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RESEARCH ARTICLE

EXPLORING HEAVY METAL TOLERANCE OF FLUORESCENT *PSEUDOMONAS* ISOLATE

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ABSTRACT

Rhizobacteria that promote the plant growth and development are called plant growth promoting rhizobacteria (PGPR). The bacterium under study, belong to PGPR and identified as *P. fluorecens strain FP18.* The present study deals with heavy metal tolerance capacity of this strain against four heavy metals. The heavy metals used in this study were Zinc sulphate, Arsenic trichloride, Mercuric chloride and Lead nitrate. The isolate exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) ranging from 50µg/ml to 300µg/ml. Heavy Metal Tolerance study indicated maximum microbial tolerance to Zinc sulphate and lowest to Lead nitrate.

INTRODUCTION

Soil is the home for variety of living microorganisms, which is getting contaminated by heavy metal pollutants such as Cd, Cu, Zn, Ni, Co, Hg, Cr, Pb and As. These heavy metal compounds cause intimidation to the environment and health because of their toxicity and non-biodegrability (Bahadir *et al.,* 2007; Pérez-Marin *et al.,* 2008; Reddad *et al.,* 2003). Some soil microorganisms are capable to degrade or transform contaminants into their less toxic forms (Vidali, 2001). The naturally occurring soil bacteria have been studied for their metal accumulation capacity from polluted sites (Clausen, 2000). Some fluorescent *Pseudomonas spp.* shown major role in biodegradation of pesticides as well as heavy metals (Wasi *et al.,* 2008). It is possible for some bacteria to survive in metal-stressed condition, because of their potential to efflux of metal ions outside the cell and diminution of the heavy metal ions to a less toxic state. Hence, by capturing such bacteria in solid or liquid inoculants preparation, the greater yield of the crops could be possible in metal containing soil. The isolate was found to be resistant to heavy metals and plant growth promoter as it is belong to PGPR. The agricultural productivity can be improved by these traits of bacterium. Therefore this study was performed to determine the heavy metal resistance patterns of bacteria which was isolated from rhizospheric soil of cotton plant.

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MATERIAL AND METHODS

Sampling sites and collection

The soil sample was collected from rhizospheric region of cotton plant from village Shivasawanga, Dist. Nagpur. The samples were collected and immediately transported in the laboratory for further experimentations.

Isolation of fluorescent Pseudomonas isolate

A soil suspension was prepared by shaking 1g of soil having 2-3 cm undamaged root pieces with tightly adhered soil in 100 ml of sterile distilled water and kept for 24hrs on a rotary shaker to release the rhizoplane bacteria. The processed samples were serially diluted from 10^{-1} to 10^{-6} and 0.1 ml of the suspension was spread on to King's medium B (KMB) agar plate and incubated at 28ºC for 48h. The occurrence of fluorescent *Pseudomonas* was examined under UV light (356 nm) by using Spectroline Ultravoilet Transilluminator.

The heavy metals used for study

The study was carried out by using four heavy metal compounds - $ZnSO_4$, $AsCl_3$, $HgCl_2$, $Pb(NO_3)_2$.

Heavy metal sensitivity profile

In preliminary studies, these heavy metal compounds were tested against the isolate to generate a toxicity sensitivity

profile (Bauer *et al.,* 1966). The overnight grown culture of fluorescent *Pseudomonas* isolate was utilized for preparing King's B agar spreadplates and according to the Kirby-Bauer test protocol incubated with selected heavy metal compounds for 24 hrs at optimum growth temperature of isolate. The zone of inhibition was noted for standardizing the sensitivity profile of test organism.

Determination of effect of heavy metal compounds on bacterial growth

The growth curve of isolate was determined by incorporating 50 µg /ml, 100 µg /ml, 150 µg /ml, 200 µg /ml, 250 µg /ml and 300 µg /ml concentration of each heavy metal and measuring the optical density at 600nm by spectrophotometer (Dynamica Halo DB - 20S).

