

**7TH INTERNATIONAL MARDIN ARTUKLU SCIENTIFIC
RESEARCHES CONFERENCE
DECEMBER 10-12, 2021**

**THE BOOK OF FULL TEXTS
ON APPLIED SCIENCES**

**EDITED BY
ASST. PROF. ABDURRAHMAN GÜMRAH**

ISBN:978-625-8423-02-0

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Issued: 25.12.2021

ISBN: 978-625-8423-02-0

CONFERENCE ID

7th INTERNATIONAL MARDIN ARTUKLU SCIENTIFIC RESEARCHES CONFERENCE

DATA AND PLACE

December 10-12, 2021

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PRESENTATION TYPE

Oral Presentation/ Virtual

NUMBER OF ACCEPTED PAPERS: 432

THE NUMBER OF PAPERS FROM TURKEY: 215

OTHER COUNTRIES: 217

EVALUATION PROCESS

All applications have undergone a double-blind peer review process

**PIONEER STUDIES ON METAGENOMIC ANALYSIS OF SAMPLES FROM
BANDED IRON FORMATION (BIF) FROM IRON ORE MINING BELT OF GOA,
INDIA**

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ABSTRACT

Goa is situated in the northwestern part of the Western Dharwar Craton (WDC) which is Asia's major metallogenic province. The iron ore of Goa are associated with greenstone and occurs as bands, reefs and lenses of Banded Haematite Quartzite (BHQ) and Banded Magnetite Quartzite (BMQ). Presence of metalliferous WDC in Goa affords sampling of auriferous materials from mining area having hitherto unexplored interesting geo-microbiological dimensions such as detection of secondary microbioform Gold. BHQ and BMQ samples explored and collected from mining areas of Goa were surface sterilized and drilled with a sterile driller to get endolithic material till a sufficient amount was obtained, sealed aseptically and sent for metagenomic analysis using Oxford nanopore sequencing. Analysis revealed that both the samples were showing association of diverse microorganisms such *Bacillus*, *Ralstonia*, *Cupriavidus*, *Burkholderia*, *Acetobacter*, Archea such as *Haloferax*, *Nitrososphaera*. This paper discusses the presence, diversity, association and the presumptive role of such microorganisms in the biogeochemical cycling of metals such as Fe, Mn, gold (Au). There are various microbiological processes involved in Banded Iron Formation deposition. The traditional model of BIF precipitation assumes the oxidation of hydrothermal Fe (II), either via abiotic oxidation by cyanobacterially produced O₂ /or biotic oxidation by chemolithotrophic bacteria. Phototrophic Fe (II)-oxidizing bacteria were discovered about two decades ago and might represent an alternative hypothesis to early BIF formation in the absence of oxygen. These bacteria could have oxidized ferrous iron to ferric iron within the photic zone of the oceans through their photosynthetic process that involves light-energy fuelled CO₂ fixation coupled to the microbial oxidation of Fe²⁺ (Konhauser et al. 2002).

Keyword: Western Dharwar Craton (WDC), Banded Haematite Quartzite (BHQ), Banded Magnetite Quartzite (BMQ), Goa, Bacteria.

Introduction

The banded rocks are scattered throughout the world and are actually ambassadors from the past because they offer clues to the environment of the early earth. Banded Iron Formation (BIF) are chemical sedimentary rocks that were precipitated throughout much of the Late Archean and Palaeoproterozoic (2.7–1.9 Ga). The stripes in the BIF represent alternating layers of silica rich quartz silica (~40–50%) and iron (~20–40%) minerals like hematite and magnetite (Barton et al., 2010). BIF have been broadly classified according to tectonic setting, size and lithology as either Algoma or Superior type. The Superior type (S-type) first appears around 3 billion years ago (Ga) during the Archean and extends to around 1.7 Ga (in the Proterozoic). In India, Iron-formations are designated as “Banded Hematite Quartzite” (BHQ) and “Banded Magnetite Quartzite” (BMQ) (Prasad et al., 2012). One of the theory tells that primitive photosynthetic blue/green algae produced oxygen as the product of photosynthesis reacted with

