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Analysis of copper tolerant rhizobacteria from the industrial belt of Gujarat, western India for plant growth promotion in metal polluted agriculture soils

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ABSTRACT

Agricultural sites irrigated for long term with water polluted by industrial effluents containing heavy metals might adversely affect the soil microbial communities and crop yield. Hence it is important to study rhizobacterial communities and their metal tolerance in such affected agricultural fields to restore soil fertility and ecosystem. Present work deals with the study of rhizobacterial communities from plants grown in copper (Cu) contaminated agricultural fields along the industrial zone of Gujarat, India and are compared with communities from a Cu mine site. Microbial communities from rhizosphere soil samples varied in the magnitude of their Cu tolerance index indicating differences in long term pollution effects. Culture dependent denaturing gradient gel electrophoresis (CD-DGGE) of bacterial communities revealed the diverse composition at the sampling sites and a reduced total diversity due to Cu toxicity. Analysis of 16S rRNA gene diversity of Cu tolerant rhizobacteria revealed the predominance of *Enterobacter* spp. and *Pseudomonas* spp. under Cu stress conditions. Cu tolerant bacterial isolates that were able to promote growth of mung bean plants *in vitro* under Cu stress were obtained from these samples. Cu tolerant rhizobacterium P36 identified as *Enterobacter* sp. exhibited multiple plant growth promoting traits and significantly alleviated Cu toxicity to mung bean plants by reducing the accumulation of Cu in plant roots and promoted the plant growth in $CuSO_4$ amended soils.

1. Introduction

Application of sewage sludge, copper (Cu) based fungicides, pesticides and usage of water polluted by industrial effluents act as sources of long term Cu contamination in agricultural soils, thus affecting the agroecosystem. Cu is one of the essential metals required by biota, but above threshold concentrations it is toxic to all life forms. Cu is considered as a pollutant and it tends to accumulate in the environment making remediation process difficult. Metal accumulation in soils can bring about long term changes in soil characteristics and microbiota. In soils, Cu has adverse impacts on microbial biomass (Frostegard et al., 1996), N-mineralisation, C-mineralisation (Dumestre et al., 1999); and brings about shifts in the microbial community structure, (Smit et al., 1997; Tom-Petersen et al., 2003; Ranjard et al., 2006; Brandt et al., 2010; Sharaff and Archana, 2015), extinction or reduction of certain plant beneficial bacteria (Brandt et al., 2006; Laguerre et al., 2006) and negatively affects symbiotic relationship by rhizobia with their host plant (Sharaff and Archana, 2016), thus affecting the agroecosystems (Giller et al., 1998). At elevated levels of Cu, plants do not exhibit visible symptoms of toxicity; however Cu tends to accumulate in edible parts of plants and in consequence, on consumption can pose a health risk to humans and animals (Xiong and Wang, 2005). Notably Cu has been reported to accumulate in certain important crop plants like cabbage (*Campestris*) (Xiong and Wang, 2005), rice (*Oryza sativa*) (Xu et al., 2006), cow pea (*Vigna unguiculata*) (Kopittke and Menzies, 2006) and wheat (*Triticum* spp.) (Guan et al., 2011).

Plant growth promoting (PGP) rhizobacteria are the key players inhabiting the rhizosphere and are directly or indirectly involved in plant growth promotion via secretion of certain regulatory chemicals in the rhizosphere (Lugtenberg and Kamilova, 2009). In metal contaminated agricultural soils, an effective method has been advocated for reducing metal toxicity to plants by colonizing them with metal resistant PGP bacteria (Zhuang et al., 2007; Khan et al., 2009). Metal resistant rhizospheric microbes often facilitate the process of phytoremediation in metal hyper accumulator plants by increasing availability of the metal ions as well as by promoting plant growth (Wenzel, 2009). However very few studies have focused on alleviation of metal toxicity by introducing metal tolerant plant growth promoting bacteria that reduce metal accumulation in plants (Burd et al., 2000; Vivas et al., 2006; Madhaiyan et al., 2007). This would be ideal for reducing the entry of metal ions in the food chain in case of crop or fodder plants growing in metal polluted soils. Hence the assessment of metal toxicity

