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Effect of Various Factors on Cadmium Tolerance of *Cupriavidus* sp. HMT 35

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Abstract: ***Heavy metal pollution poses a serious threat to environment disturbing the natural flora and fauna of the nearby area. These contaminated sites can provide efficient pool of microbial diversity to be used for bioremediation of contaminated sites. The present study was conducted to isolate a potential cadmium tolerant strain. Cupriavidussp. HMT 35 strain was isolated from heavy metal contaminated soil collected from Zawar, Udaipur, India. Cupriavidussp. HMT 35 was found cadmium tolerant and showed fairly higher MIC value (1500µg/ml). Cupriavidussp. HMT 35 showed maximum cadmium tolerance in nutrient broth adjusted to pH 7.0 at 37° C after an incubation period of 48h. The isolate has a potential to tolerate high concentration of cadmium. It can be further explored for its possible use in remediation and restoration of contaminated land.***

Keywords: *Cupriavidus*sp., Cadmium tolerance, bioremediation, heavy metal tolerance

1. Introduction

The mining activity has produced a huge amount of metal-rich wastes, caused serious environmental pollution and soil degradation in the world [1, 2]. Excessive concentrations of heavy metals especially cadmium acts as a significant toxic factor to biota in the environment [3]. Cadmium may decrease metabolic activity and diversity as well as affect the qualitative and quantitative structure of microbial communities [4]. Chemical methods for removal of cadmium are quite expensive [5]. Bacterial pool can be an option for effective remediation of cadmium contaminated soil. Several workers have isolated heavy metal tolerant bacteria from various contaminated sites [6, 7]. The common heavy metal tolerant bacteria as reported in previous studies were found to be member of Enterobacteriaceae, Pseudomonadaceae and Flavobacteriacea family including *Pseudomonas* spp., *Alcaligenes* spp., *Enterobacter* spp. etc. [8, 9]. The possible reasons for the high metal tolerance ability of bacteria surviving in metal-contaminated environments is attributed to some detoxifying mechanisms developed by these strains such as accumulation and adsorption ion exchange on the cell surface, binding and complexation by exopolysaccharides, bioaccumulation and sequestration in cell compartments or heavy metal efflux [10-12].

The present study was designed to study the effect of various factors on cadmium tolerance of *Cupriavidus*sp. HMT 35 isolated from contaminated soil collected from Zawar mining site, Udaipur.

2. Materials and Methods

Bacterial strain and maintenance

*Cupriavidus*sp. HMT 35 strain was isolated from heavy metal contaminated soil collected from Zawar, Udaipur, India. Isolate was identified using 16S rRNA sequencing. The strain was maintained on nutrient agar and activated by growing in nutrient agar at 37° C for 24h.

Determination of minimal inhibitory concentration

The bacterium was streaked on nutrient agar supplemented with increasing concentrations of cadmium chloride ranging from 100µg/ml to 1500 µg/ml with a difference of 100µg/ml. The petri plates were inoculated with the test culture and incubated at 37°C for 48 h. The colonies were observed after 48 h of incubation.

Effect of various factors on levels of bacterial tolerance to cadmium

The effect of various factors such as temperature, pH, duration and organic matter was studied on levels of bacterial tolerance to cadmium chloride.

Effect of temperature: The nutrient broth containing cadmium chloride was inoculated (1% inoculum) with the bacterial culture. The incubation was done at three different temperatures (10ºC, 37ºC, 45ºC) on a rotary shaker at 175rev/min for 48h. The growth was recorded in terms of optical density at 600nm.

Effect of pH: The bacterial culture was inoculated (1% inoculum) in nutrient broth with different pH values (4.0, 7.0, and 9.0) and supplemented with varying concentrations of cadmium chloride. The incubation was carried out on a rotary shaker at 175rev/min at appropriate temperature for 48h. The growth was recorded in terms of optical density at 600nm.

Effect of duration: The bacterial culture was inoculated (1% inoculum) in nutrient broth with appropriate pH and supplemented with varying concentrations of cadmium chloride. The incubation was carried out on a rotary shaker at 175rev/min and appropriate temperature for 24h, 48h and 72h. The growth was recorded in terms of optical density at 600nm.

