larvae on polluted mulberry leaves. (ii) Plantation of mulberry plants in fields away from the roadside, where the risk of automobile pollution is less. (iii) Looking for silkworm races which are least affected by feeding them with polluted mulberry leaves. (iv) Making the farmers aware about the ill-effects of rearing silkworm larvae on polluted mulberry leaves. (v) Strengthening the industry at the gross root level by encouraging unemployed youth to take it up as a profession. (vi) Upgradation of the existing infrastructure to national and international levels to make it more promising. (vii) Introduction of better techniques to control the occurrence of microbial diseases which are posing a risk to the survival of this vital industry in the valley.

The effect of automobile pollution on the rearing of silkworm larvae is well documented. Future studies should encompass the toxicological effects of heavy metals on silkworm pathology with specific reference to the immunological degradation caused by these toxic elements when they enter the body of the silkworm via the mulberry leaves. The immunological disruption caused by these xenobiotic compounds can further aggravate the susceptibility of silkworm larvae towards various microbial infections. Various promising steps need to be taken to address the debacle of this vital industry in the Kashmir valley. A systematic approach is the need of the hour to bestow the industry with its lost grandeur. Prompt actions need to be taken to boost the potential of this industry to play a major role in the economy of the state.

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Siderophores of haloalkaliphilic archaea from Lonar lake, Maharashtra, India

Under iron-limiting conditions, most prokaryotes, certain fungi and some monocotyledonous plants are known to produce low molecular weight (often <1000 Da), high-affinity chelating agents that solubilize, by sequestering ferric ion from the environment and transporting it into the cells[1,2] through specific receptors[3,4]. These molecules are known as siderophores and are typically found in iron-deficient cultures[5]. The name siderophore derives from the Greek for 'iron carrier'. Siderophore production by bacteria is considered an important component of bacterial machinery for iron sufficiency and is likely to be more important for survival and growth in a competitive soil environment usually deficient in soluble iron[6,7]. Siderophores are produced as free ligands in cell cultures or in the natural environment, where they preferably bind to Fe^{3+}. However, they also exhibit affinity to other metals.

Historical development of siderophore studies so far has shown that some bacteria and fungi can produce more than one type of siderophore[4,8]. It has likewise been shown that a broad range of structural variations exist within different siderophore classes[3,7]. In 1979, Armstrong and van Baalen[9] isolated the first hydroxamate type of siderophore from the culture of *Agmenellum quadruplicatum* PR6 (*Synechococcus* PCC7002). Over the past decade, several marine siderophores from cultured organisms have been structurally characterized.

As very little is known about assimilation of iron in archaea, the present study was undertaken to catalyse siderophore production by six haloalkaliphilic archaea isolated from Lonar Lake, a saline–alkaline meteorite impact crater lake in Buldhana district, Maharashtra, India (lat. 19°58′; long. 76°36′). All the six organisms are extreme haloalkaliphiles with a minimum salt (NaCl) requirement of 20% and optimum pH 8.0–9.0.

Screening for siderophores was done with the CAS (chrome azural S) test, performed by incorporating the dye in solid Tindal medium on which the cultures were spot inoculated and incubated at 40°C for 4 h. The transformation of blue to yellow colour[10,11] around the growth of organisms threw up four isolates, *Natrinema* sp. SSBJUP-1, *Natrialba wudunaoensis* SSBJUP-2, *Natrialba chahannaoensis* SSBJUP-3 and *Natronobacterium innermongoliae* SSBJUP-4 as producing siderophores.

It must be noted here that glassware used in all the studies was decontaminated of all traces of iron by soaking

Table 1. Chemical characterization of siderophores

Test organism	Peak at (420–450 nm)	Csaky test	Tetrazolium test
Natrinema sp. SSBJUP-1	+	+	+
Natrialba wudunaoensis SSBJUP-2	+	+	+
Natrialba chahannaoensis SSBJUP-3	+	+	+
Natronobacterium innermongoliae SSBJUP-4	+	+	+

+, Positive.

