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Arsenic toxicity amelioration in rice soils by plant beneficial microbes

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ABSTRACT

Over 50 million people are affected by groundwater arsenic (As) contamination beyond the prescribed safe limit of 10 µg/L across various regions in the Ganga-Brahmaputra basin of India. Among all, south-eastern Asiatic countries especially India and Bangladesh are most severely affected. Rice is the staple food for millions of people of this region and among many other crops, rice grains are champion in accumulating very high As. Irrigation of As-contaminated groundwater for rice cultivation has resulted in high deposition of As in topsoil and uptake in rice grain posing a serious threat to the sustainable agriculture. In addition, cooking and parboiling with As-contaminated water increases the As burden through dietary intake along with processed food items. Studies have shown that various factors like As solubility, bioavailability, microbial colonization, and uptake in the rice rhizosphere decides the fate of As transportation in rice. In this context, the use of plant growth promoting (PGP) microbial community members having both plant beneficial properties (increased production of phytohormone, enzymes, Siderophore, N_2 fixation, P solubilization, disease resistance, mineral solubilization, etc.) and As amelioration or detoxification activities (biosorption, accumulation, volatilization, enhanced adsorption) can be a suitable alternative for sustainable rice farming in As-hotspots.

Key words: *Arsenic, groundwater, rice, microbial communities, plant growth promotion*

