Research Paper Environmental Science

Characterization of Cadmium Resistant *Pseudomonas Pseudoalcaligenes* Strain AR-5 Isolated from Industrial Effluents and its Possible use in Bioremediation

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ABSTRACT

In the present study, the cadmium contaminated industrial effluents were selected for the isolation of Pseudomonas sp. Different types of 24 isolates were obtained from effluent treatment plants of industry from Nagpur district of Maharashtra (India). All isolates show high resistance to different heavy metals. On the basis of maximum resistance, 10 isolates were selected for further study. These isolates show resistance from 120 to 300 µg/lit. Pseudomonas pseudoalcaligenes strain AR-5 is shown maximum cadmium resistance as compare to other isolates. These isolates show maximum growth at temperature range from 35°C to 45°C. Thus it can be used for bioremediation at this temperature range.

KEYWORDS Cadmium, Pseudomonas pseudoalcaligenes, industrial effluent, bioremediation.

INTRODUCTION

In India, day by day industrial growth is rapidly increases. It results in increase of quantity of substantial industrial effluents. These effluents discharged into natural water sources or into open land. These effluents may include metals like Copper, Cadmium, Chromium, Nickel, Zinc, Lead, etc. Although, industrial effluents are treated before discharge, but these treated effluents may contain the heavy metals resistant microorganisms. Because, before treatment these organisms achieve resistance as they want to survive in the heavy metals contaminated effluent. It causes number of environmental problems including threat to plants, animals and other living beings. Continuous discharge of industrial effluents can change species diversity due to their accumulative behavior and number of studies shows identical results (Jayaraman, 2003; Kathiresan, 2000; Kenner, 1974; Malami, 2014; Morris, 1998). Most of the industries play an important role in heavy metal pollution in water bodies. On the basis of contamination of metal, the resistance type was selected. Cadmium contaminated effluents were used for the isolation of *Pseudomonas* species Hence this study includes the characterization of cadmium resistant pseudomonas pseudoalcaligenes strain AR-5 as it can be used for bioremediation.

MATERIALS AND METHODS

Collection of Samples:

Different industrial areas were randomly selected for this study. The samples were collected from effluent treatment plants and sewage treatment plants. From these areas the samples were analyzed for heavy metals contamination. (CPCB, 1996)

Heavy Metal Analysis:

Analysis of the cadmium contents in the effluent samples were done by Atomic Absorption Spectrophotometer (AAS). We used the 700B method of EPA (Environmental Protection Agency). The AAS detects the presence of heavy metals. Three trials were run on each effluent sample in every replicate of the heavy metal (NEERI, 1991).

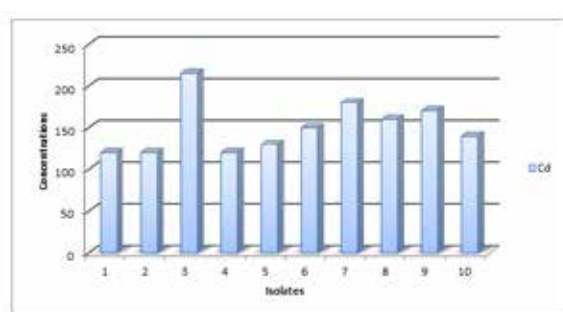
Screening of bacteria for metal resistance:-

Samples were poured on Luria Bertani (LB) agar plates with supplements of different concentration of cadmium. All the plates incubated at 37°C for 24 - 96 hrs. Isolated colonies were selected and sub-cultured on the MacConkey agar plates for purification. (Cheesbrough, M. 2000). The isolates are characterized on the basis of morphology and some biochemical characteristics. The pure cultures were identified based on their biochemical activity and by Bergey's manual of determinative bacteriology, Holt, J.G. *et al* (1994)

RESULTS AND DISCUSSION

The water analysis is carried out as per the methods described by APHA, (2005). The maximum cadmium containing effluents gives the isolates of maximum resistance to cadmium. Different isolates of *Pseudomonas* species shows the cadmium resistance. Variations of cadmium resistance to different isolates at different locations were shown graphically in Figure-1. But, isolate – 3 shows maximum resistance confirmed as *Pseudomonas pseudoalcaligenes*. This isolate was confirmed by 16S rRNA gene sequencing and registered in NCBI gene bank as *Pseudomonas pseudoalcaligenes* strain AR-5. The Accession number of strain is KP980627.

Figure-1: Resistance of different isolates at different concentrations of cadmium



Further, effects of temperature on growth rate of *Pseudomonas pseudoalcaligenes* strain AR-5 were studied. It shows maximum growth at 35 °C. Although it found that the optimum temperature range for pseudomonas pseudoalcaligenes strain AR-5 is 30°C to 45°C and this is shown in Figure-2.

Figure-2: Growth rate of *Pseudomonas peudoalcaligenes* strain AR-5 at different temperature in 48 hrs.



CONCLUSIONS:

Pseudomonas peudoalcaligenes strain AR-5 was able to withstand high level of toxicity of metals. Also, this strain can survive at wide range of temperature starting from 30°C to 45°C. These microorganisms were screened for microbial control of mosquito employing their degradative ability at various microbial cell loads (Omoya *et al*, 2009).

Conclusively, the presence of cadmium in water bodies can be referred to the heavy metal concentrations in the effluent. It may be toxic to different living animals, plants, human beings and organisms. It proves to be an essential need of biodegradation for effluents treatment. This species response to 45°C with stress, but can survive at higher temperatures (Bihong *et al*, 2005). Therefore, it is helpful at higher temperature for bioremediation.

The combined expression of metal tolerance and antibiotic resistance is caused by selection resulting from the metals present in the environment (Nakahara *et al*., 1977). This shows that there is a close association between metal resistance and antibiotic resistance. The potential impact of increased antibiotic resistance is due to metal contamination. Timoney (1978) shows heavy metal and antibiotic resistance in bacterial flora. *Pseudomonas pseudoalcaligenes* commonly associated with the skin & upper respiratory tract infections. If these microbes get entered in the atmosphere, their genes get distributed worldwide. Thus, we would like to suggest that the potential impact of metal polluted locations in human life may be much greater. As Trivedy *et al* (1986) describes chemical and biological methods for water pollution studies, regular surveillance of the effluent in industrial sites is a must if the risk of disease due to such antibiotic resistant organisms is to be avoided.

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