Indian Journal of Biotechnology
Vol 16, January 2017, pp 106-109

A study on phosphate uptake by *Acinetobacter* sp. in presence of arsenate under aerobic condition

Asha Lata Singh* and Vipin K Singh

Bioremediation Laboratory, Department of Botany, Banaras Hindu University, Varanasi 221 005, India

Received 7 February 2015; revised 14 November 2015; accepted 22 November 2015

Phosphate is the main hindrance for the removal of arsenic from the arsenic contaminated waste water. Therefore, phosphate removal from contaminated water has become imperative for the successful removal of arsenic. In the present study, an attempt was made to remove phosphate from the waste water by *Acinetobacter* sp. in the presence and absence of arsenate. When phosphate (25 ppm) containing synthetic solution was treated with *Acinetobacter* sp. at pH 6 at ambient temperature under aerobic condition, the bacterium was able to remove 71.88% (17.97 ppm) phosphate. However, in the presence of arsenate (5 ppm), only 54.24% (13.56 ppm) phosphate uptake was observed from the waste water by *Acinetobacter* sp. Thus the presence of arsenate (5 ppm) inhibited phosphate uptake by 17.64%. The phosphate uptake by *Acinetobacter* sp. follows the Michalis-Menten kinetics. In the presence and absence of arsenate, the maximum velocity (V_{max}) of phosphate uptake was 1.07 and 1.03 μM mg^{-1} h^{-1}; while the kinetic constant (K_m) was 1.13 and 0.37 mM, respectively. Consequently, arsenate was observed as competitive inhibitor for the phosphate uptake. The data thus underlines the significance of *Acinetobacter* sp. for the removal of phosphate along with arsenate.

Keywords: *Acinetobacter* sp., arsenate, phosphate, waste water

Introduction

Arsenic contaminated ground water is one of the major threats for human health. Arsenic exists in the aquatic environment in two forms, *viz*., arsenite [As(III)] and arsenate [As(V)]. The former is more toxic than latter by its methylated forms. Its presence in the ground water more than permissible limit (0.01 ppm)[1] causes serious diseases like eczema of skin/mucous membranes, hyperkeratosis of palms and soles, warts, leukemia, acute renal failure, encephalopathy, sensory disorders, neuropathy and cancer, rhinopharyngitis, pulmonary insufficiency, interstitial fibrosis, melanosis, hypertension and peripheral vascular disease, Klinefelter syndrome[2-7]. Thus arsenic acts as a silent killer. In arsenic polluted water, phosphate is found in abundace[8]. Arsenate, being an analogue of phosphate, employs the same transport pathway as phosphate does and consequently checks the phosphate uptake[9]. Subsequently, arsenate is transported through the phosphate transport system and partially blocks protein synthesis of microbes and consequently inhibits phosphate uptake[10]. Further, free-phosphate favors the arsenic toxicity in ground water. This is mainly due to competition between phosphate and arsenic for sorption on iron in ground water[11]. Thus phosphate removal from contaminated water is imperative for the successful removal of arsenic.

Though several physico-chemical methods are available for the removal of phosphate from ground water but they produce secondary sludge and are, therefore, not ecofriendly[12-14]. Now-a-days bacterial tools have been used as the best source for removal of phosphate from the contaminated water[15]. In the present investigation, *Acinetobacter* sp. was isolated from the arsenic contaminated water and its potential was tested for the removal of phosphate in the presence and absence of arsenate.

Materials and Methods

Isolation and Identification of Bacteria

Ground water samples have been collected from arsenic and phosphate contaminated ground water from Ballia district, Uttar Pradesh, India. The ground water (1 mL) was diluted 100 times with double distilled water and inoculated on solid agar medium of composition: glucose (1%), peptone (0.5%) and yeast extract (0.5%), at ambient temperature. Colonies developed on solid agar plates within 24 h were picked up and inoculated separately in liquid medium. Several individual colonies were checked for its

*Author for correspondence:
Tel: +91-9935658465
ashalata65bhu@rediffmail.com

capacity to remove arsenic and phosphate. The most efficient bacterial colony was selected and mass cultivated for the removal of phosphate and arsenate from ground water. The selected bacterium was identified as *Acinetobacter* sp. (MTCC No.10497) from the Institute of Microbial Technology, Chandigarh, India.

