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Degradative Plasmid and Heavy Metal Resistance Plasmid Naturally Coexist in Phenol and Cyanide Assimilating Bacteria

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Abstract: Problem statement: Heavy metals are known to be powerful inhibitors of xenobiotics biodegradation activities. Alleviation the inhibitory effect of these metals on the phenol biodegradation activities in presence of heavy metals resistant plasmid was investigated. **Approach:** Combination of genetic systems of degradation of xenobiotic compound and heavy metal resistance was one of the approaches to the creation of polyfunctional strains for bioremediation of soil after co-contamination with organic pollutants and heavy metals. **Results:** A bacterial strain *Pseudomonas putida* PhCN (pPhCN1, pPhCN2) had been obtained. This bacterium contained two plasmids, a 120 Kb catabolic plasmid that encode for breakdown of phenol (pPhCN1) and pPhCN2 plasmid (100 Kb) that code for cadmium and copper resistant. Cyanide assimilation by this bacterium was encoded by chromosomal genes. The inhibitory effect of cadmium (Cd^{2+}) or copper (Cu^{2+}) on the degradation of phenol and cyanide by *P. putida* strains PhCN and PhCN1 (contained pPhCN1) were investigated. The resistant strain PhCN showed high ability to degrade phenol and cyanide in presence of Cd^{2+} or Cu^{2+} comparing with the sensitive strain PhCN1. In addition, Cd^{2+} or Cu^{2+} was also found to exert a strong inhibitory effect on the C23O dioxygenase enzyme activity in the presence of cyanide as a nitrogen source. **Conclusion:** The presence of heavy metal resistance plasmid alleviated the inhibitory effect of metals on the phenol and cyanide assimilation by resistant strain.

Key words: Bacteria, cadmium, copper, plasmid, phenol degradation

INTRODUCTION

Forty percent of hazardous wastes on the Environmental Protection Agency's (EPA) National Priority List (NPL) are co-contaminated with organic and heavy metals pollutants that pose healthy hazards to humans and wildlife[12,23]. Common organic pollutants at these sites include phenol, Polycyclic Aromatic Hydrocarbons (PAHs), chlorinated solvents, cyanide, herbicide and pesticides, while common heavy metal contaminants include arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc. Isolation of bacterial strains that are able to degrade more than one organic pollutants such as phenol and cyanide, is becoming increasingly important for decontaminating polluted soil, sledges and ground water[7,13]. The use of these microorganisms may face various problems, including poor survival, substrate accessibility[16], or the presence of inhibitory compounds. Heavy metals are known to be powerful inhibitors of biodegradation activities[2,25-27, 29], thus, their presence may impair the biodegradation of aromatic compounds in polluted sites[5,15,16,24,25,29-32,34]. Situations where simultaneous contamination by heavy metals and organic compounds are present can be expected and have been detected in industrial areas[1]. For this reason, there is increasing interest[33] in bacterial strains that degrade aromatic compounds and tolerate toxic metals. It has previously been shown that strains of *Alcaligenes eutrophus* bearing plasmids of metal resistance and plasmids of biodegradation of polychlorinated biphenyls and 2,4-dichlorophenoxyacetic acid degrade these xenobiotics more effectively in the presence of nickel or zinc as compared with sensitive strain[6,32]. However, to date, there have been no data on the interaction of genetic systems of aromatic compounds degradation, cyanide assimilation and metal resistance and their effect on physiology, biodegradation efficiency and the activity of the key enzyme in multifunctional strains.

The objective of this study was to examine the physiological and biochemical features of phenol degradation in variants of the soil strain *P. putida* PhCN sensitive and resistant to heavy metals, in presence of three toxicants, cadmium (Cd^{2+}), copper (Cu^{2+}) and cyanide, as a nitrogen source.

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MATERIALS AND METHODS

Chemical: Phenol, Agarose and ethidium bromide were purchased from Sigma (St. Lous, MO, USA). Sodium cyanide, cadmium chloride ($CdCl_2$), nickel chloride ($NiCl_2$), zinc chloride ($ZnCl_2$) and copper chloride ($CuCl_2.2H_2O$) were procured from Merck (Darmstadt, Germany). Bacteriological media were purchased from Difco (Difco Laboratories, Detroit, Michigan) and Oxoid (Oxoid Inc., Nepean, Ontario). Reagents compounds used were of the highest grade commercially available.

