36]. This background along with the results obtained from our proteomic study suggested that both membrane proteins may play some role in phenol uptake by this strain.

3.3.4. Nitrogen metabolism

The 2DE profiles of the phenol-stressed strain showed a decreased abundance of the nitrogen regulatory protein P-II and glutamine synthetase (Table 2.6.1). In general, P-II protein is up regulated during stationary phase and nutrient-limiting conditions, favoring the ammonium acquisition and assimilation into glutamine by glutamine synthetase [37]. The down regulation of P-II has been described in another *Acinetobacter* strain as a mechanism to balance the uptake of nitrogen and carbon in order to avoid wasting of metabolic energy under stress [38].

The increased concentration of some enzymes responsible for amino acids metabolism also suggested no nitrogen limitation in *A. guillouiae* SFC 500-1A under the tested conditions (Table 2.6.2). Among them, the enzyme imidazole glycerol phosphate synthase plays a central role in normal nitrogen metabolism through histidine biosynthesis [39]. Additionally, D-alanine-D-alanine ligase and dihydrodipicolinate reductase are involved in the synthesis of peptidoglycan precursors (alanine and meso-diaminopimelate). Peptidoglycan is essential for cell wall stabilization in gram-negative bacteria and helps to decrease cell permeability to hydrophobic compounds. Modification of bacterial envelope composition caused by exposure to aromatic compounds is well documented [40,41].

3.3.5. Transcription and translation processes

In cells of *A. guillouiae* SFC 500-1A grown on phenol, the expression levels of proteins involved in synthesis processes were variable. For example, the concentration of 30S ribosomal protein S1 and RNA polymerase (Table 2.7), as well as various enzymes involved in the amino acids metabolism (Table 2.6.2) was increased, but the abundance of 50S ribosomal protein L9 and transcription termination factor NusA was decreased. A similar behavior was observed in strains of *A. baumannii* exposed to antimicrobial agents, which has been related to the typical increase in generation time and lag phase observed when bacteria are exposed to toxic chemicals [38,42].

3.3.6. Stress response

Heat shock proteins, starvation proteins and molecular chaperones are commonly induced in bacteria in response to environmental stress [43]. Aromatic compounds such as phenol and organic solvents are known to be toxic to bacterial cells, as they can damage the cell membrane through lipids peroxidation and proteins release [44–46]. Thus, the strong expression of the peroxidase AhpC, the osmotically inducible protein OsmC and the chaperone DnaK may be a possible defense strategy of *A. guillouiae* SFC 500-1A against phenol toxicity

Table 2
Differential abundance of proteins identified by MALDI-ToF MS in *A. guillouiae* SFC 500-1A exposed to phenol and phenol plus Cr(VI).