INTRODUCTION

Natural arsenic (As) contamination of groundwater in the Asian and South-East Asiatic countries used for drinking and irrigation has posed severe threat to health of millions of people relying on groundwater as primary resource (Fendorf et al., 2010). Various geochemical factors are found to be involved in mobilization of solid phase sedimentary As (As sorbed on Fe-oxy-hydroxide, Al/Mn oxides, silicates, aluminosilicates, etc.) into the groundwater of Bengal plain (particularly West Bengal, India and Bangladesh) (Paul et al., 2015). The As-laden groundwater has been used extensively for irrigation in the intensive rice cropping systems of this region, which has resulted in increased concentration of As in top soil and bioaccumulation of toxic As species (As^{3+}, As^{5+} as inorganic and methylated species as organic) in the rice grain as well as other plant parts. Rice is the staple crop across the world and major daily diet of the population of Southern and South-Eastern Asiatic countries mainly India, Bangladesh and China. Several studies have unanimously agreed upon the fact that in addition to the As-contaminated drinking water, consumption of rice is another potential source of dietary As intake. It is not only the consumption of rice grains but also food products based on rice, which contributes toward additional As exposure to individuals residing in the As-hotspot areas (Rahman and Hasigawa, 2011). Several investigators have examined the positive correlation among As in the irrigation water, and its transport to soil, and rice in the Indo-Gangetic plains of India and a direct positive correlation between As concentration in soil and rice crop is noticed. Although a nominal level of As (4 to 8 µg/g) is found to be present in rice soil, irrigation of As-contaminated water increase the concentration upto 83 µg/g (Williams et al., 2006). Researchers have shown that groundwater used for dry season irrigation in Bangladesh contains As in the range of 76-436 µg/L and in countries residing along the Ganges-Brahmaputra Meghna (GBM) plain, the concentration exceeds to 4000 µg/L (Rahman et al., 2006). Compared to other cereals, rice crop accumulate higher amount of inorganic As species. Mehrag et al. (2009) proposed a modelled cancer risk of As from rice in India, Bangladesh, China, by taking daily intake of inorganic arsenic in rice and a risk factor into account. It has been estimated that for fixed consumption of 100 g rice/day by a man of 60 kg weight, the median cancer rate was 22 per 10,000 people (highest in Bangladesh), followed by 15 per 10,000 (in China), and 7 per 10,000 (in India). It was speculated that cancer due to intake of As-rich rice was about 100-200 times higher than the WHO standard (1 per 100,000 people) for all the South-Eastern Asiatic countries. Thus, As-rich rice would be a potential health risk for the millions of inhabitants of As-affected Indian states, most particularly West Bengal, Bihar, Uttar Pradesh and Assam. A number of studies from West Bengal, India and other states have reported high As concentrations in rice. Arsenic concentration in rice in the range of 0.09-0.66 mg kg^{-1}, and 0.04-0.61 mg kg^{-1}, 0.08-0.55 mg kg^{-1} from various blocks of Murshidabad district of West Bengal have been reported by Roychoudhury et al. (2003). As concentrations between 0.02 and 0.40 mg/kg in rice from Kolkata, West Bengal was reported. On an overall basis, a total 0.01-0.70 µg/g As has been reported in rice grains of different parts of India. As concentration within 0.02-0.36 mg kg^{-1} in rice grains have been reported for Bengal delta region (Chatterjee et al., 2010). Recently, high levels of As (0.06-0.78 mg kg^{-1}) in rice of Bengal has been reported, posing a serious health issue to the millions of people in the Bengal plain. Among all species, As^{3+} is found to be more toxic than As^{5+}, followed by organic forms: $As^{3+} > As^{5+} >$ MMA > DMA. On an average, the inorganic As in rice grain is around 50 % of total As, and within range of 10-90 %, while rest fraction is methylated forms (DMA, MMA). Inorganic As species are found to be taken up via phosphate and silica (silicic acid) transport pathways, whereas uptake of organic As species is still unknown. Under flooded paddy soil condition, rice is noted to be a champion in taking up and translocation of As (mostly As^{3+}) into the xylem sap after it gets reduced in the root zone (Su et al., 2010). This uptake is mediated mostly by nodulin 26-like intrinsic proteins (NIPs), Si-influx transporter (OsLsi1) and Si-efflux transporter (OsLsi 2) (Ma et al., 2008). Besides, membrane intrinsic proteins (PIP), members of plant aquaporins, and mostly phosphate uptake system are found to be the major routes for As (V) transport into the rice plant (Meharg, 2004) because of ionic competition between phosphate and arsenate oxyanions. It has been estimated that among all parts, As accumulation in root is higher upto 75 times than grain and 28 times than shoot (Rahman et al., 2007) and the reason is still unidentified. The redox state formed in the rhizosphere and formation of Fe-plaque (as Fe-oxides) on root surface have profound effect on As bioavailability to rice plant. In conventional flooded rice condition, changes in Eh (redox potential), pH, and mineral chemistry influence the As speciation, mobility, and bioavailability in the root-rhizosphere regions. The predominant soil Fe-oxy-hydroxide minerals *viz*., hematite, ferrihydrite, goethite with large specific surface area facilitates the adsorbtion and/or co-precipitation of As^{5+} and As^{3+} (Pan et al., 2014) and anoxic environment prevailing in flooded condition enhances the reductive dissolution of these minerals releasing more As^{3+} in soil-water system, thus increases its availability (Khanam et al., 2020 and references therein) Rhizodeposition, incorporation of stubbles and roots increases the dissolved organic carbon (DOC) in lowland rice fields which promotes the formation of organo-As complex (Williams et al., 2011). Some reports have also highlighted the role of Fe-Mn oxides, phosphates, organic matter, soil texture, irrigation practices, land use pattern, etc. as additional factors for aggravating such processes (Sahoo and Kim, 2013). Under aerobic conditions (aerated soil), As^{3+} oxidize to As^{5+} that sorb onto Fe-oxy-hydroxide (Smedly and Kinniburgh, 2002), hence remain unavailable. Recent geomicrobiological studies have proved the crucial role of diverse rhizospheric microbial communities with As solubilizing or immobilizing potential with metal biotransformation abilities, hence altering the chemical speciation and bioavailability of As to the plants (Khanam et al., 2020). In this context, plant growth promoting (PGP) microorganisms through diverse array of plant beneficial mechanisms (N_2 fixation, P solubilization, mineral dissolution, disease resistance, siderophore and hormone production, improved soil quality, etc.) might be a suitable alternative to ameliorate As toxicity in arseniferous soil and rice crop, ultimately reducing the bioavailability of As to the plants.Hence, plant growth promoting microorganism with their abilities to ameliorate As toxicity in soil and rice as a suitable and sustainable technology of rice farming in As-affected Bengal plain is presented in this article.

