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# Molecular Identification of Bacteria and Bioaccumulation of Rare Earth Elements of Chavara

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**Rare earth soils are precious, but very common minerals across Arabian coast, especially in Chavara, Kollam District, Kerala. Rare earth soil, water, sediment, biofilm were collected from Chavara. 27 bacterial cultures were isolated from the collected five samples. Morphological and biochemical characterization were done for the isolates and also confirmed with molecular studies using 16S rRNA sequencing method. *Bacillus cereus, Bacillus* sp., *Lysinibacillus* sp., *Exiguobacterium* sp., *Bacillus* sp, *Pseudomonas* sp. were identified in Chavara samples. Bioaccumulations of rare earth elements were studied by employing FT-IR analysis and showed more accumulation of Ziercem and Ilmnate than other elements used. It had shown that, as in previous studies many microbial species identified in this study are also involved in bioaccumulation of rare earth elements. The accumulation of rare earth elements may have an additional application in field of agriculture.**

**Key words:** 16S rRNA sequencing, FTIR, rare earth elements.

The rare earths are a group of 17 elements composed of 15 Lanthanides, Scandium and Yttrium. The Lanthanides denote a group of 15 elements with atomic numbers between 57 and 71. The rare earths occur in many other minerals and are recoverable as by-products from phosphate rock and from spent Uranium leaching. Rare earths are characterized by high density, high melting point, high conductivity and high thermal conductance. A number of rare earth minerals contain Thorium and Uranium in variable amounts but Thorium and Uranium do not constitute essential components in the composition of the minerals. In India, Indian Rare Earths Ltd. (IRE), a Government of India Undertaking under the administrative control of Department of Atomic Energy and Kerala Minerals and Metals Ltd. (KMML), a Kerala State Government Undertaking are actively engaged in mining and processing of beach sand minerals from placer deposits. Several REEs (Rare Earth Elements) are not very "rare" and accurately dispersed in a variety of forms, especially as accessory minerals in granites, pegmatites, gneisses and related common types of rocks (Greenwood and Earnshan, 1984). Current literature deals with REEs in primary and secondary soil minerals, concentrations in surface soils, factors influencing adsorption, solubility and transport in soils including weathering and transformations of REE minerals, and vertical distribution in soil profiles (Tyler, 2004). Of particular note are the rare earth elements (REE) whose similar physicochemical properties suggest predictable fractionation in natural systems. Over the past three decades, REE geochemistry has become a powerful tool in both igneous and aqueous geochemistry. However, in spite of their wide use, little is known about the

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biogeochemistry of the REE. Considering the ubiquity of organisms and biofilms in natural environments this is a major shortcoming. The characteristics of REE complexation with organic ligands and REE uptake by the marine macro algae *Ulva lactuca* L. was studied (Stanley and Byrne, 1990). One of the features that make the REE so useful in studies of altered igneous rocks, elastic sediments, and soils is there immobility compared to other (trace) elements. However, it appears that this immobility is primarily due to the effectiveness with which the REE, once they are mobilized during fluid-rock interaction, are immobilized again due to incorporation into secondary minerals or sorption onto particle surfaces. The overall mobility of the REE during fluid-rock interaction, however, can be expected to increase dramatically when ligands are available that form strong (polydentate) REE solution-complexes and thereby significantly increase the REE transport capacity of the fluid. Because organic compounds such as polycarboxylic and aminocarboxylicacids are known to form very stable complexes with the REE, organic acids and siderophores may drastically increase REE mobility during fluid-rock interaction produced by microbes, perhaps changing REE distribution patterns. In India there is no study has been carried out on the relationship between rare earth elements and bacteria. Since llminate, Rutile, Zirconium is semi conducting constituents in soils in rare earth soils. The study on bacteria distribution is needed to investigate the adoptive behavior of microbes. In the present study, bioaccumulation and bacterial distribution of the soil in rare earth eco-systems have been investigated.

The basic investigations on microbiological analysis will be useful to agricultural industries. The rare earth soils are used as manure in China, but there is no research on agricultural aspect in India. Besides, it is expected that since rare earth soil has an inhibitive action against corrosion, the rare earth soil can be used as pipeline filter material. The water from rare earth environment can also be used as coolant in cooling water system because it consists of rare earth particles. DAE, Mumbai and CECRI (Karaikudi) have started some research on the survey of bacterial distribution in rare earth soil.