Preparation of Stock Solution

Stock solutions of arsenic and phosphate were prepared, following standard rocedures using sodium arsenate (Na_2HAsO_4) and dipotassium hydrogen phosphate anhydrous (K_2HPO_4), Merck, India.

Phosphate Uptake by Bacterium

Bacterial cells were harvested at mid exponential phase by centrifugation at 12,000 rpm for 5 min and suspended in phosphate of different concentrations, *viz*., 5, 10, 15, 20, 25 ppm, containing growth medium of composition: glucose (1%), peptone (0.5%) and yeast extract (0.5%). In order to study uptake of phosphate under acidic, neutral and alkaline conditions, experiments were carried out at pH 6, 7 and 8 using 5 mg dry wt/mL of bacterium. Subsequently, 500 µL samples were drawn regularly at 12 h interval, centrifuged and the supernatant was discarded. Cellular phosphate concentrations were determined by testing the pellets with a standard method[16]. Samples were analyzed by Spectrophotometer at 660 nm wavelength (OD_{660}).

Removal of Arsenate by Bacterium

Synthetic solution of 1 to 5 ppm arsenate was prepared in distilled water. *Acinetobacter* sp. (biomass 15 mg dry wt/mL) was harvested at mid exponential phase and treated with synthetic solution of 1 to 5 ppm arsenate for 90 min at pH 6. 1 mL sample was drawn at regular 15 min interval, centrifuged and supernatants were analyzed for arsenic content with the help of Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer AAnalyst 800 model).

Phosphate Uptake by Bacterium in Presence of Arsenate

Bacterial cells (15 mg dry wt/mL), harvested at mid exponential phase, were treated with 25 ppm of phosphate and arsenate (1, 2, 3, 4 & 5 ppm) containing solution to study the interaction of arsenate with the site of phosphate transport. Subsequently, sample of 500 µL was drawn at 10 h time intervals up to 48 h, centrifuged and supernatant solutions were analyzed for phosphate following the standard method[16].

Results and Discussion

Analysis of arsenic contaminated ground water samples, collected from Ballia, showed the presence of 25 ppm phosphate. Further, phosphate removing bacterium was isolated from the same contaminated ground water and identified as *Acinetobacter* sp. Phosphate uptake by *Acinetobacter* sp. was carried out at neutral, alkaline and acidic pH. The maximum uptake of phosphate (4.15 ppm) was observed at pH 6 as compared to pH 7 and pH 8 (Fig. 1). Therefore, further experiments for phosphate removal were carried out at pH 6 only. The concentration dependence of phosphate uptake by *Acinetobacter* sp. (15 mg/mL) was studied in the synthetic solutions of phosphate at concentrations of 5, 10, 15, 20 and 25 ppm for 48 h. From the respective concentrations (5, 10, 15, 20, 25 ppm) of synthetic phosphate solutions, the maximum removal of phosphate was observed upto 4.39, 6.72, 9.28, 12.87 and 17.97 ppm within 24 h. However, no significant increase in uptake was observed beyond 24 h (Fig. 2). The results clearly show that the uptake of phosphate increased with the increase in phosphate concentration of the solution and thus removal of phosphate by *Acinetobacter* sp. was concentration dependent[17].

In the presence of arsenate (1, 2, 3, 4 & 5 ppm), phosphate uptake by *Acinetobacter* sp. was observed upto 16.33, 15.68, 14.89, 14.14 and 13.56 ppm from the 25 ppm of synthetic phosphate solutions within 24 h. Beyond 24 h, however, no significant uptake of phosphate was observed (Fig. 3). Thus *Acinetobacter* sp. was efficient to remove 71.88% (17.97 ppm) phosphate from 25 ppm phosphate containing synthetic solution at pH 6 at ambient temperature under aerobic condition. However, in the presence of 5 ppm arsenate, only 54.24% (13.56 ppm) phosphate uptake was observed from the synthetic solution by *Acinetobacter* sp. Comparison of phosphate uptake by *Acinetobacter* sp. in the presence and absence of arsenate clearly showed that cellular phosphate uptake was reduced 17.64% in the presence of 5 ppm arsenate (Figs 2 & 3).These results are in agreement with the experimental data of *Escherichia coli*[18], *Streptococcus faecalis*[9] and *Neurospora crassa*[19]. The phosphate transport in bacteria occurs by two distinct systems. In one system, inorganic phosphate transport (Pit) occurs under low affinity and low selectivity for ATP and operates only at high phosphate concentration. The second system is a specific transport system (Pst)[18]. It has high affinity and high selectivity for ATP and operates at low phosphate and

