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Growth of Non-symbiotic Nitrogen Fixing Microaerophiles from Lonar Crater on Inorganic Medium of Martian Soil Simulant Components

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Lonar Crater situated in the village Lonar (Lat. 19058', Long. 76034') in Buldhana District of Maharashtra State, India is the only one in the world formed by meteorite impact in basaltic rock. Formed some 52,000 years ago, it is today a closed basin lake that is saline and alkaline, rich in carbonates and bicarbonates. As it constitutes an extreme environment (alkalinity) and as there are no previous reports of nitrogen fixing bacteria from there, a study was undertaken to isolate and characterize nitrogen fixing bacteria adapted to this environment. Nitrogen fixing bacteria could be considered to constitute an important group of primary producers in an ecosystem due to their ability to make available a very important nutrient to other species. Though vast majorities of the species known today are chemoorganotrophs, there are many autotrophic nitrogen fixers also known. Several such bacteria have also been discovered in environments that resemble the Martian environment as well. Considering the fact that the Lonar Lake is of meteorite origin, an extraterrestrial object, it was decided to test the ability of the nitrogen fixers isolated from here to grow on Martian simulated soil. This paper presents results of that study. Of the 32 bacterial isolates obtained, only four were capable of growing on the simulated Martian soil component extract medium that was lacking in both nitrogen as well as carbon. This meant they were both fixers of atmospheric nitrogen as well as carbon. This holds encouraging signs to the search for extraterrestrial life using such organisms as possible indicators.

Key words: Lonar, Meteorite, Nitrogen fixers, chemoorganotrophs, simulated, Martian Soil, Extraterrestrial.

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Nitrogen fixing bacteria could be considered to constitute an important group of primary producers in an ecosystem due to their ability to make available a very important nutrient to other species. Though vast majorities of the species known today are chemoorganotrophs, there are many autotrophic nitrogen fixers also known[2-4]. Several such bacteria have also been discovered in environments that resemble the Martian environment as well[5].

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MATERIAL AND METHODS

Seventeen littoral soil samples were collected from sites around the perimeter of the Lonar Lake and transported to the laboratory in sterile polyethylene bags. Non-symbiotic nitrogen fixing bacteria were enriched and isolated using modified (pH 10.5 and supplemented with 2.0% NaCl) Ashby's Nitrogen free Mannitol medium by the standard technique. Incubation was done in an anaerobic jar at 28°C for a week. Colony and morphological characters of the isolates was done by conventional methods. The isolates were preserved on slopes of the same medium at 4°C. Their nitrogen fixing potential was studied by cultivating them in liquid medium of the same composition and determining the nitrogen content generated in the medium after their growth[6].

The isolates were then transferred to simulated Martian soil component (Fe_2O, MgO, SiO, CaO, Al_2O_3 and TiO) extract medium7 solidified with 2.5% Agar agar to test their ability to grow and fix atmospheric nitrogen on it. Incubation of the plates was done at 28°C and 4°C.These characters were studied as described above.

RESULTS AND DICUSSION

Of the 32 bacterial isolates obtained, only four were capable of growing on the simulated Martian soil component extract medium. Their colony and morphological characters are noted in Table 1.

Isolate SW-I formed reddish brown colonies while the remaining three appeared chalky white on the same medium. The four isolates show one to be a Gram negative rod (SW-I) while three others (S-I, SW-II and SE-I) are Gram positive filamentous rods. From their characters observed on Ashby's Mannitol medium the three Gram positive bacteria are known to be Actinomycetes[8].

Table 2 and Fig. 3 show results of the study on their nitrogen fixing potential. It is evident that SW-II is the most potent of the four, while S-I is the weakest. However, the interesting point to note here is the fact that all the four are able to fix nitrogen even in the simulated Martian soil extract medium that is N as well as C free.

Table 1. Colony Characteristics of the isolates on Martian soil component extract medium

Sample Name	Size	Shape	Colour	Margin	Elevation	Opacity	Consistency
SW-I	1mm	Circular	Reddish Brown	Entire	Convex	Opaque	Mucoid
S-I	1mm	Circular	Chalky White	Entire	Flat	Opaque	Dry
SW-II	1mm	Circular	Chalky White	Entire	Convex	Opaque	Dry
SE-I	0.5mm	Circular	Chalky White	Entire	Convex	Opaque	Dry

Table 2. Nitrogen fixing potential of the isolates (growth on Martian soil extract medium)

S. No.	Isolate No.	Nitrogen Content of the medium (%)
1	SW-I	0.103
2	S-I	0.029
3	SW-II	0.215
4	SE-I	0.042
5	Control (Uninoculated Broth)	0.009