## MATERIAL AND METHODS

**Sample collection**

Soil, sediment, biofilm and water samples were collected from rare earth environment of Chavara in sterile polythene cover and brought to the laboratory in an icebox to avoid microbial contamination and proliferation during transport.

**Bacterial isolation**

The samples were serially diluted using 9ml sterile saline and total viable bacterial counts were enumerated by pour plate method technique, using the Nutrient agar medium. Duplicate plates were also maintained.

**Bacterial identification**

Morphologically dissimilar and well-isolated colonies were randomly selected and streaked onto the Nutrient agar medium, Thiobacillus agar medium, plates to obtain pure cultures. After noting the colony morphology along with color, pigmentation, shape, consistency etc., the selected pure colonies were sub cultured in Nutrient agar slants. Sub cultures of bacterial strains were made once in 30 days to keep the bacterial strain viable. The bacterial strains isolated from soil samples were identified up to generic level by employing the standard morphological and biochemical characteristics described in Bergey's manual of systemic bacteriology (Holt *et al*., 1994).

**Genomic DNA isolation**

Bacterial isolates were sub-cultured in Luria Bertani broth and genomic DNA was isolated by employing Lysozyme, SDS and Phenol- Chloroform method followed by Wawer and Muyzer 1995.

**PCR amplification, cloning and sequencing of 16S rRNA genes**

16S rRNA genes of the bacterial isolates were amplified with genomic DNA isolates as template and 8F and 1490R primers (Teske et al., 2002) in the following composition and amplification cycle. Each reaction mixture contained 2 µl of template DNA (100 ng), 0.5 µM of two primers, and 25 µl of Enzyme Master Mix (Bioron). The PCR program consisted of an initial denaturation step at 94°C for 5 min, followed by 30 cycles of DNA denaturation at 92°C for 30 sec, primer annealing at 50°C for

1 min, and primer extension at 72°C for 2 min was carried out in Thermal Cycler (Thermo Hybaid). After the last cycle, a final extension at 72°C for 20 min was added. The PCR products were purified by QIAquick PCR purification kit as described by the manufacturer and cloned using QIAGEN PCR cloning plus kit as described by the manufacturer. Clones were selected and isolated plasmids with insert were sequenced with M13 Sequencing Primers using ABI Biosystems automated sequencer.

**Sequence analysis and phylogenetic tree construction**

Nucleotide database was searched with the sequences obtained with NCBI BLAST (Blastn) tool (http://www.ncbi.nlm.nih.gov/BLAST) (Altschul *et al.,* 1997). Multiple sequence analysis was carried out using CLUSTALW (Higgins wt al., 1994) and further NJ plot (Saitou and Nei, 1987) and PHYLODRAW (Choi et al., 2000) were employed for constructing phylogenetic tree. A bootstrap analysis was performed to validate the reproducibility of the branching pattern.

**Bioaccumulation study of rare earth elements**

Sterile nutrient broth was prepared in 500ml conical flask and 1g of sterile rare earth soil was added to all the conical flasks. One of the conical flasks was kept as control where the other two were inoculated with bacterial cultures isolated and bacterial isolates with elements (MSP feed, Ilmnite, Garnet coarse, Garnet normal, Old Zircon, Ziercem and Rutile)**.** All the flasks were maintained at room temperature for five days incubation. Then the broth with the bacterial culture and control were taken and centrifuged at 10,000 rpm for 10 minutes, to pellet out. All the pelleted samples were analyzed by FTIR spectrum for knowing the physiological changes in the above samples and characterization was done by employing model NDXUS-672 model. The spectrum was taken in a mixed IR 400 -4000 cm--1 with 16 scan speed and was recorded using ATR (Attended Total Reflectometer).