The tripartite interaction: soil, plant-rhizosphere, and microbial communities

Soil offers a dynamic and complex microenvironment for activities of wide array of microorganisms because of which microbial diversity in soil is much greater than that found in other environments. The microbial diversity and activities is directly related to the soil types, vegetation cover, and climatic factors. The plant root surface (rhizoplane) and the region associated with it (rhizosphere) are known to be important sites for microbial growth. It has been found that the microbial diversity and metabolism in this rhizosphere region is much higher and has agricultural attention due to the beneficial role of these microbes for higher agricultural productivity and sustainability (Kumar et al., 2017). The microbes found in the rhizosphere (soil-plant root interface) are known as plant root colonizing microorganisms. Diverse types of plant and microbe interactions occur in this zone (commensalism, mutualism and parasitism, wholly called symbiosis), where the microbe and the plant establish the capacity to communicate with each other. Large numbers of active metabolites of both high and low molecular weight are found to be secreted from the plant root to the vicinity of the root-soil zone (termed as root exudates, Table 1), which act as signal molecules (either as carbon/nitrogen/electron sources for microbial nutrition ultimately leading to increased microbial biomass and activity (Nadeem et al., 2014). The whole process involves tripartite -way signalling cross talk between soil, plant root and microbes that employ molecular lexicons. Microorganisms colonizing plant root and other parts can be broadly divided into two classes: an epiphyte that lives on the surface of plants and endophyte that colonize internal plant tissues. Researchers have suggested that plants-microbe interaction takes place at three different layers *viz*., endosphere, phyllosphere, and rhizosphere. Among all, the most active engagement of microorganisms occur in rhizosphere and found to be involved in contributing beneficial traits to the plant as well as soil, hence termed as Plant Growth Promoting Rhizobacteria (PGPR). The plant's overall health status depends on the root-colonizing and endophytic beneficial microbial composition.

Table 1. Various actively excreted compounds by rice root for microbial activities

Class of compounds	Compounds
Volatile	Acetoin, CO_2, Ethanol, Ethylene, Iso-amyl alcohol, Isobutanol, Isobutyric acid
LMWCs	Amino acids, Nucleotides, Organic acids, Sugars, Vitamins
HMWCs	Enzymes, Polysaccharides

Source: Prescott and Klein's (2007)

LMWCs: Low molecular weight compounds; HMWCs: High molecular weight compounds.

Microbial candidates of rice plant growth promotion

There are several microorganisms that colonize the root of the plant and they include algae, protozoa, bacteria and fungi but bacteria are the most abundant among them and found to be 1011 to 1012 per gram of the soil. They have been classified according to their impact on promoting yield, plant growth and the way they interact with roots. Except some plant pathogenic members, many bacterial members are known for their beneficial properties. In the Eubacterial domain, it ranges from low G+C mol % gram-stain-positive *Bacillales* (*Bacillus*, *Clostridium*), high G+C mol % gram-stain-positive *Actinobacteria* (*Arthrobacter, Micromonospora, Streptomyces, Streptosporangium*, and *Thermobifida*), moderate G+C mol % gram-stain-negative *Proteobacteria* (*Pseudomonas, Acinetobacter, Aeromonas, Alcaligenes, Azospirillum, Azotobacter, Burkholderia, Enterobacter, Gluconacetobacter, Klebsiella, Serratia*, etc.) are considered as most important PGPR members because of their beneficial effect on several cereal crops by enhancing soil fertility (Gupta et al., 2015). In addition, other effective PGPR strains include rhizobial species *viz*., *Azorhizobium, Allorhizobium, Mesorhizobium, Bradyrhizobium, Sinorhizobium* and *Rhizobium*. The major beneficial traits achieved through these microbes are enhancement of seedling growth, promotion of shoot and root growth, tiller numbers, dry/fresh weight, nutrient accumulation, increased biological N_2 fixation rate, dry matter accumulation in grain, phytohormone synthesis, etc. Among the several food crops of international demand, cultivation of rice is most preferred as it is the staple food in several developing countries including countries of South and South-Eastern Asia and Africa. The use of chemical fertilizer is the most important input required for rice cultivation because of its high demand of N, P, and K nutrient based fertilizers. For instance, in India and Bangladesh, about 55-70 % of the total cropped land and 70-82 % of the irrigated land are used for rice cultivation. The high demand of these chemical fertilizers, ultimately leads to health hazards and environmental pollution. In order to make rice cultivation organic and sustainable without the use of chemical fertilizers, implementation of plant beneficial microbes (PGPR) is the need of the hour. Various candidate PGPR bacteria have been used by several investigators indicating the major beneficial traits achieved related to rice growth and is presented in Table 2.

Plant growth promotion and As toxicity amelioration: Microbial candidates

The mechanisms of plant growth differ between microbial species or strains and their metabolic versatility towards specific host plants or niche partitioning. So, typically not a single mechanism is accountable for promoting plant growth. PGPR enhance plant growth by direct and indirect mechanisms, or a combination of both. The detailed mechanism of plant growth is presented in Fig. 1. Among most beneficial traits, biological nitrogen fixation, phosphate solubilization, phytohormone production, disease suppression and induction of systemic resistance,

Table 2. Use of PGPR organisms for enhancing rice productivity.

