1. INTRODUCTION

1.1. Arsenic: A toxic element in the environment

Arsenic is a ubiquitous metalloid composing approximately 0.00015% of the earth's crust and in the recent years, pollution by arsenic has become a serious issue of concern in view of its toxicity to humans (Polya et al., 2019). Arsenic is known as 'king of poison' as it is a highly toxic element ranking number one in the priority list of hazardous substances and disease registry defined by World Health Organization (WHO). It is also been classified as Group I carcinogen by International Agency for Research on Cancer (IARC, 2022). In natural water, predominantly, two highly toxic forms of arsenic exist which include arsenate (As^{5+}) and arsenite (As^{3+}). Comparatively, arsenite is more toxic than arsenate (Fendorf et al., 2010). A worldwide population of more than 45 million people mainly in developing countries from Asia is at high risk of exposure to greater than 50 $\mu g\ L^{-1}$ of arsenic concentration which exceeds the maximum permissible limit (10 $\mu g\ L^{-1}$) of arsenic in drinking water fixed in WHO guidelines (Singh et al., 2016; WHO, 2018). The use of arsenic contaminated ground water for irrigation leads to the accumulation of arsenic in crops in different degrees depending on the species and variety. Consumption of arsenic contaminated drinking water and crops has been considered as the main routes for its entry into human food chain. In addition, consumption of seafood and livestock-based food products such as meat and milk from arsenic contaminated regions also contributes to the food chain transfer and contamination (Singh et al., 2023).

From its geochemical reservoirs, arsenic is continuously released into the biosphere by a variety of natural and anthropogenic human-made processes. The natural sources of arsenic include sedimentary deposits/rocks, geothermal activity, coal and volcanic activities. Human activities leading to arsenic pollution in the soil and water includes the inappropriate production and discard of arsenic containing industrial chemicals, widespread use of arsenic containing pesticides and phosphate fertilizers, emissions and drainage from smelting and mining of arsenic containing minerals and fossil fuel combustion (Ali et al., 2019).

Several studies report the toxic effects of arsenic in both plants and humans. In plants, adverse effects of arsenic include decreased chlorophyll content, reduced transpiration and photosynthetic capacity, lipid peroxidation, chlorosis and reduced reproductive capacity which ultimately impairs its physiology and metabolism (Chandra et al., 2018; Maghsoudi et al., 2020). Arsenic consumption in humans leads to several health risks such as skin disorders, cancers, melanosis, hyperkeratosis, lung disease, peripheral vascular diseases, gangrene, diabetes mellitus, hypertension, and ischemic heart disease (Dutta, 2023). Additionally, chronic exposure to inorganic arsenic increases the risk of diabetes mellitus, adverse pregnancy outcomes, and even skin cancers, lungs, and urinary bladder (Patel et al., 2023).

1.2. Siderophores in the soil

Siderophores (Greek *sidero* meaning iron and *phore* meaning carrier) are small, organic, iron (Fe^{3+}) chelating compounds with molecular weight ranging between between 500 to 1500 Da that are produced by microorganisms such as bacteria and fungi as well as by plants under iron limited conditions (Ghosh et al., 2020). Iron is an essential element required for the normal physiological functions of all living. However, its abundance is limited due to the rapid oxidation of its soluble ferrous form (Fe^{2+}) to insoluble ferric (Fe^{3+}) form. Thus, microorganisms secrete siderophores which have high affinity for Fe^{3+} form of iron and Fe^{3+}-siderophore complex is formed. These complexes are recognized by outer membrane siderophore receptor or siderophore binding proteins and then transported inside the cell through siderophore-mediated Fe transport system. Inside the cell, Fe^{3+} is released from the complex and converted into soluble ferrous iron form (Fe^{2+}) (Saha et al., 2016). With this iron chelating property, siderophore producing bacteria play a significant role in increasing plant growth directly and indirectly participate in pathogen control (Kumar et al., 2018). Khalid et al. (2015) have stated that siderophores produced by plant growth promoting rhizobacteria (PGPR) help in the fortification of iron to plants thereby improving soil fertility and plant growth. Siderophore producing bacteria have also been reported to slow down the growth of plant pathogens especially fungi by limiting the iron concentration. By sequestering the iron tightly, siderophores reduce the bioavailability of iron for plant pathogens and facilitate their killing (Ahmed and Holmstrom, 2014).

