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Protein Profiling of *Thiobacillus ferrooxidans* and *Pseudomonas fluorescens* Mutants during Metal Extraction

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Abstract

Proteins are specific for the specific task associated with the cell. The metal extracting bacteria, *Thiobacillus ferrooxidans* and *Pseudomonas fluorescens* have two different mechanisms for the metal extraction. One extracts the metals by donating electrons and by doing self-oxidation and another is accumulating the metals onto its cell surface which is negatively charged. Therefore to differentiate the task of metal extraction protein profiling was done and compared. The water soluble proteins were analyzed through SDS-PAGE. There was no significant difference in the profiles of both.

Keywords: Protein Profile; Metal extraction; *Thiobacillus*; *Pseudomonas*; Microbes; Mineral ore; Industrial waste.

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1. Introduction

Metal-microbe interaction is a routine process in environment [1]. Soil is the source, where microorganisms interact with metals [2]. Metals (alkaline, alkaline earth and heavy metals) influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity [3]. Metal tolerance is a common characteristic of microbes which reside in the mineral ores and industrial wastes [4]. Mineral ores as well as industrial waste contain large amount of metal ions which directly or indirectly affect the growth, development, and life cycle of the microbes [5]. To overcome such problems microbes develop series of polypeptide chains (induced protein) [4,6]. The heavy metal induced specific polypeptides play an imperative role in metal ion homeostasis in cyanobacteria [7]. Some bacterial strains are also known to synthesize cysteine rich low molecular weight polypeptides which play an

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important role in biosorption of these metals ultimately resulting in immobilization of toxic metals there by protecting their vital metabolic processes by enzymes [8,9]. These proteins alleviate or reduce damage to the cell by acting as chaperons that bind to and stabilize unfolded or nascent proteins and as carrier molecules [9]. Certain heavy metal tolerant bacteria such as *Escherichia coli*, *Shewanella*, *Alcaligenes*, *Pseudomonas*, *Bacillus* and *Vibrio* species and numerous species exhibited metal induced synthesis of low molecular weight cysteine rich polypeptides (metallothioneins) which bind with specific metals such as cadmium and copper making them unavailable to bacterial cell [10,11]. The objective of this study was to evaluate the differentially expressed proteins of *Thiobacillus ferrooxidans* mutant UV-26 and *Pseudomonas fluorescens* mutant UV-24 when exposed to varied concentration of heavy metals.

2. Experimental

2.1. Material and methods:

In order to study the protein profile at the time of metal extraction the proteins were isolated from the bacteria, *Thiobacillus ferrooxidans* and *Pseudomonas fluorescens*, (isolated from bauxite ore mines at Roha), mutant strains which were engaged with metal extraction process. The water soluble proteins were extracted from the bacterial cells and analyzed by using SDS-poly acrylamide gel electrophoresis. The positions and molecular weight of the separated protein bands were determined. The following steps were followed for the protein profiling.

2.2. Culturing cells

In the present investigation two bacterial mutants of *Thiobacillus ferrooxidans* (UV-mutant 26) and *Pseudomonas fluorescens* (UV- mutant 24) were used to detect the response to various metal ions or how bacteria interact with metals. These mutant strains were grown in separate culture media, 9K and King's medium, respectively. The 9K medium was composed of [ammonium sulphate 3.0 g/L, magnesium sulphate 0.5 g/L, potassium hydrogen phosphate 0.5 g/L, potassium chloride 0.1 g/L, calcium nitrate 0.01 g/L, ferrous sulphate 21.0 g/L, distilled water, 1000 mL, pH 2.0 (pH was adjusted with 10N H_2SO_4)] [12] . The Kings medium was prepared by mixing protease peptone 20.0 g/L, glycerin 15 mL, magnesium sulphate 5.0 g/L, dipotassium hydrogen phosphate 2.5 g/L, agar agar 20 g/L, distilled water 1000 mL and pH was adjusted at 7.2 as reported [13]. The experiment was carried out in Erlenmeyer flasks containing 40 mL quantity of each medium. The incubation was done at 30 ± 2°C. The culture in 9K medium was incubated for 1 to 4 weeks until growth was observed microscopically or until a chemical change occurred in the medium compared with an un-inoculated control.