Effect oftween 80 supplementation: The bacterial culture was inoculated (1% inoculum) in nutrient broth with appropriate pH and supplemented with varying concentrations of cadmium chloride. In addition, varying concentrations (0.01, 0.1, 1mg/ml) of tween 80 was also

supplemented to nutrient broth. The incubation was carried out on a rotary shaker at 175rev/min and appropriate temperature and duration. The growth was recorded as O. D. at 600nm.

All the experiments were done in triplicates and statistical analysis was done using ANOVA.

3. Results

The bacterium was streaked on nutrient agar supplemented with increasing concentrations of cadmium chloride ranging from 100µg/ml to 2000 µg/ml with a difference of 100µg/ml. The petri plates were inoculated with the test culture and incubated at 37°C for 48 h. The well-defined colonies were observed after 48 h of incubation. *Cupriavidus*sp. HMT 35 showed fairly higher MIC value (1500µg/ml).

The effect of various factors such as temperature, pH, duration and organic matter on the cadmium tolerance level of *Cupriavidus*sp. HMT 35was studied.

Effect of temperature: *Cupriavidus*sp. HMT 35 was inoculated in nutrient broth supplemented with varying concentrations of cadmium chloride (0, 8×10^2, 16×10^2 and 24×10^2 µg/ml) and incubated at different temperatures (10ºC, 37ºC, 45ºC) to determine an optimum temperature at which highest metal tolerance can be seen. The incubation was carried out on a rotary shaker at 175rev/min for 48h. The growth was recorded in terms of optical density at 600nm.

Analysis of variance (ANOVA) revealed that when the cadmium concentration was applied at different temperature the difference between concentration, between temperature and their interaction was significant. The effect of temperature on cadmium tolerance (growth) level *Cupriavidus*sp. HMT 35 is presented in Fig.1. The isolate was able to grow well at 37ºC but exhibit very little growth at 10ºC and 45ºC. The growth of isolate was gradually decreased with an increase in concentration of cadmium chloride ($CdCl_2$) for all the three temperatures (10ºC, 37ºC, 45ºC). No growth was observed in nutrient broth supplemented with 24×10^2 µg/ml of $CdCl_2$ even when incubated up to 96 h (which is indicated by the almost constant O. D. values) The isolate showed better tolerance (growth) at 37ºC for all the concentrations of cadmium chloride as compared to other incubation temperatures. Therefore, 37ºC temperature was found to be the best at which maximum tolerance (maximum growth) to cadmium was observed by *Cupriavidus*sp. HMT 35.

Effect of pH: The cadmium tolerant *Cupriavidus*sp. HMT 35 was inoculated in nutrient broth with different pH values (4.0, 7.0, 9.0) and supplemented with varying concentrations of cadmium chloride (0, 8×10^2, 16×10^2 and 24×10^2 µg/ml). The incubation was carried out on a rotary shaker at 175rev/min at 37ºC for 48h. The growth was recorded in terms of optical density at 600nm.

Analysis of variance (ANOVA) revealed that when the cadmium concentration was applied at different pH the difference between concentration, between pH and their interaction was significant. The data obtained are presented in Fig.2. It is clear from figure that the isolate was able to grow well in nutrient broth with pH 7 and showed less growth in nutrient broth adjusted to pH 4 and pH 9. The growth of isolate was gradually decreased with an increase in concentration of cadmium chloride ($CdCl_2$) for all the three pH values (pH 4, pH 7, pH 9). No growth was observed in nutrient broth supplemented with 24×10^2 µg/ml of $CdCl_2$ even when incubated up to 72 h.

*Cupriavidus*sp. HMT 35 showed maximum cadmium tolerance (maximum growth) in nutrient broth adjusted to pH 7.0.