Siderophore synthesis occurs by two different pathways depending on the chemical nature of the molecule: the non-ribosomal peptide synthetase (NRPS) pathway and the NRPS-independent siderophore (NIS) synthetase pathway (Kramer et al., 2020). In the NRPS pathway, the synthesis is carried out large multimodular enzyme complexes that activate and assemble a wide range of amino acids, leading to the formation of structurally highly variable peptides. In the NIS pathway, several enzymes, such as monooxygenases, decarboxylases, amino and acetyltransferases, amino acid ligases, and aldolases are involved in the synthesis of the building blocks that are finally assembled to form the siderophore. However, siderophore biosynthesis is tightly regulated at transcription level by the presence of iron. For bacterial regulation of siderophore production, the ferric uptake regulator (Fur) participates. It contains a regulatory binding site for Fe(II), which triggers Fur dimerization and subsequent binding to DNA at specific sites (Fur Box) to block transcription of the biosynthetic gene (Heffernan, 2023).

According to the nature of functional groups that are used as ligands for iron chelation, siderophores can be categorized into four main types; i) catecholates such as enterobactins and bacillibactin which posses a cathecholate group as ligand, ii) hydroxamates such as deferoxamines and putrebactin containing a hydroxyl group, iii) carboxylates such as rhizobactin and staphyloferrin with carboxyl group as ligand and iv) mixed type such as pyoverdin and heterobactin which have more than one iron chelating ligands (Khan et al., 2018). However, some bacteria may secrete more than one type of siderophore and they may have multiple absorption pathways for iron acquisition (Kramer et al., 2020).

Though scavenging of iron is the primary role of siderophore in the environment, siderophore has also been found to complex with other essential elements like molybdenum, manganese, nickel and cobalt, and supply them to microorganisms (Braud et al., 2009a; Dimpka et al., 2009).

1.3. Bioremediation

Bioremediation can be defined as the process of using living organisms to transform hazardous contaminants in soil/water to nonhazardous waste products (Sharma et al., 2019). Various group of microorganisms (bacteria, fungi and algae) are efficiently involved in remediating several types of environmental pollutants which include heavy metals, pesticides, dyes, hydrocarbons, plastics etc. (Ali et al., 2019; Koutra et al., 2023). Bioremediation also involves the use of plants that can accumulate high concentrations of heavy metals in the shoots from the contaminated soil, enabling the removal of contaminants from the soil, a process known as phytoremediation (Wan et al., 2023). Many bioremediation processes depend on aerobic environments, however, anoxic surroundings may also allow for bacterial metabolism of pollutants (Syed et al., 2021). Bioremediation is only efficient in environments that support microbial proliferation and activity, thus, its implementation frequently requires the modification of environmental factors to promote microbial growth and pollutant degradation/transformation (Lawniczak et al., 2020). On the basis of method applied, bioremediation can be of two types: *in-situ* and *ex-situ* bioremediation. In *in-situ* method, biological agents are applied on the polluted environment (water or soil) and bioremediation occurs on that particular spot (Ghangrekar et al., 2020). *Ex-situ* bioremediation is the process of cleaning up pollutants after they have been transported to an appropriate location by pumping or excavation (Tripathi et al., 2021).

The dissemination of arsenic contamination on a global scale has emerged as a primary concern due to the potential threats it poses to the environment as well as to the human health. Several conventional technologies have been implemented for arsenic removal from soil which include chemical methods like soil washing and immobilization, and physical methods like soil replacement, soil cover, turnover and attenuation and electro-kinetic remediation (Wang et al., 2019; Al-Makishah et al., 2020). Similarly, for arsenic contaminated water, remediation techniques like oxidation, coagulation-flocculation and membrane filter techniques are mostly used (Nicomel et al., 2016; Criscuoli and Figoli, 2019; Mohammed and Wan Dagang, 2019). However, these conventional technologies face some disadvantages which include waste generation, high cost and inefficiency in some cases (Li et al., 2022). Moreover, phytoremediation technology is also less considered for arsenic removal due to their high cultivation cost (Roy et al., 2015). Therefore, microorganism-based bioremediation techniques are an alternative treatment technique that is safe, low-risk, economically feasible, flexible, and environmentally sound (Hare et al., 2020).

