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Actinobacteria of Magnesite Mines Habitat: Diversity, Siderophore Production and Its Antagonistic Potential

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Abstract: The magnesite mines habitats in the world can be considered as an inexhaustible resource for biotechnology that has not been well exploited. In the present study, eight soil samples were collected from magnesite mines located in Chalk Hills region of Salem, South India for the isolation of actinobacteria. Actinobacterial count of magnesite soil ranged from 3.0 to 16 x 10^3 cfu/g of dry soil. Fifteen actinobacterial strains were selected from the total isolates based on their cultural characteristics and were screened for siderophore production, antibacterial activity and enzyme activity. All actinobacterial isolates produced siderophores in Iron free succinate agar medium (pH 8.0) as evidenced by positive reaction in O-CAS assay. Among the actinobacterial isolates tested for antagonism, strain A-6 showed broad spectrum of activity against test bacterial pathogens. Screening for enzyme activity revealed that 100% of isolates showed amylase and L-glutaminase activity, followed by 93.33% L-asparaginase and protease, 66.66% cellulase, and 46.66% lipase activity. Detection of chemical nature of siderophore produced by all the actinobacterial strains revealed them as carboxylates. Quantitative estimation of siderophores by CAS shuttle assay revealed the yield of 10% to 73% siderophore units. Studies carried out *in-vitro* revealed that the antibacterial activity of crude siderophore obtained from the potent actinobacterial strain A-6 was by way of chelation of iron resulting in its unavailability to the test bacterial pathogens. Based on phenotypic characteristics and 16s rRNA gene sequence analysis the potent actinobacterial strain A-6 has been identified as *Streptomyces mutabilis*. Phylogenetic tree was made by neighbor-joining method. The present study is the first report on carboxylate type of siderophore production in actinobacteria isolated from Indian magnesite mines. The *in-vitro* antagonistic action of siderophores of *Streptomyces mutabilis* has given a new focus of attempts to exploit the siderophore systems for the treatment of human infections.

Key words: Magnesite mines, Siderophores, Antagonism, *Streptomyces mutabilis*

I. INTRODUCTION

Iron is an essential micronutrient for almost all organisms in the biosphere, including bacteria, fungi and plants. It plays an essential role in respiration, nitrogen fixation, DNA and chlorophyll biosynthesis and other important enzymatic systems [1]. Although iron is the second most abundant transition metal on earth, the solubility of iron is very low at physiological pH as it forms insoluble stable complexes (10^{-17}M) of ferric oxyhydride (FeOOH), severely limiting the bioavailability of iron [2]. To sequester and solubilise ferric iron many microorganisms utilize an efficient system consisting of low molecular weight (< 1000 Da) compounds with high iron affinity, termed Siderophores [3,4,5]. Microbial siderophores are a structurally and architecturally diverse group of molecules and can be classified on the basis of the chemical structure of the functional group that interacts with Fe (III) in to hydroxamates, catecholate, carboxylates and mixed type [6, 7]. Besides microbial nutrition, siderophores also play a critical role in the expression of virulence and development of biofilms by different microorganisms.

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Siderophores and their substituted derivatives have a large number of applications in medical sciences. The most important application is selective drug delivery, a Trojan horse strategy, to defeat drug resistant bacteria. Microbial siderophores are also used in the treatment of malaria caused by *Plasmodium falciparum*, iron overload diseases like hereditary haemochromatosis (HHC), sickle cell anaemia, thalassemia major and Friedreich's Ataxia. Furthermore, iron chelators such as dexrazoxane, O-trensox, desferriexochelins, desferrithiocin, tachpyridine, have been found useful in cancer therapy [8].

