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Screening and Isolation of Metallic Nanoparticle Synthesizing Bacteria from Industrial Effluent

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ABSTRACT

The primary sources of heavy metal pollution today are a variety of operations in the metal processing and electroplating industries. Inexpensive microbes can be a good substitute for heavy metal subtraction from metal-contaminated sites. The present study was focused on the isolation and screening of metal-tolerant bacterial strains from the electroplating effluent. A total of eight bacterial colonies were isolated from electroplating effluent. Out of eight isolated colonies: only two were more cadmium tolerant than other isolated strains based on their growth. The two (Cd-7 and Cd-13) selected strains were optimized at various environmental conditions like pH (4 to10), temperatures (27 to 47°C) and different metal concentrations (20-300 mg/l). The ideal pH for both strains' growth was found to be eight, the ideal temperature was found to be 37°C and the ideal metal dose was found to be 200 ppm. The biosynthesis of Cd nanoparticles was confirmed by the change in colour of media treated with metal solution. This resulted from a noticeable decrease in cadmium salts. When metal solution was added, the solution's colour changed to a light brown for the control, and the bacteria's growth peaked after 72 of stirring. The maximum absorbance on a UV-VIS spectrophotometer at 380 nm also gave surety of the presence of cadmium nanoparticles. The screened, heavy metal-resistant microbes could be effective and valuable for treating industrial waste water.

Key words: Cadmium nitrate, nanoparticles, heavy metals, bacterial strains

INTRODUCTION

Synthesis of metal nanoparticle solvents, capping agents, toxic chemicals and risky controlled conditions is required in conventional methods, which also increases environmental pollution and cost. The biogenesis of nanoparticles by using micro-organisms is a good alternative to overcome pollution and the cost (Chugh *et al.,* 2021). In recent times, the population has increased hastily, and industrial establishments demand has also increased for the fulfilment of human needs by creating problems like over-exploitation of available resources and increased pollution (land, air and water). A substantial environmental problem is heavy metal pollution of soil and waste water (Ajibade *et al.,* 2020). Industrial waste water and sewage sludge affect human and the environment due to permanent toxicity (Zhang *et al.,* 2021). In the environment, there are different types of heavy metal sources for instance: agricultural, natural and atmospheric sources in industrial or domestic waste water. Different events like excavating, electroplating, metallurgical, casting operations and farming ensured polluted wide spread areas of world; such as Japan, Indonesia and China generally by heavy metals for instance Cd, Pb, Cu and Zn (Ahirwar, 2016; Borah *et al.,* 2020). In the industrial environment, among all the heavy metals, Cd is toxic through the ingestion and inhalation. It can cause acute and chronic dope by diffusion and persist in the environment for many years. By the magnification, ultimately Cd accumulates in the body via food chain. In tobacco Cd is also present, smoking further contributive to human exposure. It has long half-life in the human body exceptionally due to its salient toxicological property. When it is once absorbed, it irretrievably gathers in the human body, mainly in kidneys and other main organs like lungs as well as liver. Due to its astonishing amassed assets, it is highly lethal metal which can disem body (the