Bacterial strains, plasmids and culture conditions: Bacterial strains and plasmids used in this study are shown in Table 1. *P. putida* strain PhCN was isolated from Agriculture soil contaminated with different pesticides. This stain completely degraded phenol and cyanide as a sole source of carbon and nitrogen respectively[9]. PhCN1 (pPhCN1), PhCN2 (pPhCN2) and PhCNM (plasmidless) strains were the cured derivatives of wild type strain *P. putida* PhCN[9]. All isolates were grown aerobically at 30°C on a rotary shaker at 150 rpm in Luria-Bertani (LB) medium[27] or Tris-buffered (MSM), which was prepared according to Mergeay *et al*.[20]. All isolates were maintained either on nutrient agar slants at 4°C or in 20% (v/v) glycerol solution at -80°C.

Effect of the presence of heavy metals on the growth of *P. putida* PhCN and its derivatives: Bacterial cells were pre-grown in MSM with phenol (1 mM) and cyanide (0.5 mM) in Erlenmeyer flasks at 28°C (150 rpm) until an optical density (OD_{600}) of about 0.5 was reached. This culture (2.2×10^5) was used to inoculate Erlenmeyer flasks containing 1 mM phenol and ammonium and/or cyanide as the sole source of carbon and nitrogen, respectively. The concentrations of studied heavy metals were adjusted to 10 or 100 µM. The initial OD_{600} was adjusted to about 0.05. Control flasks contained free metals medium inoculated with the same studied strains. All cultures were incubated on an orbital shaker (150 rpm) at 28°C. OD_{600} nm was determined periodically and used as the measure of growth to construct growth curves and assess heavy metal effects on biodegradation as described by[18,34]. All tests were done in triplicate.

Analytical methods: The phenol degradation was determined by monitoring the change in phenol concentration[10]. At different intervals of incubation, 1 mL samples were taken and transferred to an Eppendorff tube containing 25 µL of 2% 4-aminoantipyrene and 50 µL of 2 M ammonia. After mixing, 25 µL of 8% potassium hexacyanoferrate (III) was added. The suspension was centrifuged at 5,000×g for 2 min and absorbance at 500 nm was measured and compared with phenol standards curve.

Cyanide determination: Cyanide concentration was determined by a modification of picric acid as described by Myers *et al*.[21]. Linear calibration curve was obtained with the standard cyanide solution as follow: Aliquots 0.05 mL of cyanide-containing solution (after centrifugation at 15,000 g for 10 min at 4°C) was added to 0.1 mL aliquots of solution containing 0.5% (w/v) picric acid and 0.25 M Na2CO3. The resulting solution were placed in boiling water-bath for 5 min, diluted to 1 mL with 0.85 mL distilled water and cooled in tap water for 30 min. The absorbance was read at 520 nm against a blank of distilled water and picric acid reagent.

Determination of Minimal Inhibitory Concentration (MIC) of Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} against studied bacterial variants: Minimum inhibitory concentrations (MIC) of metals for each isolate was determined by the plate-dilution method as adopted by Mergeay *et al*.[20]. The metals Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} were used as $CdCl_2$, $CuCl_2.2H_2O$, $NiCl_2$ and $ZnCl_2$, respectively, in various concentrations ranging from 0.001-8 mM. The plates were incubated at 28-30°C for 72-96 h. The concentration of metal which permitted growth and beyond which there was no growth was considered as the MIC of the metal against the strain tested. Carbon free Tris-salt medium (MSM) with 1.5% agar, containing phenol and ammonium or cyanide as a sole source of carbon and nitrogen respectively, was also used for detection of tolerance against these metals. The concentration of added phenol and cyanide in the media was 1 and 0.5 mM, respectively.

Table 1: Bacterial strains and plasmids

Strains or plasmid	Relevant characteristic(s)a	Reference or source
***Pseudomonas putida*:**		
PhCN	Ph^+, Cd^r, Cu^r, wild type pPhCN1, pPhCN2 carrier	[9]
PhCN1	Ph^+ a cured mutant of PhCN, pPhCN1 carrier	[9]
PhCN2	Cd^r, Cu^r a cured mutant of PhCN, pPhCN2 carrier	Present study
PhCNM	Ph^-, Cd^-, Cu^-a cured mutant of PhCN, Plasmidless	[9]
Plasmids		
pPhCN1	120 Kb Ph^+ catabolic plasmid of *P. putida* PhCN	[9]
pPhCN2	100 Kb Cd^r, Cu^r,, heavy metal resistant plasmid of *P. putida* PhCN	Present study