Actinobacteria are a group of gram positive bacteria play a quite important role in natural ecological system and they are also profile producers of siderophores, antibiotics, antitumor agents, therapeutic enzymes, enzyme inhibitors, immune modifiers, antiparasitic agents, herbicides, insecticides and growth promoters [9]. *Streptomyces* is the largest genus of Actinobacteria most commonly produce some type of trihydroxamate siderophore known as desferrioxamine [10] but reports have described isolates capable of producing enterobactin [11] the characteristic siderophore of Enterobacteriaceae, which was not excreted into the external environment but remained in the bacterial biomass. Others have described the *Streptomyces* siderophores coelichelin [12] and griseobactin [13], while the novel heterobactin siderophores have been found in Rhodococcus [14] and Nocardia [15].

Choice of natural materials like soil in researches is based on the assumption that samples from widely diverse locations are more likely to yield novel microorganisms and therefore hopefully, novel metabolites as a result of the geographical variation. The list of novel actinobacteria and products found in microbiologically poor explored areas of India, China and Australia also suggests that a careful exploration of new habitats might continue to be useful [16,17,18,19,20].

Presently, there is little documented information on the biodiversity of actinobacteria and its bioactive potential in magnesite mines. With this view, the present study was focused to produce the siderophores by using actinobacterium, isolated from magnesite mines habitat and to study the antagonistic potential of produced siderophores.

II. MATERIALS AND METHODS

A. Sampling Strategies and Processing

About 500 gram of magnesite soil samples were collected during March 2010 at the depth of 20 cm from eight stations of magnesite mines located in Chalk hills, Salem, South India (latitude 11.73º and longitude 78.13º) by using sterile hand trowel. The distance of sampling stations from each other was 200 metre. The samples were transported in sterile polyethylene bags to the laboratory within 1 hour of collection. Each sample was crushed, thoroughly mixed and sieved through a 2 mm pore size mesh to get rid of large debris [21]. The sieved soils were used for physico-chemical and microbiological analysis.

B. Physico-Chemical Analysis of Soil

Soil electrical conductivity (EC) and pH were determined using the saturation paste extract EC and saturation paste pH methods [22]. Soil organic carbon was estimated using titration method of Walkley and Black [23]. Total nitrogen content was determined following Kjeldahl method [24]. The available phosphorous content in soil samples were estimated using chloro-stannous reduced molybdophosphoric blue colour method [25].

C. Pretreatment of Soil and Isolation of Actinobacteria

The magnesite soil samples were air dried for one week and kept at 45ºC for 1 hour to minimize bacterial contaminants [26]. One gram of the soil sample was aseptically added to 9 ml of sterile buffer consisting of 0.5% KH_2PO_4, 0.5% K_2HPO_4; pH 7.0 [27]. The suspension was vortexed and serially diluted up to 10^{-6} dilution. An aliquot of 0.1 ml from suitable dilution was taken and spread on Starch casein agar medium supplemented with cyclohexamide (100 mg/l) and nalidixic acid (20 mg/l) to inhibit the growth of fungi and bacteria respectively. The pH was adjusted to 8±0.2 prior to autoclaving. The agar plates were incubated at 25ºC to 28ºC under aerobic condition and the colonies

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were observed for 30 days. Morphologically different actinobacteria were preserved both as slant culture in ISP-2 medium and as glycerol stock in 20% glycerol [28].

D. Screening of Actinobacteria for Siderophore Production by O-CAS Assay

Siderophores were detected by using the Overlaid Chrome Azurol Sulphonate (O-CAS) assay [29]. The medium for a litre of overlay as follows: Chrome azurol S (CAS) 60.5 mg, hexadecyltrimethyl ammonium bromide (HDTMA) 72.9 mg, Piperazine - 1,4-bis(2-ethanesulfonic acid) (PIPES) 30.24 g and 1mM $FeCl_3.6H_2O$ in 10 mM HCl 10 ml. Agarose (0.9%) was used as gelling agent. 10 ml of CAS medium was applied over Iron free succinate agar medium (grams/liter: K_2HPO_4 6.0; KH_2PO_4 3.0; $MgSO_4,7H_2O$ 0.2; $(NH_4)_2SO_4$ 1.0; Succinic acid 4.0; Agar 20.0; pH 8±0.2) containing cultivated actinobacterial isolates to be tested for siderophore production. After a maximum period of 15 minutes, siderophore production was indicated by a change in colour from blue to purple/yellow/orange in the overlaid medium.