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various biological systems), usually at the normal doses that are much lower than most toxic metals (Zhang *et al.,* 2020). Straight techniques function to sort out the problem of industrial water loaded with heavy metals and polluted soil by chemical (precipitation, neutralization) or physical (ion exchange, membrane separation, and electro dialysis and activated carbon adsorption) methods (Rajasulochana and Preethy, 2016). Further, these practices are expensive and not ecofriendly. So, there is a requirement for inventive treatment expertise for the deletion of heavy metal ions from soil, water bodies and waste water. Diverse microbes have been planned as efficient and inexpensive alternative in removal of heavy metals from soil and water (Medfu Tarekegn *et al.,* 2020). A managed spontaneous, bacteriological progression to reduce, break down or renovate pollutants to less toxic or harmless forms, by improving, eliminating and rejecting pollutants from environment is called bioremediation (Kumar *et al.,* 2018). The microbes have the abilities to reduce, decontaminate and accrued the detrimental inorganic and organic compounds by using chemical contaminants as an energy source (Ahirwar *et al.* 2016; Gu, 2020). So, the nanotechnology is expected to revolutionize both science and society. Nanotechnology includes interfering work at very small scale to provide useful materials and devices. By using this technology, the significant material helps to solve major problem in easy way. Nanotechnology is observed to significantly affect the economy, science and day to day life in 21st century and it may become one of the life lines to the next industrial generation (Kushwaha *et al.,* 2015; Rajasulochna and Preethy, 2016). The fortitude of heavy metal conflict of bacteria and growth studies is the objective of this study to exploit these isolates for crack down of industrial affected soil and waste water (Ahirwar, 2016). The biosynthesis of metallic nanoparticles is a more significant research branch in nano-technological activities. In the several biological activities, the microbes play an important role directly or indirectly. The nanoparticles which are biologically synthesized have wide range of application viz., biolabelling biosensors, in treatment of cancer and in appliances of medical coating and in heavy metals bioremediation (Kushwaha *et al.*, 2015; Kumari *et al.,* 2020).

MATERIALS AND METHODS

The industrial effluent samples were collected from electroplating industry of Rohtak (LPS) and Gurugram (JBM). By using sterilized screw cap canes, the waste water samples were collected and transported immediately to the laboratory in an ice-cubes kit and the soil samples were collected near the outlet of the industries and collected in the jip-lock plastic polybags. The temperature of the samples of waste water was preserved at about 4°C to avoid contamination and to stay samples for more longer time (Hayat *et al.,* 2015; Agunbiade and Moodley, 2016; Punjabi *et al.,* 2017).

The parameters viz. pH, TDS, colour, COD and BOD of the samples of waste water were carried out as per the method following Mustapha and Halimoon (2015) and the values were compared with effluent standard recommended by WHO, the maximum permissible limit for industrial effluent (WHO, 2017; Table 1).

Table 1. Physico-chemical characterization of electroplating industrial effluents

Parameters	Permissible* limit	JBM, Gurugram	LPS, Rohtak
pH	9	6.23	3.5
Colour	Colourless	Yellowish green	Dark green
TDS	1000	1498	13632
COD	300	682	680
BOD	30	332	334
Cd	0.003	18.14	16.80

*All the units in mg/l except pH, Indian standard IS :10500.

By using distilled water and labolene, all glasswares were thoroughly washed and rinsed. After wards, the glass ware was allowed to dry in a hot air oven for 3 h at 60°C for sterilization. The deionized water was used in serial dilutions, then autoclaved for 15 min at 121°C. With the help of 75% alcohol, the workplace was cleaned prior to and after every experiment. All the experiments were conducted in duplicate.

The bacteria were isolated on nutrient agar amended with 20 mg/l of Cd nitrate. The isolated bacteria were heavy metal tolerant. The nutrient agar was sterilized in an autoclave at 121°C for 15 min and allowed to cool to 40-45°C. Then in Laminar air flow, the metals were affixed in the sterilized nutrient agar and poured into petri-plates and waited for solidification. 0.1 ml of the serially diluted waste water was spread on agar plates for incubation at 37°C for 48-72 h in incubator. Different colonies appearing in different morphology were selected for further sub-culturing (Nagarajan *et al.,* 2015; Punjabi *et al.,* 2017; Bhateria and Dhaka, 2019). The streak plate method was used for sub-culturing of microbes (Fig. 1).

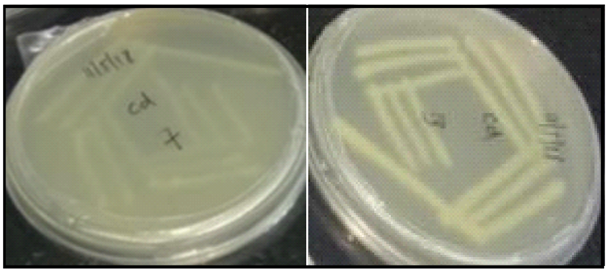

Fig. 1. Screened metal tolerating strains.