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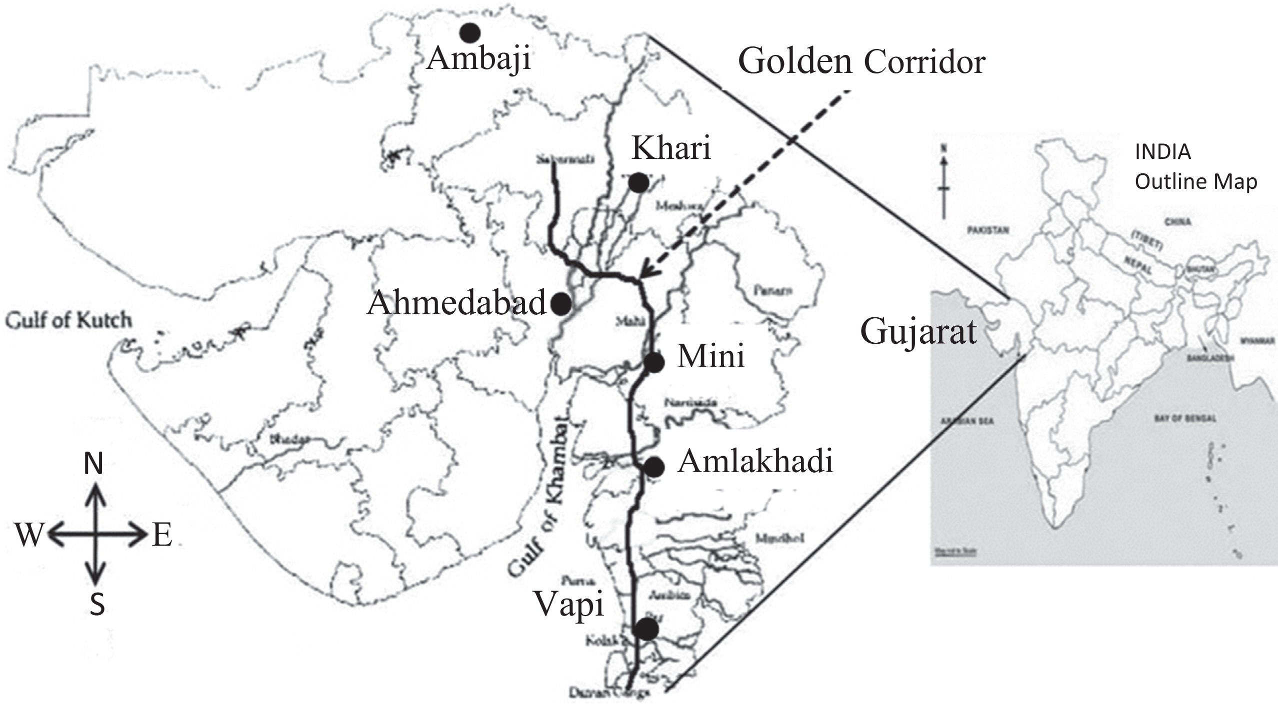

Fig. 1. Location of sampling sites in the Gujarat state, western India. Sites are indicated as Amlakhadi (Ankaleshwar, Bharuch district), Mini (Vadodara district), Khari (Ahmedabad district) and Ambaji (Cu mining area, Banaskantha district). The thick line running along the sites indicates the industrial belt of Gujarat, known as the Golden corridor spanning regions from Vapi to Ahmedabad.

on rhizobacteria in metal polluted agricultural soils is of importance for evaluating the toxicity levels and screening potential metal resistant PGP rhizobacteria. This strategy can indeed pave a new direction for crop protection that reduces the risk of consumption of metal contaminated food crops for human and animal health.

