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Effect of Heavy Metals and Antibiotics on Siderophores Producing Bacterial Isolates

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In this study, siderophore-producing bacterial species have been isolated from soils contaminated with diesel oil. In *P. aeruginosa* (P-7) production of siderophores was inhibited at concentrations of $ZnSO_4$ and $CoCl_2$ of 2000 μM, while *B. subtilis* (BS-1) has increased siderophores production at similar concentrations of $CoCl_2$ and $ZnSO_4$. The same bacteria have increased the production of siderophores in presence of streptomycine (512 mg/ml). In *B. subtilis* the production of siderophores was inhibited by cefuroxime. The results show the effect of metals on the efficiency of siderophore production by bacteria for potential application in bioremediation of metal-contaminated iron-deficient soils in the microbial assisted phytoremediation processes. The results include the effect of metals on siderophore production efficiency of the bacteria used for the bioremediation of metal-contaminated soils. In conclusion, it has been found that heavy metals and antibiotics significantly effect the production of siderophores by bacteria.

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1. Introduction

Siderophores, iron-binding ligands, are produced by microorganisms under conditions in which the amount of iron is limited [1]. Many other metals and amino acids were found to suppress or stimulate siderophore production in bacteria [2, 3]. Effect of metals on the efficiency of siderophore production by bacteria is essential for potential applications in bioremediation of metal-contaminated iron-deficient soils, especially in the microbe-assisted phytoremediation processes [4]. Metal-resistant siderophore-producing bacteria play an important role in the successful survival and growth of plants in metal-contaminated soils [5]. During our studies on siderophore-producing bacteria from the soil contaminated with diesel oil we have investigated the influences of heavy metals ($CoCl_2$ and $ZnSO_4$) and antibiotics (cefuroxime and streptomycine) on siderophore production.

2. Materials and methods

2.1. Isolation and identification of the isolate

The bacterial strain, used in this study, was isolated from soils contaminated with diesel oil. The selected isolates *P. aeruginosa* (P-7) and *B. subtilis* (BS-1) were grown on nutrient agar and their cultural, morphological and biochemical characteristics were observed.

2.2. Screening for siderophore production

The production of siderophore by the isolates was detected using chrome azurol sulphonate (CAS) assay, as reported by Schwyn and Neiland [6], in iron-free succinic acid medium consisting of 6.0 g/l of K_2HPO4, 3.0 g/l of KH_2PO4, 0.2 g/l of $MgSO_4{\cdot}7H_2O$, 1.0 g/l of NH_4SO_4, and 4.0 g/l of succinic acid, at 28 °C under constant shaking at 120 rpm for 24–48 h [7]. After the incubation, cell density was measured at 640 nm by using double-beam UV-visible spectrophotometer (Shimadzu 1240, Japan).

2.3. Determination of minimal inhibitory concentrations

P. aeruginosa and *B. subtilis* (6×10^7 cells/ml) were grown in succinate medium externally supplemented with 700-2000 μM of compounds of heavy metals like $CoCl_2$ and $ZnSO_4$ at 28 °C at 120 rpm [8] and with cefuroxime and streptomycine (32, 64, 128, 256 and 512 mg/ml). We have studied the influence of different concentrations of bacterial growth inhibitors on siderophores production in succinate medium. The strains were grown in succinate medium with different concentrations of the following inhibitors: $CoCl_2$, $ZnSO_4$, cefuroxime and streptomycine.

3. Results and discussion

3.1. Siderophore production and detection

Change in color of CAS agar and CAS reagent from blue to orange-red indicated the ability of *P. aeruginosa* and *B. subtilis* to produce and excrete siderophores. Detection of siderophores and the change in color of the CAS reagent was due to the fact that siderophores, present in the supernatant, chelate the iron from the CAS reagent, which results in color change from blue to orange-red [9].