RESULTS AND DISCUSSION

By using standard methods, the common parameters were characterized. The results of physico-chemical analysis of electroplating effluents of Jai Bharat Maruti Ltd. (JBM) Gurugram and Lakshmi Precision Screws Ltd. (LPS) Rohtak, Haryana, India. The pH was observed 6.23 and 3.5 in the wastewater samples from JBM, Gurugram, and LPS, Rohtak, respectively (Table 1). The LPS, Rohtak sample indicated acidic nature due to low pH. If the pH of samples becomes highly acidic or more basic, the number of microbes also reduces slowly. Most of the microbes cannot tolerate the levels of pH more than 9.5 and lower than 4.0 (Peng *et al.,* 2020; Qu *et al.,* 2020; Verma and Balomajumder, 2020; Gayathri *et al.,* 2021; Aragaw *et al.,* 2022). Generally, for good bacterial growth, the optimum pH is between 6.5 and 7.5. The most important parameter of industrial effluents, like BOD and COD, was similarly measured. Both parameters behaved as an indicator of pollution. The measurement of biodegradable pollutants was done by BOD test, while the oxidizable pollutants by the COD test. Generally, the COD is measured 2 to 3 times more than the BOD of waste water (Santos and Boaventura, 2015). The COD value was moderately high i.e. 682 and 680 mg/l in the waste water samples of JBM, Gurugram, and, LPS, Rohtak, respectively, due to the presence of a higher quantity of dissolved solids in the effluents. The BOD value was found moderate i.e. 332 and 334 mg/l in the waste water samples of JBM, Gurugram, and LPS, Rohtak, respectively, due to the presence of a lower quantity of decomposable material in the waste water. The cadmium ion conc. was found to be 18.14 and 16.80 mg/l in waste water of JBM, Gurugram, and LPS, Rohtak, respectively. Other researchers reported similar results (Moersidik *et al.,* 2020).

The bacterial colonies were grown in huge. For further study, only eight strains were selected, these were Cd tolerant. But in these eight strains, only two strains (Cd-07 and Cd-13) gave the best results (Table 2). The bacterial strains Cd- 07 and Cd-13 showed maximum growth that 380 nm wave length. There were different bacterial colony growth, which had morphological dissimilarities Choosing the best single colony and further streaking the colony in the same media for purification of isolates. Finally, found the pure growth of metal tolerant bacterial strain. The bacterial isolates were investigated for nanoparticle synthesizing potential. The bacterial strains were grown up on Nutrient Agar Media (amended with cadmium nitrate) initially at neutral pH.

On the basis of colour and OD range, two

Table 2. Screening of isolates and selection of wave length for bacterial growth

Wave length (nm) → Strains ↓	370	380	390	400	410	420
Control	0.412	0.459	0.412	0.392	0.389	0.373
3	2.263	2.308	2.208	2.063	2.012	1.964
4	1.722	1.813	1.648	1.559	1.497	1.445
5	1.279	1.342	1.203	1.132	1.070	1.029
7	2.309	2.368	2.239	2.090	2.034	1.976
10	1.533	1.596	1.466	1.372	1.288	1.234
12	1.854	1.947	1.768	1.661	1.599	1.535
13	2.392	3.348	2.294	2.147	2.102	2.052
14	2.188	2.257	2.107	1.974	1.905	1.840

bacterial strains were screened out for Cd nanoparticle synthesis. Screening of isolates and selection of wavelength for bacterial growth for Cd (incubation time 72 h) out of total eight bacterial strains the strain no.07 and 13 were selected on the basis of best growth.

A change in the colour of media treated with $(CdNO_3)_2$ was observed, confirming Cd salts' reduction to their nanoparticles. The colour of the solution was found to be light brown with respect to control after 72 h. of agitation. The OD taken by the UV-VIS spectrophotometer gave maximum absorbance at 380 nm, confirming the presence of Cd nanoparticles. The incubation time for the bacterial strain 07 and 13 w.r.t. control the bacterial growth was observed after 72 h (Fig. 2). So, on the basis of the result of best bacterial growth, the optimum incubation time of 72 h was observed.