the iron ions and formed magnetite (Fe_3O_4), an iron oxide (Konhauser, 1998). The preservation of bacteria in rocks is applicable to the search for the oldest evidence of life on earth found in Precambrian stromatolites and banded iron deposits (Byerly et al., 1986). Goa is situated in the north-western part of the metallogenic archaic Western Dharwar Craton (WDC) thus auriferous samples from the mining and non-mining areas can be used as a source for isolating microorganisms. Whether they are exposed to extremes of heat or cold, or buried deep beneath the Earth's surface, microorganisms have an uncanny ability to survive under these conditions. This ability to survive has fascinated scientists for nearly a century, but the recent development of metagenomics and 'omics' tools has allowed us to make huge leaps in understanding the remarkable complexity and versatility of extremophile communities. Metagenomics is the analysis of DNA from a mixed population of organisms and initially involved the cloning of either total or enriched DNA directly from the environment (eDNA) into a host that can be easily cultivated (Handelsman, 2004). Advances in next generation sequencing (NGS) technologies allow isolated eDNA to be sequenced and analyzed directly from environmental samples (Shokralla et al., 2012). Metagenomic studies of extreme temperature habitats such as Subsurface microbial mat sample from Hishikari gold mine (Japan) Sequence screening (Nunoura et al., 2005) as well as subsurface oil reservoir sample Norwegian Sea (Kotlar et al., 2011) has been reported. Microbial diversity from the continental shelf regions of the Eastern Arabian Sea by metagenomic approach (Sachithanandam et al., 2020; Shu et al., 2021; Methé 2021; Yaman et al., 2020). There has been no record of metagenomic analysis of BIF associated microbes from Goa. This research fills the gap and obtains a better understanding of bacterial diversity associated with BIF from Goa.

Materials and Methods

Regional geologic setting

Goa is situated in the north western part of the metallogenic archaic Western Dharwar Craton (WDC). The Dharwar Craton is divided into Eastern and Western Cratons wherein Goa is situated in the north western part of the WDC which includes Sanvordem, Bicholim, and Vagheri Formations (Dessai 2011). Samples used for the studies were Banded Hematite Quartzite (BHQ) from Velgeum, Surla North Goa and Banded Magnetite Quartzite (BMQ) from Sacorda, south Goa. (Fig 1 and Fig 2).

Sample preparation

The obtained samples were photographed, surface sterilized using 70% ethanol treatment on the exolithic surface of the samples and flame sterilized (Hirsch et al., 1995). Samples were scraped using sterile scraper till the sufficient amount is obtained and collected on a sterile aluminum foil and transferred into the sterile tube. The samples were labels as A1 and A2. A1-Banded Hematite Quartzite, A2-Banded Magnetite Quartzite).

Metagenomic studies and Bioinformatics analysis

Metagenomic studies were carried out using 16s & 18s Metagenomics by Oxford Nanopore MinION SAC Code: 998111 at Triyat Genomics (A division of Triyat Scientific Co.) Nagpur, Maharashtra, India. Protein nanopores are tiny holes that in nature forms gateway across membranes. In this technology nanopores are embedded into a synthetic membrane based in an electrophysiological solution and ionic current is passed through the nanopores. Molecules such as DNA/RNA moves through the nanopores they cause disruption in current which is measured in signal trace. During sequencing the nanopore analysis the entire fragment of DNA or RNA. The methodology followed includes DNA isolation metagenomics, 16s primer amplification, library preparation and Bioinformatics.

Results

The representative BHQ and BMQ samples used in the studies are represented in the figure 1 and 2. Sterile samples were successfully obtained in a sufficient amount. It was found that both the samples were showing association of microorganisms.

Diversity of BHQ associated microorganisms

The number of sequence reads from the BHQ were 37,618. In case of BHQ, reads analysed were 18,809, reads classified were 669 whereas the remaining reads were unclassified and were 18,140 in number. BHQ sample showed presence of 71% Bacteria, 27% Eukaryota and 1% archaea (Fig 3). Diversity of Bacteria found associated with the BHQ samples is given in the table 1.



Fig 1: BHQ sample



Fig 2: BMQ sample

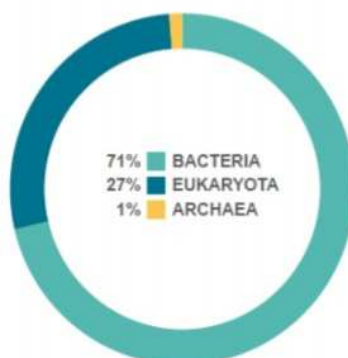


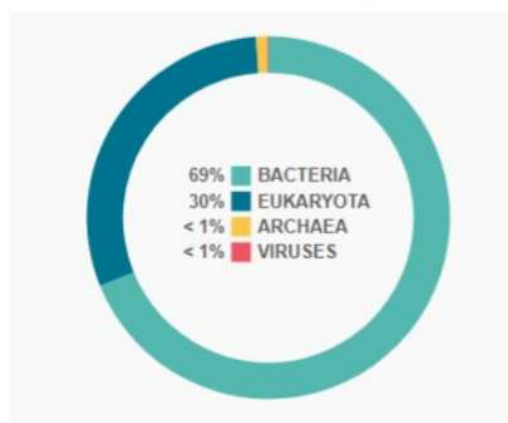
Fig 3: Percentage wise classification of microorganisms at different levels in case of BHQ