PGPR used	Beneficial traits on Rice
Bacillus subtilis Ljb-4, *Lysinibacillus xylanilyticus* GDLY1, *Alcaligenes faecalis* B17, *Bradyrhizobium japonicum* HHB-02, *Rhizobium etli* bv. *mimosae* Mim-1	Enhancement of seedling growth, promotion of root growth, tiller numbers, plant dry weight, nutrient accumulation and biological N_2 fixation rate.
Pseudomonas aeruginosa BHUJY16, *P. aeruginosa* BHUJY20 *P. fluorescens* BHUJY21, *P. putida* BHUJY23	More effective and boost-up growth attributes, yield and nutrient uptake
Azospirillum BYJ3	Improved rice growth and productivity
Azospirillum brasilense CW903, *Burkholderia pyrrocinia* CBPB-HOD, *Methylobacterium oryzae* CBMB20	Increased shoot and root length, nutrient uptake, increase in germination rates, plant height, dry matter production in rice seedlings.
Pseudomonas sp.	Increased IAA levels
Azospirillum sp.	Increased grain yield and N content
Bacillus sp., *Paenibacillus* sp.	Significant increase in the root and shoot growth
Azospirillum lipoferum	Increased root length, root surface area and root volume
Pseudomonas species	Higher ability of resistance to bacterial and fungal root pathogens
Azospirillum amazonense	Grain dry matter accumulation (7-11.6%), the number of panicles (3-18.6%) and N accumulation in grain (3.5-18.5%)
Burkholderia vietnamensis	Increased grain yield
Herbaspirillum sp. B501	Increased dry and fresh weight, Increased N fixation and phytohormone synthesis
Azospirillum sp., *Pseudomonas* spp.	Increased shoot biomass and grain yield, Increased N fixation and phytohormone synthesis
Azospirillum brasilense A3, *Bacillus circulans* P2, *Bacillus magaterium* P5, *Bacillus* sp. Psd7 *Streptomyces anthocysnicus*, *Pseudomonas aeruginosa* Psd5, *Pseudomonas pieketti* Psd6, *Pseudomonas fluorescens* MTCC103	Increased rice grain yield
Herbaspirillum seropedicae Z67	Higher plant colonization, increased Nitrogenase activity, plant dry weight with higher N content
Azospirillum sp., *Aeromonas veronii*, *Enterobacter cloacae*	Increase in root area, plant biomass and N fixation.
Rhizobium leguminosarum	Promotion of root and shoot growth, seedling vigor and grain yield
Burkholderia sp., *Herbaspirillum* sp.	Increase in fresh weight of rice plant, net increase of N content in grains
Rhizobium leguminosarum	Higher seedling growth and grain content
Burkholderia vietnamiensis Azoarcus	Increased shoot and root weight and leaf surface, increased N fixation and phytohormone production