2.2 Adding metal ions:

Three metal ions were studied [by using metal salts such as ferrous sulphate, aluminum potassium phosphate, titanium solution and silicon chloride (Merck,USA)], were inoculated in the range of 100 µg/mL and 200 µg/mL of each. Un-inoculated media were served as control for *Thiobacillus ferrooxidans* and *Pseudomonas fluorescens* mutants.

2.3 Protein extraction

The 24 h grown cells were harvested by centrifugation at 5000 rpm for 10 min and used for extraction of proteins. The pellets were re-suspended and washed three times in double distilled water. The concentrated cells were disrupted by sonication with a sonicator two times for 30 sec with 1 min on ice in between. Samples were vortexed thoroughly, and shaken. Cell debris was removed by centrifugation at 10,000 rpm for 10 min at 4°C and supernatant was used as sample for SDS-PAGE analysis.

2.4 SDS-PAGE analysis:

The 10 µL of SDS (10%) was added to protein sample (90 µL) and mixed with 100 µL loading dye (containing 1% SDS, 25% glycerol, 0.1% 2-mercaptoethanol and 100 mg bromophenol blue). This sample was heated in boiling water for 5 min and then used for electrophoresis. Electrophoresis was performed at constant voltage (150 V) for 4.5 h. The gels were fixed in 10 % TCA for 30 min. Then the gels were stained with staining solution for 5 h and washed briefly in destaining solution (40 mL acetic acid and 230 mL ethanol were mixed well and made to the final volume to 500 mL by adding distilled water till the background became clear. The gels were photographed and molecular weight of every band was determined using gel documentation system. The Gene snap software was used for molecular weight determination [14].

3. Results and Discussion

Proteomic technologies are powerful tools for examining alterations in protein profile [15]. The protein profile of the metal extracting bacteria mutants, *Thiobacillus ferrooxidans* mutant UV-26 and *Pseudomonas fluorescens* mutant UV-24 was analyzed carried in two concentrations of metal ions (1 mg/mL) for all the four metals, Fe, Al, Ti and Si along with control (no addition of metal ions). The bands obtained were analyzed and compared. Mutants of *Thiobacillus ferrooxidans* and *Pseudomonas fluorescens* showed quite similar protein profile pattern. The protein molecular weight was ranged from 10.00 to 120.00 KDa (Tables 1 and 2). The number of bands varied from 10 to 24. Hence it was concluded that in the presence of different types of metals, mutants of *Thiobacillus ferrooxidans* UV-26 and *Pseudomonas fluorescens* UV-24 did not show any significant difference in their protein profile pattern (Fig. 1). Similar results were obtained

by [16,17] when *Thiobacillus* inoculated with cadmium and nickel. On the basis of above result it was noticed that in presence of metals these bacteria produces a minor types of proteins. For critical analysis and comparison further work is needed [17].

Table 1. Molecular weight of each band of proteins observed during metal extraction for *Thiobacillus ferrooxidans* mutant UV-26.

Lane-1	Lane-2	Lane-3	Lane-4	Lane-5	Lane-6	Lane-7	Lane-8	Lane-9
120.5	118.5	119.2	114.0	117.2	120.2	118.2	121.0	118.8
94.8	92.4	90.2	98.2	96.2	94.2	98.4	92.4	91.6
83.4	81.6	78.2	76.4	77.8	82.4	90.2	81.2	82.8
74.8	72.4	68.4	64.3	71.2	72.8	76.4	72.6	76.2
66.0	65.2	63.4	60.2	64.8	65.4	70.5	64.2	68.4
48.2	48.2	42.4	40.2	46.7	49.7	68.1	46.7	46.1
45.4	40.3	40.2	38.4	44.2	47.4	49.4	42.1	42.6
43.8	43.4	42.4	32.2	41.2	45.2	46.2	39.8	40.2
29.5	28.4	27.5	26.5	29.0	42.9	36.7	26.7	32.4
15.2	14.2	14.6	14.4	15.6	36.2	26.8	16.8	16.8
15.0	14.5	14.9	12.4	15.2	30.4	18.3	14.6	14.2
14.3	13.5	13.0	12.0	14.2	26.5	15.5	14.0	14.0
12.6	12.2	12.0	11.2	12.4	18.8	14.4	12.8	12.8
10.2	10.2	10.6	10.2	10.2	14.4	12.6	10.9	10.4
10.0	10.0	10.5	10.0	10.6	12.2	10.8	10.4	10.0