## RESULTS

The counts of bacteria from soil, water, sediment, and biofilm plate were tabulated in Table 1. The biochemical characterization of the

**Table 1.** Bacterial counts of Chavara samples

| S.No. | Samples | No. of viable CFU |
|---|---|---|
| 1. | Water | $59 \times 10^5$ CFU/ml |
| 2. | Sediment | $65 \times 10^5$ CFU/gm |
| 3. | Soil | $82 \times 10^5$ CFU/gm |
| 4. | Biofilm | $78 \times 10^5$ CFU/gm |

**Table 2.** Biochemical characters of Chavara isolates (water sample)

| S. | Name of the test | Test microorganism | | | | | |
|---|---|---|---|---|---|---|---|
| No. | performed | CW1 | CW2 | CW3 | CW4 | CW5 | CW6 |
| 1. | Morphology | Rod | Rod | Rod | Rod | Rod | Rod |
| 2. | Gram staining | + | + | + | + | + | + |
| 3. | Motility | + | + | + | - | + | + |
| 4. | Indole test | + | + | + | - | + | + |
| 5. | Methyl red test | - | - | - | - | - | - |
| 6. | Voges Proskaue test | - | - | - | + | - | - |
| 7. | Citrate test | + | + | + | + | + | + |
| 8. | Starch hydrolysis | + | + | + | + | + | + |
| 9. | Gelatin hydrolysis | - | - | - | + | - | - |
| 10. | Lipid hydrolysis | - | - | - | - | - | - |
| 11. | Catalase | + | + | + | + | + | + |
| 12. | Oxidase test | + | + | + | + | + | + |
| 13. | Fermentation | + | + | + | + | + | + |
| | a. Acid test | | | | | | |
| | b. Gas test | - | - | - | - | - | - |

