Jahangirnagar University J. Biol. Sci. **8**(1): 9-15, 2019 (June)

Characterization of rhizospheric and non rhizospheric bacteria from arsenic contaminated soil

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Abstract

The rhizosphere soil has a large and various number of microorganisms especially the bacteria. This experiment was conducted at the department of Botany, Jahangirnagar University, Savar, to investigate the rhizospheric and non-rhizospheric bacteria from arsenic contaminated soil. *Pteris vittata* was treated with different concentrations of arsenic ranges from 5000 to 10000 ppm in the pot. The experimental result indicated that the negative correlation between arsenic concentration and rhizospheric soil bacteria. Highest number of bacteria (8.6×10^8 cfu/g) were found in rhizospheric soil (control), while lowest numbers of bacteria (4.0×10^7 cfu/g) were found in the non-rhizospheric soil with 10000 ppm arsenic. Thirty bacteria were isolated from rhizospheric and non-rhizospheric soil samples. Out of thirty samples *Bacillus* and *Pseudomonas* were selected on the basis of morphological and biochemical nature. The present study concluded that the arsenic has an adverse effect on the growth of rhizospheric soil bacteria.

Key words: Characterization *Pteris vittata*, rhizospheric and non-rhizospheric soil

INTRODUCTION

Arsenic (As) is a metalloid element known as poison and its contamination is one of the promising public concerns in the whole world due to its toxic and carcinogenic nature (Singh *et al*., 2015). High concentration of arsenic has adverse effect on human health and environment (Hettick *et al*., 2015). In nature, arsenic can be found in four oxidation condition, among four oxidation states arsenate [As(V)] and arsenite [As(III)] are the abundance form in the environment. As(V) is known as phosphate analogue and is mixed with cellular processes, like oxidative phosphorylation and ATP synthesis, on the other hand, As (III) binds to the sulfhydryl groups and change the protein function (Oremland & Stolz, 2005).

Widespread use of ground water for irrigation is a source of arsenic contamination in food chain (Huq *et al*., 2006) and the presence of arsenic in food chain (Duxburya *et al*., 2003; Das *et al*., 2004) affects human health directly or indirectly.

Arsenic also affects the soil microorganisms. The natural levels of soil arsenic range from 0.1 to 40 mg/kg in various countries (García-Sánchez *et al.*, 2010). Anthropogenic sources like pesticide, insecticide, mining, coal combustion etc. generally exceed natural

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level of arsenic in the environment (Mesa *et al.*, 2017). More than 10,000 arsenic contaminated sites have been reported in Australia (Smith *et al.*, 2008).

On the other hand, microbial interaction plays a major role in the plant growth (Lampis *et al.*, 2015). Bacterial mechanisms increase the plant growth by using bacterial metabolites, like indole-3-acetic acid (IAA), different kinds of acid production, soil acidification, solubilization of phosphates and methylation (Lebeau *et al.*, 2008; Ma *et al.*, 2011). Bacteria colonize the plant tissue and promote plant growth through mechanisms such as IAA, phosphate solubilization and production or supplying necessary vitamins to plants for their growth and development (Ryan *et al.*, 2008, Kuffner *et al.*, 2010). Rhizosphere bacteria can act as biocontrol agents against pathogenic microorganisms and help is nitrogen fixation (Weyens *et al.*, 2009). A sustainable technology for cleaning As-contaminated soils is phytoremediation, which is defined as the use of plants to remove or reduce toxic concentrations of hazardous substances in the environment (Weis *et al.*, 2004). Therefore, the current study was undertaken to investigate the effect of arsenic on rhizospheric and non-rhizospheric soil bacteria.

MATERIALS AND METHODS

Collection of samples: Rhizospheric and non-rhizospheric soil of *Pteris vittata* were collected from different pots containing and without containing arsenic. Then the soil was treated with 5000, 6000, 7000, 8000, 9000 and 10000 ppm arsenic. Sample was collected on the duration of 7, 14 and 21th days interval. Soil was collected very carefully and preserved at 4°C.