Various mechanisms have been implemented by microorganisms for the bioremediation of arsenic from the environment. These may include the arsenic uptake and accumulation with the help of cell surface moieties which can bind arsenic by complex formation, and thereby facilitate its entry inside the cell (Garbinski et al., 2019; Zhang et al., 2022). The other mechanisms include oxidation and reduction where, in case of oxidation, arsenic is converted from its more lethal form (AsIII) to less noxious form (As V) through arsenite oxidase as a detoxification mechanism (Sonthiphand et al., 2021) while reduction involves alteration of As V to As III through arsenate reductase (Wang et al., 2021). The fate of arsenic can also be altered by methylation, resulting in methylated arsines through arsenite methyl transferase (Chen and Rosen, 2020) and demethylation in which methylated arsenic species are degraded to non-toxic volatile arsines through ArsI C-As lyase (Nadar et al., 2022). Some recent studies report the application of different groups of microorganisms (singly or in combination) in the bioremediation of arsenic which includes; *Bacillus thuringiensis and Paenibacillus glucanolyticus* (Banerjee et al., 2020), *Humicola* sp (Tripathi et al., 2020), *Dunaliella salina* (Wang et al., 2020), *Pseudomonas aresenitoxidans* (Pipattanajaroenkul et al., 2021), *Noviherbaspirillum denitrificans* (Wu et al., 2021), *Serendipita indica* (Shukla et al., 2023).

1.4. Biocontrol in plant disease management

Agricultural practices are facing serious problems in crop production because of the plant pathogens causing several types of plant disease. To circumvent these problems, agricultural crops are commonly treated with synthetic fungicides, which despite their effectiveness in controlling diseases caused by phytopathogens, are not sustainable alternatives due to their high economic and environmental costs (Sharma et al., 2009). These synthetic chemicals leave harmful residues in the soil, water, and the atmosphere as well as induce resistance in phytopathogens (Villarreal-Delgado et al., 2018). Therefore, there is a need to develop some efficient and environment friendly technologies in order to eliminate or reduce the application of synthetic fungicides in agriculture (Santoyo et al., 2012). Thus, the use of biocontrol agents (BCA) has emerged as a promising alternative to achieve this goal and a wide array of microbial BCAs have been developed for the management of fungal and bacterial diseases of plants (Santoyo et al., 2016).

Bacterial biocontrol agents (BCA) use a wide variety of mechanisms (either one or in combination) for prevention of plant disease, interacting directly or indirectly with the pathogen (Kohl et al., 2019; Legein et al., 2020). The direct interaction of BCAs occurs through the secretion of antimicrobial metabolites like as lipopeptides, bacteriocins, antibiotics, biosurfactants, cell-wall degrading enzymes or volatile compounds which hinders the metabolic activity of pathogens and inhibits their growth. They can compete with the pathogens for nutrients and space where highly competitive BCAs may colonize and survive in the infection site and uptake nutrients more efficiently than the pathogens. An example of such uptake system is the low molecular weight siderophores with affinity for ferric ion. They can also interfere with the quorum sensing (QS) system of the pathogens by enzymatically degrading or inhibiting the synthesis of signal molecules with the help of QS inhibitors like lactonases, pectinases and chitinases which degrades QS signal molecules, thereby imparing pathogen infection and reducing the infection in plants (Kalia et al., 2019). Apart from direct interactions, BCAs can protect plants indirectly, either by triggering the defense response (inducing resistance) or promoting plant growth (Elnahal et al., 2022). As a result of elicitation of resistance, structural barriers build up and the host undergoes a variety of biochemical and molecular defense reactions, providing protection against a variety of infections.

Biological control has achieved some promising advancements, particularly with the successful application of a number of antagonistic biocontrol agents (BCAs), specially *Pseudomonas* sp., *Bacillus* sp., *Burkholderia* sp., and *Trichoderma* sp. against pathogens like *Agrobacterium radiobacter* var *radiobacter*, *Erwinia* sp., *Fusarium* spp., *Rhizoctonia solani, Phytophthora* spp. and *Pythium* spp. which are responsible for causing different foliar and soilborne diseases (Lahlahi et al., 2022).