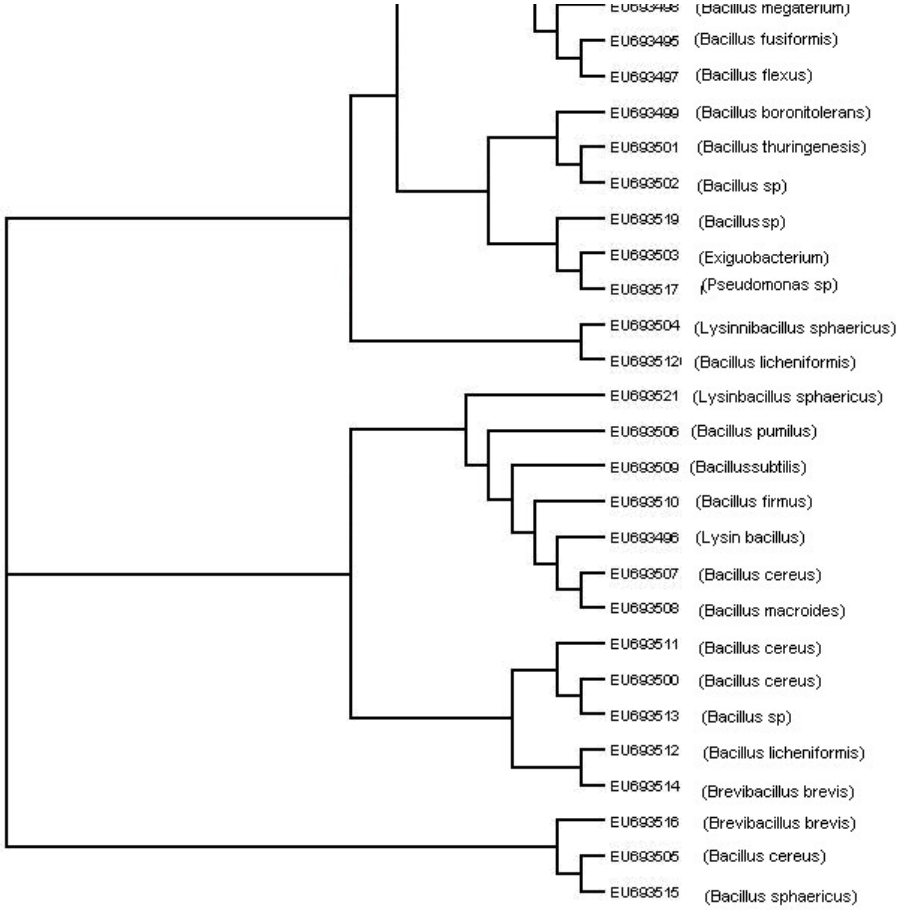


**Fig. 1.** Phylogenetic tree of 16S rRNA sequences of Chavara bacterial isolates

isolates from the collected water, soil, sediment and biofilms of Chavara were tabulated (Tables 2, 3, 4 and 5). The sequence obtained was searched in nucleotide database using BLAST (Altschul *et al.,* 1997*)* software in NCBI server. BLAST results showed that the sequences were having similarity with listed microorganisms and the sequences were deposited in Genbank using Bankit software and assigned accession numbers (Table 6). The phylogenetic tree (Fig. 1) showed the relational information between the sequences of the isolates. Centrifuged sample pellets from the eight systems were used for FT- IR analysis. The FTIR spectra of the systems FA to FH with different elements were presented in the figures 2A-2G. In the first system FA; when compared with control system, strong peaks of –OH and –C=C and carbonyl peak C=O were observed in cultures and elements system. In the systems FB and FC; C=O carbonyl peaks were not observed in cultures and elements system (Garnet coarse). In the system FD; either no significant peaks were observed or missed, but concentration of the molecules were decreased. In the system FE; -C=O acid group peak was not observed where as in the systems FF and FG; -OH and –NH intense peaks were observed in cultures with elements (Ziercem and Ilmnate). In the system FH; -C-H alkane and C=C aromatic peaks were observed in cultures with element M.S.P feed.

## DISCUSSION

It has been shown that Lanthanum, Europium and Terbium were accumulated during growth, between inner and outer membrane of the

cell envelope (periplasmic space) of *Escherichia coli* (Bayer, 1991). On the other hand, they may influence the environment by producing mineral acids, chelating agents such as siderophores, or by-products of the metabolism (organic acids etc.,). For example, the interaction between a mycobacterial siderophore (mycobactin) and Europium (Andres *et al*., 2003a) ions have been shown by or Spectrophotometric approach. Moreover, some siderophores such as ferrioxamine B could deplete europium fixation by goethite or boehmite (Andres *et al*., 2003b). Biosorption encompasses the uptake of metals by the whole biomass (living or dead) through physico-chemical mechanism such as adsorption, ion exchange or surface precipitation. The process

**Table 3.** Biochemical characters of Chavara isolates (Sediment sample)

| S. No. | Name of the test performed | Test microorganism | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | CS1 | CS2 | CS3 | CS4 | CS5 | CS6 | CS7 |
| 1 | Morphology | Rod | | | | | | |
| | Rod | Rod | Rod | Rod | Rod | Rod | | |
| 2 | Gram staining | + | + | + | + | + | + | + |
| 3 | Motility | + | - | + | + | + | + | + |
| 4 | Indole test | + | - | + | + | + | + | + |
| 5 | Methyl red test | - | + | - | - | - | - | - |
| 6 | Voges Proskauer test | + | - | - | - | - | - | - |
| 7 | Citrate test | + | + | + | + | + | + | + |
| 8 | Starch hydrolysis | - | + | - | + | + | + | + |
| 9 | Gelatin hydrolysis | - | - | - | - | - | - | - |
| 10 | Lipid hydrolysis | - | - | - | - | - | - | - |
| 11 | Catalase | + | + | + | + | + | + | + |
| 12 | Oxidase test | + | + | - | + | + | + | + |
| 13 | Fermentation | + | + | + | + | + | + | + |
| | a. Acid test | | | | | | | |
| | b. Gas test | - | - | - | - | - | - | - |

**Table 4.** Biochemical characters of Chavara isolates (Sediment sample)

| S. No. | Name of the test performed | Test microorganism | | | | | |
|---|---|---|---|---|---|---|---|
| | | CO1 | CO2 | CO3 | CO4 | CO5 | CO6 |
| 1. | Morphology | Rod | Rod | Rod | Rod | Rod | Rod |
| 2. | Gram staining | + | + | + | + | + | + |
| 3. | Motility | + | + | + | + | + | - |
| 4. | Indole test | + | + | + | + | + | - |
| 5. | Methyl red test | - | - | - | - | - | + |
| 6. | Voges Proskauer test | - | - | - | - | - | - |
| 7. | Citrate test | + | + | + | + | + | + |
| 8. | Starch hydrolysis | + | + | + | + | + | + |
| 9. | Gelatin hydrolysis | - | - | - | - | - | - |
| 10. | Lipid hydrolysis | - | - | - | - | - | - |
| 11. | Catalase | + | + | + | + | + | + |
| 12. | Oxidase test | + | + | + | + | + | + |
| 13. | Fermentation | + | + | + | + | + | + |
| | a. Acid test | | | | | | |
| | b. Gas test | - | - | - | - | - | - |

