Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2014, 5(1):277-281

ISSN: 0976-8610
CODEN (USA): AASRFC

Isolation of a novel iron oxidizing bacteria from the iron scraps of a steel industry

***Suparna Deepak, Ankita Patil and Arti Matele**

Department of Biotechnology, Karmaveer Bhaurao Patil College, Vashi, Navi Mumbai, Maharashtra, India

---

ABSTRACT

An iron-oxidizing bacterium has been isolated from the iron scraps of a steel industry. It is an aerobic obligate chemolithoautotroph. Growth occurs optimally at pH 4 and 45 to 50ºC in a mineral medium containing 9% $FeSO_4$ but lacking organic carbon. The organism grows as cocci in clusters with sheath around each cell. The isolate is morphologically and physiologically distinct from other well characterized iron-oxidizing bacteria. The 16S rRNA sequencing suggests an uncultured bacterium clone B9-70.

Keywords: iron-bacteria, chemolithoautotroph, iron scrap.

INTRODUCTION

Many bacterial species have been found in iron deposits [1] but only a few pure cultures have been isolated. Iron deposition may result from the activities of both chemoautotrophic bacteria which use ferrous oxidation as an energy source, and heterotrophs which can cause the precipitation of ferric compounds either following utilization of iron-chelating compounds or by changing the oxidation-reduction potential of the environment.

It is very important to study the physiology of iron oxidation to remove excess iron from the water bodies and similar environments [2]. Thus, it will help in the environmental management. Also, microorganisms which are capable of transforming metals and inorganic compounds such as, iron, copper, uranium, sulphur, manganese etc. can be utilized not only for extracting minerals from poor ores.

The objective of this study was to isolate iron oxidizing bacteria from scrap iron found in a steel factory. The isolated strain was further characterized for its optimum growth.

MATERIALS AND METHODS

Sample: Iron scrap materials from a steel company at Khopoli, Raigad, Maharashtra was used for the isolation of iron oxidizing bacteria. The metal piece was scrapped out in phosphate buffer saline and 1 mL was inoculated into 200ml of liquid media.

Isolation: Two different mediums were used and their compositions were as follows:

Media A: 0.3 gm of $(NH_4)_2SO_4$, 0.01 gm of KCl, 0.05 gm of K_2HPO_4, 0.05 gm of $MgSO_4.7H_2O$, 0.01 gm of $Ca(NO_3)_2$ was added in 100 ml of distilled water and this salt solution was autoclaved.

Media B: 0.2 gm of NH_4SO_4, 0.2 gm of $CaCO_3$, 0.04 gm of $MgSO_4$, 0.002gm of KH_2PO_4 and 0.8 gm of $KHC_8H_4O_4$ was added in 100 ml of distilled water and autoclaved.

To both the above salt solutions, 100 ml of 18% $FeSO_4.7H_2O$ was sterilized separately and added to have a final concentration of 9% $FeSO_4.7H_2O$ in the medium. The pH of the media was adjusted to 4.1 using 10N H_2SO_4. Medium A had a lot of precipitate. Medium B had less precipitate which could be filtered out easily to give a clear yellow coloured medium. The broths were incubated at 37^{o}C. After 72 hours of incubation, media B started showing turbidity and after 1 week it turned dark brown with some precipitate at the bottom of the flask. Media A did not show any change even after 3 weeks. So, media B was chosen for the further study. After 1 week, one loopful of the enriched media B was streaked on the same media solidified with agar and incubated at 37^{o}C. After 3 days, dark yellow coloured powdery growth was observed on the plates. The culture was subcultured many times.

Morphological Characterization: Colony morphology of the isolates was recorded after growing them on media B at 37^{o}C for 72 hours, depending on growth. Overall shape, size, opacity, elevation, margin, texture were recorded [3]. Cell morphology was observed under oil immersion lens of microscope after Gram staining. Cell shape and arrangement were recorded.

Optimization of growth conditions: The optimum temperature and pH required for the growth of the bacterial isolate was determined. For determination of optimum temperature, 0.1 ml of freshly prepared suspension of the bacterial isolate was inoculated in 10 ml of media B and incubated at 10^{o}C, 24^{o}C, 37^{o}C, 50^{o}C and 65^{o}C. After an incubation period of 72 hr, the growth was checked in the form of turbidity. The uninoculated sterile broth tube was kept as negative control. For determination of optimum pH, 5 conical flasks each containing 20 ml of nutrient broth were prepared and their pH was adjusted at 2.0, 4.0, 6.0, 8.0 and 10.0 then autoclaved. These flasks were inoculated with 0.2 ml of fresh culture. After an incubation period of 72 hr, their absorbance was checked colorimetrically.

Utilization of organic carbon source*:* Carbohydrate utilization pattern of the strain was tested by supplementing 10 different tubes of media B with 0.1% and 0.5% each of glucose, lactose, maltose, fructose and sucrose.