1.5. Brinjal: a common nutritious vegetable

Brinjal (*Solanum melongena* L.) with other names such as eggplant, aubergine, guinea squash, belonging to the Solanaceae family, is one of the most popular and productive vegetables grown worldwide and extensively in India. Among the Indian states, West Bengal is the largest producer of brinjal with different varieties under cultivation (APEDA 2017-2018). Variations are observed in case of color, sizes, shapes and taste of the fruit. The fruit can be ovoid to long club shaped with colors such as yellow, white, green and purple skin coloring to almost black. It has great importance as a vegetable in the human diet as it is very low in calories and can supply vitamins, proteins and fundamental minerals including iron, calcium, potassium, magnesium, nutritious fiber, protein, and antioxidants. Moreover, this vegetable is also rich in anthocyanin chemicals which show protective effects against diabetes, cancer, cardio vascular disease (Naeem and Ugur, 2019).

Several factors contribute to the low production of brinjal which include aberrant climatic conditions, soil fertility, seeds quality, and plant diseases. Among these factors, disease due to pathogen attack is one of the main constraints for the low yield and quality of this crop. Wilt is one of the most harmful diseases among all the diseases of brinjal that causes great economic loss in brinjal production. Brinjal is susceptible to many wilt diseases like verticillium wilt, fusarium wilt and bacterial wilt caused by *Verticillium dahlia*, *Fusarium oxysporum f. sp. melongenae* and *Ralstonia solanacearum* respectively (Chaterjee et al., 2021). *F. oxysporum f. sp. melongenae* is the most destructive pathogen that causes wilt in brinjal and the severity of wilt is 10%-90% (Sahoo, 2022). The fungus invades into the roots of the host brinjal-through the feeding of nematodes pinholes, natural-and other-wounds. After invasion, it penetrates in the xylem vessels of plant which transports water and nutrients from roots towards crown and also produces toxins as it proliferates through plant. Thus, it limits the nutrients and water transport in the vessel, and its macro and micro conidia are implicated in this process. Initially, wilting symptoms can be observed in isolated patches containing less or more circular outlines which gets enlarged with the disease progression. Eventually, the crown start bending and plants die. Improper development of root system occurs followed by vascular vessel discoloration and wilting (Abdel-Monaim et al., 2014).

Numerous management strategies are widely adopted to reduce disease incidences in brinjal such as cultural operations, biological controls, resistant varieties, soil solarization, crop rotation & less availability of soil moisture (Ishaq et al., 2023). In recent years, biological control agents have been successfully implemented in the wilt disease management owing to their rapid growth, easy handling and aggressive colonization in the rhizosphere (Theradimani and Swaminathan, 2018). However, very few reports of effective management of *Fusarium* wilt by *F. oxysporum* in brinjal are available (Chakraborty et al., 2021). Thus, there is a need for exploration of different biocontrol agents which can be successfully used as an alternative to different chemical fungicides for effective management of *Fusarium* wilt in brinjal caused by *F. oxysporum.*

1.6. Objectives

Arsenic contamination in soils can cause serious threat to human health as it can enter into human food chain through different agricultural crops grown in such areas. In India, West Bengal is one of the most contaminated states with several reports of arsenic toxicity. Thus, there is an immense need for efficient arsenic remediation strategies. Nowadays, bioremediation using microorganism is gaining much importance as compared to the conventional methods for heavy metal remediation since it is cost effective and ecofriendly. Another problem which is in focus since many years is the agricultural loss due to the attack by plant pathogens. Biocontrol agents are being extremely used as an alternative to the use of synthetic chemicals for the better management of crop production. Among the BCAs, bacteria which produce siderophores primarily for iron acquisition can be relevant in combating arsenic toxicity in agricultural soils. Moreover, very few studies have been done regarding the application of siderophorogenic bacteria in the bioremediation of arsenic and in West Bengal such studies will be of outmost significance.

Therefore, considering above factors, through this study we aimed to focus on the rhizosphere inhabiting bacteria which can be applied as a potent multifunctional agent for arsenic bioremediation as well as in the biocontrol of plant pathogens. With this aim, the present study was designed with following objectives:

1. Isolation of siderophorogenic bacterial strains from agricultural soils and their screening for antifungal activity and arsenic resistance.

2. Identification and phylogenetic analysis of the selective isolates based on 16S rRNA gene sequence.

3. Evaluation of antagonistic potential of selected bacterial strains against plant pathogenic fungi.

4. Characterization of siderophore and metal complexation studies between siderophore and arsenic.

5. Analyzing the presence of genetic determinants of arsenic resistance and antifungal property in the bacterial isolates.

6. Batch study for arsenic removal from the soil by potent isolate.

7. Studying the biocontrol potential of selected bacterial strain under greenhouse conditions.