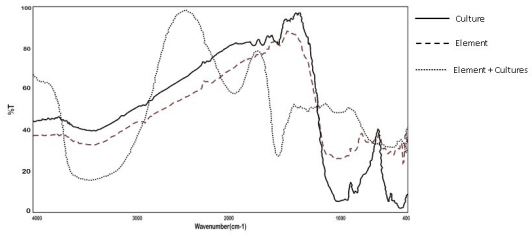


**Fig. 2A.** FTIR analysis of Chavara isolates with MSP feed

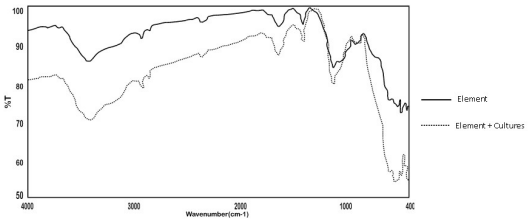


**Fig. 2B.** FTIR analysis of Chavara isolates with Ilmanite

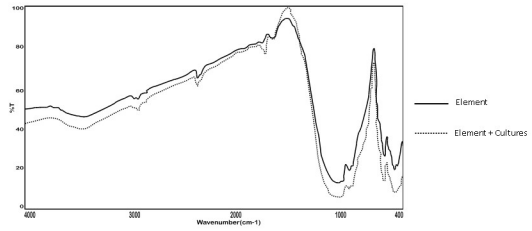


**Fig. 2C.** FTIR analysis of Chavara isolates with Garnet Coarse

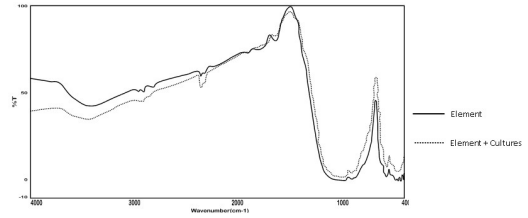


**Fig. 2D.** FTIR analysis of Chavara isolates with Garnet Normal

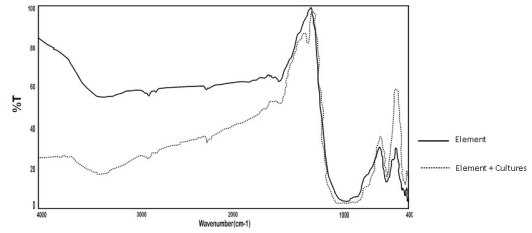


**Fig. 2E.** FTIR analysis of Chavara isolates with Ziercem

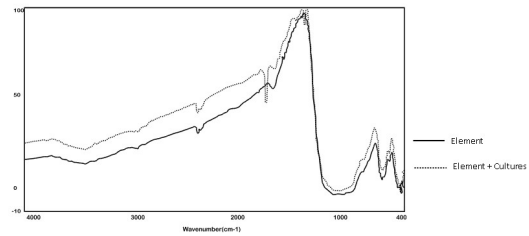


**Fig. 2F.** FTIR analysis of Chavara isolates with Old Zircon

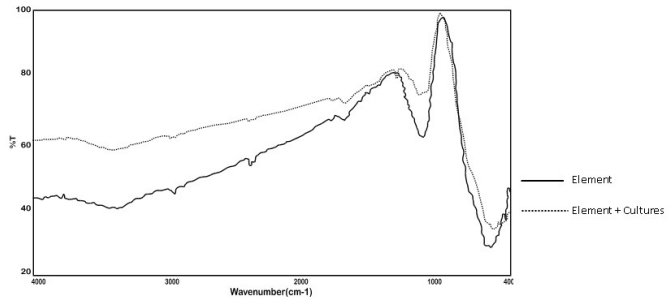


**Fig. 2G.** FTIR analysis of Chavara isolates with Rutile

**Table 5.** Biochemical characters of Chavara isolates (Soil sample)

| S. No. | Name of the test performed | Test microorganism | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CB1 | CB2 | CB3 | CB4 | CB5 | CB6 | CB7 | CB8 |
| 1. | Morphology | Rod | Rod | Rod | Rod | Rod | Rod | Rod | Rod |
| 2. | Gram staining | + | + | + | + | + | + | + | - |
| 3. | Motility | + | - | + | - | + | - | + | - |
| 4. | Indole test | + | - | + | - | + | - | + | - |
| 5. | Methyl red test | - | + | - | + | - | - | - | + |
| 6. | Voges Proskaue test | - | - | - | - | - | - | + | - |
| 7. | Citrate test | + | + | + | + | + | + | + | + |
| 8. | Starch hydrolysis | + | + | + | + | - | + | - | + |
| 9. | Gelatin hydrolysis | - | - | - | - | - | - | - | - |
| 10. | Lipid hydrolysis | - | - | - | - | - | - | - | - |
| 11. | Catalase | + | + | + | + | - | + | - | + |
| 12. | Oxidase test | + | + | + | + | + | + | + | + |
| 13. | Fermentation | + | + | + | + | - | + | - | + |
| | b. Gas test | - | - | - | - | - | - | - | - |

takes place on the cell wall with rapid kinetics. Rare Earth Element (REE) is often used in industry for the production of glass additives, fluorescent materials, catalysts, ceramics, lighters, superconductors, magnets or condensers. They are even more widely used in agriculture. Forestry and aquaculture in which they are found in fertilizers or animal food. Some authors (Diatloff *et al*., 1995) have shown that REE Lanthanum and Cerium could have a negative effect as the root elongation of corn and mungbean. A