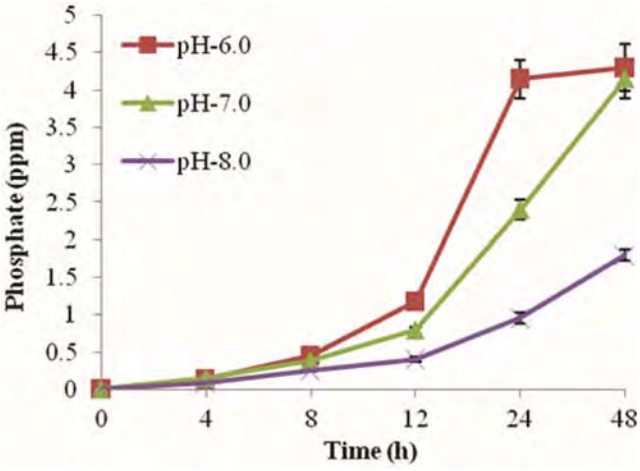

Fig. 1 — Effect of pH on phosphate removal by *Acinetobacter* sp. (5 mg dry wt/mL).

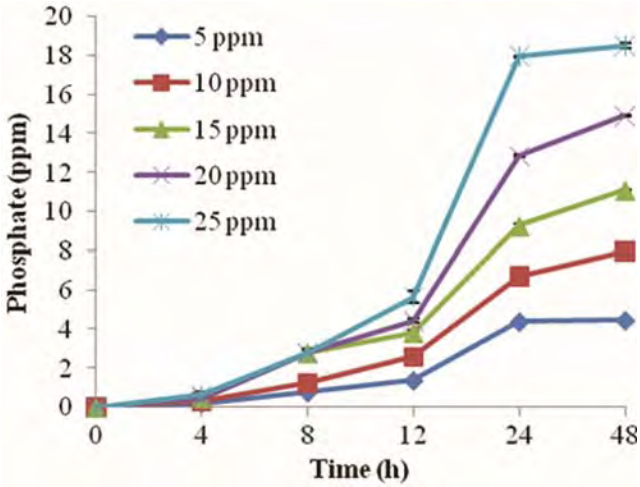

Fig. 2 — Phosphate uptake by *Acinetobacter* sp. (15 mg dry wt/mL).

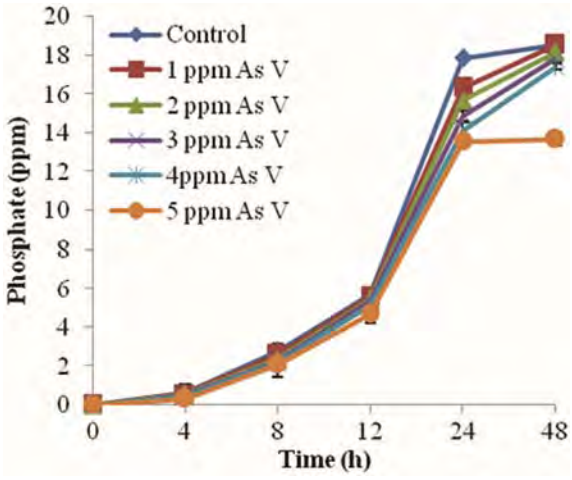

Fig. 3 — Phosphate uptake in presence of arsenate by *Acinetobacter* sp. (15 mg dry wt/mL).

high arsenate concentration[18,20]. As observed in arsenate bound enzyme structures, the arsenate has the same binding mechanism as that in the case of phosphate. Arsenate being an inhibitor of oxidative phosphorylation depletes the ATP pool[18]. The inhibition of Pi (niorganic phosphate) transport by arsenate has been well documented in bacteria, in which arsenate resistance has been used to select mutants defective in Pi transport[20-24]. This is due to interaction of arsenate with phosphate site due to their similar transport site. Since *Acinetobacter* sp. was isolated from arsenic contaminated water, therefore, it was also found efficient for the arsenate removal, which could be due to its resistant character. In an experiment it was observed that *Acinetobacter* sp. could remove arsenate respectively up to 2.67 ppm within 15 min with 15 mg dry wt/mL biomass (Fig. 4). Thus *Acinetobacter* sp. can be used for the removal of both phosphate and arsenate from contaminated ground water.