Preparation and crude enzyme extract: The effect of Cd^{2+} or Cu^{2+} was studied at the same concentration, previously mentioned for studying the effect on growth and phenol degradation. *P. putida* strain PhCN and its derivatives were grown overnight in MSM containing 0.5 mM phenol. Then, the culture was diluted in fresh LB medium, LB medium plus 0.5 mM phenol, or MSM containing either ammonium or KCN as a sole nitrogen source and 0.5 mM phenol as a carbon source. Cells were harvested by centrifugation at 10,000×g for 10 min at 4°C. The pellets were washed twice in 50 mM phosphate buffer and were disrupted sonically at 4°C. Cellular debris was removed by centrifugation (12,000×g for 20 min at 4°C). The enzymes activity were measured by incubating the crude enzymes extract with 100 µM catechol or phenol in 33 mM Tris-HCl (pH 8.0), containing 1.3 mM EDTA and 3.3 mM 2-mercaptoethanol, at 25°C. The activity of catechol-2,3-dioxygenase was determined from the rate of α-oxymuconic semialdehyde ormation (α = 375 nm, E = 33.4 µM cm^{-1}). The activity of catechol-1,2 dioxygenase was determined from the rate of cis-cis muconate formation (α = 260, E = 16.9 µM cm^{-1}). The specific enzyme activity was expressed in nano moles of the cofactor consumed or the product formed in 1 min 1 mg^{-1} of total bacterial protein. The protein concentration was determined spectrophotometrically according to Lowery *et al*.[17].

Plasmid analysis: Plasmids were analyzed from cells grown in liquid media under selective conditions given by either metals or the carbon source. Plasmids of *P. putida* PhCN strains and its derivatives were analyzed according to the method of Kado and Liu[14]. Standard DNA techniques were used as described previously[28]. For plasmids analysis, 0.7% (wt/vol) agarose gels with Tris borate-EDTA buffer were employed. The size estimate of the isolated plasmid was obtained by comparing relative mobility on agarose gel with standard molecular markers.

RESULTS AND DISCUSSION

Native plasmids in *P. putida* PhCN: Plasmid DNA analysis of *P. putida* strain PhCN cultures grown on phenol and cyanide indicated the presence of two large plasmid elements[9]. Plasmid curing experiments produced a plasmid-free strain named as PhCNM, a variant containing the 120 kb plasmid (pPhCN1) named as PhCN1 and a strain harboring 100 kb plasmid (pPhCN2) named as PhCN2. Based on the fact that subsequent plasmid curing experiments demonstrated that the ability to utilize phenol was encoded by the 120 kb plasmid, whereas the ability to utilize cyanide appeared to be encoded by the chromosome[9].

Each plasmid in these variants was remained stable and retained the determined phenotype phenol degradation (ph^+) and/or heavy metal resistance (cd^r/Cu^r) after several passages in non selective medium. In the wild type strain PhCN (contained pPhCN1, pPhCN2), both plasmids were also maintained stably and retained the determined phenotype of ph^+ and Cd^r/Cu^r after several passages in a non selective medium.

Minimal Inhibitory Concentration (MIC) of Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} against studied bacterial variants: In the preliminary screening, the ability of *P. putida* PhCN and its derivatives; PhCN1, PhCN2 and PhCNM, to form confluent growth in the presence of incremental concentrations of studied metal salts was evaluated. On complete medium (LB), the wild type strain PhCN exhibited the highest resistance to $Cd^{2+}$and Cu^{2+}in comparison with the other variants, PhCN1and PhCNM (Table 2). However, the MICs of Zn^{2+} and Ni^{2+} for tested variants were very close.