published review on REE toxicity, has reported that Cerium could be a potent antiseptic drug for gram-negative bacteria and fungi (Hirano and Suzuki, 1996). Moreover, some Lanthanide ions are produced in nuclear fission and could be dispersed in the environment like$^{140}$ La or$^{141}$ Ce/ $^{143}$Ce in the case of the Chernobyl accident in 1986 (Andres *et al*., 2003a). But in India, there is no work available on the interaction between biology and rare earth elements.

The distribution of *Klebsiella* sp. and *Bacillus* sp were noticed in Goa sediment and reported as phosphate solubilizers by Desouza (2000). These species were also reported in Chavara Waters. It can be assumed that alkaline phosphatase production and ability to solubilize inorganic phosphate may be due to the above microbes in phosphorites sediment. The influence of cations ($A1^{3+}$, $Ca^{2-}$, Na, K) and anions ($N0_3^-$, $S0_3^{2-}$, Cl) in the solution on the biosorption performance has been studied (Andres *et al.,* 2003b). Aluminium was noticed as more inhibitive ion for the fixation of rare earth element namely Europium, Lanthanum and Ytterbium. The industrial use of low cost biosorbents like microorganism has been of increasing interest in environmental remediation. The optimization of the biosorption conditions, the location of rare earth element binding sites and the studies of the sorption capacities of immobilized cells are good argument for using biosorption in the industrial removal of heavy metal from solutions. *Staphylococcus* sp*., Staphylococcus epidermidis, Pseudomonas aeroginosa* were used for the bio adhesion to Zirconium by Bucznski (2003). They demonstrated that these three bacteria preferred the zirconium than SS and suggested that the adsorption depends upon the surface of the material.

Ozaki *et al.,* (2005) studied the interaction of rare earth elements between *Pseudomonas sp*. and organic ligands. They noticed Eu (III) adsorbs on bacterial cells in the presence or organic ligands with low chelating ability. Merroun *et al.,* (2003) also noticed the fixation of Lanthanum by *Myxococcus xanthus* by extra Cellular polymeric substances. The fixing of heavy metals was higher and this microorganism could be used as model of bacteria