Kinetic parameters for phosphate transport in the presence and absence of arsenate by *Acinetobacter* sp. were determined. The data pertaining to kinetics of phosphate obtained are furnished in Table 1. The results show that the maximum velocity (V_{max}) for phosphate uptake in the presence of arsenate was

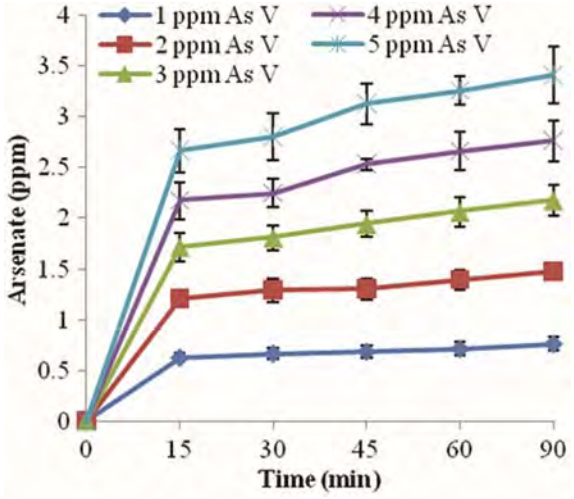

Fig.4 — Arsenate removal in absence of phosphate by *Acinetobacter* sp. (15 mg dry wt/mL).

Table 1 — Kinetic parameters of phosphate transport in absence and presence of 5 mg/L arsenate by *Acinetobacter* sp.

Phosphate transport in absence of arsenate	
V_{max} (µM of phosphate mg^{-1}fresh wt h^{-1})	1.034
K_m (mM phosphate)	0.37
Phosphate transport in presence of arsenate	
V_{max} (µM of phosphate mg^{-1}fresh wt hr^{-1})	1.068
K_m (mM phosphate)	1.13

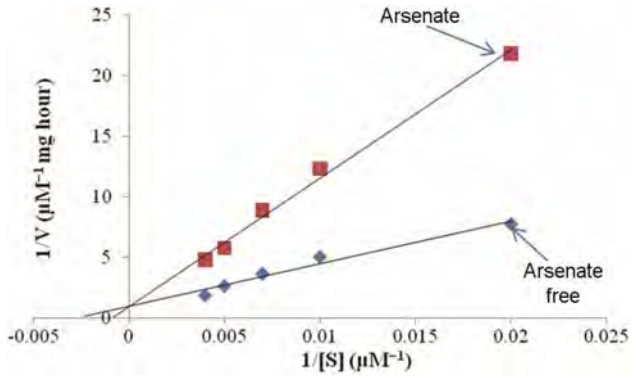

Fig. 5 — Kinetics of phosphate transport by *Acinetobacter* sp. in absence and presence of arsenate

1.07 μM mg^{-1} h^{-1}, while V_{max} in the absence of arsenate was 1.03 μM mg^{-1} h^{-1}. The kinetic constant (K_m) in the presence and absence of arsenate was observed to be 1.13 and 0.37 mM, respectively (Table 1). Thus the phosphate uptake by *Acinetobacter* sp. follows the Michalis-Menten kinetics. The Lineweaver-Burk plot was also drawn by taking 1/S phosphate in the presence and absence of arsenate *vs* 1/V phosphate uptake. The plot shows the competitive inhibition (Fig. 5) of phosphate uptake by arsenate in *Acinetobacter* sp.

Conclusion

Acinetobacter sp. (15 mg dry wt/mL) was found efficient for the removal of 17.97 ppm phosphate within 24 h. However, phosphate uptake decreased (13.56 ppm) in the presence of arsenate. Since *Acinetobacter* sp. was isolated from arsenic and phosphate contaminated water, it was found competent to remove 2.67 ppm arsenate. Thus *Acinetobacter sp.* can be used for the removal of phosphate in the presence of arsenate.

Acknowledgement

The authors (ALS & VKS) thankfully acknowledge the Head, Department of Botany, Banaras Hindu University, for extending the necessary facilities. The financial assistance (Project No. P-01/572) provided by University Grant Commission, New Delhi is also thankfully acknowledged.

References

1 WHO, *Guidelines for drinking water quality: Recommendations*, vol 1, 2nd edn (World Health Organization, Geneva) 1993, p 188.