Utilization of ferrous salts: The selected isolates were tested for their growth responses in different iron salt containing media by the disc diffusion method [4]. Isolates were seeded by spread plate method in media B exclusive of $FeSO_4.7H_2O$ and then the paper discs impregnated with selected salt solutions [*viz.* $FeSO_4.7H_2O$; $Fe_2(SO_4)_3$ and $C_6H_8FeO_7.NH_3$] were placed on the surface of the agar plates. Each disc was 6 mm in diameter and the concentration of salt was 50 µg/disc. After 24 h of incubation, the plates were observed and the zones of inhibition and stimulation were recorded.

Utilization of sulphur: The medium used for testing the utilization for sulphur was similar to that used in the report by [5] and has the following composition (per L): 4.0 mL of sodium lactate, 1.0 g of yeast extract, 0.1 g of ascorbic acid, 0.2 g of heptahydrate magnesium sulphate, 0.01 g of potassium hydrogen phosphate (anhydrous), 10.0 g of sodium chloride, 0.1 g of ferrous ammonium sulphate hexahydrate, 15.0 g of agar [6].

Identification of bacterial isolate: The bacterial isolate was sent for identification by 16S rRNA sequencing at NCCS, Pune. The 16S rRNA gene sequence of the isolate was aligned with reference 16S rRNA gene sequences of the European Microbiological Laboratory (EMBL), GenBank and the database of Japan using the BLAST algorithm [7] available online (http://blast.ncbi.nlm.nih.gov/).

RESULTS

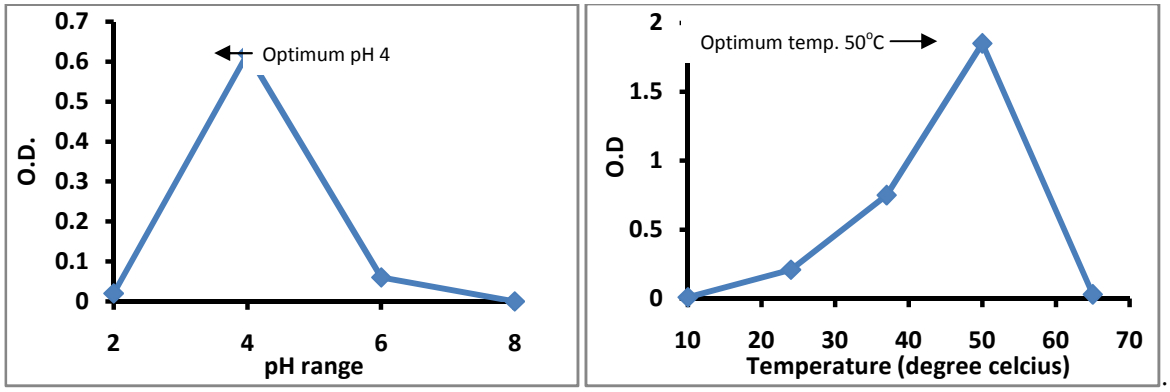
Isolation of the iron-oxidizing bacteria: Growth was observed only in the media B. When a loopful of the enriched broth was isolated on the same solidified medium, a dark yellow coloured matt growth was observed on the plates after an incubation of 72 hours. Individual separate colonies were not obtained. But only one type of growth was observed hence, providing with the pure culture of the isolate. The media tend to dry up very fast even after refrigeration. So, frequent subculturing had to be done.

(a) (b)

Fig 1: (a) Enriched medium B after 3 weeks. (b) The isolated culture on the solidified medium after 72 hours

Morphological Characteristics: Gram staining showed gram positive cocci in clusters. But these cocci had a sheath on the outer surface which appeared lightly stained. The growth was powdery, opaque and flat in elevation.

Optimum of growth conditions:



(a) (b)

Fig 2: (a) The growth pattern at different pH. (b) The growth pattern at different temperatures

Utilization of organic carbon source*:* When the isolate was inoculated in the medium B broth supplemented with 0.1% and 0.5% of glucose, lactose, maltose, fructose and sucrose, no growth was observed in any of the tubes even after incubation for 3 weeks.

Utilization of ferrous salts: The isolate could utilize all the 3 types of the ferrous salts. Growth was observed only near the disc impregnated with the ferrous salts. Best growth was observed around $FeSO_4.7H_2O$ followed by $Fe_2(SO_4)_3$ and then $C_6H_8FeO_7.NH_3$.

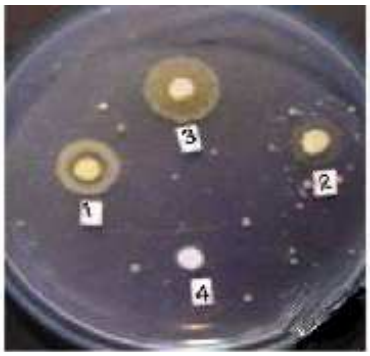


Fig 3: The growth pattern with different ferrous salts

Disc 1 was impregnated with $Fe_2(SO_4)_3$, disc 2 with $C_6H_8FeO_7.NH_3$ and disc 3 with $FeSO_4.7H_2O$. Disc 4 was control which was impregnated with saline.