E. Screening of Actinobacteria for Antibacterial and Enzymatic Activity

Actinobacterial isolates were grown on ISP-2 medium for 14 days. Agar cylinders (5 mm in diameter) were then taken with hollow punch and deposited on the surface of Mueller-Hinton agar plates (Himedia, Mumbai) previously seeded with the following bacterial pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, S*almonella paratyphi* B, *Shigella dysenteriae*, *Shigella sonnei* and *Proteus mirabilis*. Plates were incubated at 37ºC for 24 hours and the zone of inhibition around the plug were examined. Bacterial cultures used in the present study were obtained from Gokulam hospitals (P) Ltd., Salem, India. Actinobacterial isolates were also screened for protease, lipase, amylase, cellulase, L-asparaginase and L-glutaminase enzyme activity by spot inoculation method [30].

F. Production of Siderophores

All the glass wares were soaked overnight in 6 M HCl to remove traces of iron present and rinsed 10 times with distilled deionized water before use. Media used in the present study were also prepared with distilled deionized water. Seed cultures were prepared by growing the actinobacterial isolates on Oat meal agar [31] at 28ºC for 14 days. After incubation, the whole aerial mycelium were scrapped by a sterile inoculation loop and then suspended in 10 ml of sterile distilled deionized water. Aliquots of 2.5 ml of the spore suspension were inoculated in to 500 ml Erlenmeyer flasks containing 250ml of Iron free succinate media [32] consisting of grams/liter: K_2HPO_4, 6.0; KH_2PO_4, 3.0; $MgSO_4.7H_2O$, 0.2; $(NH_4)_2SO_4$, 1.0; Succinic acid, 4.0 and pH adjusted to 8.0 prior to sterilization. The flasks were then incubated at 25ºC to 28ºC under aerobic condition on a rotary shaker (Remi, India) for 7 days. At the end of fermentation, fermentation broths were centrifuged in a refrigerated centrifuge (Remi, India) at 6000 rpm and 4ºC for 15 minutes and the supernatant were subjected to qualitative and quantitative estimation of siderophores and detection of chemical nature.

G. Estimation of Siderophores

Qualitatively siderophore production was detected by Chrome Azurol Sulphonate (CAS) assay [33]. The change in colour from blue to orange after 2-4 minutes on addition of 1 ml of CAS solution to 1 ml of culture supernatant indicated siderophore production. Quantitative estimation of siderophore was done by CAS shuttle assay [34] in which 0.5 ml of cell free culture supernatant was mixed with 0.5 ml of CAS reagent and absorbance was measured at 630 nm against a reference consisting of un inoculated Iron free succinate medium and 0.5 ml of CAS reagent. Siderophore produced was calculated by using following formula,

% Siderophore units = [(Ar - As) / Ar] x 100

Where, Ar is the absorbance of reference and As is the absorbance of sample at 630 nm.

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H. Determination of Functional Groups of siderophores

The chemical nature of siderophore produced was examined for catecholate nature by $FeCl_3$ test [1] and Arnow's test [35] and hydroxamate nature by $FeCl_3$ [1], while carboxylate nature was determined by Spectrophotometric test [36].

I. Determination of Antagonistic Potential of Siderophore by Agar Well Diffusion Method

The cell free culture supernatant of iron free succinate medium inoculated with Actinobacterial strain A-6 was subjected to bioassay against bacterial pathogens by agar well diffusion method. Wells of 8 mm diameter were punched in Mueller-Hinton agar plates seeded with test organisms. Then, 100 µl of crude siderophore was added in these wells. Control wells contained sterile saline. Plates were incubated at 4°C for three hours, followed by 24 hours of incubation at 37°C. The same experiment was repeated using Mueller-Hinton agar incorporated with 10 to 200 mM $FeCl_3$. The zone of inhibition in millimetre diameter were read and taken as the activity against the test pathogens.

