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Possible bioremediation of arsenic toxicity by isolating indigenous bacteria from the middle Gangetic plain of Bihar, India

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ABSTRACT

In middle Gangetic plain, high arsenic concentration is present in water, which causes a significant health risk. Total 48 morphologically distinct arsenite resistant bacteria were isolated from middle Gangetic plain. The minimum inhibitory concentration (MIC) values of arsenite varied widely in the range 1–15 mM of the isolates. On the basis of their MIC, two isolates, AK1 (KY569423) and AK9 (KY569424) were selected. The analysis of the 16S rRNA gene sequence of selected isolates revealed that they are belong to the genus *Pseudomonas*. The $AgNO_3$ test based microplate method revealed that isolates, AK1 and AK9, have potential in transformation of arsenic species. Further, the presence of *aoxR, aoxB* and *aoxC* genes in the both isolated strain AK1 and AK9 was confirmed, which play an important role in arsenic bioremediation by arsenite oxidation. Isolated strains also showed heavy metal resistance against Cr(IV), Ni(II), Co(II), Pb(II), Cu(II), Hg(II), Ag(I) and Cd(II).
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1. Introduction

Arsenic is a toxic metalloid, widely distributed due to natural and anthropogenic activities in the environment. It occurs in four oxidation states (+5, +3, 0, and −3) although the arsenate (AsV) and arsenite (AsIII) are the most common forms and AsIII is more toxic than AsV [1,2]. Drinking water and food are the main sources of exposure of arsenic to the consumers, including animals and humans. Arsenic species are deposited in the skin, lungs, kidney, liver, etc. and cause several severe diseases by oxidative stress, altered DNA methylation, altered DNA repair, mitochondrial damage, proliferation of cell, tumour promotion and co-carcinogenesis [1,3]. It is reported that conventional methods such as oxidation or reduction, chemical precipitation, filtration, ion exchange, reverse osmosis and evaporation recovery of cleaning contaminated water are too much expensive and laborious [4]. So, there is need to develop eco-friendly and low cost technique to mitigate the arsenic contamination.

It is well documented that bioremediation is a cost-effective and a comparatively innocuous alternative to physical methods for heavy-metal remediation [3–6]. Bioremediation of arsenic species by microbial community involves their reduction, oxidation, intracellular bioaccumulation and methylation [6]. The arsenite-oxidising ability to the bacteria is provided by *aox* operon. The expression of the *aox* operon is controlled by AoxR, after expression of the *aox* operon AoxAB complex is synthesized and exported to the periplasm. The AoxAB complex, an arsenite oxidase is involve in the oxidation of the AsIII into AsV [6–8].

Ganga river, originating from the Himalaya, is one of the natural source of arsenic in the Gangetic plain of Bihar. But there is no proof regarding the natural emission of arsenic in the Ganga plain so far [9]. However, the arsenic is released in the Gangetic plain of Bihar by the natural processes in groundwater from holocene sediments containing clay and silt [10,11]. The concentration of arsenic in Gangetic plain of Bihar found above the permissible limit of 10 ppb [12]. So, there is an urgent need of remediation of these contaminated areas.

As far as our knowledge, study related to bioremediation of arsenic toxicity by employing arsenite-oxidising bacteria from arsenic contaminated groundwater of the middle Gangetic plain, Bihar, India is not available. So considering the importance of work

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in present study, we describe the stimulation of the indigenous bacteria for bioremediation of arsenic toxicity. The bacterial isolates were also evaluated for other heavy metal resistance such as Cr(IV), Ni(II), Co(II), Pb(II), Cu(II), Hg(II), Ag(I) and Cd(II).

2. Material and methods

2.1. Sample collection

The water samples were collected before the rainy season from handpumps of 12 different arsenic contaminated sites of Gangetic plain in the Bihar region. Water samples were collected in two different storage bottles, one sample was treated with 2–3 drops of nitric acid to prevent the metal from precipitation, adsorption and microbial degradation. Another water sample was used for isolation of bacteria hence kept as it is at ice. The selected sites were namely Karja (25°39′09.2″N 84°42′27.7″E), Pararia (25°24′05.9″N 84°34′41.8″E), Bhakhura (25°38′21.1″N 84°09′00.2″E), Semaria (25°40′27.8″N 84°43′26.6″E), Keshopur (25°39′54.7″N 84°43′06.6″E), Maner Thana (25°38′51.6″N 84°53′07.6″E), Danapur (25°34′56.4″N 85°02′36.9″E), Badhora (25°22′08.0″N 84°59′58.1″E), Danapur (Auto-stand) (25°38′14.1″N 85°02′42.4″E), Maner Thana (Lodipur) (25°38′52.0″N 84°53′06.8″E), Akauna (25°07′18.5″N 85°24′16.6″E), Science College (25°37′05.6″N 85°10′10.2″E) (Fig. 1). The samples were kept in sterile sample collection plastic bottles and preserved at 4 °C for further use.