| Spot | Protein name and classification | Abbreviation | Accession code | Theoretical | | Mascot results | | | Average fold change* Phenol/ Control | Ph + Cr (VI)/ Control | Ph + Cr (VI)/ Phenol |
|---|---|---------------|----------------|-------------|----------|----------------|----------|------------------|--|-----------------------------|----------------------------|
| | | | | pI | MW (KDa) | Score | Coverage | Matched peptides | | | |
| 1 Phenol degradation | | | | | | | | | | | |
| 1 | Phenol hydroxylase (sub DMS) | PH(DMS) | gi 2605613 | 4.7 | 39.7 | 226 | 70 | 21 | +6.6 | +3.7 | -1.7 |
| 2 | Phenol hydroxylase (sub P5) | PH (P5) | gi 490859324 | 4.7 | 39.8 | 161 | 42 | 13 | +6.5 | +3.0 | -2.1 |
| 3 | Phenol hydroxylase | PH | gi 490859323 | 5.5 | 13.9 | 127 | 60 | 7 | +8.4 | ND | -4.1 |
| 4 | Phenol hydroxylase (sub P1) | PH (P1) | gi 490859317 | 5.8 | 38.6 | 196 | 50 | 17 | +12.3 | +11.3 | ND |
| 5 | Catechol 1,2-dioxygenase | CAT A | gi 490859513 | 4.9 | 34.6 | 166 | 64 | 16 | +19.9 | +11.2 | -1.8 |
| 6 | Muconate cycloisomerase 1 | CAT B | gi 490859524 | 5.5 | 39.9 | 109 | 24 | 7 | +17.7 | +12.7 | ND |
| 7 | Muconolactone delta-isomerase | CAT C | gi 490958629 | 5.7 | 11.4 | 101 | 48 | 7 | +32.5 | +32.9 | ND |
| 8 | 3-oxoadipate CoA-transferase (sub. A) | CAT I | gi 674995151 | 5.3 | 24.3 | 172 | 63 | 17 | +26.9 | +19.9 | -1.3 |
| 2 Carbon metabolism | | | | | | | | | | | |
| 9 | Isocitrate lyase | IL | gi 490994972 | 5.3 | 59.8 | 209 | 34 | 20 | +2.0 | ND | -2.8 |
| 10 | 2-oxoglutarate dehydrogenase (E1) | OGD 1 | gi 490855238 | 5.8 | 106.1 | 364 | 44 | 39 | ND | -4.5 | -6.3 |
| 11 | 2-oxoglutarate dehydrogenase (E2) | OGD 2 | gi 490855236 | 5.2 | 43.5 | 229 | 49 | 21 | -1.4 | -2.1 | -1.5 |
| 12 | Succinyl-CoA synthetase (sub. β) | SCS | gi 490855232 | 4.9 | 41.8 | 254 | 68 | 26 | -1.9 | -2.1 | ND |
| 13 | Succinate dehydrogenase | SQR | gi 490855242 | 5.7 | 59.7 | 203 | 37 | 20 | ND | +2.4 | ND |
| 14 | Enolase | ENO | gi 490857888 | 4.9 | 46.2 | 237 | 48 | 18 | ND | -2.2 | -1.8 |
| 15 | Aconitate hydratase 2 | AH 2 | gi 490858864 | 5.0 | 95.1 | 356 | 44 | 33 | ND | -1.9 | -2.0 |
| 3 Energetic processes, cofactors and electron carriers | | | | | | | | | | | |
| 16 | ATP synthase (sub b) | ATPS β | gi 490861379 | 5.1 | 50.3 | 173 | 50 | 17 | +4 | +3.2 | ND |
| 17 | ATP synthase (sub b) | ATPS β | gi 490794371 | 5.1 | 50.3 | 105 | 32 | 10 | ND | +3.1 | +3.1 |
| 18 | ATP synthase (sub a) | ATPS α | gi 490861381 | 5.2 | 55.6 | 226 | 39 | 21 | -2.3 | ND | ND |
| 19 | ATP synthase (sub d) | ATPS δ | gi 490961892 | 4.6 | 19.3 | 93 | 44 | 5 | ND | +1.8 | +2.2 |
| 20 | Electron transfer flavoprotein (sub a) | ETF | gi 490855183 | 4.9 | 31.4 | 248 | 89 | 20 | +19 | +6.8 | -2.0 |
| 21 | Pyridoxine 5'-phosphate oxidase | PDX H | gi 490856558 | 5.7 | 25.6 | 138 | 67 | 10 | +2.2 | +2.3 | ND |
| 22 | 6,7-dimethyl-8-ribityllumazine synthase | RIB 4 | gi 490856707 | 5.8 | 16.4 | 189 | 75 | 14 | +4.2 | +3.4 | ND |
| 23 | Riboflavin synthase (sub a) | RIB 5 | gi 490863253 | 5.4 | 23.8 | 103 | 46 | 7 | ND | +2.4 | +1.7 |
| 24 | ATP-binding protein | PSTB | gi 490863632 | 5.4 | 56.5 | 258 | 51 | 22 | +2.2 | +1.7 | -1.3 |
| 4 Metabolism of fatty acids and lipooligosaccharides | | | | | | | | | | | |
| 4.1 Fatty acids synthesis/oxidation | | | | | | | | | | | |
| 25 | Acyl-CoA synthetase | ACS | gi 674994296 | 5.5 | 61.8 | 108 | 24 | 10 | +2.8 | +2.0 | ND |
| 26 | Acetyl-CoA carboxylase | ACC | gi 690996038 | 5.4 | 50.8 | 134 | 28 | 12 | +2.0 | +2.0 | ND |
| 27 | Acetate kinase | ACK | gi 736601335 | 5.9 | 44.2 | 123 | 37 | 10 | +2.6 | +2.7 | ND |
| 28 | Acyl-CoA dehydrogenase | ACD | gi 736607308 | 5.7 | 37.2 | 113 | 41 | 13 | ND | +2.4 | +2.8 |
| 29 | Pyruvate dehydrogenase (sub. E1) | PDH | gi 493630557 | 5.4 | 102 | 138 | 15 | 13 | ND | +2.2 | +1.8 |
| 30 | Pyruvate dehydrogenase (acetyl-transf) | PDH | gi 490856570 | 5.3 | 101 | 303 | 47 | 39 | ND | +2.6 | +2.1 |
| 31 | Long-chain fatty acid-CoA ligase | ACSL | gi 490858943 | 5.6 | 60 | 244 | 57 | 23 | ND | -2.5 | -2.9 |
| 32 | Acetyl-CoA carboxylase (biotin carrier) | ACC | gi 490857748 | 4.9 | 14.7 | 104 | 45 | 7 | ND | -2.8 | -2.1 |
| 4.2 Methylcytrate cycle | | | | | | | | | | | |
| 33 | Methylisocitrate lyase | MICL | gi 490858658 | 4.9 | 32.6 | 123 | 36 | 12 | +181 | +68 | -2.5 |
| 34 | 2-methylisocitrate dehydratase | MICDH | gi 490860929 | 5.1 | 95.9 | 206 | 29 | 21 | +2 | ND | -1.9 |
| 4.3 PHA synthesis/degradation | | | | | | | | | | | |
| 35 | Succinyl-CoA:3-ketoacid-CoA transferase | SCOT | gi 490859391 | 4.9 | 25.3 | 116 | 54 | 10 | -6.7 | -3.4 | ND |
| 36 | 3-hydroxybutirate dehydrogenase | HBDH | gi 674995203 | 5.6 | 27.6 | 162 | 46 | 13 | -2.9 | -2.2 | ND |
| 37 | 3-ketoacyl-ACP reductase | FAB G | gi 490861494 | 4.8 | 27.1 | 143 | 53 | 11 | -2.1 | +2.4 | +5.2 |
| 4.4 Synthesis of capsular LPS and EPS | | | | | | | | | | | |
| 38 | D-arabinose 5-phosphate isomerase | KDS | gi 490862304 | 5.5 | 35.8 | 118 | 34 | 9 | ND | +2.4 | +1.5 |
| 39 | UDP-glucose 4-epimerase | GALE | gi 490961831 | 5.1 | 37.3 | 252 | 67 | 22 | ND | +3.6 | +3.7 |
| 5 Membrane proteins and transporters | | | | | | | | | | | |
| 40 | Glucose-inducible porin | OPR B | gi 490861972 | 5.6 | 47.3 | 154 | 39 | 11 | +2.3 | ND | -1.8 |