RESULTS

Isolation of fluorescent *Pseudomonas* bacteria

The test organism used in this study was isolated from rhizospheric region of cotton plant from village Shivasawanga, Dist. Nagpur. On the basis of standard microbiological, biochemical and molecular tests, the bacterial strain was identified as the *P. fluorecens strain FP18* (Fig. 1).

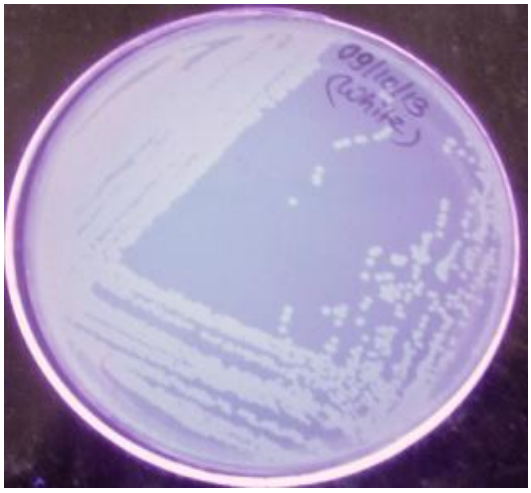
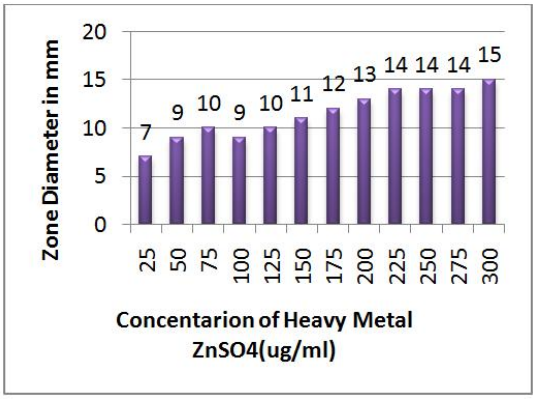


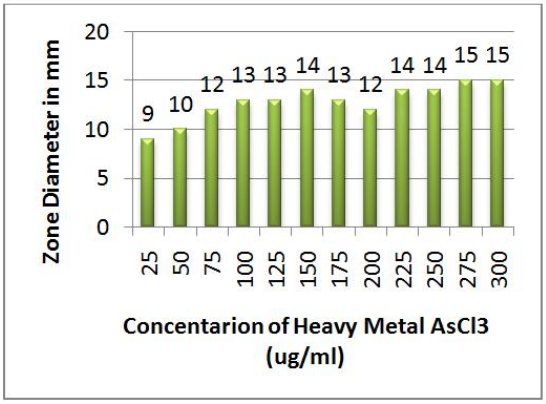
Fig. 1. Fluorescent *Pseudomonas* isolate under UV light

Preliminary screening of the test organisms against heavy metals

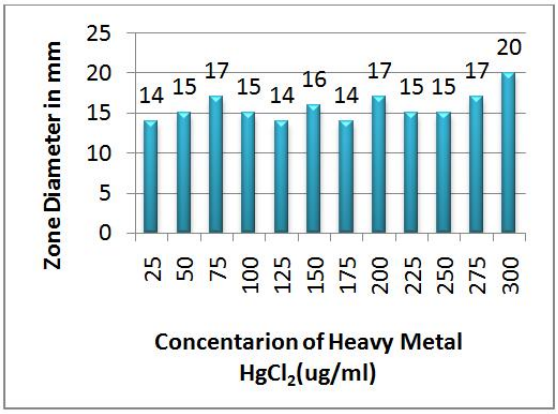
The isolate was tested for sensitivity towards four different heavy metals by Kirby-Bauer test method. The bacterial isolate was treated with the four heavy metal – $ZnSO_4$, $AsCl_3$, $HgCl_2$, $Pb(NO_3)_2$. The growth of bacterial isolate was analyzed on the basis of zone of inhibition against different concentration of heavy metals used. When the isolate was tested against heavy metals- $ZnSO_4$ and $AsCl_3$, no zone or slightest zone was found as compared to the control up to the conc. 300 ug/ml, indicating good growth of isolates even after addition of contaminants. While greater zone of inhibition for heavy metal $HgCl_2$ and $Pb(NO_3)_2$ indicated sensitivity of the isolate for these heavy metals [Fig. 2 (A-D)].



