Annals of Plant and Soil Research 16 (3): 268-270 (2014)
Short Communication

ISOLATION, SCREENING AND CHARACTERIZATION OF ARSENIC RESISTANT BACTERIA FROM ARSENIC CONTAMINATED SOILS OF HOOGLY, WEST BENGAL

ANSHU SINGH, IZHARUL HAQ AND ADESH KUMAR*[1]

Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar Central University, Lucknow, U.P., India
Received: May, 2014; Revised accepted: August, 2014

Arsenic (As) is the most common toxic metal widely occurring in the environment which possess serious hazardous impacts not only for human health but also from broader ecosystem viewpoint from last few decades. The two worst arsenic affected areas in the world are Bangladesh and West Bengal, India. In both the areas, the source of arsenic is geological in origin. Arsenic was first identified in West Bengal, India, in the month of July in 1983 when the first cases of arsenicosis were identified in 16 patients from one village of a district. In West Bengal 7 district viz Murshidabad, Malda, Burdwan, Nadia, Hoogly, 24 Parganas (North and South) are the most severely affected. About 38.4% of the area of West Bengal and about 44.4% of the total population appear to be affected by arsenic poisoning. Around 6 million people in West Bengal and more than 46 million people in Bangladesh are estimated to be at risk from drinking water with arsenic above 50 mg/L (Wang & Mulligan, 2006). Drinking of arsenic contaminated water is responsible for the development of hyper pigmentation, skin cancer, liver cancer, circulatory disorders, and other ailments. To prevent various adverse impacts of Arsenic (As), the United States Environmental Protection Agency (USEPA) promulgated the new arsenic rule that lowered the maximum contaminant level (MCL) in drinking water to 10µg/1 (10 ppb) for both community and non-transient, non-community water systems. The presence of arsenic in the environment may thus lead to the enrichment of arsenic resistant bacteria. Bacteria belonging to the genera *Acidithiobacillus*, *Bacillus*, *Deinococcus*, *Desulfitobacterium* and *Pseudomonas* have already been reported to be resistant to arsenic (Singh *et al.*, 2001; Suresh *et al.*, 2004). Therefore, it is necessary to remove arsenic from the contaminated environment to achieve the above MCL of arsenic in water.

Table 1: Morphological characteristics of Arsenic resistant bacterial isolates

Isolates	Color	Shape	Elevation	Margin	Texture
AR-1	Off-white	Circular	Raised	Entire	Smooth dull
AR-2	Dark yellow	Circular	Flat	Entire	Smooth shiny slimy
AR-3	Orange light	Irregular	Raised	Curled	Rough dull
AR-4	Yellow light	Circular	Convex	Entire	Smooth shiny
AR-5	Yellow	Circular	Flat	Lobate	Smooth shiny slimy
AR-6	Pale yellow	Circular	Convex	Entire	Smooth dull
AR-7	Cream	Circular	Raised	Undulate	Rough dull
AR-8	White	Circular	Convex	Entire	Smooth shiny transparent
AR-9	Off-white	Circular	Flat	Entire	Smooth shiny
AR-10	Golden yellow	Circular	Raised	Lobate	Smooth shiny
AR-11	Pink light	Circular	Raised	Entire	Smooth shiny
AR-12	Pink	Circular	Convex	Entire	Smooth shiny
AR-13	White	Circular	Flat	Erose	Smooth dull
AR-14	Cream	Circular	Convex	Erose	Smooth dull
AR-15	White	Circular	Flat	Undulate	Smooth shiny

Four soil samples were collected from Hoogly, West Bengal, where soil, sediment and ground water in the area have been contaminated with arsenic for many years. The collected soil samples were placed in plastic bags and kept at 4^{o}C until further analysis. The pH of soil sample was determined by pH meter. The medium used for isolation was nutrient agar (0.5 % peptone, 0.5 % NaCl, 0.3 % beef extract and 1.5 % agar, pH 7.0). The serial dilution agar plating method was used for isolation of bacteria from soil sample. Growth media was sterilized by autoclaving at 121^{o}C for 25 minutes. Sodium arsenate [Na-As (V)] was filter sterilized and added to the medium for the initial concentration of 1000, 5000, 10,000, 15,000, 25,000

**Corresponding author: adesh.kumar88@yahoo.com*
[1]Department of Plant Molecular Biology & Genetic Engineering, Narendra Deva University of Agriculture & Technology Kumarganj Faizabad 224229

and 50,000 mg/L. One gram of each soil sample was dissolved in 10 ml of distilled water and shaken for 3 minutes. Soil suspension (0.1ml) was inoculated on nutrient agar plate containing sodium arsenate of different concentration and incubated at 37^{o}C for 24 to 48 hours. Bacterial isolates that could tolerate the highest arsenate concentration were selected and characterized by their morphological features and biochemical properties. Bacterial isolates were grown on nutrient agar with the different concentrations of sodium-arsenate and incubated at 30^{o}C until colony was observed. Gram Stain and cell morphology was investigated under microscope. Biochemical properties of isolates were tested according to Bergey's Manual of Systematic Bacteriology (Krieg, 1984). The following properties were determined: motility, citrate utilization, starch hydrolysis, casein hydrolysis, catalase test, oxidation /fermentation (O/F), acid production from carbohydrates: glucose, lactose and sucrose. Bacterial isolates were also tested for different concentration of heavy metal like chromium, zinc and nickel in nutrient medium.