(continued on next page)

Table 2 (continued)

| Spot | Protein name and classification | Abbreviation | Accession code | Theoretical | | Mascot results | | | Average fold change* Phenol/ Control | Ph + Cr (VI)/ Control | Ph + Cr (VI)/ Phenol |
|--|---|--------------|----------------|-------------|----------|----------------|----------|------------------|--|-----------------------------|----------------------------|
| | | | | pI | MW (KDa) | Score | Coverage | Matched peptides | | | |
| 41 | Outer membrane protein 38 | OMP 38 | gi 490860976 | 5.7 | 37.2 | 182 | 45 | 14 | +2.7 | -5.2 | -14 |
| 42 | Family type VI secretion protein | EVP B | gi 490961779 | 5.0 | 55.5 | 10 | 47 | 17 | -1.9 | -2.2 | ND |
| 43 | Outer membrane protein W precursor | OMP W | gi 514347513 | 5.6 | 21.9 | 126 | 39 | 8 | ND | +6.3 | +4.4 |
| 6 Nitrogen metabolism | | | | | | | | | | | |
| 6.1 Nitrogen assimilation | | | | | | | | | | | |
| 44 | Nitrogen regulatory protein P-II | NRP PII | gi 490863245 | 5.4 | 12.2 | 202 | 83 | 13 | -1.8 | -3.3 | -1.8 |
| 45 | Glutamine synthetase | GLNS | gi 490862518 | 5.1 | 52.4 | 217 | 51 | 22 | -5.3 | -2.2 | -2.0 |
| 6.2 Amino acids metabolism | | | | | | | | | | | |
| 46 | D-alanine-D-alanine ligase | DDL | gi 490856584 | 4.9 | 33.6 | 171 | 53 | 13 | +2.1 | +1.6 | -1.3 |
| 47 | Dihydrodipicolinate reductase | DAP B | gi 490856795 | 5.5 | 28.5 | 165 | 44 | 11 | +++ | +++ | -2.1 |
| 48 | tRNA dimethylallyltransferase | MIAA | gi 490858824 | 5.5 | 36.2 | 133 | 35 | 9 | +2.7 | +2.7 | ND |
| 49 | Branched-chain aminotransferase | BCAAS | gi 490861095 | 5.9 | 34.5 | 182 | 49 | 13 | +2.6 | +2.1 | ND |
| 6.2.1 Aromatic amino acids | | | | | | | | | | | |
| 50 | Imidazole glycerol phosphate synthase | HIS F | gi 490954769 | 5.3 | 27.3 | 196 | 63 | 15 | +9.3 | +8.7 | ND |
| 51 | Tyrosyl-tRNA synthetase | TYRS | gi 490856878 | 5.4 | 44.9 | 250 | 61 | 19 | +1.5 | +2.7 | +1.7 |
| 52 | Fumarylacetoacetase | FAA | gi 490855304 | 5.5 | 48.8 | 182 | 36 | 14 | -2.2 | -7.3 | -3.5 |
| 53 | DAHPS synthase | DAHPS | gi 736607028 | 5.7 | 39.1 | 179 | 59 | 15 | ND | +2.4 | +2.2 |
| 54 | 4-hydroxyphenylpyruvate dioxygenase | HPPD | gi 490855316 | 4.9 | 40.1 | 276 | 58 | 31 | ND | -2.1 | -1.7 |
| 55 | Histidine ammonia-lyase | HAL | gi 490859758 | 5.2 | 54.8 | 164 | 36 | 19 | ND | -4.6 | -3.0 |
| 6.2.2 Glutamate | | | | | | | | | | | |
| 56 | Glutamine amidotransferase | GAT | gi 490855407 | 5.3 | 21 | 131 | 54 | 8 | ND | +2.0 | +1.8 |
| 57 | Glutamate dehydrogenase | GDH | gi 490859749 | 5.7 | 46.5 | 143 | 43 | 13 | ND | -3.3 | -3.5 |
| 6.2.3 Sulfur-containing amino acids | | | | | | | | | | | |
| 58 | S-adenosylmethionine synthase | MET K | gi 490857422 | 5.5 | 42.3 | 184 | 55 | 18 | ND | +3.2 | +4.0 |
