# Proteins Involved in the Plant Growth Promotion of *Pseudomonas orientalis* in the Presence of Cadmium. https://doi.org/10.17952/37EPS.2024.P1275

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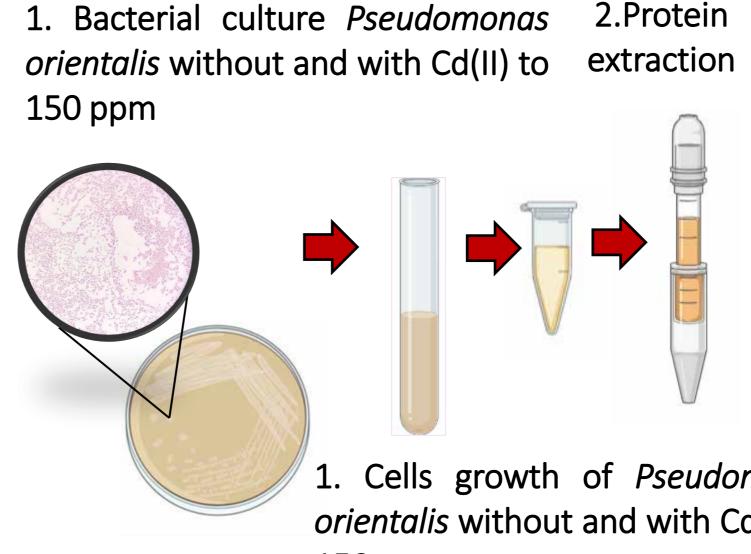
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### 1. INTRODUCTION

Cadmium is considered a heavy metal that has effects on human health and also on ecosystems. In the soil, it reduces fertility and agricultural productivity. Bacteria have different stress tolerance mechanisms due to the presence of this metal, including the production of siderophores, proteins and exopolysaccharides. The objective of this study was to identify *Pseudomonas* orientalis B06CM strain [1] proteins in response to cadmium using nano LC/MSMS proteomic analysis of the bacterial extract with and without cadmium at a concentration of 150 ppm. The bioinformatic analysis of the proteins was carried out with the Gene Ontology (GO) annotation from the UniProt database, which allowed the protein to be classified into three categories such as Molecular Function, Biological Process and Cellular *Component*. These findings contribute to the fulfillment of sustainable development objectives 3 and 15.

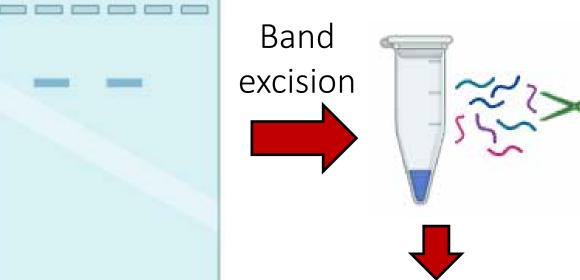


# 2. MATERIALS AND METHODS



3. Electrophoresis (Tricine-SDS-PAGE) -based protein separation

4. In-Gel Protein digestion with trypsin



5. nano LC-MS/MS Analysis.

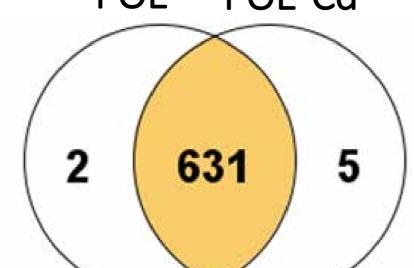
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# 3. RESULTS AND DISCUSSION

### **Protein Identification**

The results obtained allowed us to identify 5 differentially present proteins, which may be related to the effect of tolerance on the growth of bacteria in the presence of cadmium as shown in figure 1. POE POE-Cd

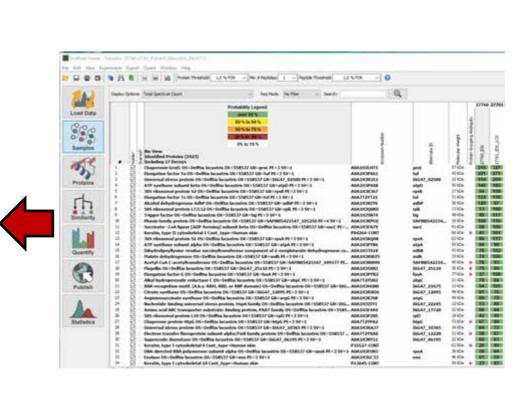
Figure 1. Venn diagram provides an overview of key proteins of *Pseudomonas orientalis* extract without (POE) and with Cd(II) to 150 ppm (POE-Cd).