(a)



(b)



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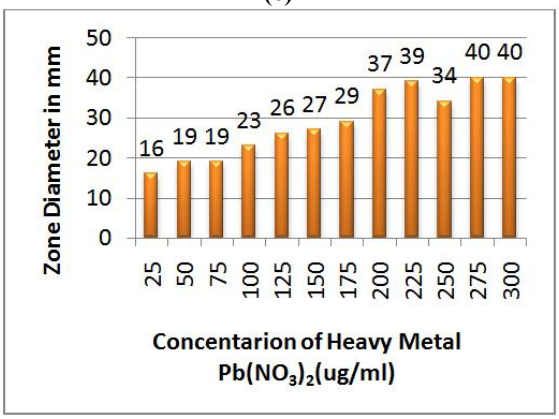

(d)

Fig. 2. Interpretation of zone of inhibition showed by isolate against different heavy metal compounds (A-D).

Table 1. Spectrophotometric values of cell density measurement for *P. fluorecens strain FP18*

S.No.	Heavy metal compounds	O.D. 600nm at (24 hrs)						O.D. 600nm at (48 hrs)					
	Concentration of heavy metal (ug/ml)	50	100	150	200	250	300	50	100	150	200	250	300
	Control(without heavy metals)			1.05						1.74			
1.	ZnSO4	1.09	1.00	0.92	0.91	0.89	0.85	1.12	1.09	1.99	0.98	0.91	0.84
2.	$AsCl_3$	1.10	1.00	0.98	0.91	0.85	0.81	1.02	1.01	0.93	0.92	0.87	0.81
3.	HgCl2	0.98	0.91	0.82	0.78	0.71	0.69	0.94	0.90	0.93	0.88	0.73	0.62
4.	$Pb(NO_3)_2$	0.88	0.81	0.74	0.69	0.64	0.58	0.71	0.65	0.59	0.43	0.38	0.23

Spectrophotometric analysis

The effect of heavy metal compounds on cell density of *P. fluorecens strain FP18* was analysed by spectrophotometer. Heavy metal compounds $ZnSO_4$ and $AsCl_3$ had little or no effect, while $HgCl_2$ and $Pb(NO_3)_2$ had adverse effect on the cell density after 24 hrs to 48 hrs of incubation with heavy metal compounds. These results showed that the bacterial candidate under study was found to be resistant to heavy metals $ZnSO_4$ and $AsCl_3$ while sensitive to $HgCl_2$ and $Pb(NO_3)_2$ (Table 1).

In the present scenario, screening of multiple heavy metal compounds, the results showed maximum microbial tolerance to Zinc sulphate and lowest to Lead nitrate. The isolate showed minimum inhibitory concentration (MIC) for $ZnSO_4$ and $AsCl_3$ upto conc. 300 µg /ml while MIC for $HgCl_2$ was upto 250 µg /ml and for $Pb(NO_3)_2$ upto 200 µg /ml.

Research reports suggested that diverse group of free-living soil bacteria can improve host plant growth and lessen toxic effects of heavy metals on the plants (Belimov *et al.,* 2004; Wani 2008). Osborne *et al.* (2010) isolated plant growth promoting rhizobacteria and evaluated metal tolerance concentration (MTC) of PGPR strains which were able to survive till 300 mg/L on Cd amended minimal medium (Osborne *et al.,* 2010).

Conclusion

Majority of soil microorganisms have ability to survive in metal contaminated soil and it is found that, they promote plant growth by decreasing the heavy metal toxicity. The experimental results of the bacterium under study, clearly revealed that the isolate is tolerant to heavy metal contaminations and enhanced PGPR activity in metal containing soil.

The selection of such microorganisms which are heavy metal tolerant and plant growth promoter can be speed up agricultural productivity in metal containing soil also.

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