| 59 | Methionyl-tRNA formyltransferase | FMT | gi 736609637 | 5.3 | 34.7 | 119 | 42 | 10 | ND | +2.7 | ND |
| 60 | Homocysteine methyltransferase | HMT | gi 754745926 | 6.7 | 13.9 | 128 | 50 | 8 | ND | +4.0 | +2.2 |
| 61 | Methionine synthase | MET E | gi 490957454 | 5.4 | 38.9 | 173 | 45 | 14 | ND | -2.8 | -1.8 |
| 7 Transcription and translation | | | | | | | | | | | |
| 62 | DNA-directed RNA polymerase (Sub. b) | RNA POL | gi 490856432 | 5.4 | 152 | 103 | 14 | 15 | +3.8 | +2.2 | ND |
| 63 | DNA-directed RNA polymerase (Sub. b) | RNA POL | gi 497799882 | 5.3 | 152 | 138 | 12 | 16 | ND | — | — |
| 64 | 30S ribosomal protein S1 | 30S S1 | gi 514347225 | 5.0 | 61.4 | 102 | 43 | 27 | +2.2 | +7.2 | +3.2 |
| 65 | 50S ribosomal protein L9 | 50S L9 | gi 490857291 | 5.7 | 15.7 | 155 | 68 | 11 | -1.9 | -40 | -21 |
| 66 | Transcription termination factor NusA | NUS A | gi 490856294 | 4.5 | 54.9 | 270 | 45 | 24 | -2.4 | -2.7 | ND |
| 67 | Transcriptional regulator Crp | CRP | gi 490859097 | 4.9 | 26.7 | 217 | 69 | 18 | ND | -2.3 | -1.9 |
| 68 | 5'-nucleotidase surE | SUR E | gi 490859229 | 4.8 | 28.3 | 172 | 53 | 12 | -2.2 | -1.9 | ND |
| 69 | RNA-binding protein | RBP | gi 490863328 | 5.7 | 87.2 | 505 | 60 | 44 | ND | -7.0 | -8.4 |
| 70 | Elongation factor Tu | EF TU | gi 490861613 | 5.1 | 43.1 | 156 | 47 | 18 | ND | -2.6 | -2.2 |
| 71 | Trigger factor | TF | gi 490864495 | 4.8 | 49.6 | 289 | 39 | 21 | ND | -2.0 | -1.6 |
| 72 | Prolyl-tRNA synthetase | FPR | gi 736607424 | 5.0 | 63.1 | 271 | 53 | 27 | ND | -2.2 | ND |
| 73 | Phosphoribosyl formylglycinamide synthase | FGAR | gi 490960834 | 5.0 | 140.1 | 142 | 20 | 17 | ND | -2.4 | -1.8 |
| 8 Stress processes and antioxidant response | | | | | | | | | | | |
| 74 | Chaperone protein DnaK | DNA K | gi 491176517 | 4.7 | 69.6 | 195 | 26 | 13 | +2.8 | ND | -3.1 |
| 75 | Osmotically inducible protein C | OSM C | gi 490856544 | 5.9 | 14.9 | 104 | 49 | 6 | +2.4 | ND | ND |
| 76 | Peroxidase Ahp | AHP C | gi 736606013 | 5.4 | 23.9 | 133 | 40 | 8 | +2.0 | +3.0 | +1.4 |
| 77 | Ferredoxin-NADP reductase | FPR | gi 490859866 | 5.2 | 29.3 | 142 | 55 | 14 | ND | +2.1 | +2.5 |
| 78 | Fe-S protein assembly chaperone HscA | HSC A | gi 490858594 | 5.2 | 67.8 | 106 | 42 | 19 | ND | +4.0 | +1.4 |
| 79 | Recombinase RecA | REC A | gi 490857713 | 5.4 | 37.7 | 134 | 72 | 17 | ND | +3.1 | +1.7 |
| 80 | Dihydroliipoamide dehydrogenase | DLD | gi 490864439 | 5.8 | 50.7 | 114 | 23 | 8 | ND | +2.5 | +2.1 |
| 81 | Alkyl hydroperoxide reductase (Sub C) | AHP C | gi 490859781 | 5.0 | 20.8 | 141 | 51 | 9 | ND | +2.5 | +1.5 |
| 9 Cell regulation and chemotaxis | | | | | | | | | | | |
| 82 | AraC family transcriptional regulator | ARA C | gi 490856041 | 5.7 | 38.5 | 355 | 69 | 22 | +46.0 | +64.5 | +1.4 |