J. Taxonomy of Potential Actinobacterial Strain

The potential Actinobacterial strain A-6 was identified based on the following characteristics, viz. aerial mass colour, melanoid pigment, reverse side pigment, spore chain morphology and assimilation of carbon sources, and also by comparing the characteristics with the keys given by Nonomura [37]. The chromosomal DNA of actinobacterial strain A-6 was extracted using GenElute Bacterial Genomic DNA kit (Sigma). The 16s rDNA fragment was amplified by using PCR with *Taq* DNA polymerase and primers 27f (5^1 AGTTTGATCCTGGCTCAG 3^1) and 1492r (5^1ACGGCTACCTTGTTACGACTT 3^1). The conditions for thermal cycling were as follows: denaturation of the target DNA at 94°C for 4 minutes followed by 30 cycles at 94°C for 1 minute, primer annealing at 52°C for 1 minute and primer extension at 72°C for 1 minute. At the end of the cycling, the reaction mixture was held at 72°C for 10 minutes and then cooled to 4°C. PCR amplification was detected by agarose gel electrophoresis and was visualized by UV fluorescence after ethidium bromide staining [38].

The PCR product obtained was sequenced by an automated sequencer (Genetic Analyzer 3130, Applied Biosystem, USA). The same primers as above were used for this purpose. The sequence was compared for similarity with the reference species of bacteria contained in genomic database banks, using the NCBI BLAST available at http://www.ncbi-nlm-nih.gov/. A phylogenetic tree was constructed with the Molecular Evolutionary Genetics Analysis (MEGA) software version 5 using the Neighbor-Joining algorithm [39, 40]. Tree topologies were evaluated by bootstrap analysis based on 1000 resamplings of the neighbour joining data set [41]. The evolutionary distances were computed using the Maximum Composite Likelihood method [42].

III. RESULTS AND DISCUSSION

A. Physico- Chemical and Microbiological Characteristics of Magnesite Mines Soil

Physical, chemical and microbiological characteristics of magnesite mines soil have been represented in Table 1. Soil sample collected from station-1 was found to have sandy clay loam texture, whereas soil of the other stations is of sandy loam texture. Soil EC, pH, N, P and K differed remarkably among eight different sampling stations. Physical and chemical analysis of the soil clearly indicates that the nutrients, pH, temperature and humidity were different from the normal ecosystem. Isolation of Actinobacteria has always been faced with difficulties in comparison to their competitors like other bacteria and fungi [43]. However, the recovery rate of Actinobacteria was increased by pre-treatment of the samples by air drying for 1 week and incubating at 45ºC for 1 hour. Use of Starch casein agar medium supplemented with cyclohexamide (100 mg/l) and nalidixic acid (20 mg/l) was crucial in inhibiting contaminating fungi and bacteria. Total actinobacterial count of magnesite soil was in the range of 3.0 - 16 x 10^3 cfu/g. The survival of low number of actinobacteria in such harsh and challenging habitation might be due to their adaptation to the extreme environment. A total of 15 different actinobacterial colonies having characteristic features such as leathery, powdery

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appearance with convex, concave, or flat surface and colour ranging from white, gray to pinkish and yellowish were selected for the study.

B. Screening of Actinobacteria for Siderophore Production, Antibacterial and Enzymatic Activity

Siderophore production of all the actinobacterial isolates were studied by cultivating in iron free succinate agar medium and detection by Overlaid Chrome Azurol Sulphonate (O-CAS) Assay (Figure1). The actinobacterial isolates were screened for antibacterial activity by agar plug method. Out of fifteen actinobacterial isolates, strain A-6 showed maximum inhibition of 14 mm against *Escherichia coli* and *Proteus mirabilis* respectively, 13 mm against *Klebsiella pneumoniae*, 12 mm against *Staphylococcus aureus*, 11 mm against *Salmonella typhi*, 10 mm against *Shigella dysenteriae* and *Shigella sonnei* respectively. The magnesite soil actinobacterial strain A-6 was selected for its broad spectrum of antimicrobial activity and enzyme activity (Figure 2) for further studies.