Table 1: List of Microorganisms found associated with the BHQ samples

Microorganisms found associated with the BHQ samples	
<i>Bacillus, Ralstonia, Burkholderia, Acetobacter, Enterobacter, Marinobacter, Aeromonas, Halomonas, Acinetobacter, Desulfuromonas, Chromobacterium, Actinomyces, Rhizobium, Rhodospirillum, Altererythrobacter (Proteobacteria), Novosphingobium, Sphingorhabdus, Achromobacter, Thermodesulfobium</i>	Bacteria
<i>Haloferax, Nitrososphaera</i>	Archaea
<i>Colletotrichum, Aspergillus, Schizosaccharomyces</i>	Eukryota

BMQ associated microorganisms

In case of BMQ sample the total number of sequence reads were 72,758. In case of BMQ, reads analysed were 36,379, reads classified were 1,016 and reads unclassified were 35,363. Sample showed presence of 69% of Bacteria, 30% of Eukaryota, <1% of archaea and <1% viruses (Fig 4). Diversity of Bacteria found associated with the BMQ samples is given in the table 2.

**Fig 4:** Percentage wise classification of microorganisms at different levels in case of BMQ**Table 2:** List of Microorganisms found associated with the BMQ samples

Microorganisms found associated with the BMQ samples	
<i>Bacillus, Streptomyces, Ralstonia,, Acetobacter, Burkholderia, Stenotrophomonas, , Rhodospirillum, Desulfococcus, Nitratiruptor, Acetohalobium ,Wenyingzhuangia, Mucilaginibacter, , Bradyrhizobium, Rhodoplanes, Methylobacterium, Rhizobium, Rhodobacter, Granulibacter ,Haematospirillum, Novosphingobium, Sphingomonas, Achromobacter,Cupriavidus, Polynucleobacter Janthinobacterium, Thioalkalivibrio, Methylococcus, Methylomonas, Halomonas, Aggregatibacter, Corynebacterium, Tessaracoccus, Alicyclobacillus, Desulfotomaculum,</i>	Bacteria
<i>Haloferax, Nitrososphaera</i>	Archaea

<i>Aspergillus, Malassezia, Lobosporangium, Batrachochytrium, Schizosaccharomyces, Penicillium, Fusarium, Magnaporthe, Grosmannia, Thielavia, Candida, Agaricus, Phycomyces, Fonsecaea, Penicillium, Trichophyton, Paracoccidioides, Verticillium, Chaetomium,</i>	Eukaryota
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Discussion

It was found that both the sample BHQ and BMQ showed the association of microorganisms. Metagenomic analysis serves as a useful technique to study the microbes associated with the BIF samples of Goa. This is the first study on the metagenomic analysis of BIF of Goa. In both the samples the bacterial association was high as compared to Eukaryotes, archaea similar studies has been reported earlier (Rea et al., 2016). Overlapping of bacterial community is shown in fig 5. Metagenomic analyses can provide extensive information on the structure, composition, and predicted gene functions of diverse environmental microbial assemblages. One strength of the metagenomic approach is in enabling researchers to investigate the phylogenetic and functional diversity of microorganisms at the community level, independent from cultivation-associated biases (Schloss and Handelsman, 2003; Cowan et al., 2005, 2006). Metagenomics (16S amplicon sequencing) and DGGE analysis of bacterial diversity of acid mine drainage has been studied earlier (Yaman et al., 2020). For instance, *Bacillus cereus*, a common soil bacterium, has been shown to act as a biogeochemical indicator for concealed mineralization. Resident organotrophs are likely to metabolize a wide range of complex organic compounds commonly found in placer environments, e.g. low and high molecular weight organic acids especially, *Acinetobacter* spp., *Burkholderia* spp. (Xie and Yokota 2005; Liou et al., 2008; Mroziak and Piotrowska-Seget 2010). Microorganisms such as *Burkholderia*, *Methylobacterium*, *Acinetobacter*, *Rhodobacter* the recruitment of phototrophic and heterotrophic Gram-negative bacteria are involved in EPS production. The proliferation and growth of biofilm community including heterotrophic and metalophilic species such as *Rhodobacter* sp, *Rhizobium* sp, *Achromobacter* sp. *Methylobacterium*, *Achromobacter*, play role in mobilization, detoxification and re-precipitation of secondary Au; and *Acinetobacter* play role in seeding of dispersal cells with release of nano-particle and Au complexes (Rea et al., 2016). Association of *Chromobacterium* with samples can be useful source of natural cyanide production which can be applied in Biomining (Campbell et al., 2001; Liang et al., 2014). Such microorganisms reported in this study can help to know their role in formation of BIF. Acidophilic microorganisms capable of oxidizing iron and sulfur can be employed in industrial processes to recover metals from minerals. Biosensors can be developed on how microorganisms interact with gold complexes which may further support exploration. This knowledge of bacterial diversity can be Useful in Biomining, biogeochemistry, bio metallurgy of heavy metals.