(continued on next page)

Table 2 (continued)

| Spot | Protein name and classification | Abbreviation | Accession code | Theoretical | | Mascot results | | | Average fold change* Phenol/ Control | Ph + Cr (VI)/ Control | Ph + Cr (VI)/ Phenol |
|--|---------------------------------|--------------|----------------|-------------|----------|----------------|----------|------------------|--|-----------------------------|----------------------------|
| | | | | pI | MW (kDa) | Score | Coverage | Matched peptides | | | |
| 83 | Carbon storage regulator | SCR A | gi 490922332 | 5.9 | 9.9 | 114 | 56 | 11 | ND | -3.6 | -4.0 |
| 84 | Chemotaxis protein CheY | CHE Y | gi 490860813 | 4.9 | 27 | 191 | 75 | 16 | -1.4 | -2.1 | ND |
| 10 Metabolism of aromatic compounds | | | | | | | | | | | |
| 85 | Quercetin 2,3-dioxygenase | QR | gi 736602565 | 5.1 | 35.4 | 159 | 41 | 12 | + 2.4 | ND | -1.6 |
| 86 | Quercetin 2,3-dioxygenase | QR | gi 490856510 | 5.3 | 31.9 | 127 | 53 | 14 | + 6.6 | ND | -3.0 |
| 87 | Nitroreductase | NR | gi 736609117 | 5.2 | 21.8 | 310 | 64 | 20 | + 5.5 | + 2.3 | -2.3 |

*Fold change is the ratio of protein abundance between the treatments. “+” and “-” indicate increased and decreased proteins, respectively. “+++” indicates the spots that only appeared in phenol and phenol plus Cr(VI) treatments, not in the control. “-” indicates the spots that disappeared in phenol plus Cr(VI) treatment. ND: there were no significant differences in the relative abundance of a spot between two conditions.

(Table 2.8).

The alkyl hydroperoxide reductase AhpC is one of the best-characterized enzymes able to detoxify organic hydroperoxides in bacteria. It has been reported that AhpC acts as a key antioxidant protein involved in the survival of *Acinetobacter* strains exposed to different stressors [47,48]. Recently, the combined activity of AhpC with the osmotically inducible protein OsmC has been demonstrated in other microorganisms as a defense mechanism against organic hydroperoxides [49].

Moreover, the induction of DnaK protein reflects the presence of misfolded proteins in the cytoplasm of *A. guillouiae*. This Hsp-70 chaperone plays an important role in the protection of newly formed proteins in bacteria under stress conditions, such as exposition to aromatic compounds [45,47].

Following phenol treatment, the content of the AraC transcriptional regulator was around 50-fold increased (Table 2.9), suggesting the essential role of this protein in the response of *A. guillouiae* SFC 500-1A to phenol. In this sense, many transcriptional regulators for aromatics degradation in bacteria belong to AraC family [18]. They have also been involved in the resistance to oxidative stress agents, antibiotics and organic solvents [50].

3.3.7. Remediation of aromatic compounds different from phenol

The abundance of quercetin 2,3-dioxygenase and nitroreductases, which are involved in catabolism of flavonoids and nitroaromatic compounds, was increased after phenol addition (Table 2.10). Such enzymatic induction by structural analogues has been already detected in aromatic-degrading microorganisms and is usually employed as an acclimation strategy [51].

As it is well known, quercetin 2,3-dioxygenase catalyzes the breakdown of some flavonoids to generate protocatechuate [52]. Flavonoids are important compounds for plant-microorganism associations and cellular signaling. Bacteria able to degrade flavonoids have been pointed as good candidates for rhizosphere colonization and, consequently, may be suitable for assisted phytoremediation [53]. In addition, nitroreductases have a central role in the activation of nitroaromatic and nitroheterocyclic compounds for their further bioremediation, with influence on the environment and human health and significant biotechnological and medical potential [54].