Isolation of soil bacteria and bacterial load determination: Soil bacteria were isolated by using serial dilution methods (MacLowry *et al.*, 1970). Nutrient agar medium was used for isolation of bacteria and microbial load determination. Microbial load was determined from the total number of discrete colonies counted after incubation. Isolated colonies were counted in colony formation unit as Number of cfu/volume plated dilution factor. The bacterial colonies were observed to study various characters viz. colour, form, elevation, margin, surface etc.

Purification and selection: Bacterial colonies were cultured on different selective and differential media such as: MacConkey, Bouillon agar, King’s B media. Different biochemical tests (Casein test, Fermentation test, Indole test, Starch hydrolysis test, Catalase test, API 20E) were also performed for bacterial identification. Results of the morphological, cultural and biochemical tests of selected isolates were analyzed following Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1986).

RESULTS AND DISCUSSION

Isolated bacteria from arsenic contaminated soils were grown in nutrient agar with different concentrations of 5000, 6000, 7000, 8000, 9000 and 10000 ppm of arsenic. Highest number of bacteria (8.6×10^{8} cfu/g) grown in control soil and lowest number of bacteria (8.0×10^7 cfu/g) were found in 10000 ppm of rhizospheric soil with 10000 ppm arsenic.

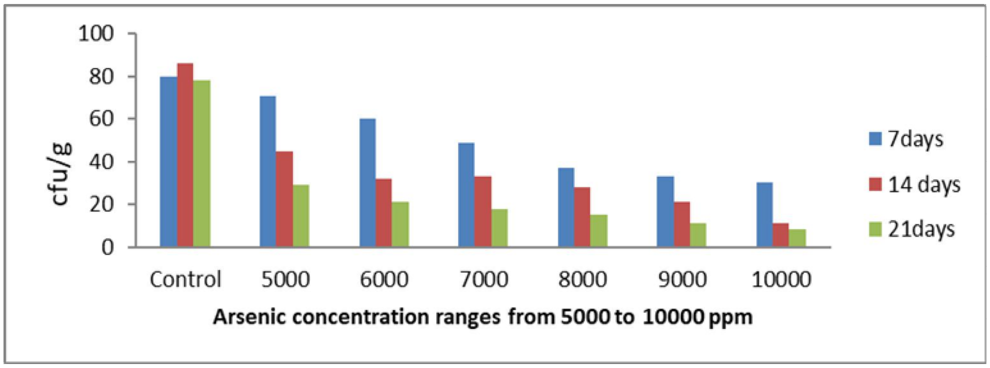

Fig. 1. Bacterial abundance in the rhizospheric soil of different concentration of arsenic (5000 to 10000 ppm) at different intervals

The results of graphical analysis of bacterial abundance in the non-rhizospheric soil of different concentration of arsenic are presented in Fig. 2. Highest number of bacteria (8.2×10^7 cfu/g) was found in control and lowest (4.0×10^7 cfu/g) were recorded from non-rhizospheic soil contain 10000 ppm of arsenic. The results have clear indication that arsenic has negative relationship with bacterial abundance in the soil. Besides Fayiga *et al.* (2007) shows that arsenic has adverse effect on soil bacterial community and phytoremediation process helps to increase the bacterial community in the arsenic contaminated soil). Microorganisms are far more sensitive to heavy metal stress than soil animals or plants growing on the same soils. Evidence suggested that heavy metal have an extreme effect on soil microbial communities (Giller *et al.*, 1998).