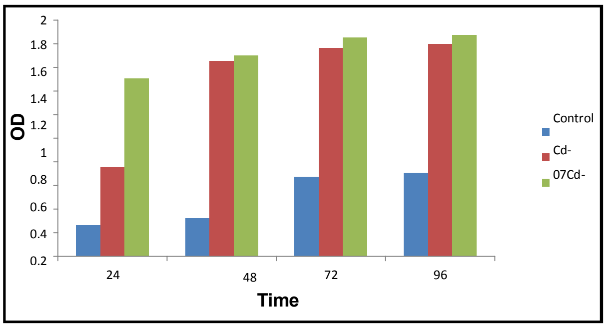

Fig. 2. Incubation time for best bacterial growth.

The microbial concentration becomes slow if the pH of the water was very acidic or alkaline. Generally, for good bacterial growth, pH between 6.5 and 7.5 was the optimum range of pH observed. The observed microbes cannot tolerate pH levels more than 9.5 or lower than 4.0 (Peng *et al.*, 2020; Qu *et al.*, 2020; Verma and Balomajumder, 2020; Gayathri *et al.*, 2021; Aragaw *et al.*, 2022). From the pH range of 4-10, the optimum pH was found to be eight, which showed the maximum growth of bacteria (Fig. 3).

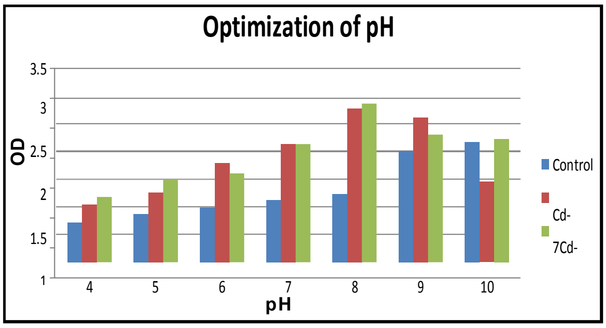

Fig. 3. Bacterial growth at different pH.

The bacterial strains were grown at different temperatures ranging from 27 to 47°C. The bacterial strains showed maximum growth at 37°C after 72 h of incubation time. At temperature 37°C the bacterial growth observed was maximum at the absorbance 380 nm (Fig. 4).

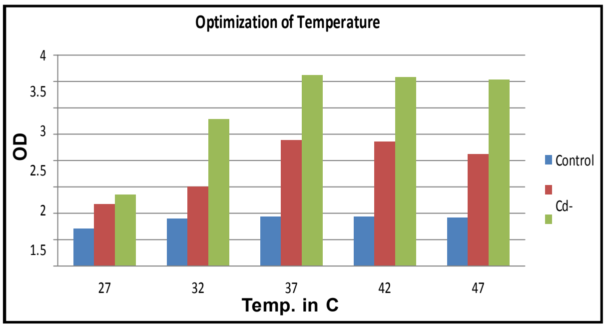

Fig. 4. Bacterial growth at different temperature.

The bacterial growth and the nanoparticles synthesis also depended on metal ion concentration. The metal dose was given from 20 to 300 ppm. But both strains showed maximum growth at 200 ppm dose (Fig. 5).

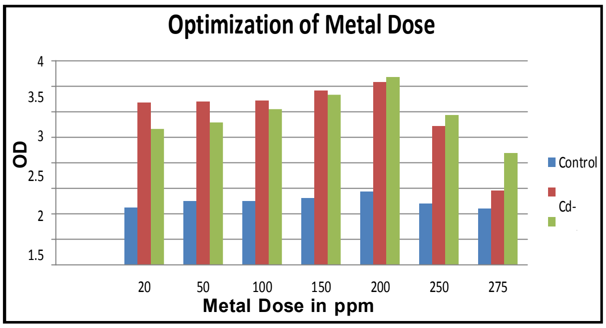

Fig. 5. Bacterial growth at different metal doses.