It has been previously demonstrated that the interference of a high phosphate content in the usual minimal medium with a metal effect can lead to the over estimation of the MIC[20]. Therefore, in the present study, levels of resistance in wild type strain PhCN and its cured derivatives to Zn^{2+}, Cu^{2+}, Ni^{2+} and Cd^{2+} were determined on Tris-medium supplemented with glucose as the sole carbon and energy source. As presented in Table 2, for the resistance variant PhCN , MIC of Cd^{2+} (1.5 mM) and Cu^{2+} (1.2 mM) were much higher than in sensitive strain PhCN1 (0.03 and 0.02 mM for $Cd^{2+}$and Cu^{2+}, respectively). No significant difference in the MICs of Zn^{2+} and Ni^{2+} among the four strains were observed. Furthermore, the MICs of Cd^{2+}, Zn^{2+}, Cu^{2+} and Ni^{2+} for tested strains in the Tris-medium were lower than those observed in the LB medium (Table 2). This result was in harmony with those mentioned by Mergeay *et al*.[20]. They stated that MICs of heavy metals in Tris-medium were lower than those observed in a rich medium. On the other hand, the MICs of Cd^{2+}, Zn^{2+}, Cu^{2+} and Ni^{2+} in presence of phenol and cyanide as sole carbon and nitrogen sources, respectively, for tested strains were lower than those observed in the Tris-medium supplemented with glucose and ammonium as sole carbon and nitrogen sources, respectively (Table 2). These results are consistent with findings of[4] that the MICs of metals for catabolic strains in the presence of aromatic compounds as the only source of carbon are only slightly lower than those evaluated in the presence of glucose or malate.

Table 2: MICs of heavy metals for Pseudomonas putida PhCN and its derivatives in different cultural conditions

Bacteril strains	MIC of Cd^{2+} (mM)				MIC of Cu^{2+} (mM)				MIC of Zn^{2+} (mM)				MIC of Ni^{2+} (mM)			
	LB	MS+G	MS+Ph	MS+CN+Ph	LB	MS+G	MS+Ph	MS+CN+Ph	LB	MS+G	MS+Ph	MS+CN+Ph	LB	MS+G	MS+Ph	MS+CN+Ph
PhCN	2.00	1.50	1.00	0.800	1.80	1.20	1.00	0.85	1	0.9	0.60	0.50	1.2	0.9	0.6	0.4
PhCN1	0.05	0.04	0.03	0.015	0.04	0.03	0.02	0.01	1	0.8	0.02	0.01	1.2	0.8	0.6	0.4
PhCN2	2.00	1.50	0.00	0.000	1.80	1.20	0.00	0.00	1	0.8	0.00	0.00	1.2	0.9	0.0	0.0
PhCNM	0.04	0.04	0.00	0.000	0.04	0.04	0.00	0.00	1	0.7	0.00	0.00	1.2	0.8	0.0	0.0

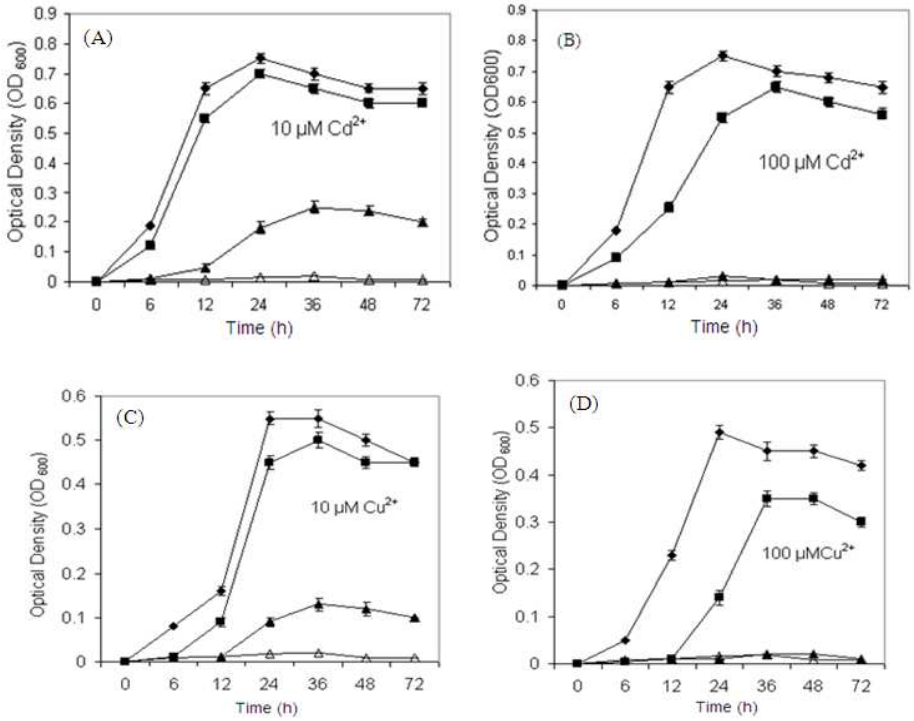

Fig. 1: Effect of cadmium and copper concentrations on the growth of *Pseudomonas putida* PhCN and its derivatives in MSM medium amended with phenol and ammonium as a sole carbon and nitrogen sources respectively. Strains symbols: (♦): PhCN without metals (control); (■): PhCN; (▲): PhCN1; (Δ): PhCNM

