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Metal uptake potential of four methylotrophic bacterial strains from coal mine spoil, exploring a new possible agent for bioremediation

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HIGHLIGHTS

- Metal removal potential of four methylotrophic bacteria have been studied.
- Bacteria were identified through biochemical and 16SrRNA gene sequence method.
- The methylotrophic population was less tolerant to the tested metals.
- The bacteria showed similarity with *Pseudomonas*, *Methylophilus*.
- Highest metal uptake for Cd and lowest for Cr.
- Metal uptake potential varied with strain as well as metal species.
- It suggests that methylotrophic bacteria can be used as accumulator of Cd.
- Study suggests that these bacteria can be used for metal removal.

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ABSTRACT

The abundance of total metal tolerant culturable bacteria and methylotrophic bacteria was numerated in the barren mine spoil. Metal resistant methylotrophic bacteria were isolated and identified through biochemical testing and 16SrRNA gene sequence analysis. The metal uptake potential of the isolates was determined. The size of the methylotrophic bacteria was significantly low (10^4) compared to total bacterial population (10^8). The methylotrophic population was less tolerant to the tested metals viz., Cd, Cu, Cr, Ni and Zn. The metal resistant isolates showed close similarity with *Pseudomonas*, *Methylophilus* and *Methylobacterium* sp. Lower concentration (5–10 μM) of al the tested metals was growth stimulatory. MIC values were lowest for Cd and highest for Zn. At the same time, MIC value was highest for *Pseudomonas* strain and lowest for *Methylobacterium*. Highest metal uptake was observed for Cd and lowest for Cr. Metal uptake potential varied with strain as well as metal species. There was no relation between the intracellular metal accumulation and metal adsorbed to the bacterial cell wall. Lowest adsorption capacity was exhibited by the *Pseudomonas* strain. Highest adsorption was for Cu and lowest for Ni. Metal uptake was

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rapid during first 4 h, and then after rate of uptake declined. It suggests that methylotrophic bacteria can be used as potential accumulator of Cd.

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1. Introduction

Heavy metal pollution is one of the most common problems associated with coal mine spoil (Mishra et al., 2008a; Wong, 2003). Several heavy metals such as Cd, Cu, Cr, Ni and Zn have been reported from coal mine spoil (Juwarkar and Jambhulkar, 2008; Mishra et al., 2008b). These heavy metals are toxic, bioaccumulative and persistent in nature hence can affect the flora and fauna of the ecosystem. A number of health effects have been reported to be caused by these heavy metals (Ref). These metals can adversely affect the number, diversity and activity of soil organisms, resulting in to decreased soil organic matter decomposition and N mineralization (Wong, 2003). These heavy metals are associated with a number of direct and indirect consequences if they are retained in soil or water of an ecosystem. Usually treatment of these heavy metals requires their removal through some technologies. Currently adopted technologies used for heavy metal treatment are reverse-osmosis, ion-exchange, electro-dialysis, adsorption etc. Most of these technologies are quite costly, energy intensive and metal specific. However

Nevertheless, a range of metal resistant bacterial groups are found in coalmine spoil. Microbial community in mine spoil is usually represented by several Gram-positive (such as *Bacillus*, *Arthrobacter*, and *Corynebacterium*) as well as Gram-negative bacteria (such as *Pseudomonas*, *Alcaligenes*, *Ralstonia*, and *Burkholderia*) (Wuertz and Mergeay, 1997; Kozdrój and van Elsas, 2001; Ellis et al., 2003). Besides, there are reports indicating the presence of another specialized group of bacteria called methylotrophs (utilizing C_1 compounds as carbon and energy source) in coal mine spoil (Piotrowska-Seget et al., 2005). The ability of methylotrophs to tolerate a certain degree of desiccation and scavenge trace amounts of nutrients explains their presence in such harsh environment (Piotrowska-Seget et al., 2005; Pasamba et al., 2007). They play an indispensable role in the ecosystem functioning by actively participating in various biogeochemical cycles. There are few studies indicating the metal tolerance nature of methylotrophic bacteria. De Marco et al. (2004) isolated number of *Methylobacterium* and *Methylophilus* strains highly resistant to As, Cd, Cr, Hg and Pb. Many endophytic *Methylobacterium* species resistant to Ni were isolated from Ni accumulating *Thalpsi goesingense* (Idris et al., 2006). Madhaiyan et al. (2007) isolated *Methylobacterium oryzae* from rice field, which reduces nickel and cadmium toxicity and promotes growth of tomato (*Lycopersicon esculentum* L.). Metal resistance in these groups of organisms may be result of either increased influx of metal accompanied with transformation or efficient efflux system to remove the toxic concentration of metal out from cellular interior. Methylotrophs isolated from such environment can be used as an alternative to chemical and physical methods of metal removal. The robust growth of methylotrophs on the cheap nitrogen source (methanol obtained from fermentation) and their ability to grow in range of physical and environmental conditions make them potential metal bioaccumulator. The response of methylotrophic strains towards meat toxicity and their metal uptake potential is vital for their application in remediation of metal polluted sites. However, studies involving their metal uptake potential have rarely been performed. We have investigated the effect of metal tolerance on methylotrophic population size compared to total bacterial population. Further, the four methylotrophic strains were isolated from the mine spoil and their growth kinetics was studied towards the Cd, Cu, Cr, Ni and Zn. Besides, an attempt was made to study the metal uptake potential of the isolated strains as affected by the contact time and metal concentration.

2. Material and method

2.1. Study site and soil analyses

Samples from surface coalmine spoils (0–10 cm) were collected from Bina coalmine area (24°11' N, 82°38' E), India during November 2005. Dump site was barren and two years old. Proportions of sand, silt and clay were determined by employing sieves of different mesh sizes. Bulk density of soil was determined by measuring the weight of dry soil of a unit volume. Water-holding capacity (WHC) was determined using perforated circular brass boxes as described by Piper. Soil moisture was estimated by drying the soil samples at 105 °C for 48 h. Soil pH was measured by employing a digital pH meter (Control Dynamics, India). Soil organic C and total N were analyzed by dichromate and macrokjeldahl method, respectively (Walkley, 1947; Jackson, 1958). Ammonium-N (NH_4-N) and nitrate-N (NO_3-N) were estimated by the method of Koroleff (1983) and Jackson (1958), respectively. Na^+, K^+ and Ca^{+2} were extracted from soil in ammonium acetate solution following repeated leaching procedure. The solution was filtered through Whatman No. 42 filter paper and the solution was diluted to 50 ml with distilled water and metal concentrations were determined in the filtrate with an atomic absorption spectrophotometer (Perkin-Elmer model 2130, USA) fitted with a specific lamp for a particular metal using appropriate drift blanks.

For the analyses of metal in soil, 0.5 g of dried samples was digested with 15 ml of HNO_3, H_2SO_4, and $HClO_4$ in 5:1:1 ratio at 80 °C till the mixture became transparent (Allen et al., 1986). Metal concentrations were analyzed as described earlier. Quality control measures were adopted to assess the reliability of data. Readings from blank and drift standards (Sisco Research Laboratories Pvt. Ltd., India) were taken after each five samples to calibrate the instrument. Precision and accuracy of analysis were also ensured through repeated analysis of samples against National Institute of Standards and Technology standard reference material (SRM 1570) for all the tested heavy metals.

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