Table 2. Molecular weight of each band of proteins observed during metal extraction for *Pseudomonas fluorescens* mutant UV-24

Lane-1	Lane-2	Lane-3	Lane-4	Lane-5	Lane-6	Lane-7	Lane-8	Lane-9
118.5	116.5	114.2	114.0	117.2	114.5	116.4	118.6	116.0
96.8	92.4	94.2	96.2	96.2	92.4	98.2	90.2	94.6
83.4	81.6	78.2	76.4	77.8	82.2	79.8	86.2	80.8
74.8	71.4	68.4	64.3	71.2	71.2	71.2	71.4	76.4
66.0	64.2	62.4	60.2	64.8	68.4	68.2	65.4	66.2
44.2	48.2	42.4	40.2	46.7	42.6	49.5	41.2	41.4
45.4	40.3	40.2	38.4	44.2	40.2	47.6	46.4	38.4
43.8	43.4	42.4	32.2	41.2	36.2	41.2	40.8	36.4
22.5	28.4	27.5	26.5	29.0	24.8	26.8	29.8	21.4
15.2	14.2	14.6	14.4	15.6	14.8	16.4	21.4	16.8
15.0	14.5	14.9	12.4	15.2	14.0	15.8	16.5	15.9
14.3	13.5	13.0	12.0	14.2	12.6	14.8	15.6	14.6
12.6	12.2	12.0	11.2	12.4	12.0	14.0	14.3	12.5
10.2	10.2	10.6	10.2	10.2	10.8	12.8	12.8	12.0
10.0	10.0	10.5	10.0	10.6	10.2	10.9	10.9	10.5

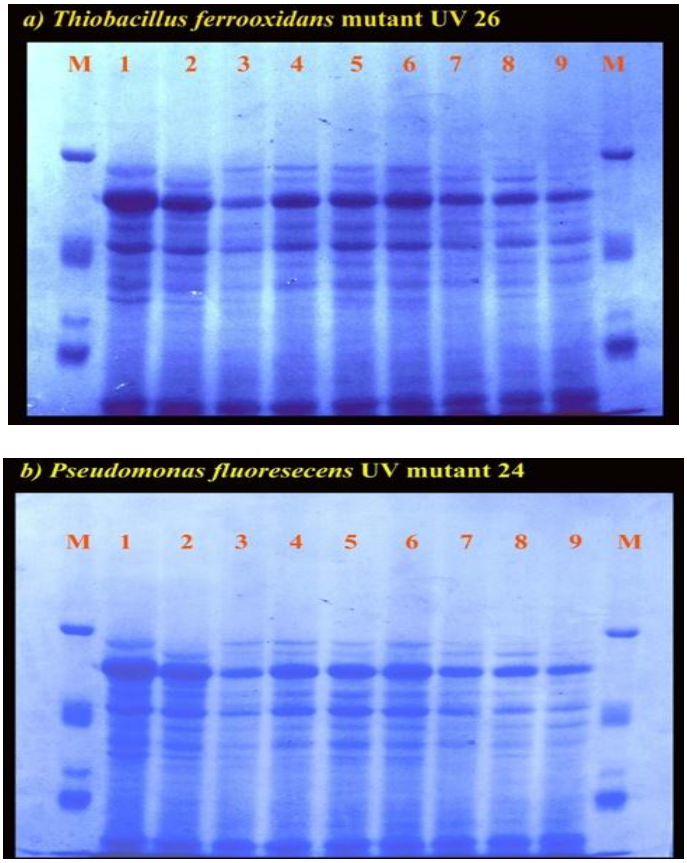

Fig. 1. SDS-PAGE profile of *Thiobacillus ferrooxidans* mutant UV 26 and *Pseudomonas fluorescens* mutant UV 24 (Lane 1-Control, Lane 2-1 mg/ml Fe, Lane 3- 2 mg/ml Fe, Lane 4-1 mg/mL Al, Lane 5-2 mg/mL Al, Lane 6-1 mg/mL Ti, Lane 7-2 mg/mL Ti, Lane 8-1 mg/mL Si, Lane 9- 2 mg/mL Si.

4. Conclusion

The protein profiling of the *Thiobacillus ferrooxidans* and *Pseudomonas fluorescens* shows that the pattern of proteins during the extraction of metals is not variable even after increasing concentration of the metal salts.

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