Growth kinetics:

Biodegradation of the phenol in the absence of heavy metals: Preliminary assays were performed to evaluate the growth rate of wild type strain PhCN and its derivatives PhCN1 on MSM medium free from heavy metals and supplied with phenol and ammonium or cyanide as a sole source of carbon and nitrogen, respectively. The results shown in Fig. 1A-D indicated that both strains which harbored a catabolic plasmid pPhCN1 were able to degrade the compound with similar rate. However, grown on MSM medium supplied with phenol and cyanide revealed a decrease in the growth rate as well as the rate of phenol consumption comparing with that supplied with ammonium salt as a nitrogen source. Similar findings were reported by[3] for *Pseudomonas* sp. grown on phenol and cyanide.

Biodegradation of the phenol in the presence of the cadmium: The effect of cadmium on growth parameters and phenol consumption by resistant strain PhCN (pPhCN1, pPhCN2) and sensitive strain PhCN1 (pPhCN1) was investigated. Cadmium at concentration of 10 µM, did not affect the growth rate of PhCN, whereas, the growth of sensitive strains was significantly reduced comparing with that of the cadmium free medium (Fig. 1A). Moreover, the addition of cadmium at a concentration of 10 µM did not change the rate of phenol consumption by resistant strain PhCN, whereas Phenol consumption by sensitive strain PhCN1, was significantly reduced. compared to that of the free-cadmium MSM (Fig. 2A)[2,30].

Increasing concentration of cadmium by ten-fold (100 µM) caused a complete inhibition of growth in

sensitive strain PhCN1 (Fig. 1B), whereas, the growth and Phenol consumption by resistance strain PhCN was slightly reduced compared to that of the free-cadmium MSM (Fig. 1B and 2B). Previously a similar finding has been reported for the multifunctional strains of *Alcaligenes eutrophus* that bear plasmids of resistance to bivalent cations, pMOL28 (cnr-Co^r N^r) and pMOL30 (czc-Co^r Zn^r Cd^r) and catabolic plasmids of biodegradation of polychlorinated biphenyls, pSS50 (BpH^+/Cbp^+) and 2,4-dichlorophenoxyacetic acid, pJP4 (Tfd^+) are effectively degraded the above pollutants in the presence of metals[6,19].

Biodegradation of phenol in presence of copper: Growth response of the PhCN and its variant in the presence of cupric chloride was shown in Fig. 1. At low concentration (10 µM) in MSM supplemented with phenol and ammonium as a sole source of carbon and nitrogen, respectively, the growth rate of resistant strain PhCN did not affected, whereas the growth rate of the sensitive strain PhCN1 was significantly reduced (Fig. 1C). Phenol consumption by resistance strain PhCN was less active compared to the MSM free from copper (Fig. 2C), whereas, phenol consumption by sensitive strain was significantly reduced (Fig. 2C). This results was in agreement with previous results of Lin *et al.*[16] which showed that copper exhibited high toxicity at relatively low concentrations of 10 mg L^{-1}.

A ten-fold increase of copper (100 µM) in MSM supplemented with phenol and ammonium as a sole source of carbon and nitrogen source respectively, caused a long lag-phase (12 h) and required long time (48 h) for complete phenol consumption by resistance strain PhCN (Fig. 1D and 2D). However , the addition of copper at a concentration of 100 µM to MSM was completely inhibited the growth of sensitive strain (Fig. 1D and 2D)[31].

Biodegradation of cyanide in presence of cadmium: The inhibitory effect of heavy metals on bacterial growth and cyanide degradation were investigated. As shown in Fig. 3A, addition of cadmium (10 µM) to MSM containing glucose and cyanide as the sole source of carbon and nitrogen, respectively, showed a slightly decrease in the growth rate and cyanide consumption by resistant strain PhCN (Fig. 3A and 4A), whereas, induced a significant decrease in bacterial growth and retarded cyanide consumption by sensitive strain PhCN1 (Fig. 3A and 4A).