Effect of duration: The cadmium tolerant *Cupriavidus*sp. HMT 35 was inoculated in nutrient broth with pH 7 and supplemented with varying concentrations of cadmium chloride (0, 8×10^2, 16×10^2 and 24×10^2 µg/ml). The incubation was carried out on a rotary shaker at 175rev/min and 37ºC for 24h, 48h and72h. The growth was recorded as O. D. at 600nm. Analysis of variance (ANOVA) revealed that when the cadmium concentration was applied at different duration the difference between cadmium concentration, between duration and their interaction was significant. The data obtained for the effect of duration on cadmium tolerance (growth) level of *Cupriavidus*sp. HMT 35 are presented in Fig.3. It is clear from figure that the tolerance (growth) of the isolate increased up to 48h of incubation period for all concentrations of cadmium chloride. After that the growth was gradually decreased up to 72 h of incubation period for all the concentrations of cadmium chloride. No growth of the isolate in broth supplemented with 24×10^2µg/ml of $CdCl_2$ even when incubated up to 72h which is indicated by the almost constant O. D. values. Hence, the duration of 48h is considered as the best duration for the growth of this isolate at all the concentration of cadmium chloride. *Cupriavidus*sp. HMT 35 showed maximum tolerance to cadmium (maximum growth) when incubated for 48h of duration.

Effect of tween 80 supplementation: The cadmium tolerant *Cupriavidus*sp. HMT 35 was inoculated in nutrient broth with pH 7 and supplemented with varying concentrations of cadmium chloride (0, 8×10^2, 16×10^2 and 24×10^2 µg/ml). In addition, varying concentrations (0.01, 0.1, 1mg/ml) of tween 80 was supplemented to this nutrient broth. The incubation was carried out on a rotary shaker at 175rev/min and 37ºC for 48h. The growth was recorded as O. D. at 600nm.

Analysis of variance (ANOVA) revealed that when varying concentration of cadmium was applied at different concentrations of tween 80, the difference between concentration of cadmium was significant. The data obtained to study the effect of varying concentrations of tween 80 on cadmium tolerance (growth) ability of *Cupriavidus*sp. HMT 35 is presented in Fig.4. It is clear from figure that there was an increase in the tolerance (growth) of the isolate as compared to control when different concentration of tween 80 (0.01, 0.1, 1mg/ml) were added to nutrient broth supplemented with varying concentrations of cadmium chloride. The fairly high tolerance (maximum growth) of the

isolate was observed on addition of 0.1mg/ml of tween 80 as compared to the other concentrations of tween 80 used in the study. No growth was observed for the isolate in nutrient broth supplemented with 24×10^{2}µg/ml of $CdCl_2$ even after addition of tween 80 which is indicated by the almost constant O. D. values.

Hence it was found that 0.1mg/ml of tween 80 increases the cadmium tolerance (growth) ability of *Cupriavidus*sp. HMT 35.

4. Discussion

Microorganisms tolerant to stress due to heavy metals can bioremediate soils with metal contamination [13]. We have identified a bacterial strain that can hold high levels of tolerance to cadmium. *Cupriavidus*sp. HMT 35 exhibited MIC of 1500µg/ml against cadmium chloride. Many workers previously studied MIC of *Cupriavidus*and reported it variably. Zhang *et al., [*14] reported it to be 7mM for cadmium by *Cupriavidusnecator*YR2. These results are in accordance to our results in the present study. The isolate *Cupriavidus*sp. HMT 35 showed remarkable tolerance against $CdCl_2$ at 37ºC in nutrient broth with pH 7 on addition of 0.1% tween 80 when incubated for 48h. The effect of various factors such as temperature, pH, incubation period, presence of certain organic compounds etc. on heavy metal tolerance capability of bacteria is strain specific. Shamim and Rehman [15] studied the effect of temperature and pH on growth of cadmium tolerant *Klebsiellapneumoniae* strain CBL isolated from waste water collected from industrial areas of Shekhupura (Pakistan) on LB agar supplemented with 100µg of Cd^{+2}. They found that 30ºC temperature, pH 7 and 12h of incubation period were best conditions for maximum growth of the strain. Doge*net al* [16] studied the effect of organic acids (citric acid, glucoronic acid, alginic acid) on growth and exopolymeric (EPS) substance production. They reported that all organic acids increased the growth and EPS production.