**Table 6.** Sequence similarity analysis and assigned accession numbers in NCBI database

| S. No | Sample Name | BLAST Results | Similarity with database sequences % | GenBank Accession Number |
|---|---|---|---|---|
| 1. | CW1 | *Bacillus fusiformis* | 99 | EU693495 |
| 2. | CW2 | *Lysini bacillus* | 100 | EU693496 |
| 3. | CW3 | *Bacillus flexus* | 99 | EU693497 |
| 4. | CW4 | *Bacillus megaterium* | 99 | EU693498 |
| 5. | CW5 | *Lysini bacillus boronitolerans* | 99 | EU693499 |
| 6. | CW6 | *Bacillus cereus* | 100 | EU693500 |
| 7. | CS1 | *Bacillus thuringenesis* | 99 | EU693501 |
| 8. | CS2 | *Bacillus sp* | 100 | EU693502 |
| 9. | CS3 | *Exiguobacterium* | 99 | EU693503 |
| 10. | CS4 | *Lysinibacillus sphaericus* | 99 | EU693504 |
| 11. | CS5 | *Bacillus cereus* | 99 | EU693505 |
| 12. | CS6 | *Bacillus pumilus* | 99 | EU693506 |
| 13. | CS7 | *Bacillus cereus* | 100 | EU693507 |
| 14. | CO1 | *Bacillus macroides* | 100 | EU693508 |
| 15. | CO2 | *Bacillus subtilis* | 100 | EU693509 |
| 16. | CO3 | *Bacillus firmus* | 100 | EU693510 |
| 17. | CO4 | *Bacillus cereus* | 100 | EU693511 |
| 18. | CO5 | *Bacillus licheniformis* | 100 | EU693512 |
| 19. | CO6 | *Bacillus sp* | 99 | EU693513 |
| 20. | CB1 | *Brevibacillus brevis* | 99 | EU693514 |
| 21. | CB2 | *Bacillus sphaericus* | 100 | EU693515 |
| 22. | CB3 | *Brevibacillus brevis* | 100 | EU693516 |
| 23. | CB4 | *Pseudomonas sp* | 99 | EU693517 |
| 24. | CB5 | *Bacillus cereus* | 99 | EU693518 |
| 25. | CB6 | *Bacillus sp* | 99 | EU693519 |
| 26. | CB7 | *Bacillus cereus* | 100 | EU693520 |
| 27. | CB8 | *Lysinibacillus sphaericus* | 100 | EU693521 |

CW- Water sample of Chavara, CS- Sediment sample, CO-Soil sample and CB- Biofilm sample.

- Lanthanide interactions. FT-IR spectroscopy revealed strong involvement of cellular carboxyl and phosphate groups in lanthanum binding by the bacterial biomass of *Pseudomonas sp* (Kazy and Susanta 2006). In the present study the influence of rare earth elements on bacterial reveals that rare earth enhances the production of acid, and aromatic nuclei which can be noticed in FTIR spectrum. It can be assumed that rare earth Ziercem and Ilmnate induces the bacteria for the production of carboxylic acid. Zhao and Zhu (2004) also noticed promotion of indole alkaloid production in *Catheranthus roseus* cell cultures by rare earth elements.

Hence, the present investigation suggests that the interaction between bacteria and rare earth elements are needed for future technology development in agriculture. On the other hand, literature information on the environmental effects, bioaccumulation and bioavailability of REEs has received scant attention until very recent years. Starting from the late 1990s, increasing effort has been directed to the environmental effects of REEs, and some of the recent reports include the study of REE accumulation in plant (Ichihashi and Morita, 1992). Promotion of indole alkaloid production in *Catharanthus roseus* cell cultures for rare earth elements was studied (Zhao

and Zhu, 2004). Ozaki *et al*., (2005) investigated the interaction by rare earth elements with bacteria and organic ligands such as malic acid, citric acid, a siderophore, cellulose, chitin and chitosan. Malic acid formed complexes with Eu III, but degradation of malic acid was observed. Zhang and Shang, (2001) reported the rare earth elements in soil and accumulation by wheat with rare earth fertilizer application. Significant accumulation of REEs was found in both the root and shoot parts of wheat.

Hence as preliminary study, bacteria were enumerated and identified in fresh water and soil sediment. Infrared spectroscopy revealed that rare earth elements induce the bacteria for the production of excess carboxylic acid and it is expected that the production of carboxylic acid chelate the available phosphorites in rocks as $P0_4$ and supplies to the plant, which will be useful for future research for developing countries like India and also that the base line data will be useful to investigate on the development of manure from rare earth environment.