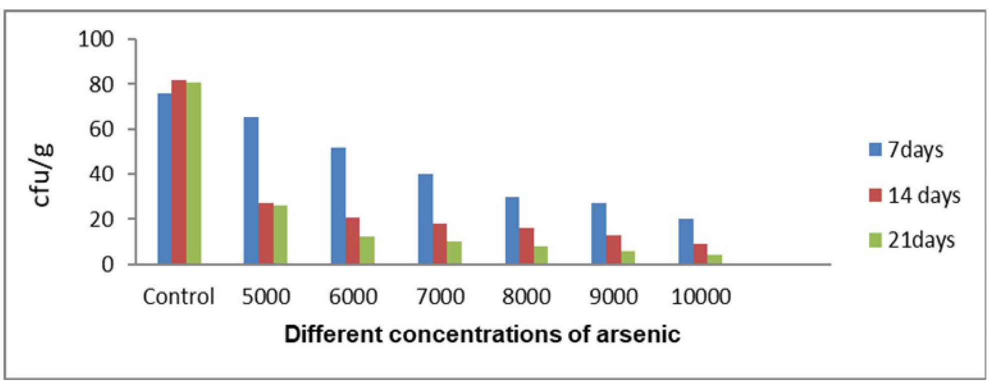

Fig. 2. Bacterial abundance in the non-rhizospheric soil of different concentration of arsenic at different intervals

Microorganisms and plants have developed several essential or adaptive mechanisms to mix with as enabling them to resist and metabolize it (Mesa *et al.*, 2017). The rhizosphere soil is the source of high variety of microorganisms which play an important role in the enhancement of the plant growth. Interaction of plant and microbe helps to improve the plant growth (Prasad *et al.*, 2014). Rhizosphere bacteria influence plant growth by changing the soil chemical properties and interact with plant roots and this influence can be beneficial, neutral, or deleterious (Russell 1977, Doi *et al.*, 2007). Isolated bacteria from rhizosphere soils have showed the beneficial impact on plants by direct effect on nitrogen fixation (Han *et al.*, 2005).

In the study thirty bacteria were isolated from rhizosphere and non-rhizosphere soil zone. The isolated was designed as NZ_1 to NZ_{30}. From the selective media NZ14, NZ15, NZ17, NZ27, NZ28 were identified by using morphological and biochemical test (Plate 1). *Bacillus* (Table 1) *Pseudomonas* was recognized during the experiment. Kloepper *et al.* (1989) isolated a large number of rhizospheric soil bacteria and identified among which the common species of *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Arthobacter*, *Burkholderia*, *Alcaligens*, *Bacillus* and *Serratia*.

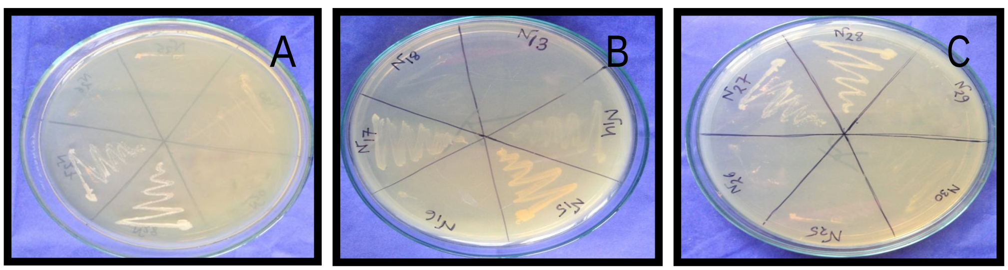

Plate 1. Photographic plates show the presence and absence of isolated bacteria on king's B media. Here A and C show the off white and white color of NZ_{27}, NZ_{28}. B shows the off white color of NZ_{14}, cream color of NZ_{17}, orange color of NZ_{30}

In the present study *Bacillus* and *Pseudomonas* were isolated through their typical colony characteristics on to the specific culture media. *Bacillus* sp. gave white colored colony on Bouillon agar and showed positive test in starch hydrolysis, endospore staining test (Plate 2). *Pseudomonas* sp. showed positive biochemical test in API 20 E system during the experimental time (Plate 3).

