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## RESEARCH ARTICLE

# ENIGMATIC BEHAVIOR OF *BACILLUS LICHENIFORMIS* DAS-2 IN MITIGATING THE ARSENIC (AS) CONTAMINATED REGION OF EASTERN INDIA

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### ABSTRACT

Bihar state (Part of eastern India) is a part of Bengal Delta located at 85° 32' E and 25° 11' N latitude of the earth. This region is less industrialized and 70% of the people are dependent for their livelihood on agriculture. The major source of irrigation is contaminated underground water, so form of Arsenic passes through food chain to human beings. This is through both primary and secondary production. Chakraborti et. al., 2004 had reported adverse effect of Arsenic in the form of skin lesion and neurological disorder from the people of Simaria ojha-patti(a village) of Bihar state. *B.licheniformis* DAS-2 also degrades the different types of waste product and shows both reductase as well as keratinolytic activity. The above enigmatic property of this rhizospheric bacteria is that it converts more toxic form of pentavalent Arsenic (AsV) such as arsenate to less toxic form of trivalent Arsenic (AsIII) called as arsenite because it secretes an enzyme arsenate reductase. Although this bacterial strain also shows keratinolytic properties. It degrades the the keratin containing animal waste product such as feather, hairs, hoofs, Nails etc. Since keratin protein is essential for the making cosmetics, leather industry. Feather waste is a major animal food stuff polluted the environment that is degraded by *B.licheniformis* DAS-2. Eventhough it is mesophilic, gram positive, nonpathogenic, rhizospheric in nature but it shows above unique properties. So this is the novel characteristics of this bacterial strain. Soil with long term exposure of As (V) and As (III) may result in this evolution of diverse arsenic resistant strains. Relative abundance of different form (III and V) in the soil is influenced by microbial transformations. Transformation of arsenic in different chemical species might be the best survival strategy of soil bacteria in arsenic contaminated environment. So it may concluded that this bacterial strain involved in enigma of toxic environment.

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## INTRODUCTION

*B.licheniformis* DAS-2 is mesophilic, gram positive, nonpathogenic, rhizospheric in nature but it shows above unique properties. So this is the novel characteristics of this bacterial strain. Soil with long term exposure of As (V) and As (III) may result in this evolution of diverse arsenic resistant strains. Relative abundance of different form (III and V) in the soil is influenced by microbial transformations. In eastern India reported as the most arsenic contaminated region on the Earth, due to the more toxicity of arsenic in ground water, where 36 million people are exposed to the risk of arsenic contamination (Nordstrom, 2002). Bihar state is a part of Bengal Delta located at 85° 32'E longitude and 25° 11'N latitude on the Earth. Majority of the population of Bihar depends upon agriculture for their livelihood. They are getting polluted with arsenic, due to bio-magnified level of arsenic in their food, via irrigation water. Arsenic has been reported as carcinogenic (Rosen, 1971) and has a wide range of adverse health effects including skin lesions (Chakraborti et al., 2004)

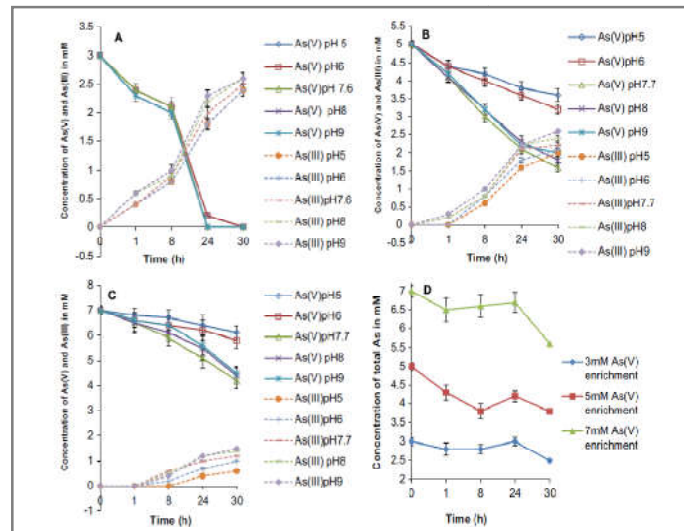
and neurological disorders. The sources of arsenic are both natural and anthropogenic. Inorganic arsenic occurs in various oxidation states including As (V) [arsenate], As(III) [arsenite], elemental arsenic (0) and As(III) arsenide. Among them trivalent and pentavalent arsenic are most common forms but, the trivalent form is most toxic. Transformation of arsenic in different chemical species might be the best survival strategy of soil bacteria in arsenic contaminated environment. So it may concluded that this bacterial strain involved in enigma of toxic environment. The resulting products of hydrolysis of keratin have also proven that, it is useful for the production of fertilizer, cosmetics, biomedical (decontamination), textiles (fibers modification), bioactive hydrolysates (Brandelli et al., 2010; Brandelli et al., 2015). Present study might be the new report on one rhizospheric bacterium *B.licheniformis* DAS-2, showing capacity of biodegradation of feather wastes.

## MATERIALS AND METHODS

*B.licheniformis* DAS-2 isolated from the rhizosphere of a wild plant for the above usage and identified by 16sr RNA. The

nucleotide sequences of isolate have been deposited in Gen Bank under the accession number KF664028. Cell cultures were taken at different time intervals during lag, log and stationary phases (from 0 to 32h). Optical density (OD) was measured at 600nm. Quantification of As(V) and As(III) was done by digesting the residual media/cell biomass by nitric acid method (APHA, 2005; Tripti *et al.*, 2014) and estimated the arsenic by azureB method (Cherian and Narayana, 2005) with some modifications (Tripti *et al.*, 2014). Biochemical analysis for measuring chemical toxicity (Dehydrogenase activity) was done by Resazurin test (Liu, 1981). Purification of extracellular keratinases by ammonium sulphate and SDS-PAGE.

## RESULT AND DISCUSSION



Removal of As(V) from the growth media and reduction of As(V) to As(III) by *Bacillus licheniformis* DAS-2. Y-Axis represents the concentration of As(V) and As(III) in residual media at different time point (X-axis) of growth phase at different concentration of As(V) enrichment (A) 3mM As(V), (B) 5mM As(V), (C) 7mM As(V) and (D) showing total arsenic in residual media at different time point of growth phase at different concentration of As (V) enrichment. All values are mean of three replicates and standard errors (SE) are presented as error bars (7).



Degradation of white chicken feather after Bacterial incubation.

## Conclusion

*Bacillus licheniformis* DAS-2 is a native soil bacteria which was isolated from the arsenic contaminated region located at 85° 32' E longitude and 25° 11' N latitude on the Earth. It is unique that the bacterium had shown the capability to tolerate both As(V) [MIC 8 mM] and As(III) [MIC 6 mM]. The bacteria had also removed/uptaken good enough amount of As(V) and As(III) (i.e. 100%) from the growth medium particularly at the lower concentration of arsenic enrichment. As(III) was determined in the same media which was previously enriched by As(V) only, along with concentration of As(V) was found decreasing from the media. This phenomenon signified transformation/reduction of uptaken/removed As(V) into As(III) which might be one of the survival strategy of *B. licheniformis* DAS-2 to tolerate arsenic toxicity. Results support the conclusion that enigma of the arsenic toxicity in *B. licheniformis* DAS-2 which can play better role in amelioration of arsenic contamination.

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