C. Estimation and Determination of Functional Groups of Siderophores

The Chrome Azurol Sulphonate (CAS) assay [33] was used to assess siderophore production by actinobacterial isolates during growth in Iron free succinate medium. After 7 days incubation, pH of the culture supernatant was adjusted to neutrality and equal amount of CAS reagent was added to it. A colour change from blue to orange was observed which indicated the presence of siderophores. Un-inoculated Iron free succinate medium was used as a negative control and remained blue in the assay (Table 2). Quantitative estimation of siderophore was done by CAS shuttle assay [34]. Actinobacterial strains grown in iron free succinate medium with pH adjusted to 8.0 was found to yield 10% to 73% siderophore units. Among the fifteen isolates that produced siderophores, maximum siderophore production was seen in Actinobacterial strain A-6. Minimum siderophore yield revealed that the media components were not conducive for the maximum production of siderophores (Figure 3). Detection of functional groups of siderophores indicated that all the fifteen actinobacterial strains produced carboxylate type of siderophores as evidenced by positive Shenker's test and negative for $FeCl_3$ test and Arnow's test (Table 2). Our findings clearly demonstrated the presence of carboxylate siderophores in actinobacteria of magnesite mines habitat. Hydroxamates are produced by bacteria and fungi, catecholates only by bacteria, while novel carboxylate (= complexone) siderophores by fungi belonging to *Mucorales*, bacteria as *Rhizobium meliloti*, *Staphylococcus hyicus*, halophilic archaea [44] and the magnesite soil actinobacteria.

D. Determination of Antagonistic Potential of Siderophores

The antagonistic potential of crude siderophore obtained from Actinobacterial strain A-6 against various target bacterial pathogens is given in Table 3. Two possible explanations can be put forth for siderophore mediated antimicrobial activity: antibiotic like activity and chelation of iron, resulting in its unavailability to test bacterial pathogens. For further confirmation, various iron concentrations were incorporated in agar plates, and the antimicrobial activity of siderophore was checked. With a gradual decrease in the zones of inhibition at 10 mM and 100 mM $FeCl_3$, the inhibition was completely reversed at 200 mM $FeCl_3$ in the medium. This proves that the antagonistic activity displayed by siderophore is by way of starving the test bacterial pathogens of iron. A phenomenon of similar nature regarding antimicrobial activity of enterochelin on *E. coli* and catecholate type siderophore from *Azospirillum lipoferum* M has been reported. The inhibitory effects of siderophores were reversed by a relatively high iron concentration in the medium [45, 46]. It was found that a marine isolate *Citriococcus* sp. KMM3890 produced a bioactive compound which was characterized as cyclic siderophore nocardamine showed antitumour activity and antimicrobial activity against *Bacillus subtilis*, *Enterococcus faecium* and *Staphylococcus aureus* [47]. It has been reported that the crude siderophore produced by yeast *Aureobasidium pullulans* HN 6.2 was able to inhibit cell growth of *Bacillus subtilis*, and two marine animal pathogens, *Vibrio anguillarum* and *Vibrio parahaemolyticus*. When the crude siderophore solution was mixed with excess of Fe^{3+}, it lost the ability to inhibit the growth of test bacterial pathogens [48].