2.2. Evaluation of total arsenic in water samples

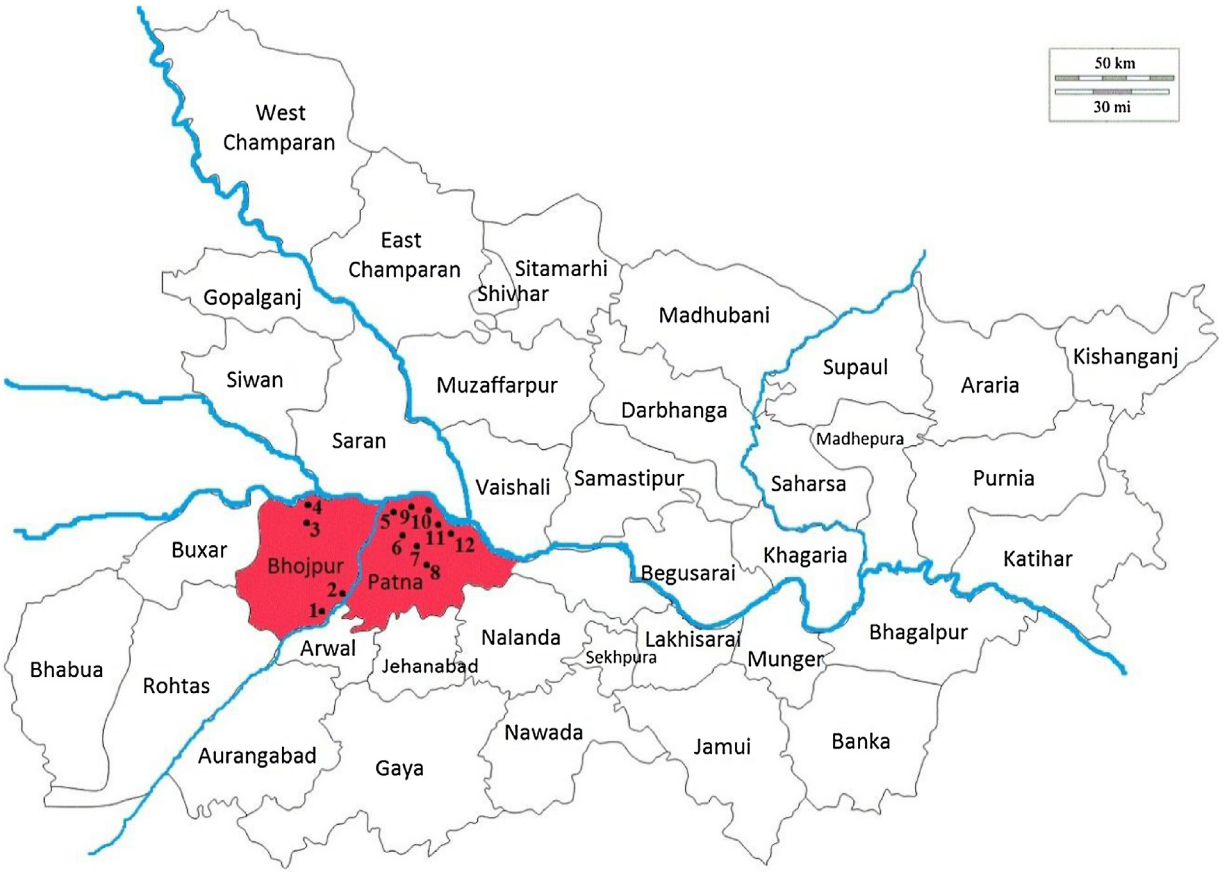
The collected water samples were analysed for total arsenic present by using MQuant Arsenic Test kit (Merk). Total arsenic concentration was measured semiquantitatively by visual comparison of the reaction zone of the test strip with the fields of a colour scale. The measuring range of the test strip was varied from 5 ppb to 500 ppb.

2.3. Isolation of arsenic resistant bacteria

For isolation of arsenic resistant bacteria, 100 μl of water samples were inoculated in 10 ml of Luria-Bertani (LB) media and incubated at 30 °C for overnight [3,4,18]. The 100 μl of revived culture was spread onto LB-agar plates containing 1.33 mM of AsIII. The plates were incubated at 30 °C for 72 h. The colonies, which showed resistance to AsIII and were morphologically different, were picked up and isolated after successful purification by growing repeatedly on LB medium and stored at 4 °C.

2.4. Evaluation of the MIC value

The minimum inhibitory concentration of AsIII for all the 48 isolates was evaluated by growing them on AsIII supplemented medium [18,21]. 2 μl of the freshly revived cultures were spotted onto LB-agar plates supplemented with increasing concentration (1.33–20 mM) of AsIII. The plates were then incubated at 30 °C for



1. Karja, 2. Pararia, 3. Bhakhura, 4. Semaria, 5. Keshopur, 6. Maner Thana, 7. Danapur, 8. Badhora, 9. Danapur (auto stand), 10. Maner Thana (Lodipur), 11.Akauna, 12. Science College

Fig. 1. Water sample collected from twelve different arsenic contaminated sites of Gangetic planes in Bihar region before rainy season. source: [13,14].

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