Thus, this proteomic study demonstrated the capability of *A. guillouiae* SFC 500-1A for degrading phenol through the β -ketoacid pathway, and also suggested its potential for bioremediation of nitro-substituted phenols and some kind of polycyclic compounds.

3.4. Proteomic variations in *A. guillouiae* SFC 500-1A exposed to phenol plus Cr(VI)

3.4.1. Phenol oxidation and carbon assimilation

The increased content of enzymes from the β -ketoacid pathway proved that *A. guillouiae* SFC 500-1A is able to metabolize phenol even in the presence of Cr(VI) (Table 2.1). Nevertheless, the differences observed in the abundance of these proteins between treatments could explain the negative effect of Cr(VI) on phenol degradation efficiency previously reported for this strain [9].

The addition of Cr(VI) also affected the central pathways of carbon assimilation. On the one hand, the level of the TCA enzymes 2-oxoglutarate dehydrogenase and succinyl-CoA synthetase decreased. On the other hand, unlike that observed in the presence of phenol, the glyoxylate shunt seems not to have occurred (Table 2.2). It is possible that the lesser rate of phenol degradation generated a lower concentration of acetyl-CoA and succinate to be incorporated into assimilatory pathways. These changes could also be a strategy carried out by this strain to decrease the flux through the electron transport chain and mitigate the oxidative damage caused by the combination of both pollutants, as it has been observed in other bacteria under stress conditions [30,55]. The induction of succinate dehydrogenase (SQR) is framed in this scenario. In this sense, Yankovskaya et al. [56] found that SQR prevents the reactive oxygen species (ROS) formation through succinate oxidation and flavins reduction in *E. coli* under aerobic conditions.

3.4.2. Cr(VI) metabolism and stress-associated response

It has been previously demonstrated that *A. guillouiae* SFC 500-1A is able to reduce Cr(VI) to Cr(III) through soluble NADH dependent chromate reductases [9]. However, these enzymes have not yet been identified. Our proteomic study revealed that its exposure to phenol plus Cr(VI) caused a significant increase in cellular concentration of enzymes involved in redox reactions, such as ferredoxin-NADP reductase (Fpr), dihydrolipoamide dehydrogenase (DLD) and alkyl hydroperoxide reductase (AhpC) (Table 2.8). These enzymes may be implicated in Cr(VI) reduction and stress mitigation and could help to elucidate what happens in the strain during the simultaneous bioremediation process.

In certain bacteria, Fpr and DLD proteins carry out a two-step Cr(VI) reduction in which the electron transfer from NADPH to Cr(VI) is mediated by FAD [57–59]. Therefore, a possible role of these flavo-proteins in the Cr(VI) reducing potential of *A. guillouiae* SFC 500-1A may be proposed. The possible overproduction of riboflavin caused by an increase in the cellular concentration of 6,7-dimethyl-8-ribityllumazine synthase and riboflavin synthase (Table 2.3) could also be related to Cr(VI) reduction to Cr(III). Riboflavin is a FAD precursor crucial

