



Detailed genomic and biochemical characterization and plant growth promoting properties of an arsenic-tolerant isolate of *Bacillus pacificus* from contaminated groundwater of West Bengal, India

Ashutosh Kabiraj^a, Urmi Halder^a, Anindya Sundar Panja^b, Annapurna Chitikineni^{c,d},
Rajeev K. Varshney^{c,d}, Rajib Bandopadhyay^{a,*}

^a Department of Botany, The University of Burdwan, Bardhaman, West Bengal, 713104, India

^b Department of Biotechnology, Molecular Informatics Laboratory, Oriental Institute of Science and Technology, Vidyasagar University, Midnapore, West Bengal, 721102, India

^c Center of Excellence in Genomics and Systems Biology, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India

^d State Agricultural Biotechnology Centre, Centre for Crop and Food Innovation, Murdoch University, Murdoch, 6500, Australia

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ABSTRACT

In this study, arsenic tolerating bacteria *Bacillus pacificus* (AKS1a) was isolated from arsenic contaminated groundwater of Purbasthali, Purba Bardhaman, West Bengal, India and its bioremediation potential was preliminary screened. This multimetal resistant strain was able to grow against more than 20 mM arsenate and 10 mM arsenite salts. The genome was more than 5.16 Mb in length, with an average of around 35.2% GC content, bearing 5403 protein coding genes. Arsenic resistant genes like *arsC*, *arsB*, *arsR*, etc. were also identified. Rapid Annotation using Subsystem Technology (RAST) identified 328 subsystems within the genome. Presence of six Genomic Islands (GIs) and five phage virus genomic parts indicated its ecological adaptations to overcome environmental stresses. The production of about 415 $\mu\text{g mL}^{-1}$ indole acetic acid (IAA), 258.0 $\mu\text{g mL}^{-1}$ gibberellic acid (GA), and 183 $\mu\text{g mL}^{-1}$ proline by the bacterium, along with nitrogen fixation ability under *in-vitro* conditions, indicate its plant growth promoting potential. This was further confirmed through rice seedling growth enhancement under arsenic stress. Beside arsenite oxidation to arsenate, its arsenic adsorption property was confirmed through X-ray Fluorescence spectroscopy (XRF), Fourier Transform Infrared spectroscopy (FTIR), and Energy Dispersive X-ray spectroscopic (EDS) analysis. Genomic comparisons among 25 different strains of *B. pacificus* showed that there are tremendous genetic differences in respect to their accessory genome content. In future, this strain can be applied as biofertilizer or biostimulant for improving rice plant growth.

1. Introduction

Arsenic and its detrimental impacts on different organisms are well established today (Sher and Rehman, 2019). Arsenic, a metalloid, is ubiquitously distributed in environment and its presence markedly dependent on biotic and abiotic factors like nature of soil, microbial population, pH, water content, etc. Background concentration of arsenic ranges from 0.1 to 40 mg kg⁻¹ in soil and <0.5 to

* Corresponding author.

E-mail address: rajibindia@gmail.com (R. Bandopadhyay).



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journal homepage: www.elsevier.com/locate/envresCopper removal capability and genomic insight into the lifestyle of copper mine inhabiting *Micrococcus yunnanensis* GKSM13Krishnendu Majhi^{a,b}, Moitri Let^a, Urmi Halder^a, Annapurna Chitikineni^{c,d},
Rajeev K. Varshney^{c,d}, Rajib Bandopadhyay^{a,*}^a Microbiology Section, Department of Botany, The University of Burdwan, Burdwan, West Bengal, 713104, India^b Department of Botany, Ananda Chandra College, Jalpaiguri, 735101, India^c Center of Excellence in Genomics and Systems Biology, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India^d State Agricultural Biotechnology Centre, Centre for Crop and Food Innovation, Murdoch University, Murdoch, 6500, Australia

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ABSTRACT

Heavy metal pollution in mining areas is a serious environmental concern. The exploration of mine-inhabiting microbes, especially bacteria may use as an effective alternative for the remediation of mining hazards. A highly copper-tolerant strain GKSM13 was isolated from the soil of the Singhbhum copper mining area and characterized for significant copper (Cu) removal potential and tolerance to other heavy metals. The punctate, yellow-colored, coccoid strain GKSM13 was able to tolerate 500 mg L⁻¹ Cu²⁺. Whole-genome sequencing identified strain GKSM13 as *Micrococcus yunnanensis*, which has a 2.44 Mb genome with 2176 protein-coding genes. The presence of putative Cu homeostasis genes and other heavy metal transporters/response regulators or transcription factors may responsible for multi-metal resistance. The maximum Cu²⁺ removal of 89.2% was achieved at a pH of 7.5, a temperature of 35.5 °C, and an initial Cu²⁺ ion concentration of 31.5 mg L⁻¹. Alteration of the cell surface, deposition of Cu²⁺ in the bacterial cell, and the involvement of hydroxyl, carboxyl amide, and amine groups in Cu²⁺ removal were observed using microscopic and spectroscopic analysis. This study is the first to reveal a molecular-based approach for the multi-metal tolerance and copper homeostasis mechanism of *M. yunnanensis* GKSM13.

1. Introduction

The term “heavy metal” refers to the group of metallic elements and metalloids with high molecular weight and atomic density greater than 5 g cm⁻³ (Dhaliwal et al., 2020). The non-degradable and persistent metals such as copper (Cu), cadmium (Cd), chromium (Cr), lead (Pb), arsenic (As), nickel (Ni), cobalt (Co), zinc (Zn), and mercury (Hg) (Kumar et al., 2021) exacerbate the negative impacts on the ecosystem. The bioaccumulation and biomagnification of these heavy metals in ecosystems pose an acute threat to the entire food chain (Durube et al., 2007; Das et al., 2022). These pollutants are cytotoxic in nature, create gastrointestinal complications, short time memory loss, mental retardation, etc. even at low concentrations (Priyadarshane and Das, 2021). In addition, adverse effects on human are generally dose-dependent and metal specific. For example, Pb causes oxidative damage by forming reactive oxygen species (ROS), Cr (VI) and Cd effect on cellular integrity, As and Hg form toxic derivatives of methyl and thiol groups, and Fe

cause lipid peroxidation (Balali-Mood et al., 2021; Priyadarshane and Das, 2021).

Among the heavy metals, copper (Cu) is considered an essential micronutrient and acts as a cofactor for multiple enzymes and proteins involved in photosynthesis, oxidation, nitrogen fixation, and other cellular metabolisms (Rehman et al., 2019). According to the World Health Organization (WHO), the permissible limit of Cu in drinking water is 2 mg L⁻¹ (Chan et al., 2022). However, several natural and anthropogenic activities such as soil erosion, leaching, mining, smelting, automobile exhaust, coal combustion, municipal compost, fertilizers, pesticides, and fungicides are the major contributors for the excessive Cu inputs into the environment (Kumar et al., 2021; Saha et al., 2022). This may cause vegetation loss, soil nutrient depletion, and human health risks (Shabbir et al., 2020). In humans, Cu toxicity generates free radicals within the cell and causes nausea, headache, vomiting, diarrhoea, respiratory infections, liver and kidney failure (Rathi and Yogalakshmi, 2021; Shabbir et al., 2020). Nevertheless, the excessive Cu

* Corresponding author.

E-mail address: rajibindia@gmail.com (R. Bandopadhyay).<https://doi.org/10.1016/j.envres.2023.115431>

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