Growth on sulphur containing media: The isolate was streaked on a sulphate media to check the utilization of sulphur. Strangely, the type of growth observed on this media was very different as compared to the media B. The colonies were white, opaque, needlike crystalline. After gram staining, same gram positive cocci in clusters were observed but without any sheath**.**

Fig 4: The white, opaque, needlike crystalline growth on sulphur containing media

Identification of bacterial isolate: The alignment results using the BLAST algorithm showed that the 16S rRNA sequence was from an uncultured bacterium clone B9-70 (Accession Number: KF010739.1).

DISCUSSION

The iron oxidizing bacteria isolated from the iron scraps showed very distinct growth characteristics which distinguish it from the other known iron oxidizing bacterias reported by other researchers. Unlike most of the other iron oxidizing bacterias, this strain is aerobic. The strain did not grow by utilizing organic substrates. The results suggest that these organisms are true or obligate lithotrophs, unable to utilize reduced organic carbon compounds as an energy source. It is a chemoautotroph which could utilize CO_2 in the form of calcium carbonate. Other autotrophic iron-bacteria which have been isolated to date are *Gallionella ferruginea* [8], *Metallogenium* sp. [9] and *Leptospirillum ferrooxidans* [10] among others, which are morphologically very distinct and different from our isolate.

Ehrlich and Brierley (1990) [11] mentioned that the optimum temperature range of iron oxidizing bacteria is generally from 45ºC to 50ºC but they are active over a wide range of temperature. Even we found similar results supporting this hypothesis as the optimum growth temperature for this isolate was also found to be around 50ºC. As reported by most researches, the isolate was thermophilic and acidophilic.

The isolate showed growth stimulation around the disc impregnated with the ferrous salts. This indicated its ability to use iron at a higher concentration. Bopp *et al.* (1983) [12] mentioned that there are several bacterial strains that contain genetic determinants of resistance to heavy metal. Dependent upon the environmental condition, this strain could grow either as iron or as sulphur bacteria. The growth characteristics are distinct under both these conditions.

CONCLUSION

The newly isolated iron-oxidizing bacterium, as suggested by the cultural characteristics and the 16S rRNA sequencing results, is an aerobic obligate chemolithoautotroph. It is moderately thermophilic and acidophilic. Further work can be carried out to study the metal transformation ability to check if it could be used to extract metal from low grade ores.

Acknowledgements
The authors gratefully acknowledge NCCS, Pune for identification of isolates by 16S rRNA sequencing. This study was supported by Karmaveer Bhaurao Patil College. We thankfully acknowledge Prof. Jogdand, the Coordinator of Department of Biotechnology, Karmaveer Bhaurao Patil College for constant support and encouragement and the Principal for providing necessary infrastructural facilities.

REFERENCES

[1] D. R. Cullimore & A. E. Mccann. *Aquatic Microbiology*, **1977**, 219-259. Edited by F. A. Skinner & J. M.

Shewan. London: Academic Press.
[2] A. D. Agate. Basic principles of geomicrobiology. Uneppune Mircen International workshop on metal microbe interactions and their applications. Agharkar Research Institute, Pune, **1995**, 102.
[3] R. M. Smibert, N. R. Krieg; Methods for general and molecular bacteriology, ASM Press, Washington DC, **1994**, 607-654.
[4] R. C. Allred. *Producers Monthly*, **1958,** 22:32.
[5] M. R. Atlas, AE Brown and LC Parks. Laboratory manual experimental microbiology. Mosby-Year Book. Inc.11830 Westly Industrial Drive, **1995**, 565.
[6] K. M. Paknikar and A. D. Agate. Laboratory manual. International Workshop on metal microbe interactions and their applications. United Nations Environmental Programme, Pune, **1995**, 84.
[7] S. F. Altschul, T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, D. J. Lipmana. *Nucleic acid program Res.*, **1997**, 25, 3389-3402.
[8] S. Kucera, & R. S. Wolfe. A selective enrichment method for *Gallionella ferruginea*, *Journal of Bacteriology*, **1957**, 74, 344-349.
[9] F. Walsh & R. Mitchell. An acid-tolerant iron-oxidizing Metallogenium. *Journal of General Microbiology*, **1972**, 72, 369-376.
[10] V. V. Balashova, I. Vedenina, G. E. Markosyan & A. Zavarzing. *Mikrobiologiya*, **1974**, 43,491-494.
[11] H. L. Ehrlich and C. L. Brierley. Microbial mineral recovery. McGraw-Hill, New York, **1990**, 450.
[12] L. H. Bopp, A. M. Chakrabarty and H. L. Ehrlich. *J. Bacteriol*., **1983**, 173: 3149-3158.