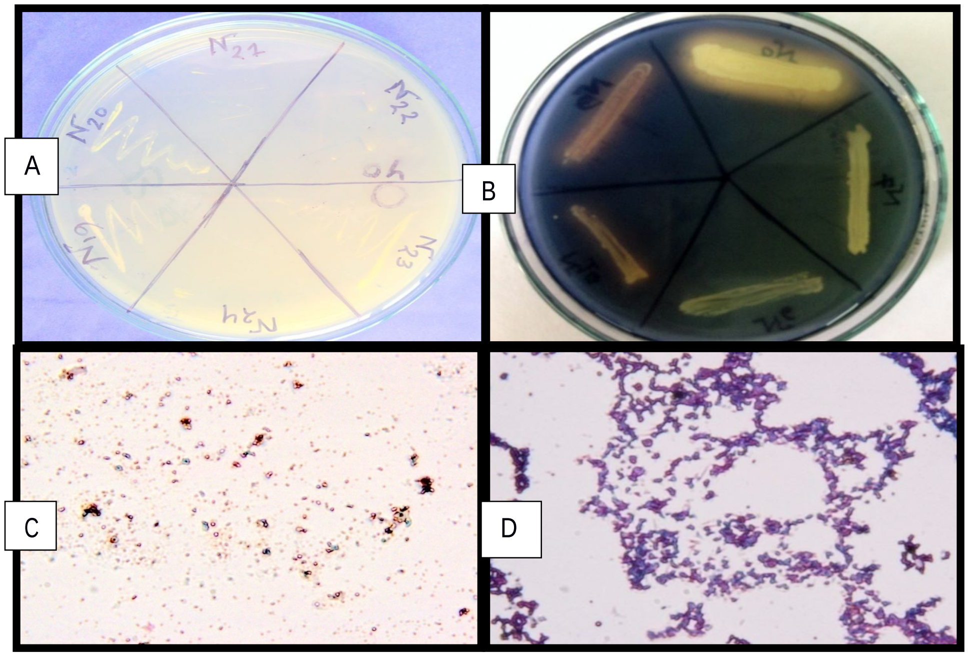

Plate 2. Bacterial colonies on Bouillon Agar media for *Bacillus* sp. (A), starch hydrolysis positive test (B), endospore staining positive test (C) and gram staining (D)

Table 1. Identification of *Bacillus* sp. (Sneath *et al*., 1986)

Criteria	Result	Identification
Shape	Rod shape	
Cell	Thick cell wall	
Gram staining	Gram positive	*Bacillus* sp.
Spore formation	Endospore	
Location of spore	Central	
Starch hydrolysis	Positive	
Growth at 50ºC	Positive	

Plate 3. Identification of *Pseudomonas* isolated from rhizospheric soil arsenic using API 20 E system

Soil bacteria that colonize plant roots and enhance plant growth are known as plant growth-promoting rhizobacteria (PGPR). PGPR are highly diverse and are used as bio control agents (Beneduzi *et al.*, 2012). A rhizosphere bacterium helps to control the root knot nematode (Zhao *et al.*, 2018). Rhizobacteria belonging to the genera *Pseudomonas* and *Bacillus* are well known for their antagonistic effects and has the ability to efficient biocontrol agents to improve cropping systems (Beneduzi *et al.*, 2012). Huang (2016) described that many bacteria are tolerant to heavy metals and play important roles in the accumulation of heavy metals. Plant growth promoting rhizobacteria *Pseudomonas aeruginosa* have a heavy metal tolerance like cadmium which might be used as an ideal candidate for bioremediation and plant growth promotion against Cd-induced stress (Huang *et al.*, 2016). Therefore, the identified *Bacillus* and *Pseudomonas*, which are arsenic resistant bacteria and also beneficial rhizosphere bacteria for plant growth and development. These two bacteria have the potential bioremediation capacity which might be used to remove arsenic from soil.

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