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E. Taxonomy of Potential Actinobacterial Strain

Characterization of potent actinobacterial strain A-6 was carried out by adopting the standard method for characterisation of *Streptomyces* [31]. The strain showed aerial and substrate mycelium with smooth spore surface. Phenotypic characteristics of the strain are given in Table 4. PCR-amplified 16s rDNA of actinobacterial strain A-6 was sequenced and analyzed for the similarities. The NCBI BLAST search program showed that the sequence data of strain A-6 had 99% identity to those of *Streptomyces mutabilis* strain NRRL ISP-5169 with a total score and E value of 2344 and 0, respectively. Based on the results of phenotypic characteristics and 16s rRNA gene sequencing analysis, actinobacterial strain A-6 was identified as *Streptomyces mutabilis.* The 16S rRNA sequence (1286 nucleotides) of actinobacterial strain A-6 was deposited in GenBank, National centre for biotechnological information (NCBI), USA under accession number KF304796. The evolutionary tree was inferred using the Neighbor-Joining method (Figure 5). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 13 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1255 positions in the final dataset.

Table 1: Microbiological and Physico-Chemical Parameters of Magnesite Mines Soil Samples

Sampling Station Code	Total Actinobacterial Count X 10^3/g Dried Soil	Physico-Chemical Parameters						
		Texture	$CaCO^3$	EC (dSm^{-1})	pH	N	P	K
S-1	08	SCL	-	0.1	8.1	42	8.0	55
S-2	04	SL	-	0.1	8.2	45	7.0	35
S-3	03	SL	-	0.5	8.3	146	6.0	35
S-4	07	SL	-	1.0	8.0	104	8.0	35
S-5	16	SL	-	0.1	8.0	32	4.5	95
S-6	10	SL	-	0.1	8.0	49	3.5	20
S-7	09	SL	-	0.1	8.0	34	2.5	25
S-8	14	SL	-	0.2	8.0	59	4.0	71

SCL: Sandy clay loam; SL: Sandy loam

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Table 2: Screening of Actinobacterial Isolates for Siderophore Production and Detection of Functional Groups

Test Strains	Siderophore Detection		Siderophore Type Determination				
			$FeCl_3$ Test		Tetrazolium Test (Hydroxamate)	Arnow's Test (Catecholate)	Shenker's Test (Carboxylate)
	O-CAS Assay	CAS Assay	Peak at 425-450 nm (Hydroxamate)	Peak at 495 nm (Catecholate)			
A-1	Orange	+	-	-	-	-	+
A-2	Yellow	+	-	-	-	-	+
A-3	Light orange	+	-	-	-	-	+
A-4	Yellow	+	-	-	-	-	+
A-5	Light orange	+	-	-	-	-	+
A-6	Orange	+	-	-	-	-	+
A-7	Yellow	+	-	-	-	-	+
A-8	Orange	+	-	-	-	-	+
A-9	Light orange	+	-	-	-	-	+
A-10	Yellow	+	-	-	-	-	+
A-11	Yellow	+	-	-	-	-	+
A-12	Yellow	+	-	-	-	-	+
A-13	Orange	+	-	-	-	-	+
A-14	Orange	+	-	-	-	-	+
A-15	Orange	+	-	-	-	-	+

+: Positive -: Negative

Table 3: Determination of Antagonistic Potential of Siderophore by Well Diffusion Method

S.No	Bacterial Pathogen	Antagonistic Activity of Siderophore (Zone of Inhibition in mm)	
		Mueller-Hinton agar Without $FeCl_3$	Mueller-Hinton agar With 200 mM $FeCl_3$
1	*Staphylococcus aureus*	14	-
2	*Escherichia coli*	19	-
3	*Klebsiella pneumoniae*	15	-
4	*Salmonella typhi*	16	-
5	*Salmonella paratyphi* B	17	-
6	*Shigella dysenteriae*	14	-
7	*Shigella sonnei*	15	-
8	*Proteus mirabilis*	18	-

- : No Antagonistic Activity

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Table 4: Phenotypic Characteristics of Potential Actinobacterial Strain A-6