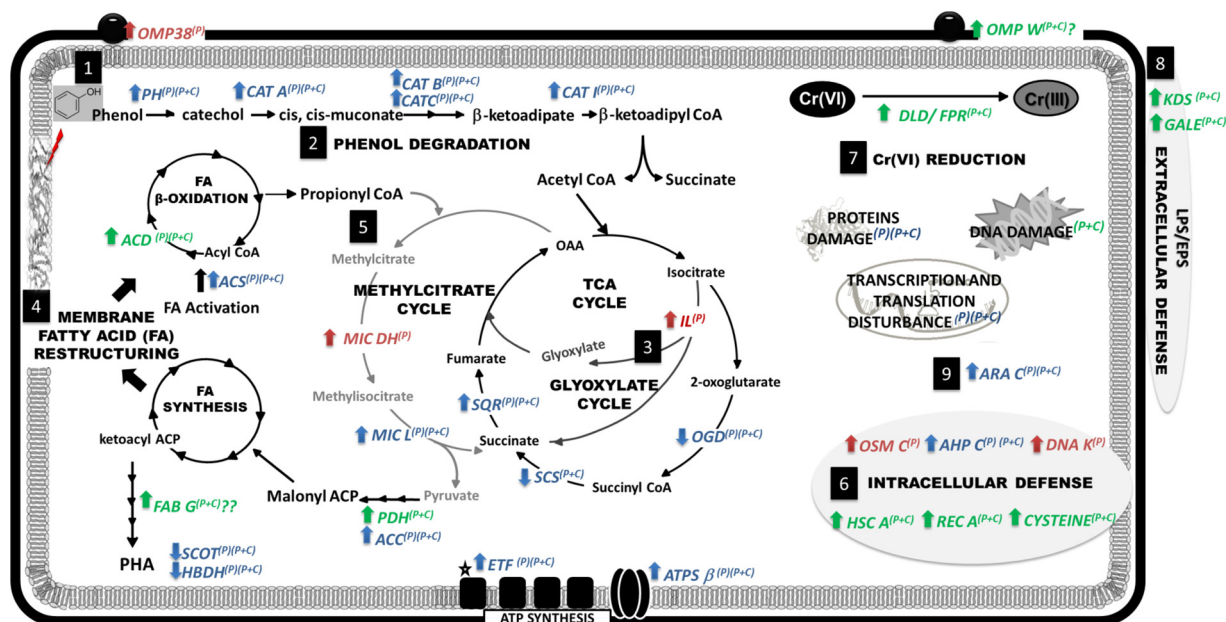


Fig. 3. Schematic representation of cellular changes in *A. guillouiae* SFC 500-1A following phenol or phenol plus Cr(VI) exposition. The increased and decreased proteins are marked by \uparrow and \downarrow , respectively. Superscripts indicate the condition in which proteins or processes registered variations: (P): Phenol, (P + C): Phenol plus Cr(VI), (P) (P + C): Both phenol and phenol plus Cr(VI). Protein abbreviations are included in Table 2. Numbers indicate: 1. Phenol uptake; 2. Phenol degradation through β -ketoadipate pathway; 3. Glyoxylate shunt; 4. Membrane restructuring; 5. Propionyl-CoA degradation by methylcitrate cycle; 6. Cellular defense against pollutants toxicity; 7. enzymatic reduction of Cr(VI) to Cr(III); 8. Production of polysaccharide matrix as extracellular response; 9. Transcriptional regulation by AraC protein.

for Cr(VI) tolerance and detoxification in different microorganisms [60–62].

It is well known that Fpr and DLD proteins produce the transient radical Cr(V), which cycles redox and creates ROS, damaging macromolecules and altering cellular processes [57,58]. In this regard, the increased abundance of RecA recombinase and HscA chaperone in cells of *A. guillouiae* SFC 500-1A would indicate damage to DNA and proteins and demonstrates the effort made by the strain to protect these macromolecules (Table 2.8). RecA belongs to SOS system, which can repair some DNA injuries produced by redox-active intermediates Cr(V/IV) and Cr(III)-DNA adducts [63]. Meanwhile, HscA is a specialized chaperone involved in [Fe-S] proteins refolding and maturation under different stress conditions [64,65]. As it can be deduced from Table 2.7, DNA transcription and translation processes were also affected in this strain like in other Cr(VI)-exposed bacteria [66].

The alkyl hydroperoxide reductase AhpC and various enzymes involved in cysteine metabolism were also increased after exposition to phenol and Cr(VI) (Table 2.6.2.3). Both AhpC and cysteine probably contribute to offset the cell damage generated during the co-remediation process considering that AhpC is a thiol-specific antioxidant and cysteine constitutes an efficient reducing agent. The involvement of AhpC in ROS scavenging and bacterial protection against multiple abiotic stresses is well documented [67] and the up-regulation of cysteine biosynthesis constitutes a defense mechanism in other chromate-stressed microorganisms [62]. Furthermore, a relation has been established between the levels and the redox state of cysteinyl groups of certain molecules and the activity of AhpC in cellular response to stress [68].

The abundance of several proteins related with PHA synthesis and mobilization was also modified in response to phenol and Cr(VI) (Table 2.4.3). This is the case of protein 3-ketoacyl-ACP reductase, a supplier of 3-hydroxyacyl-CoA for the synthesis of PHA [69], whose cell content increased 2.4 fold, while the relative abundance of SCOT and HBDH (involved in their degradation) was low, in agreement with the results obtained in the phenol treatment. These results suggest that PHA accumulation occurred when *A. guillouiae* SFC 500-1A was exposed to

both pollutants, as reported for other Cr(VI)-resistant bacteria [37]. PHA synthesis and the inhibition of their degradation are important defense mechanisms under oxidative stress conditions. Additionally, it has been demonstrated that the polymer endows bacteria with enhanced survival, competition abilities and tolerance to toxic substances [70].

In addition to intracellular response, it is known that cellular envelope and extracellular polymeric matrix play a key role in protection of Gram negative bacteria from toxic compounds. Capsular lipopolysaccharides (LPS) and exopolysaccharides (EPS) have been recognized as substantial factors for chromium tolerance [43,71]. Therefore, the high concentration of the enzymes D-arabinose 5 phosphate isomerase and UDP-glucose 4-epimerase (Table 2.4.4) could be related to some kind of mechanism for external protection against the metal, since they are essential enzymes for extracellular glycolipids and glycoproteins synthesis. The abundance of OmpW was also significantly increased in the presence of phenol and Cr(VI) when compared to control and phenol-only treatments (Table 2.5). Although the biological function of this protein remains largely uncharacterized, the participation of OmpW in bacterial protection against environmental stressors, resistance to antibiotics and virulence has been reported [72]. Similarly, the cellular concentration of AraC transcriptional regulator increased 1.4 times regarding only-phenol treated cells and was 65 times above than in control conditions (Table 2.9). This result indicates that AraC protein may play crucial role defending the strain against phenol and Cr(VI) toxicity but also regulating in the bioremediation process.