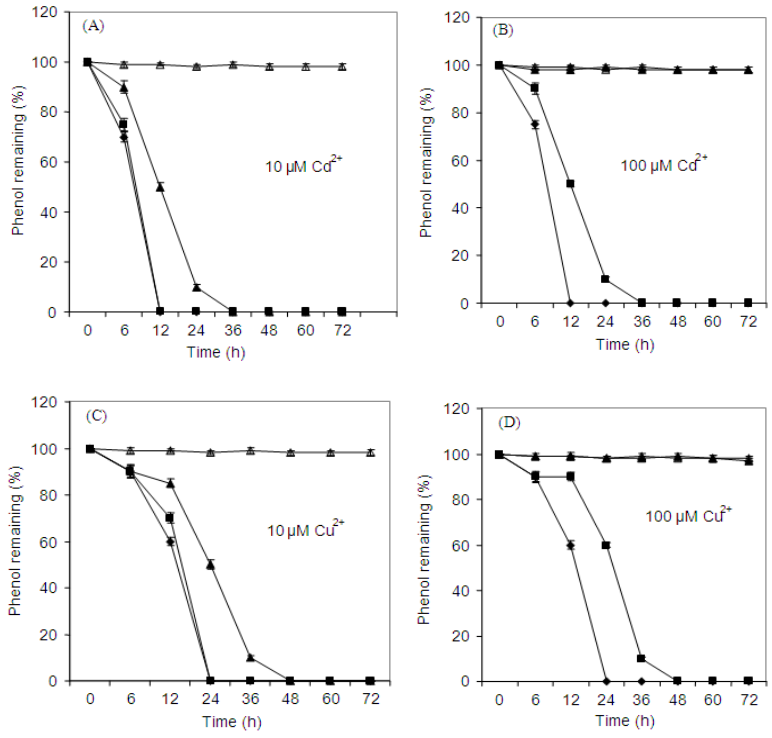

Fig. 2: Effect of cadmium and copper concentrations on the phenol utilization by *Pseudomonas putida* PhCN and its derivatives. Strains symbols: (♦): PhCN without Metals (control); (■): PhCN; (▲): PhCN1; (Δ): PhCNM

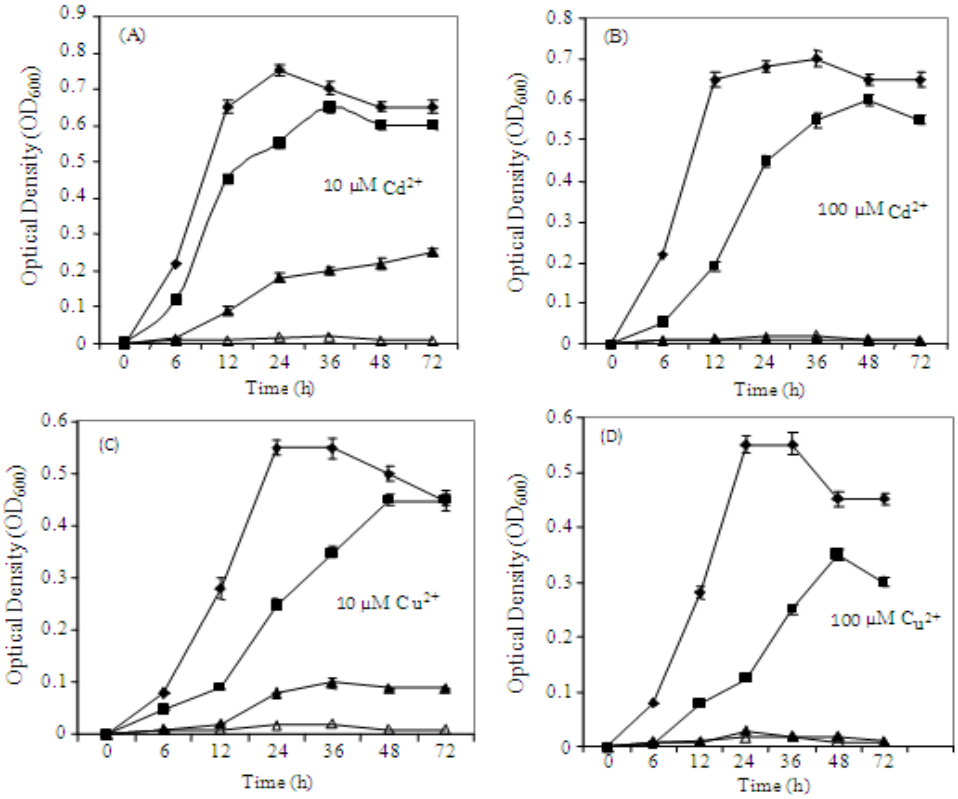

Fig. 3: Effect of cadmium and copper concentrations on the growth of *Pseudomonas putida* PhCN and its derivatives in MSM medium amended with glucose and cyanide as a sole carbon and nitrogen sources respectively. Strains symbols: (♦): PhCN without Metals (control); (■): PhCN; (▲): PhCN1; (Δ): PhCNM