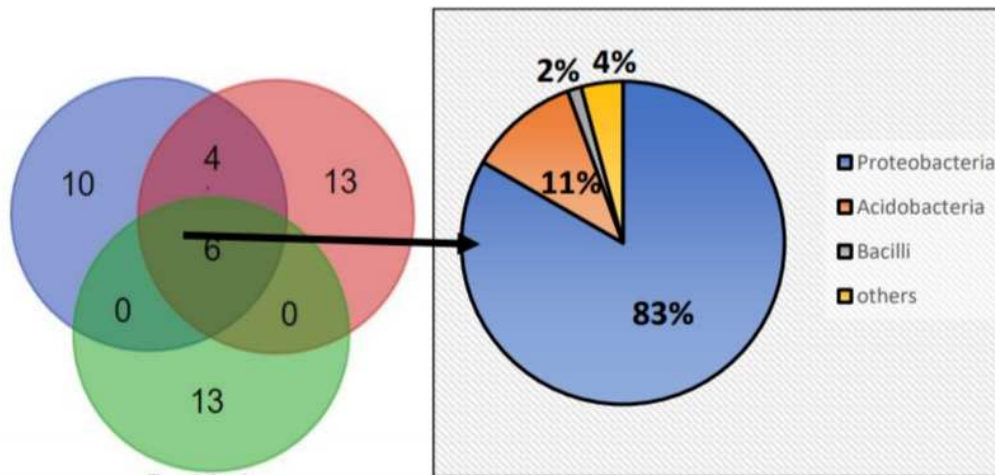


Fig 5: Overlap of the bacterial community

Conclusions

This is the first report on metagenomic studies of Banded Iron formation of Goa. There are various microbiological processes involved in Banded Iron Formation deposition. Metagenomic analysis using Oxford nanopore sequencing revealed that both the samples were showing association of diverse microorganisms. Metagenomic analyses can provide extensive information on the structure, composition, and predicted gene functions of diverse environmental microbial assemblages. An understanding of the distribution of microbial species in soils overlying mineralization can be utilized to develop bioindicator systems that assist with mineral exploration. Combined the implementation of biotechnologies in the gold mining sector is set to revolutionize the industry, leading to the greener, more efficient extraction of gold.

References

1. Byerly, G. R., Lower, D. R., Walsh, M. M. 1986. Stromatolites from the 3,300–3,500-Myr Swaziland Supergroup, Barberton Mountain Land, South Africa. *Nature*, 319(6053): 489.
2. Cowan, D., Meyer, Q., Stafford, W., Muyanga, S., Cameron, R., Wittwer, P, 2005. Metagenomic gene discovery: past, present and future. *TRENDS in Biotechnology*, 23(6): 321-329.
3. Craw, D., Lilly, K, 2016. Gold nugget morphology and geochemical environments of nugget formation, southern New Zealand. *Ore Geology Reviews*, 79, 301-315.
4. Dessai, A. G, 2011. The geology of Goa Group: revisited. *Journal of the Geological Society of India*, 78(3): 233.
5. Handelsman, J. 2004. Metagenomics: application of genomics to uncultured microorganisms. *Microbiol. Mol. Biol. Rev.*, 68(4): 669-685.
6. Hirsch, P., Eckhardt, F. E. W., & Palmer Jr, R. J, 1995. Methods for the study of rock-inhabiting microorganisms—a mini review. *Journal of microbiological methods*, 23(2): 143-167.