## REFERENCES


1. Greenwood, N.N.,and A. Earnshan, Chemistry of the elements Pergamon Presss, London 1984.
2. Tyler G., Vertical distribution of major, minor and rare elements in a Haplic Podzol, *Geoderma,* 2004; **119**: 277-290.
3. Stanley, Jr., and J.K., Byrne, R.H., The influence of solutin chemistry on REE uptake by ulva lactucal L in seawater. Geochim, *Cosmochim. Acta,* 1990; **54**: 2847-2858.
4. Wawer, C., and Muyzer, G., Genetic diversity of DesulfoVibrio spp. In Environmental samples analysed by denaturing gradient gel electrophoresis of (NiFe) hydrogenase gene fragments, *J Applied and Environmental microbiology,* 1995; **65**: 2203-2210.
5. Holt, J. G., N. R. Kreig., P. H. A. Sneath., J. T. Staley., and S.T. Williams., Bergey's Manual of Determinative Bacteriology 9th edition. Williams and Wilkins, Maryland, USA 1994.
6. Altschul, Stephen, F., Thomas L., Madden., Alejandro, A., Schäffer, Jinghui Zhang, Zheng Zhang., Webb Miller, and David, J., Lipman., "Gapped BLAST and PSI-BLAST: a new generation of Protein database search programs", *Nucleic Acids Res*, 1997; **25**: 3389-3402.
7. Bayer, M.H., Lanthanide accumulation in the periplasmic space of *E.coli* B, *J Bacteriol,* 1991; **173**(1): 141-149.
8. Andres, Y., Thonand, G., Boualam, M, and Mergeay, M., Factors influencing the biosorption of gadolinium by microorganism and its mobilization from sand, *Appl. Microbiol Biol,* 2003a; **54**: 262-267.
9. Andres, Y., Texier, A.C., and Cloiree., Rare earth elements removal by microbial biosorption: A review, *Environmental Technology,* 2003b; **23**: 1367-1375.
10. Diatloff, E., Smith, F.W., and Asher, C.J., Maha., Rare-earth elements and plant growth, 2. Responses of corn and mungbean to low concentration of lanthanum in dilute, continuously flowing nutrient solutions, *J. Plant Nutrition,* 1995; **18**: 1977-1989.
11. Hirano, and Suzuki, T., Exposure, Metabolism, and toxicity of rare earth and related compounds,*J. Enviromental,* 1996; **104**: 85-95.
12. DeSouza, M J., Phosphate solubilizing bacteria around Indian peninsulaen, *J.Marine Sci,* 2000; **29**: 48-51.
13. Bucznski, B.W., Kory, M.M., Steiner, R.P., Kittinger, T.A., and Ramsier, R.D., Bacterial adhesion to Zirconium surfaces, Colloids and Surfaces, *B: Biointerfaces,* 2003; **30**: 167-175.
14. Ozaki, T., Yoshinori Suzuki, Takuya Nankawa, Takahiro Yoshida, Toshihiko, Ohmiki Takaumi Kimura, and Arokiasamy, J.Francis, Interaction of rare earth elements with bacteria and organic legends, 2005; **3**: 1191-1195.
15. Merroun, M.L., Ben, K., Chekroun, J.M., Arias, M.T., and Gonzakz-Munoz, Lanthanum fixation by *Myxococcus Xanthus*: Cellular location and extra cellular polysaccharide observation, *Chemosphere,* 2003; **52**: 113-120.
16. Kazy, K., and Susanta, K., Lanthanum biosorption by a *Pseudomonas sp:* equilibrium studies and chemical characterization, *J.Indus.Micr and Biotech,* 2006; **33**: 773-783.
17. Zhao, J., and Zhu, W. H., Promotion of indole alkaloid production in *Catharanthus roseus* cell cultures by earth elements, *J.Biotechnol,* 2004; **22**: 825-828.
18. Ichihashi, H., and Morita, H., REEs in naturally grown plants in relation to their variation in soils, *Environ Pollut,* 1992; **76**: 157-62.
19. Zhang, S.Z., and Shang, X.Q., Speciation of rare earth elements in soil and accumulation by wheat with rare earth fertilizer application,

*Environ. Polu,.* 2001; **112**: 395-405.

20. Higgins D., Thompson J., Gibson T.Thompson J.D., Higgins D.G., and Gibson T.J., CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*., 1994; **22**: 4673-4680.
21. Saitou, N. and Nei, M., The Neighbor-Joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 1987; **4**: 406-425.
22. Choi, J.H., Jung, H.Y., Kim, H.S. and Cho, H.G., PhyloDraw: a phylogenetic tree drawing system. *Bioinformatics*., 2000; **16**: 1056-1058.
23. Teske, A., Hinrichs, K. U., Edgcomb, V., Gomez, A. V., Kysela, D., Sylva, S.P., Sogin, M. L. and Jannasch, H. W., Microbial Diversity of hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities, *Appl. Environ. Microbiol*., 2002; **68**: 1994-2007.