3.2. Minimal inhibitory concentration of metal and antibiotics

The results indicate that *P. aeruginosa* shows resistance compared to other bacteria as reported by Rachid

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and Bensoltane [10] and by Filali et al. [11]. Minimal inhibitory concentrations of the two antibiotics and tested metal salts are shown in Tables I and II. The results indicate that these strains show resistance compared to other bacteria, as reported by Filali et al. [11]. Strain P-7 was found to be the most resistant strain to the metals and antibiotics. *B. subtilis* has exhibited resistance to cefuroxime, while the same bacteria were sensitive to streptomycine. Among all preparations, the broth rich in siderophores exhibited potent antibacterial activity. The minimum bactericidal concentration of siderophore was 2000 μM for both *P. aeruginosa* and *B. subtilis.*

3.3. Effect of metals and antibiotics on bacterial siderophore production

Significant increase in extracellular siderophore production by *B. subtilis* was observed in the presence of $CoCl_2$ and $ZnSO_4$ at concentrations of 2000 μM (Table I), whereas in the present study the *P. aeruginosa* (P-7) have shown no siderophore production at concentrations of 2000 μM (Fig. 1).

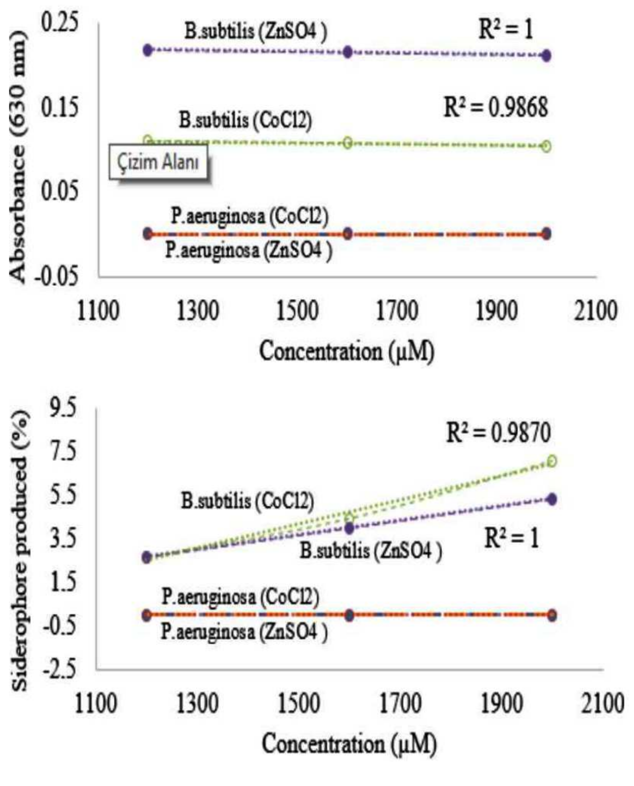

Fig. 1. Effect of $CoCl_2$ and $ZnSO_4$ on siderophore production in succinate medium by *P. aeruginosa* and *B. subtilis.*

The value of regression R^2 of the relationship between the concentrations of $CoCl_2$ and $ZnSO_4$ and the concentration of the produced siderophore was found to be higher for the UV spectroscopic method (Table I). Figure 1 shows that there exists a linear relationship between the applied concentrations of $CoCl_2$ and $ZnSO_4$ and the concentration of the produced siderophore.

TABLE I

Effect of $CoCl_2$ and $ZnSO_4$ on siderophore production in succinate medium by *B. subtilis* (BS-1).

Metal ions [μM]	Absorbance at 630 nm	R^2	Reference at 630 nm	% Siderophore produced [μg/ml]	R^2
$CoCl_2$ [μM]					
1200	0.110	0.9868	0.113	2.65	0.9870
1600	0.108		0.113	4.42	
2000	0.105		0.113	7.07	
$ZnSO_4$ [μM]					
1200	0.218	1	0.224	2.67	1
1600	0.215		0.224	4.01	
2000	0.212		0.224	5.35	

An increase of siderophore production was observed in *B.subtilis* at concentrations of $CoCl_2$ and $ZnSO_4$ of 2000 μM. $ZnSO_4$ as well as $CoCl_2$ are known to bind to siderophores and have shown a 2.67% and 5.35% decrease in growth at 1600 and 2000 μM respectively, which was suppressed at lower concentrations of 1200 and 1600 μM (Fig. 1).