7. Koehler, I., Konhauser, K., Kappler, A, 2010. Role of microorganisms in banded iron formations. In Geomicrobiology: molecular and environmental perspective :309-324. Springer, Dordrecht.
8. Konhauser, K. O, 1998. Diversity of bacterial iron mineralization. Earth-Science Reviews, 43(3-4): 91-121.
9. Kotlar, H. K., Lewin, A., Johansen, J., Throne-Holst, M., Haverkamp, T., Markussen, S., Jakobsen, K, 2011. High coverage sequencing of DNA from microorganisms living in an oil reservoir 2.5 kilometres subsurface. Environmental microbiology reports, 3(6): 674-681.
10. Liang, C. J., Li, J. Y., Ma, C. J. 2014. Review on cyanogenic bacteria for gold recovery from E-waste. In Advanced Materials Research, 878: 355-367. Trans Tech Publications.
11. Liou, J. C., DeRito, C. M., Madsen, E. L. 2008. Field-based and laboratory stable isotope probing surveys of the identities of both aerobic and anaerobic benzene-metabolizing microorganisms in freshwater sediment. Environmental microbiology, 10(8): 1964-1977.
12. Loy, A., Mandl, M., & Barton, L. L. (Eds.). 2010. Geomicrobiology: Molecular and environmental perspective. Springer Science & Business Media. <https://books.google.co.in/books?hl=en&lr=&id=Wmj7X2tjUBcC&oi=fnd&pg=PR3&dq=Barton+%2B+Banded+iron+formation&ots=nI9qN1tgQT&sig=iBFdYvNeXBr2vCY4MSkfvYRnq10#v=onepage&q=Barton%20%2B%20Banded%20iron%20format ion&f=false>
13. Mrozik, A., Piotrowska-Seget, Z, 2010. Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. Microbiological research, 165(5): 363-375.
14. Nunoura, T., Hirayama, H., Takami, H., Oida, H., Nishi, S., Shimamura, S., Horikoshi, K. 2005. Genetic and functional properties of uncultivated thermophilic crenarchaeotes from a subsurface gold mine as revealed by analysis of genome fragments. Environmental microbiology, 7(12): 1967-1984.
15. Prasad, K. S. S., Sankar, D. B., & Reddy, Y. V. 2012. Geochemistry and Origin of Banded Iron-Formation from the Granulitic Terrain of North Arcot District, Tamil Nadu, South India. Chemical Science Transactions, 1(3), 482-493.
16. Rea, M. A., Zammit, C. M., Reith, F. 2016. Bacterial biofilms on gold grains—implications for geomicrobial transformations of gold. FEMS microbiology ecology, 92(6).
17. Reith, F., McPhail, D. C., Christy, A. G. 2005. *Bacillus cereus*, gold and associated elements in soil and other regolith samples from Tomakin Park Gold Mine in southeastern New South Wales, Australia. Journal of Geochemical Exploration, 85(2): 81-98.
18. Schloss, P. D., Handelsman, J. 2003. Biotechnological prospects from metagenomics. Current opinion in biotechnology, 14(3): 303-310.
19. Shokralla, S., Spall, J. L., Gibson, J. F., Hajibabaei, M. 2012. Next-generation sequencing technologies for environmental DNA research. Molecular ecology, 21(8): 1794-1805.
20. Xie, C. H., Yokota, A. 2005. Reclassification of *Alcaligenes latus* strains IAM 12599T and IAM 12664 and *Pseudomonas saccharophila* as *Azohydromonas lata* gen. nov.,

- comb. nov., *Azohydromonas australica* sp. nov. and *Pelomonas saccharophila* gen. nov., comb. nov., respectively. International journal of systematic and evolutionary microbiology, 55(6): 2419-2425.
21. Zammit, C. M., Cook, N., Brugger, J., Ciobanu, C. L., Reith, F. 2012. The future of biotechnology for gold exploration and processing. Minerals Engineering, 32: 45-53.
22. Sachithanandam, V., Saravanane, N., Chandrasekar, K., Karthick, P., Lalitha, P., Elangovan, S. S., Sudhakar, M. 2020. Microbial diversity from the continental shelf regions of the Eastern Arabian Sea: A metagenomic approach. Saudi journal of biological sciences, 27(8): 2065-2075.
23. Yaman, B. N., Mutlu, M. B., Çelik, P. A., Çabuk, A, 2020. Metagenomics (16S amplicon sequencing) and DGGE analysis of bacterial diversity of acid mine drainage. Journal of microbiology, biotechnology and food sciences, 9(5): 932-936.
24. Shu, W. S., Huang, L. N, 2021. Microbial diversity in extreme environments. Nature Reviews Microbiology, 1-17.
25. Methé, A. A, 2021. A metagenomic and metabolomic analysis of an Ecuadorian mine tailings
26. Yaman, B. N., Mutlu, M. B., Çelik, P. A., Çabuk, A, 2020. Metagenomics (16S amplicon sequencing) and DGGE analysis of bacterial diversity of acid mine drainage. Journal of microbiology, biotechnology and food sciences, 9(5): 932-936.