Table 2. Determination of mono-, di- and trihydroxamate type of siderophores

Test organism	pH	λ_{max} (nm)	λ_{max} shift (nm)	Type
Natrinema sp. SSBJUP-1	4	430	4	Trihydroxamate
	5	432		
	6	429		
	7	431		
	8	433		
	9	433		
N. wudunaoensis SSBJUP-2	4	421	5	Trihydroxamate
	5	425		
	6	423		
	7	426		
	8	426		
	9	421		
N. chahannaoensis SSBJUP-3	4	439	6	Trihydroxamate
	5	433		
	6	436		
	7	435		
	8	434		
	9	435		
N. innermongoliae SSBJUP-4	4	423	3	Trihydroxamate
	5	424		
	6	425		
	7	423		
	8	422		
	9	425		

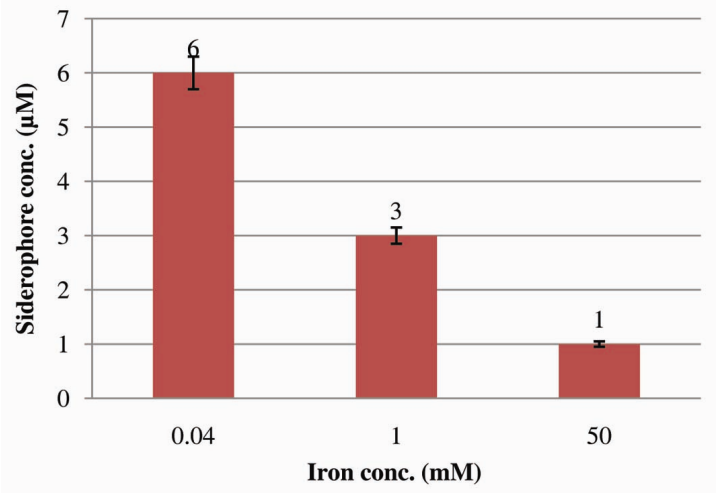

Figure 1. Siderophore production under increasing concentration of Fe^{2+} as determined by Atkin test – specific for hydroxamate-type siderophores. Desferrioxamine B was used as a standard. Results are the means of three independent experiments.

overnight in 6M HCl and rinsed several times with distilled water.

The isolates were subjected to siderophores production in TYES and Fiss glucose mineral medium supplemented with 20% NaCl and pH 8.0. The medium taken in Erlenmeyer flasks was previously decontaminated of Fe^{3+} ions with 8% hydroxyquinoline in chloroform and sterilized. The isolates were inoculated in cell densities of 10^6 cells/ml (count standardized microscopically in Neubauer chamber) in each flask and incubated at the optimum temperature of 40°C and tested for siderophore production on days 3, 4, 6, 8, 13, 15 and 16. Cells were removed by centrifugation at 10,000 rpm for 15–20 min and the supernatants subjected to determination of absorption maxima and λ_{max} shift on a UV–VIS spectrophotometer (model Systronics 119), and chemical analysis by Csaky, Tetrazolium and Arnow tests[12]. All the siderophores were found to be of the hydroxamate type, as confirmed by the absorption maxima (420–450 nm), and positive Csaky and tetrazolium tests (Table 1). Catecholate and carboxylate types of siderophore were ruled out through negative Arnow's test (data not shown). They were further confirmed to be tri-hydroxamates on the basis of λ_{max} shift results in relation to pH change (Table 2).

The previous reports on siderophores from halophilic and halophilic alkaliphiles such as *Natrialba* sp.[13] and *Halomonas campisalis*[14] indicate the production of carboxylate and simple ferrioxamine types of siderophore. The production of only the hydroxamate type by an organism that thrives under such extreme conditions of salinity and alkalinity is shown in the present study.

Siderophores are often conjugates of amino acids. Hence the siderophores were also analysed for amino acid composition after acid hydrolysis (heating in 6N HCl) followed by thin layer chromatography on 40% silica gel G and a solvent system of methanol : ammonium acetate (60 : 40) using ninhydrin as indicator, and found to comprise of three amino acids – tryptophan, tyrosine and lysine.

Tests for siderophore production in iron-sufficient conditions showed results along expected lines[15], wherein the production declined as iron sufficiency was increased in the medium (Figure 1). One hundred millilitre aliquots of TYES mineral medium amended with 20% NaCl and pH 8.0, and supplemented with various iron concentrations as 0.04 (iron stress), 1.0 and 50 mM prepared from 1 g $FeCl_3$ in 15% HCl (Merck) were used for this experiment. The aliquots were each inoculated in cell densities of 10^6 cells/ml and incubated at the optimum temperature of 40°C and tested for siderophore production by the Atkin test for hydroxamate siderophores using desferrioxamine B as standard.

Ferrioxamine types of siderophore have been shown to form stable complexes with toxic heavy metals and radionuclides. Such complexes of environmental contaminants with microbial siderophores could potentially be useful in bioremediation and biomineralization, and could prove more potent than existing models. Further characterization, however, of the siderophores produced by haloalkaliphiles is needed to adequately address the impact that these organisms would have on metal contaminant mobility in saline and alkaline environments.

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