### 1. Cells growth of *Pseudomonas* orientalis without and with Cd(II) to 150 ppm

### 7. Functional Bioinformatic Analysis

The analysis by gene ontology (Gene Ontology) was derived from the UniProt-GO database, the proteins were classified into the Biological Process, categories: Molecular Function and Cellular Component.





### 6. Protein ID

Mascot (Matrix Science, London, 2.8.0). Database UK; version *Pseudomonas orientalis* Uniprot (19009 entries). Scaffold (version Scaffold\_5.1.2) was used to validate MS/MS based peptide and protein identifications. criteria: At least 2 unique peptides identified sum score greater than

# 2X p<0,01 (0.0001% error rate).

### **Functional Bioinformatic Analysis**

Furthermore, approximately 30 common proteins were identified in the two bacterial extracts involved in the activities of nitrogen fixation, phosphate solubilization, sulfur metabolism, siderophore production, synthesis of the phytohormone indole acetic acid, metal resistance, exopolysaccharide biosynthesis and oxidative stress response (Table 1).

**Figure 2.** Gene Ontology (GO) classification of *Pseudomonas orientalis* proteins in presence to Cd(II) stress in the categories: Biological Process, Molecular Function and Cellular Component.

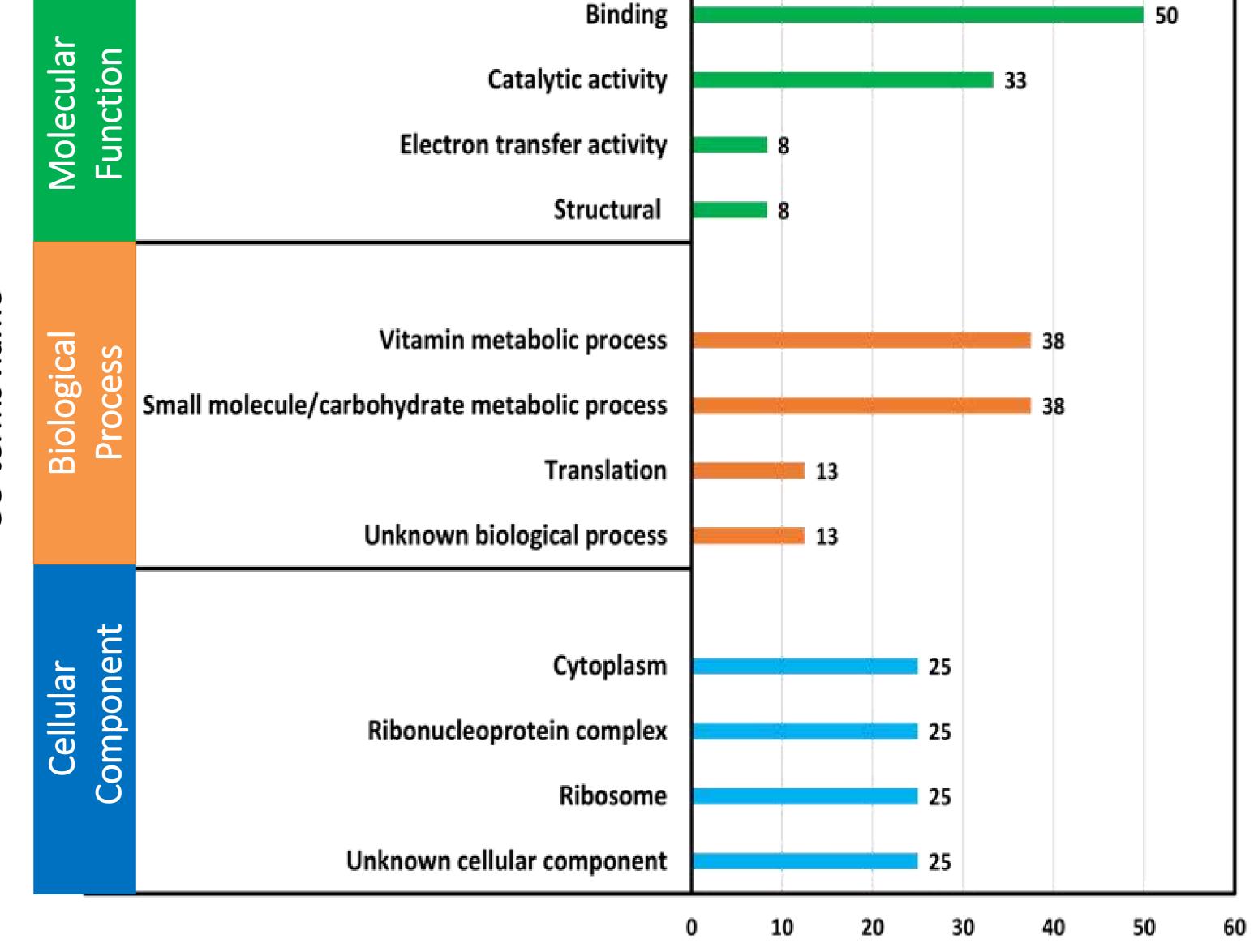
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**Table 1.** Identified proteins of *Pseudomonas orientalis* extract with Cd possibly associated with the response to this metal (green) and both extract involved in the Plant Growth Promotion of by LC-MS/MS