S.No	Characteristics	Actinobacterial Strain A-6
Micromorphology		
1	Aerial mycelium	+
2	Substrate mycelium	+
3	Spore chain morphology	Retinaculiaperti (RA)
4	Spore surface	Smooth
Cultural Characteristics		
5	Aerial mass colour	White
6	Reverse side pigment	Non distinctive
7	Soluble pigment	-
8	Melanoid pigment	-
Growth on ISP media		
9	ISP-1	Good
10	ISP-2	Good
11	ISP-3	Good
12	ISP-4	Good
13	ISP-5	Moderate
14	ISP-6	Moderate
15	ISP-7	Moderate
Carbon Utilization		
16	Glucose	+
17	Arabinose	+
18	Xylose	+
19	Inositol	+
20	Mannitol	+
21	Fructose	+
22	Rhamnose	+
23	Sucrose	+
24	Raffinose	-
25	Galactose	+
26	Salicin	-

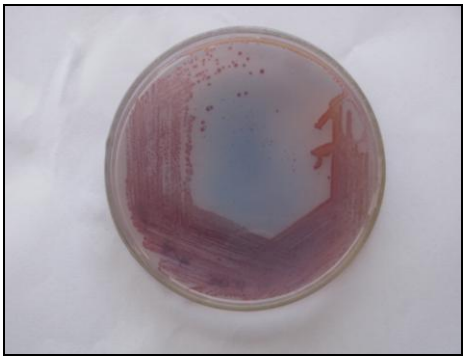

Figure 1: O-CAS Assay for Actinobacterial Strain A-6. Formation of orange colour around the colony indicates iron complexation, and therefore the production of siderophores

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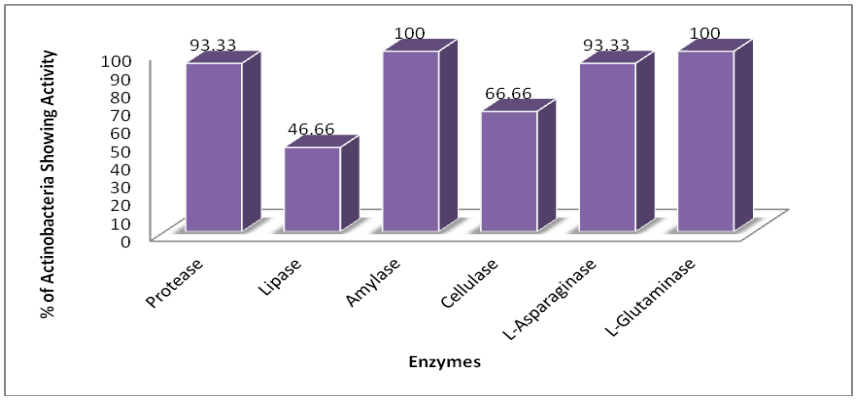

Figure 2: Enzymatic activity of Actinobacterial isolates

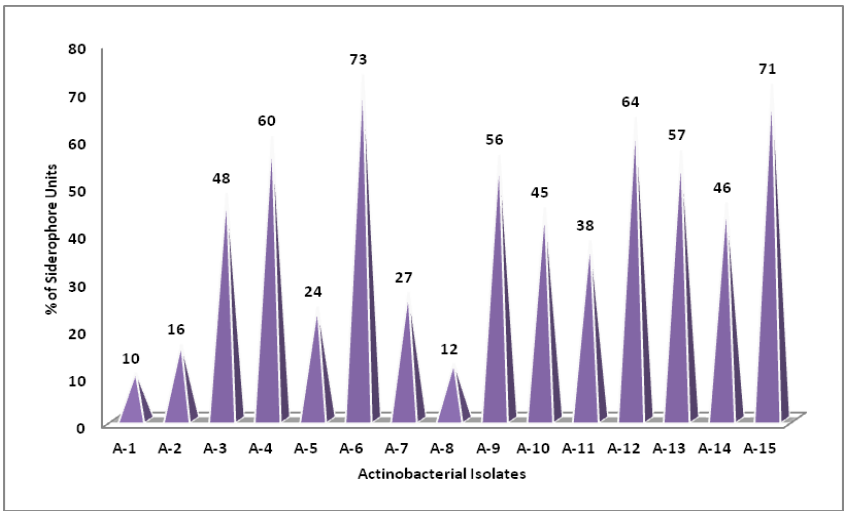

Figure 3: CAS-Shuttle assay for quantitative estimation of siderophores produced by Actinobacterial isolates