The biosynthesis of NPs was verified by change in colour or changes in precision for chromatic surveillance in every reaction flask (Fig. 6). Such colour changes were symbolic of variation in the metal ion reduction state resultig in the biologically formation of NPs (Zhang *et al.*, 2016).

The proficiency of bacteria against heavy metals resistance may be a remarkable proof that the industrial wastes are hazardous and toxic which affect human health as well as developed resistance mechanisms for bacterial growth. Therefore, the study was very useful and suggested that the direct possible effect of pollution was lesser as compared to indirect effect of metal contaminated sites in human life. Based on the results obtained the isolates Cd-3, Cd-4, Cd-5, Cd-7, Cd-10, Cd-12, Cd-13 and

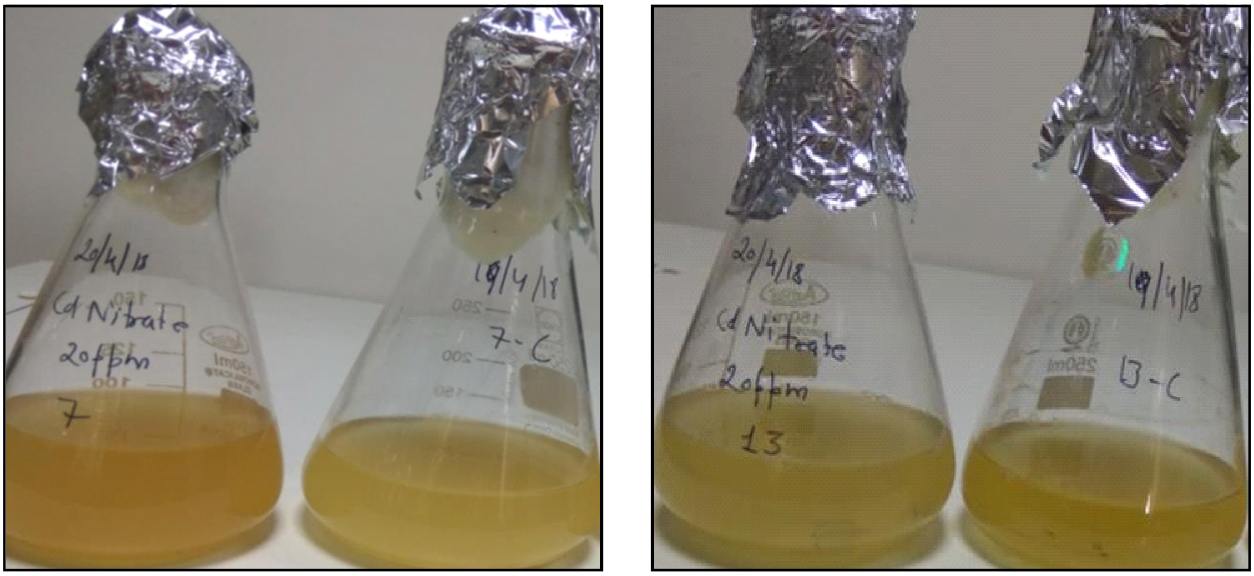

Fig. 6. Visual confirmation of nanoparticle synthesis on the basis of colour difference.

Cd-14 were able to tolerate 20-300 ppm dose of Cd nitrate. But Cd-7 and Cd-13 gave best results at various optimized conditions i.e pH, temperature and metal dose. In this study, the maximum bacterial growth, wavelength and the change in colour confirmed the reduction in metal concentration and synthesis of NPs at optimized conditions. At last, the results of this research article showed that the nanoparticles synthesizing bacterial strains used as a biotechnological, eco-friendly and cost-effective device for the effluent treatment encompassing metals such as cadmium, lead and other heavy metals for bioremediation purpose.

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