Activity	Identified protein	Entry name UniProt	Molecular mass (kDa)	
	4-hydroxytetrahydrobiopterin dehydratase	A0A2L0S3R6	46	
Tolerance in presence of cadmium	4-hydroxythreonine-4-phosphate dehydrogenase	A0A1H2G0Q6	35	
	50S ribosomal protein	A0A4Q7D568	5	
	Formyltetrahydrofolate deformylase	A0A0R2ZXH8	32	
	Ferredoxin	A0A0R3A2T1	12	
	dITP/XTP pyrophosphatase	A0A3G7X6U5	21	
Phosphate solubilization	Exopolyphosphatase	A0A0R3A3Q0	56	
	Inorganic pyrophosphatase	A0A0R2ZTP5	19	
Nitrogen fixation/Nitrogen	Cysteine desulfurase	A0A1H2FJN3	45	
ciclation	Nitrogen regulatory protein P-II family	A0A0R2ZXC0	12	
Sulfur metabolism	Sulfate ABC transporter ATP-binding protein	A0A0R2ZPQ3	37	
	Sulfite reductase	A0A2L0RY68	62	
	Sulfate transport system substrate-binding protein	A0A1H2I5V4,	37	
	Glutathione S-transferase	A0A1H2EYI0	23	
GSH biosyntesis/	S-(Hydroxymethyl)glutathione dehydrogenase /			
Metabolisms	alcohol dehydrogenase	A0A1H2HTS8	39	
	Glutathione synthetase	A0A2L0S3Q2	35	
Siderophore Biosynthesis	Bacterioferritin	A0A3G7WTM2	20	
	Ferric uptake regulation protein	A0A0R2ZTQ2	15	
	Chorismate synthase	A0A0R3A5P1	39	
	Bifunctional chorismate mutase/prephenate			
	dehydratase	A0A4Q7D396	40	
Synthesis of the phytohormone indole acetic acid	Anthranilate synthase component l	A0A3G7X759	54	
	Anthranilate synthase, component II	A0A3G7XNG7	22	
	Tryptophan synthase beta chain	A0A3G7WSN2	45	
	Anthranilate phosphoribosyltransferase	A0A0R2ZYL9	37	
	Indole-3-glycerol phosphate synthase	A0A1H2G078	30	
Oxidative stress response	Superoxide dismutase	A0A0R2ZYT5	22	
	Catalase	A0A1H2FY57	54	
	Thiol peroxidase	A0A0R3A1L2	18	
	Alkyl hydroperoxide reductase C	A0A0R2ZU27	22	
	Alkylhydroperoxidase AhpD domain protein	A0A3G7X046	20	
	Alkyl hydroperoxide reductase C	A0A0R3A3V0	20	
	Thioredoxin peroxidase	A0A1H2HY76	17	
	Alkyl hydroperoxide reductase	A0A3G7X6Y1	24	

GO terms name



## CONCLUSION

The identification and functional classification of proteins in extracts of *Pseudomonas* orientalis with and without cadmium associated with plant growth promoting traits, make it a future candidate to potentially be used as a bioinoculant in contaminated soils.

### % of identified protein

These proteins are involved with metalloproteins or proteins binding to different molecules (50%), catalytic activity (33%). In *Biological Processes* involved in metabolic processes of vitamins (38%) and small molecules or carbohydrates (38%). Regarding the Cellular Component, most proteins are part of the cytoplasm, ribosome and ribonucleoprotein complex (Figure 2).

# ACKNOWLEDGMENTS

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Universidad del Tolima

# REFERENCE

1. Ortiz Ortiz, J. C. ; Ramirez, R. A. ; Pulido, X. ; Varon López, M. 2021. Evaluación de la tolerancia a cadmio y plomo y su capacidad promotora de crecimiento vegetal de microorganismos aislados de relaves mineros . Memorias XXX Reunión Latinoamericana de Rizobiología V Conferencia Latinoamericana de Microorganismos Promotores del Crecimiento Vegetal.