Streptomycine has inhibited siderophore production in *P. aeruginosa* (Table II).

TABLE II

Effect of cefuroxime and streptomycine on siderophore production in succinate medium by *B. subtilis* (BS-1).

Antibiotics [mg/ml]	Absorbance at 630 nm	R^2 value	Reference at 630 nm	% Siderophore produced [μg/ml]	R^2 value
Cefuroxime [mg/ml]					
128	0.00	–	0.115	0.00	–
256	0.00		0.115	0.00	
512	0.00		0.115	0.00	
Streptomycine [mg/ml]					
128	0.095	0.9842	0.113	15.9	0.9846
256	0.091		0.113	19.4	
512	0.086		0.113	23.8	

A significant positive correlation was found between concentrations, measured using UV spectroscopic method, of the the streptomycine and siderophores produced by B.subtilis (Table II).

While streptomycine has increased the production of siderophores in *B.subtilis*, siderophore production was inhibited by cefuroxime (Fig. 2).

Metal-resistant siderophore-producing bacteria play an important role in the successful survival and growth of plants in contaminated soils by alleviating the metal toxicity and supplying the plant with nutrients, particularly with iron [12]. *P. aeruginosa* JAS-25 has the potential to act as an excellent biocontrol agent against phytopathogens of agricultural crops [13]. Siderophores from strain *Bacillus sp* SD12 pave the way for their utilization in the field of medicine and agriculture [14].

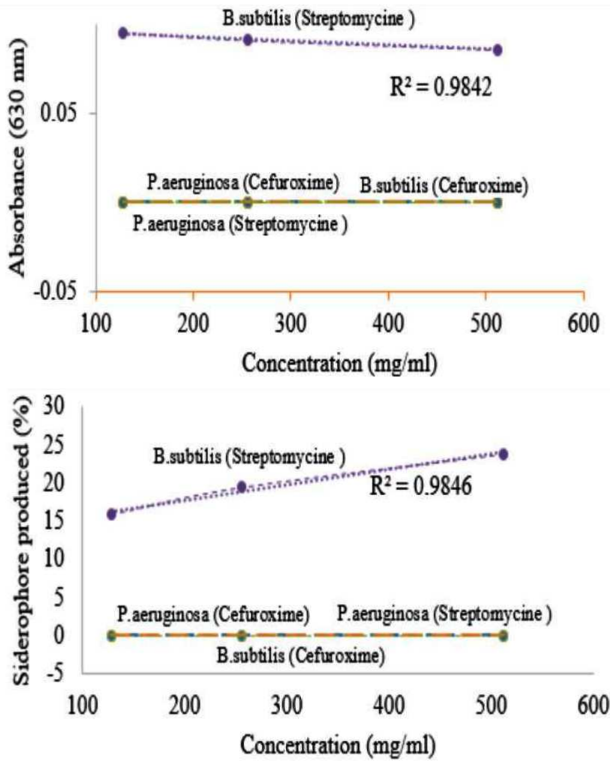

Fig. 2. Effect of cefuroxime and streptomycine on siderophore production in succinate medium by *P.aeruginosa* and *B. subtilis*.

4. Conclusions

In recent years, siderophores have attracted much attention due to appearance of different application areas. Because siderophores have the ability to bind a variety of metals in addition to iron, they have gained importance in various fields. Siderophores can be used as biocontrols, biosensors, bioremediation and chelation agents. The present study concludes that in the diesel oil contaminated soils *P. aeruginosa* and *B. subtilis*, producing siderophores, improve the plant growth and have antibacterial activity against the human clinical pathogens.

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