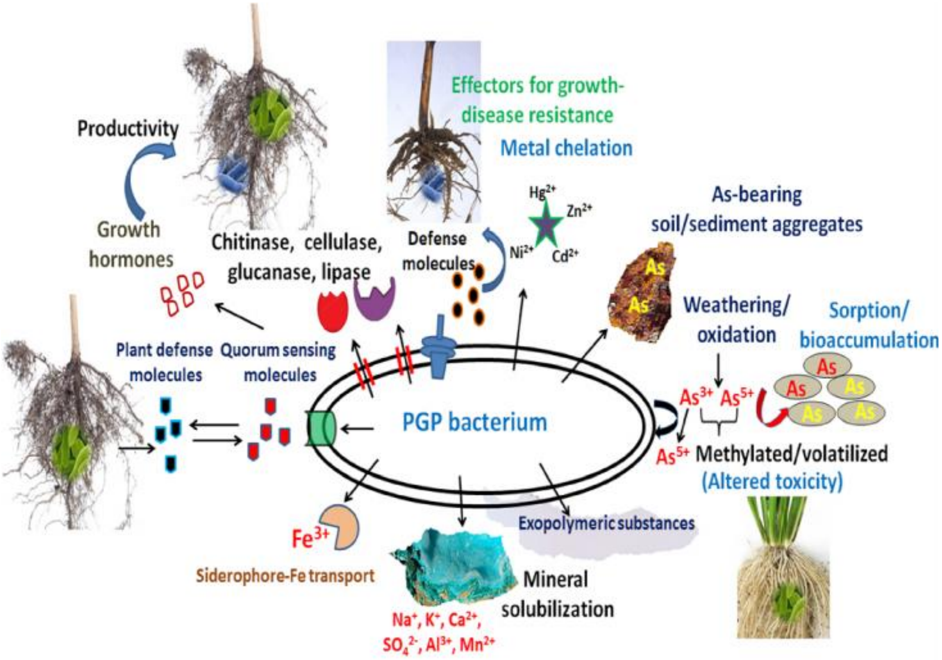

Fig. 1. Mechanisms of plant growth promotion and As toxicity amelioration by plant beneficial bacteria.

production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), etc. are the foremost, where direct benefit is provided to the plant. Besides, indirect mechanisms of plant growth promotion include siderophore production, quorum sensing (QS), signal interference, inhibition of pathogenic biofilm formation, exhibition of antimicrobial activity, production of volatile organic compounds (VOCs), minimization of toxic effects of abiotic and biotic stress including heavy metals, production of extracellular enzymes, polysaccharides, nutrient bioavailability through mineral dissolution, soil amelioration through amendment of soil organic matter and soil aggregation (Bhardwaj et al., 2014).

With respect to widespread As contamination of groundwater and its use for irrigation, the soil and the rice crop of the As hot-spot area of Bengal plain are found to be under severe As toxicity. Under such situation, use of PGPR for amelioration of As toxicity in soil and rice root rhizosphere is a suitable alternative for reducing As uptake and accumulation in rice crop. Though As is redox-active and its mobility and toxicity vary greatly with respect to environmental factors, As^{3+} (arsenite) and As^{5+} (arsenate) are most toxic forms. Arsenic transformation in soil involves oxidation, reduction, and methylation. Microbe (bacteria) mediated As transformations are found to be the crucial mechanism affecting As biogeochemical cycling. Microbial As biotransformation includes oxidation of toxic As^{3+} to less toxic As^{5+}, sequential conversion of As^{3+} and As^{5+} into their arsines (methyl derivatives) and its volatilization, sorption of either of the species onto the cell surface, biomass or exopolysaccharides (EPS), and bioaccumulation. Among all, oxidation of As3+ under aerobic conditions and volatilization of inorganic species have found to be most preferred in agricultural point of view (Chen et al., 2017). Bacterial oxidation of As^{3+} was first reported in 1918 in cattle-dipping tanks and to date many As^{3+}-oxidizing bacterial species have been isolated from various habitats and characterized. They broadly belong to chemolithotrophic As^{3+}-oxidizing, where As^{3+} acts as electron donor (energy generation) and heterotrophic As^{3+}- oxidizing as a detoxification mechanism. A number of bacterial members *viz*., *Achromobacter, Alcaligenes, Arthrobacter, Thiomonas, Pseudomonas, Rhizobium, Leptothrix, Acinetobacter, Acetobacter, Ochrobactrum, Arcobacter, Desulfovibro* etc. are known for As^{3+}-oxidation abilities involving either Aio/Aox proteins in combination with Ars (arsCB) system (Sar et al., 2017).

Table 3. Utilization of PGPR strains for amelioration of As toxicity in various crops.

PGPR organisms	Associated host plant	Beneficial traits	Mode of action	Reference
Chlorella vulgaris Nannochloropsis sp.	*Oryza sativa*	50 % lower accumulation of As in the roots and shoots	Reduced oxidative stress, As toxicity	Upadhyay et al.,
Bacillus flexus	*Oryza sativa*	Increased grain yield (35-55 %)	Siderophore production, IAA, ACC-deaminase, and phosphate solubilization	Das et al., 2016
Brevundimonas diminuta	*Orya sativa*	Reduced As^{5+} accumulationin aerial parts especially in edible parts and enhanced plant growth	Siderophore production, IAA, ACC-deaminase, and phosphatesolubilization	Singh et al., 2016
Ralstonia eutropha,Rhizobium tropici, Exiguobacterium aurantiacum	*Brassica rapa, Raphanus sativus*	Increased biomass and reduced As content (22–50%) of of vegetables	IAA and siderophore production	Zhang et al., 2011
Acinetobacter lwoffii (RJB-2)	*Vigna radiata*	*No accumulation of As in crop*	*Siderophore, IAA production, and phosphate solubilization*	*Das and Sarkar, 2018*
Methylobacterium oryzae	*Acacia farnesiana*	Decreased As uptake without reduction inbiomass and chlorophyll	Production of Auxins,cytokinins, ACC deaminase, increase in GSH concentration, and glutathione-S-transferase	Alcántara-Martínez et al., 2018
Kocuria flava Bacillus vietnamensis	*Oryza sativa flava*	Significant reduction in As^{3+} uptake and increment in rice seedling growth	Reduced bioavailability of As	Mallick et al., 2018
Pseudomonas putida and *Chlorella vulgaris* consortium	*Oryza sativa*	Significant improvement in rice growth and decline in As concentration of root and shoot	Improved antioxidants, and thiol metabolism	Awasthi et al., 2018