A ten-fold increase of cadmium (100 µM) caused a 12 h lag-phase (Fig. 3B) and cyanide consumption was less active by resistant stain PhCN compared to that of the MSM free cadmium (Fig. 4B). Under the same conditions, the growth of sensitive strain PhCN1 was completely inhibited (Fig. 3B and 4B). This may be due to the fact that when various toxicants were added together, the growth of almost bacterial isolates was very poor or absent, suggesting that the tolerance level of heavy metal resistance strains is affected by many xenobiotics[8].

Biodegradaion of the cyanide in the presence of the copper: The addition of copper at a concentration 10 µM to the MSM containing glucose and cyanide as a sole source of carbon and nitrogen respectively, produced no appreciable affect on the growth and cyanide consumption by resistance variant, while the growth of sensitive strain was significantly reduced (Fig. 3C and 4C).

A ten-fold increase of copper (100 µM) , caused a long lag-phase (12 h) and required long time (60 h) for complete phenol consumption by resistance strain PhCN (Fig. 3D and 4D). However, the addition of copper at a concentration of 100 µM to MSM was completely inhibited the growth of sensitive strain (Fig. 3D and 4D)[31].

By comparing the obtained results in Fig. 1-4, it can be concluded that, low concentration of both Cu^{2+} and Cd^{2+} (10 µM) did not exhibit any significant inhibitory effect on microbial growth of resistant variants in presence of ammonium or cyanide as a nitrogen source. However, both metals at low concentration significantly reduced the growth of sensitive strain[30]. Ten-fold increase of both metals concentration caused slightly increase a lag phase in the case of resistant variant PhCN while was completely inhibited the growth of the sensitive strain PhCN1. However, in the presence of KCN as a nitrogen source, the overall bacterial growth and phenol consumption was less than that observed when the bacteria grew in the presence of ammonium as a nitrogen source. Medium composition affects the degree and pattern of metals inhibition of organic pollutants biodegradation[11].

Table 3: Specific activities of *meta*-cleaving enzyme C23O dioxygenase in a crude extract of parent strain PhCN and its derivatives grown on different substrate. Enzyme was assayed as described in materials and methods

	Substrate (Nitrogen source)							
	In presence of ammonium ions				In presence of cyanide			
Strains	TSM+G	TSM+Ph	TSM+Ph+Cd	TSM+Ph+Cu	TSM+G	TSM+Ph	TSM+Ph+Cd	TSM+Ph+Cu
PhCN (pPhCN1,pPhCN2)	0.01	14.2	12.2	11.4	0.01	12.8	11.6	10.8
PhCN1(pPhCN1)	0.01	14.6	0.4	0.2	0.01	10.8	0.09	0.02
PhCN2 (pPhCN2)	ND	ND	ND	ND	ND	ND	ND	ND
PhCNM(Plasmidless)	ND	ND	ND	ND	ND	ND	ND	ND

Enzyme activity is given in U mg^{-1} of protein. ND: Not Detected; G: Glucose; Ph: Phenol; Cd: Cadmium; Cu: Copper