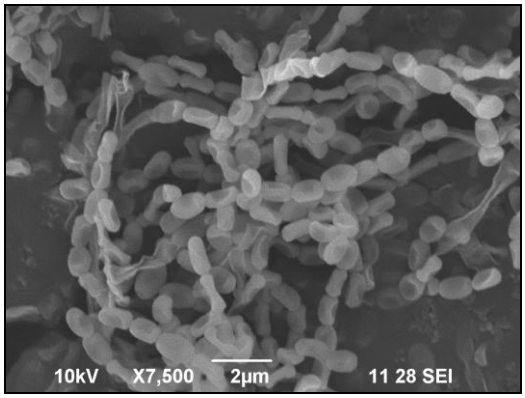

Figure 4: Micromorphology of Actinobacterial strain A-6 under Scanning Electron Microscope

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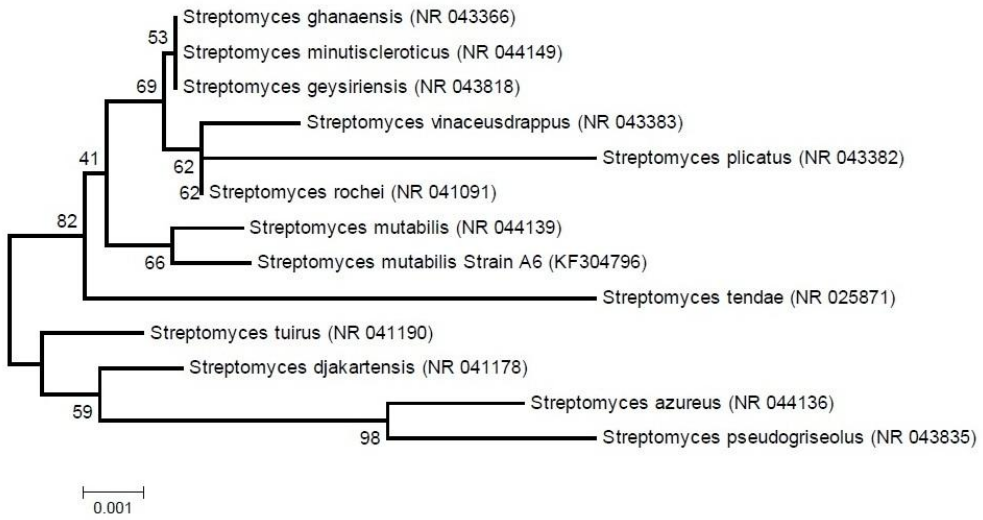

Figure 5: Neighbour-joining tree based on 16S rRNA sequences showing the relationship between *Streptomyces mutabilis* strain A6 and type species of the genus *Streptomyces*. The numbers at the nodes indicate the levels of bootstrap support based on data for 1000 replicates; only values that are greater than 50% are shown. The scale bar indicates 0.001 substitutions per nucleotide position.

C. CONCLUSION

In conclusion this paper is the first report on Actinobacteria synthesizing and excreting carboxylate type of siderophores. Screening of actinobacterial isolates for enzyme production suggests that magnesite mines soil actinobacteria are potential source of industrially useful and therapeutically important antileukemic enzymes. *In-vitro* antagonistic action of siderophores of *Streptomyces mutabilis* strongly suggests the potential application of siderophore in the treatment of human infections. This study opens an account for the research on actinobacterial siderophores with multiple applications.

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