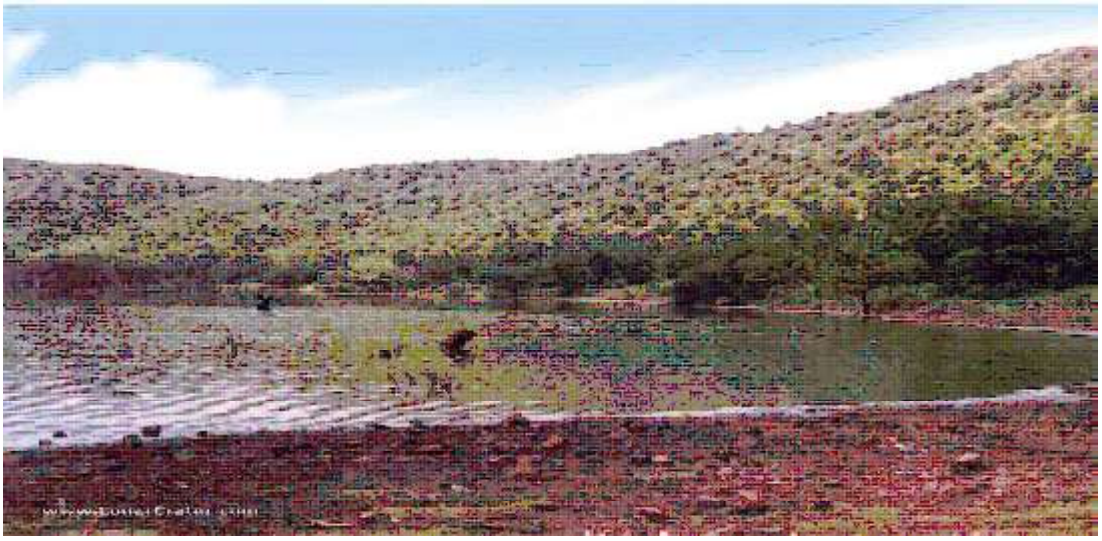

Fig. 1. A view of Lonar Lake from its bank

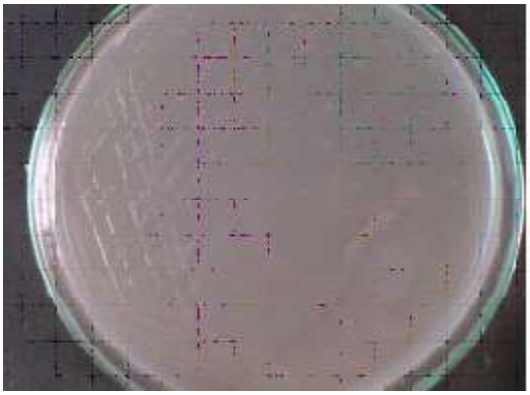

Plate 1. Colonies of isolate SW-I

Plate 2. Colonies of isolate S-I

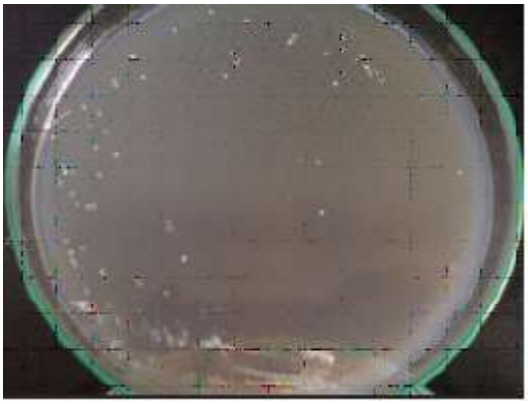

Plate 3. Colonies of isolate SW-II

Plate 2. Colonies of isolate SE-I

Fig. 2. Photographic plates 1-4

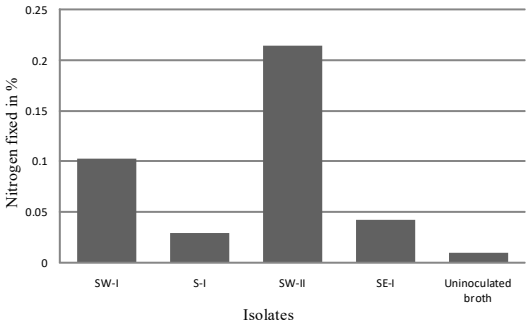

Fig. 3. Bar diagram of nitrogen fixing potential of the isolates obtained

CONCLUSION

The medium used in this study – the Martian soil component extract medium - is lacking in nitrogen as well as carbon and four nitrogen fixing bacteria isolated from the lake in the meteorite impact Lonar crater are seen capable of growing on it. The organisms therefore would appear to be capable of utilizing atmospheric carbon as well. This holds encouraging signs to the search for extraterrestrial life using such organisms as possible indicators.

However, the atmospheric conditions used in this study were anything but Martian and therefore extensive studies simulating those precise conditions are required before any prospective role can be ascribed to these organisms.

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