Overall, the current results show that phenol and Cr(VI) significantly altered the proteome profile of *A. guillouiae* SFC 500-1A so that the bacterium could develop a proper response against these environmental pollutants.

4. Conclusions

The evaluation of proteome variations in *A. guillouiae* SFC 500-1A exposed to phenol and Cr(VI) served as a useful strategy for analyzing the bacterial response to these pollutants and designing a possible

model to understand its bioremediation ability (Fig. 3).

After uptake, probably mediated by the OmpA-like porin Omp38 (Fig. 3.1), phenol is hydroxylated to catechol and further *ortho*-oxidated to β -ketoacyl-CoA, a precursor of acetyl-CoA and succinate (Fig. 3.2). The assimilation of these phenol degradation products would be associated to the bypass of TCA cycle to glyoxylate cycle (Fig. 3.3) to generate biomass and promote the cellular growth.

In spite of constituting a carbon source for many microorganisms, phenol is a strong inducer of cellular damage. In this strain, phenol toxicity would be associated to the oxidation of membrane fatty acids followed by hydroperoxides generation, damage to other structures and alteration of cellular processes. Restructuring the phospholipid composition seems to be a strategy of *A. guillouiae* SFC 500-1A to keep the membrane integrity (Fig. 3.4). Additionally, the activation of the methylcitrate cycle could be explained as a mechanism to prevent the accumulation of toxic propionyl-CoA generated during β -oxidation of odd-chain and branched-chain fatty acids (Fig. 3.5). The defense response would also include increasing the cellular concentration of hydroperoxide resistance enzymes (AhpC and OsmC) for stress mitigation and chaperone DnaK to protect newly formed proteins (Fig. 3.6).

The exposure to phenol and Cr(VI) caused more harm to the growth and energetic metabolism of the strain. Nevertheless, *A. guillouiae* SFC 500-1A was able to co-remediate these contaminants, oxidizing phenol through the β -ketoacyl-CoA pathway and removing Cr(VI) simultaneously by chromate reductases. Two flavoproteins, Fpr and DLD (Fig. 3.7), possibly associated with cofactors derived from riboflavin, could be involved in Cr(VI) reduction to Cr(III). The negative effect of both pollutants on cell growth may be related with the oxidative stress generated during the bioremediation process, strongly altering cellular processes such as transcription and translation and generating a significant damage to DNA and proteins. The assembly control of specialized [Fe-S] proteins by HscA chaperon and the activation of the SOS response that involves RecA protein for DNA repairing can be mentioned among the possible mechanisms displayed to alleviate the damage (Fig. 3.6). The oxidative stress mitigation together with the redox state regulation appears to be crucial for the survival of *A. guillouiae* SFC 500-1A exposed to both pollutants. Unlike the scenario with phenol-only treatment, in the presence of phenol and Cr(VI) the bacterium would display an active metabolism of cysteine (Fig. 3.6), a provider of thiol groups that are essential for maintaining an intracellular reducing environment. The increase in the polysaccharide matrix could also be mentioned as a bacterial response to Cr(VI), possibly involved in the extracellular protection from the metal (Fig. 3.8).

Therefore, the ability of this strain to grow in the presence of phenol and remediate it efficiently could be explained as a successful balance between its assimilation and the stress mitigation. Moreover, the bacterium showed a metabolic machine adapted for phenol assimilation even in the presence of Cr(VI) and the enzymatic potential to simultaneously reduce Cr(VI) to Cr(III). The effort to mitigate the cellular stress was more evident during the co-remediation process, resulting in less growth and a reduced energy metabolism. It is likely for the transcriptional regulator AraC, highly expressed in phenol exposed cells and even more in the presence of Cr(VI) (Fig. 3.9), to play some role in such bacterial response to contaminants. The implication of the porin OmpW in Cr(VI) resistance also remains to be clarified.

The results of this study are in agreement with previous findings that demonstrated the ability of this strain to co-remediate phenol and Cr(VI) and also provide information about potential target proteins and processes for improving the bioremediation of these pollutants. Additionally, they suggest other possible biotechnological skills of *A. guillouiae* SFC 500-1A, such as bioremediation of other phenolic compounds and synthesis of PHA, interesting to be addressed in future studies.

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