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**Title: Multiple resistance mechanisms in *Staphylococcus* sp. strain AS6 under arsenite stress and its potential use in amelioration of waste water**

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**Supplementary data (Table and Figures)**

**Table S1:** Physiochemical properties of waste water sample from where the bacterium isolated.

|  |  |
| --- | --- |
| **Physiochemical parameter** | **Results** |
| Color | Light black |
| Temperature | 32 |
| pH | 7.5 |
| Electrical conductivity (μS/cm) | 1356 |
| Turbidity (NTU) | 11.8 |
| TDS (total dissolved solids) (μg/L) | 681 |
| Arsenic concentration (μg/L) | 200 |

**Table S2: MIC of different heavy metals in isolated bacterium.**

|  |  |
| --- | --- |
| **Metal ions used** | **MIC (mM)** |
| Arsenite (As+3) | 25 |
| Arsenate (As+5) | 150 |
| Lead (Pb) | 5 |
| Cadmium (Cd) | 3 |
| Chromium (Cr) | 5 |
| Mercury(Hg) | 2.5 |
| Selenium (Se)Cobalt(Co)Nickel (Ni) | 435 |

**Table S3:** Morphological and biochemical characteristics of isolated bacterial strain *Staphylococcus* sp. strain AS6.

|  |  |
| --- | --- |
| Morphological and biochemical tests | Results |
| Form  | Circular |
| Surface | Smooth  |
| Color | Yellow |
| Margin | Entire |
| Elevation | Convex |
| Opacity  | Opaque |
| Cell shape | Cocci |
| Motility  | Non-motile |
| Gram staining | Gram positive cocci |
| Catalase | Positive |
| Oxidase | Negative |
| Coagulase | Negative |
| Urease  | Positive |
| Citrate | Positive |
| Lactose fermentation | Negative |
| H2S | Negative |
| Nitrate reduction | Positive |
| Indole | Negative |
| Methyl red | Positive |
| Voges proskauer (VP) | Positive |
| Manitol salt agar growth/color | Positive with no color change |

**Table S4:** Glutathione and non-protein thiols concentration under 15 mM arsenite stress in isolated bacterium *Staphylococcus* sp. strain AS6.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Arsenite concentration** **(mM)** | **GSH****(mM g-1 FW)** | **GSSG (mM g-1 FW)** | **GSH+ GSSG (mM g-1 FW)** | **GSH/GSSG** | **% increase in** **GSH/GSSG** | **Non-protein thiols** | **% increase in non-protein thiol** |
| 0 | 6.4 | 0.5 | 6.90 | 12.80 | 5.77:12.80\*100= 45.08 % | 1.7 | 1.3:1.7\*100 =76.47 % |
| 15 | 13.0 | 0.7 | 13.70 | 18.57 |  | 3.0 |  |

**Table S5:** Enzymatic activities of SOD, CAT, POX, and APX of *Staphylococcus* sp. strainAS6 with and without metal stress.

|  |  |  |
| --- | --- | --- |
| **Antioxidant enzymes** **U min-1 mg protein-1** | **Strain AS6 (without arsenite stress)** | **Strain AS6 (with arsenite stress -10 mM)** |
| Superoxide dismutase (SOD) | 10.44 ± 0.12 | 6.56 ± 0.26 |
| Catalase (CAT) | 14.22 ± 0.33 | 28.45 ± 0.54 |
| Peroxidase (POX) | 0.372 ± 0.03 | 0.200 ± 0.05 |
| Ascorbate peroxidase (APX) | 0.290 ± 0.01 | 0.345 ± 0.02 |

**Table S6: Genes responsible for the resistance of other metals and their putative functions.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Genes and its position** | **Product** | **Putative function**  | **Closest related sequence** | **% Query coverage** | **% Ident** |
| *czcD\_1*18465-19418 | Cobalt-zinc-cadmium resistance protein CzcD | Inorganic ion transport and metabolism | [WP\_019467694.1](https://www.ncbi.nlm.nih.gov/protein/WP_019467694.1?report=genbank&log$=prottop&blast_rank=1&RID=1VCC7044014) | 85% | 100% |
| *cadA\_1*52258-54675 | Cadmium transporting P- type ATPase | Play role in translocating cadmium and other heavy metal divalent ions | [WP\_019469244.1](https://www.ncbi.nlm.nih.gov/protein/WP_019469244.1?report=genbank&log$=prottop&blast_rank=1&RID=1VC5ZTV3014) | 99% | 100% |
| *cadA\_2*9765-12158 | Putative cadmium transporting ATPase | Translocate cadmium ions | [WP\_019467776.1](https://www.ncbi.nlm.nih.gov/protein/WP_019467776.1?report=genbank&log$=prottop&blast_rank=1&RID=1VD2NJ6D014) | 99% | 100% |
| cadC\_112175-12543 | Cadmium resistance transcriptional regulatory protein CadC | Regulate the transportation of arsenic and other divalent heavy metal ions | [WP\_011304078.1](https://www.ncbi.nlm.nih.gov/protein/WP_011304078.1?report=genbank&log$=prottop&blast_rank=1&RID=1VD93DJH01R) | 99% | 100% |
| *czcD\_2*1879-2820 | Cobalt-zinc-cadmium resistance protein CzcD | Inorganic ion transport and metabolism | [WP\_019467694.1](https://www.ncbi.nlm.nih.gov/protein/WP_019467694.1?report=genbank&log$=prottop&blast_rank=1&RID=1VCC7044014) | 85% | 100% |
| cadC\_211923-12270 | Cadmium resistance transcriptional regulatory protein CadC | Regulate the transportation of arsenic and other divalent heavy metal ions | [WP\_011304078.1](https://www.ncbi.nlm.nih.gov/protein/WP_011304078.1?report=genbank&log$=prottop&blast_rank=1&RID=1VD93DJH01R) | 99% | 100% |
| PROKKA\_0263512289-12906 | Cadmium resistance transporter | Play role in cadmium resistance or sequestration | [WP\_002472504.1](https://www.ncbi.nlm.nih.gov/protein/WP_002472504.1?report=genbank&log$=prottop&blast_rank=2&RID=1VDUU58K01R) | 99% | 99.51% |
| *CorC\_1*41022-42041 | Magnesium and cobalt efflux protein | Efflux Mg+2 and Cobalt from cell | [WP\_019469692.1](https://www.ncbi.nlm.nih.gov/protein/WP_019469692.1?report=genbank&log$=prottop&blast_rank=1&RID=1VE2651101R) | 95% | 100% |
| *CorC\_2*26889-28241 | Magnesium and cobalt efflux protein | Efflux Mg+2 and Cobalt from cell | [WP\_019469692.1](https://www.ncbi.nlm.nih.gov/protein/WP_019469692.1?report=genbank&log$=prottop&blast_rank=1&RID=1VE2651101R) | 95% | 100% |



**Figure S1:** Geographical map of District Sheikhupura, Pakistan.

 **(a)**

 **(b)**

**Figure S2:** Growth (OD) of isolated bacterium at different temperature and pH.













**Figure S3: The annotated genes responsible for other heavy metals resistance.**