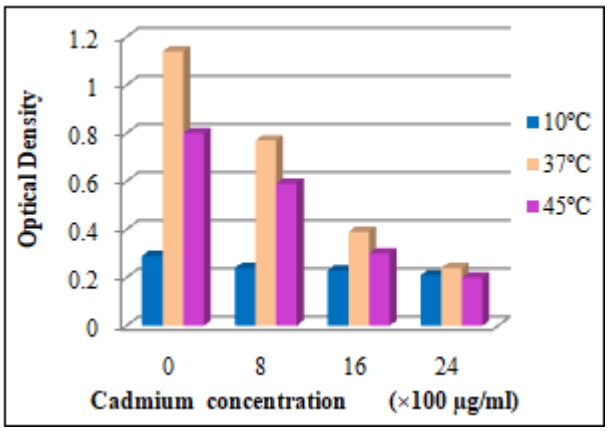

Figure 1: Effect of temperature on cadmium tolerance of *Cupriavidus*sp. HMT 35

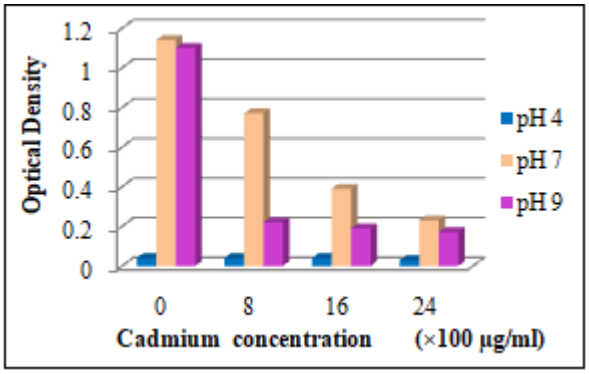

Figure 2: Effect of pH on cadmium tolerance of *Cupriavidus*sp. HMT 35

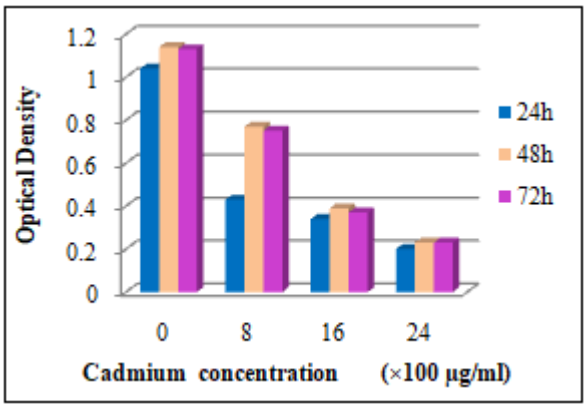

Figure 3: Effect of duration on cadmium tolerance of *Cupriavidus*sp. HMT 35

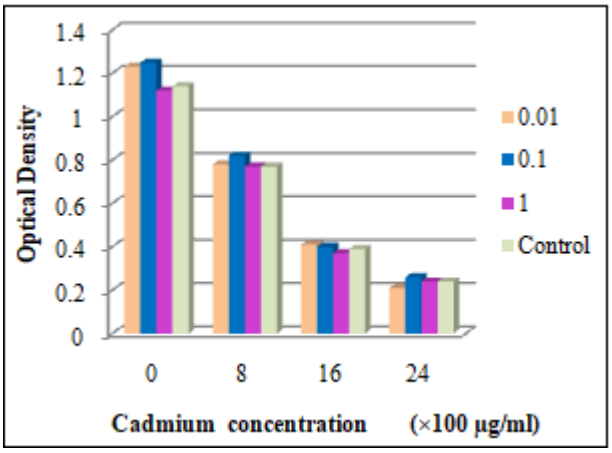

Figure 4: Effect of between 80 supplementation on cadmium tolerance of *Cupriavidus*sp. HMT35

5. Conclusion

*Cupriavidus*sp. HMT 35 is identified as a good cadmium tolerant strain. Cd detoxification will depend on the uniqueness of the bacterial strains, the biological and chemical properties of Cd formation, as well as the bacterial growth conditions. Optimization studies on isolate for effective cadmium tolerance revealed its potential to tolerate cadmium in normal environmental conditions. Further research can be carried out to use this strain in the bioremediation of contaminated agricultural soils.

6. Acknowledgement

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