2 WHO, *International programme on chemical safety (IPCS)—Environmental health criteria 8, Arsenic* (World Health Organization, Geneva) 1981.

3 Katsoyiannis I A, Zouboulis A I & Jekel M, Kinetics of bacterial As(III) oxidation and subsequent As(V) removal by sorption onto biogenic manganese oxides during groundwater treatment, *Ind Eng Chem Res*, 43 (2004) 486-493.

4 Hossain S K M & Anantharaman N, Studies on arsenic(III) biosorption using *Thiobacillus ferrooxidans*, *Indian J Environ Prot*, 25 (2005) 76-80

5 Chakraborti D, Samanta G, Mandal B K, Chowdhury T R, Chanda C R *et al*, Calcutta's industrial pollution: Ground water arsenic contamination in a residual area suffering of people due to industrial effluent discharge—An eight year study report, *Curr Sci*, 74 (1998) 346-355.

6 Singh A L, Singh V K & Srivastava A, Effect of arsenic contaminated drinking water on human chromosome: A case study, *Indian J Clin Biochem*, 28 (2013) 422-425.

7 Singh A L & Sarma P N, Removal of arsenic(III) from waste water using *Lactobacillus acidophilus*, *Bioremed J*, 14 (2010) 92-97.

8 Kish M M & Viola R E, Oxyanion specificity of aspartate-β-semialdehyde dehydrogenase, *Inorg Chem*, 38 (1999) 818-820.

9 Harold F M & Baarda J M, Interaction of arsenate with phosphate transport system in wild type and mutant *Streptococcus faecalis*, *J Bacteriol*, 91 (1966) 2257-2262.

10 Budd K & Craig R, Resistance to arsenate toxicity in the blue-green algae *Synechococcus leopoliensis*, *Can J Bot*, 59 (1981) 1518-1521.

11 Stephan J, Hug O X L & Berg M, Bangladesh and Vietnam: Different groundwater compositions require different approaches to arsenic mitigation, *Environ Sci Technol*, 42 (2008) 6318-6323.

12 De-Bashan L E & Bashan Y, Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997-2003), *Water Res*, 38 (2004) 4222-4246.

13 Donnert D & Salecker M, Elimination of phosphorus from municipal and industrial waste water, *Water Sci Technol*, 40 (1999) 195-202.

14 Bitton G, *Wastewater microbiology* (John Wiley and Sons, Inc., New York, USA) 1994, 63-65.

15 Singh A L, Singh V K, Singh S K & Tiwari S P, Arsenic contamination of ground water and its possible remedial measures, in *Recent advances in microbiology*, volume 1, edited by S P Tiwari, R Sharma and R K Singh (Nova Science Publishers, Inc., Jaunpur, India) 2012.

16 Fiske C H & Subbarow Y, The colorimetric determination of phosphorus, *J Biol Chem*, 66 (1925) 375-400.

17 Singh A L, Asthana R K, Srivastava S C & Singh S P, Nickel uptake and its localization in a cyanobacterium, *FEMS Microbiol Lett*, 99 (1992) 165-168.

18 Willsky G R & Malamy M H, Characterization of two genetically separable inorganic phosphate transport systems in *Escherichia coli*, *J Bacteriol*, 144 (1980) 356-365.

19 Burns D J & Beever R E, Kinetic characterization the two phosphate uptake systems in the fungus *Neurospora crassa*, *J Bacteriol*, 132 (1977) 511-519.

20 Rao N N & Torriani A, Molecular aspects of phosphate transport in *Escherichia coli*, *Mol Microbiol*, 4 (1990) 1083-1090.

21 Bennett R L & Malamy M H, Arsenate resistant mutants of *Escherichia coli* and phosphate transport, *Biochem Biophys Res Commun*, 40 (1970) 496-503.

22 Harold F M and Spitz E, Accumulation of arsenate, phosphate and aspartate by *Streptococcus faecalis*, *J Bacteriol*, 122 (1975) 266-277.

23 Mitchell P, Transport of phosphate across the osmotic barrier of *Micrococcus pyogenes*: Specificity and kinetics, *J Gen Microbiol*, 11 (1954) 73-82.

24 Rosenberg H & La Nauze J M, The isolation of a mutant of *Bacillus cereus* deficient in phosphate uptake, *Biochim Biophys Acta*, 156 (1968) 381-388.