Many microbial consortiums (or enrichments from diverse habitat) have also been reported to have As^{3+} oxidation abilities. Several studies have demonstrated the microbial role in As volatilization from soil to atmosphere. The process involves biomethylation of As, where sequential conversion of the inorganic As occurs to form arsines [monomethylarsine, dimethylarsine, trimethylarsine], ultimately leading to volatile form of non-toxic nature (LD_{50} of 1060 mg L^{-1}). Bacterial members belonging to *Pseudomonas*, *Sphingomonas*, *Cytophaga* and *Bacillus* are found to be reported for As volatilization in various arseniferous soil. In addition, biostimulation (amendments for microbial activities) and/or bioaugmentation (inoculation of potent indigenous strains) are found to be most beneficial for enhancing the rate and efficiency of As methylation and volatilization. Various reports have demonstrated the effect of addition of organic matter *viz*., cellulose, cattle manure, rice straw, dried distiller grain, clover, etc. on As volatilization properties of microbes in soil systems. A number of reports have also described the enhanced growth effect of biochar in rice and other crops but, not much information on application of biochar in cropping systems. Recently several As-transforming plant growth promoting bacterial strains have been isolated from rhizospheric regions of various plants. Arsenic-tolerant and transforming organisms *viz*., *Bacillus licheniformis*, *Micrococcus luteus*, *Pseudomonas fluorescens* were found to possess siderophore producing, phosphate solubilizing and nitrogen fixing properties by Ivan et al. (2017). Mesa et al. (2017) have isolated and characterized 54 rhizobacteria and 41 root endophytic As-transforming bacteria belonging to *Variovorax* spp., *Phyllobacterium* spp., *Bacillus* spp., *Brevundimonas* spp., *Ensifer* spp. etc. with several plant beneficial traits that might alleviate As toxicity in rice. Several plant beneficial Firmicutes members (*Bacillus* spp., *Lysinibacillus* spp.) have been characterized by Singh et al. (2015) from As-laden paddy soil that showed As tolerance in the range 1100-3200 mg L^{-1}. Number of studies (Table 3) have shown the successful utilization of PGPR strains for reducing the As accumulation in plants and improving the growth of plants. Exploration and utilization of these indigenous As-transforming PGP microbes can definitely alter the As availability in rice root zone and ameliorate As toxicity to rice systems in As-hit Bengal plain of India.

CONCLUSION

Several agronomic management strategies have been advised as mitigation steps to lower the bioavailability of As in rice or other crops. Among all, Alternate wet and dry method (AWD) for oxidation of As^{3+} to As^{5+} and lesser uptake, application of silica/silicic acid to compete with As^{3+}, Fe/Fe-oxide amendments for higher immobilization and root-plaque formation, biochar application for higher adsorption of As, use of As^{3+} oxidizing microbial candidates or consortia, ectomycorrhizal fungi mediated hyperaccumulation, and phytoremediation based methods have been effective. But, sustainable technologies need to be developed in future both for safe agricultural production in As contaminated environments and for remediation of the contaminated sites. Arsenic resistant/transforming PGP microbes offer a great deal of soil As management in this regard. However, extensive research would be required to advance this technology *viz*., (1) to standardize PGP microbes based strategy for different As-affected environments, (2) to identify potential PGPR organisms and/or their combination (consortium), and (3) to gain deeper insights into the genetic or molecular mechanisms of action of PGPR. The future research in developing economic PGPR formulations along with public and farmers' participation and awareness might bring effective and sustainable rice framing in As-hit Bengal province and other parts of India.

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