In western India, Gujarat state covers nearly 80% of major and medium scale industries and about 65% of small scale factories located in the stretch extending from Vapi to Ahmedabad known as the "Golden corridor" (Fig. 1). This industrial belt spans across the basins of Sabarmati, Mahi, Narmada, Tapi and Damanganga rivers representing 78.1% of the available surface water resources of the state (Labunska et al., 1999). In the "Golden corridor" Cu is one of the major pollutants in the effluents released by many industries (Jose and Hasmukh, 2001). As a result several agricultural sites are affected by polluted waters at Amlakhadi (Ankaleshwar), Mini (Vadodara), and Khari (Ahmedabad) which are tributaries to the main rivers. An understanding of the rhizosphere microbial communities of such polluted agricultural sites may lead to a comprehensive knowledge that can aid in modification of rhizospheric processes or introduction of certain native metal resistant PGP bacteria as a means of crop protection. With this view, the current study was focused on the effect of Cu pollution on rhizobacterial communities in agricultural sites along the industrial belt of Gujarat and characterization of native Cu tolerant rhizobacteria with multiple PGP traits. Additionally, the effect of Cu tolerant rhizobacteria on growth of agriculturally important crop plant *Vigna radiata,* belonging to cowpea miscellany group, under Cu supplemented conditions was assessed.

2. Materials and methods

2.1. Description of study sites and collection of soil samples

Among the four study sites, three sites included agricultural fields with a long term history of usage of polluted water for irrigation located at Ahmedabad, Vadodara, Ankaleshwar. The fourth site is Ambaji near a Cu mining area (Fig. 1, Table S1). The study sites were named after the river /common effluent treatment canal which ran alongside the fields. The disposed effluents are expected to have varied content of organic and inorganic compounds from industrial units dealing with dyes, plastics, pharmaceuticals, pesticides, petrochemicals that are located around the sampled fields. From all the locations both bulk soils as well as rhizosphere soils (from the particular plants grown during the season) were collected. Soil sample Khari was collected from a field grown with *Sorghum vulgare* plants near the Kharicut canal (N22°56′, E72°32′) in the province of Ahmedabad city. Kharicut canal receives the treated industrial effluents from Naroda, Vatva and Odhav industrial estates and are eventually disposed in the river Sabarmati. Soil sample Mini was collected from a field with *Vigna radiata* plants near the Mini river (N22°20′, E73°3.5′) in the province of Vadodara. Mini receives effluents from Nandesari industrial estate lodging small scale industries of chemicals, dyes, plastics, pharmaceuticals and pesticides. Amlakhadi soil sample was collected from a field with *Saccharum officinarum* located near the Amlakhadi river (N21°38′, E72°53′) in the province of Ankaleshwar. Amlakhadi receives the industrial effluents from Ankaleshwar, Panoli and Jagadia industrial estates that constitute the major petro chemical, dye and paint industries and are eventually disposed in the river Narmada. The fourth sample, Ambaji was obtained from an area adjacent to the Cu mine (N24°20′, E72°51′) at Ambaji located in Banaskantha district, north of the Golden corridor. Here rhizosphere samples were collected from unidentified mountain plants growing in the mining area.

Three to four soil samples (rhizosphere and bulk) were randomly collected from each study site. After carefully uprooting 4–5 plants randomly from the field plots, soil adhering to roots was collected as rhizospheric soil samples, while bulk soil was collected from adjoining soil that was not directly under the influence of plant roots. Finally the soil samples were pooled according to the field site into two sets. One set of soil samples were stored at 4 °C for further analysis and another set was used to analyze physico-chemical properties at Soil testing laboratory, Gujarat State Fertilizer Corporation (GSFC), Vadodara. Bulk soil was used to estimate bioavailable Cu by Diethylene-triamine penta-acetic acid (DTPA) method and total Cu was determined as described by