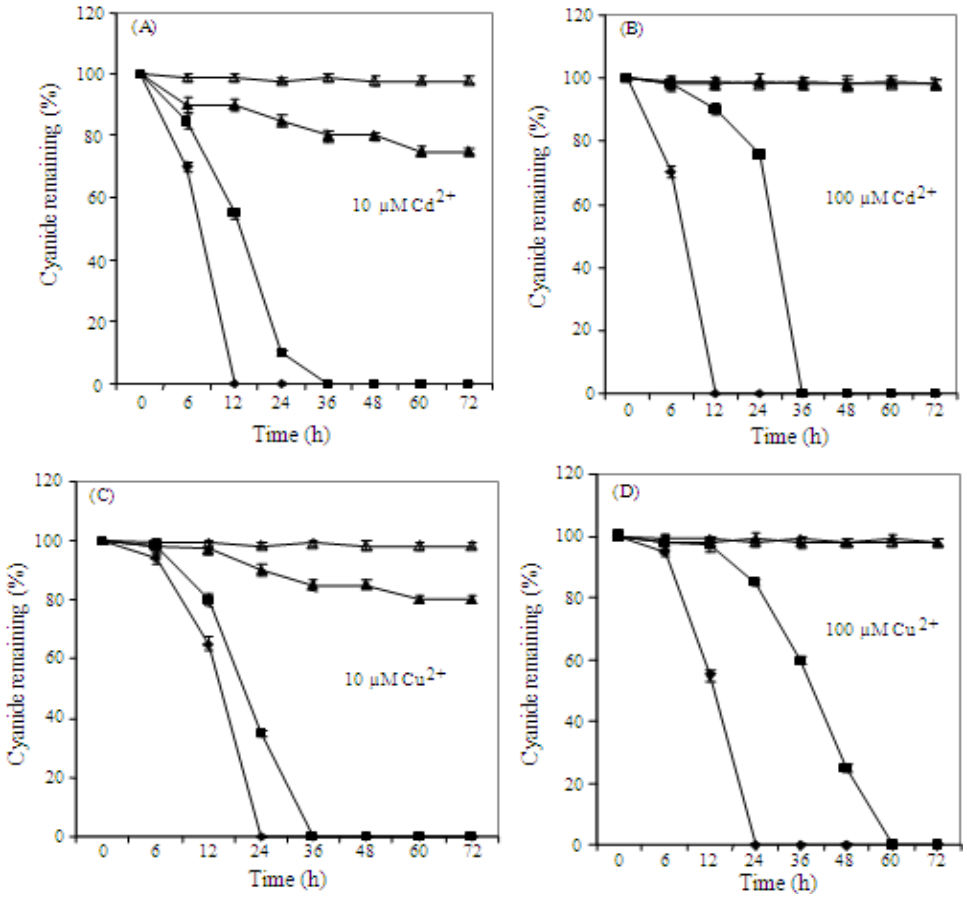

Fig. 4: Effect of copper concentrations on the cyanide utilization by *Pseudomonas putida* PhCN and its derivatives. Strains symbols: (♦): PhCN without metals (control); (■): PhCN; (▲): PhCN1; (Δ): PhCNM

Effect of metal ions on the phenol degradation enzyme activity: The C23O pathway for catechol degradation during phenol metabolism in *Pseudomonas* sp. PhCN was previously demonstrated[9]. When the activity of C23O enzyme was measured as described in Materials and Methods, no C23O activity was detected in LB medium in the absence of phenol. In the presence of phenol, the activity of C23O was negligible (Table 3).

In the absence of heavy metals, the C23O activity was high when the cells were grown in the presence of phenol and ammonium salt as a sole source of carbon and nitrogen respectively (Table 3). In the presence of KCN as a nitrogen source, the overall enzyme activity was less than that observed in the presence of ammonium as a nitrogen source (Table 3).

The effect of Cd^{2+} or Cu^{2+} on the 2,3-dioxygenase enzyme activity in the MSM containing phenol and

ammonium as a sole source of carbon and nitrogen respectively, was investigated. The results in Table 3 showed that both the metals had severely inhibitory effect on the 2,3-dioxygenase activity in the sensitive strain while they had slight effect on the enzyme activity in resistant strain PhCN[22,35]. The effect of Cd^{2+} or Cu^{2+} was also found to exert a strong inhibitory effect on the enzyme activity in the presence of cyanide as a nitrogen source (Table 3). This could be attributed to enzymes produced into the extracellular environment often face higher concentration of metals, since they are not protected by cell-associated metal-detoxification mechanisms[35].

CONCLUSION

Multifunctional strain of *Pseudomonas putida* strain PhCN that bear plasmids of resistance to bivalent cations, pPhCN2 (Cd^r, Cu^r) and catabolic plasmids of biodegradation of phenol pPhCN1 (Ph^+) are effectively degraded the above pollutants in the presence of metals as compared with sensitive strain. The effect of Cd^{2+} or Cu^{2+} was also found to exert a strong inhibitory effect on the C23O dioxygenase enzyme activity in the presence of cyanide as a nitrogen source. However, the presence of heavy metal resistance plasmid alleviated the inhibitory effect of metals on the enzyme activity in resistant strain. These findings demonstrate the possibility of combining the plasmids of phenol catabolism, plasmids of resistance to heavy metals and systems of chromosomal gene encoded for cyanide assimilation. This approach can be used for the creation of polyfunctional bacterial strains effective for bioremediation of soil polluted by phenol in combination with cyanide and heavy metals.

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