AR= Arseinic Resistant

The pH of soil samples collected from area containing arsenic contaminated soil was ranged from 6.2 to 9.1. A total of twenty four bacterial strains were isolated from nutrient agar medium at different sodium-arsenate concentrations, only 15 bacterial strains found able to grow in the presence of 15,000 mg/L of sodium-arsenate. The result suggested that the isolates may have developed metal resistance systems in an attempt to protect sensitive cellular components. It was observed that most of the isolates had yellow color, circular-shaped, translucent colony with flat plateau, smooth and shiny surface. Some isolates had off-white, pale orange, pink and cream colored colonies (Table1). In general microbial ability to grow at high metal concentration is found coupled with a variety of specific mechanisms of resistance and environmental factors. Mechanism of resistance by microorganisms include microbial surface sorption, enzymatic transformation, precipitation by oxidation/reduction reaction and biosynthesis of metal binding proteins or extracellular polymer, whereas environmental factors may include the surrounding pH and redox potential, metal speciation, soil particulates and soluble organic matters (Srinath *et al.*, 2002; Zouboulis *et al.*, 2004). Screened arsenic-resistant bacteria were grown on nutrient agar plates with heavy metals like zinc, nickel and chromium at different concentrations. The results suggested that six isolates were resistance to zinc at 350 mg/L concentration, ten isolates were resistance to nickel at 400 mg/L concentration and one isolate shown resistance to chromium at 20 mg/L concentration. Morphological and biochemical characteristics of all 15 isolates were determined according to Bergey's Manual of Systematic Bacteriology (Krieg, 1984) and results showed that six isolates were Gram positive coccoid-shaped bacteria, two isolates were Gram positive bacilli-shaped bacteria, five isolates were Gram negative rod-shaped bacteria and two isolates were Gram negative coccoid-shaped bacteria. The variable carbon source utilization pattern was found among the arsenic resistant bacteria as given in Table 2. Twenty bacterial isolates were found to tolerate 1000 mg/L of sodium-arsenate in nutrient agar medium and

Table 2: Biochemical Characteristics of arsenic resistant bacterial isolates

Isolates	Biochemical characteristics									
	Gram stain	Cell shape	Motility	Citrate Utilization	Starch Hydrolysis	Catalase Test	Casein Hydrolysis	Lactose	Sucrose	Mannitol
AR-1	-	Cocci	-	-	+	-	-	-	+	-
AR-2	-	Rod	-	+	-	-	+	-	+	-
AR-3	+	Cocci	+	+	-	+	-	-	-	+
AR-4	+	Cocci	+	-	+	+	-	+	+	+
AR-5	+	Cocci	-	+	+	+	+	-	+	+
AR-6	+	Cocci	+	-	-	+	-	-	-	+
AR-7	+	Cocci	-	+	-	+	-	-	-	+
AR-8	-	Rod	+	-	+	+	-	-	-	-
AR-9	-	Cocci	+	+	-	+	-	-	-	-
AR-10	-	Rod	+	-	+	+	+	-	+	-
AR-11	-	Rod	-	-	-	+	-	-	-	-
AR-12	+	Cocci	+	-	+	+	-	-	-	+
AR-13	+	Bacilli	+	-	+	+	-	-	+	+
AR-14	+	Bacilli	-	+	-	+	-	-	+	+
AR-15	-	Rod	-	-	-	+	-	-	-	-

+= Positive - = Negative

out of these; fifteen isolates were able to grow with the addition of 15,000 mg/L of sodium-arsenate. The isolates do not produce extracellular polymeric-like substances to remove large amount of arsenate from the cultivation medium are probably due solely to the cellular absorption mechanism. In order to fully appreciate the arsenic remediation potential of these isolates, further studies on the use of viable or non-viable biomass as adsorbing material, optimizing the bio-adsorption conditions, the possible recycling of this adsorbing material, and optimizing of the adsorbing and desorbing conditions need to be investigated.

REFERENCES

Krieg, R.N. (1984) Bergey's Manual of Systematic Bacteriology. Williams & Wilkins, Baltimore, pp. 837-942.

Singh, PRS., Mishra, SK and Mahadevan, A (2001) Detection and analysis of chromosomal arsenic resistance in *Pseudomonas fluorescens* strain MSP3. *Biochemical Biophysical Research Commun*ications **280**: 1393– 1401.

Srinath, T, Verma, T, Ramteke, PW and Garg, S K (2002) Chromium (IV) biosorption and bioaccumulation by chromate resistant bacteria. *Chemosphere.* **48**:427-435.

Suresh K, Prabagaran S.R, Sengupta, S and Shivaji S (2004) *Bacillus indicus* sp. nov., an arsenic-resistant bacterium isolated from an aquifer in West Bengal, India. *International Journal of Systematic and Evolutionary Microbiology* **54**: 1369–1375.

Wang, S. and Mulligan CN (2006) Occurrence of arsenic contamination in Canada: sources, behavior and distribution. *Science of Total Environment* **366**: 701-721.

Zouboulis, AI, Loukidou, MX and Matis, KA. (2004) Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils. *Process Biochemistry*. **39**: 1-8.