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Microbial Diversity of Coal Mine area soil and Water and their Pathogenicity

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Introduction: Nature is very diverse particularly with respect to the microbial flora. The prokaryotic diversity of the nature is an important tool for mankind. As they can be used extensively for a wide variety of purposes. The unique property of prokaryotes is the ability to utilize a large number of organic and inorganic molecules for their energy source. The present study provided a large number of isolates ranging from 35-40, when different samplings from coal and water components were carried out. Since most of the isolate were similar in their colony characterization on Nutrient Agar, there was a need of primary screening on visible bases. 23 isolates were screened off and subjected to conventional biochemical identification techniques. However 18 out of 23 showed positive growth on Isolation Medium for Iron bacteria. The overall study suggested the presence that, the Iron bacteria are quite a dominant species in Gondegaon Coal Mine area.

Soil is the richest source of microbes and shows extreme diversity particularly in the group of prokaryotes and lower eukaryotes. Mine area water, and the minerals are not very favourable for all prokaryotes to grow and adapt in the unusual physiochemical conditions prevailing in them. The pH conditions in mine soils near 5 hence most of the transition metals tend to remain in their higher oxidation state. Such changes usually do not favour mineral transition and transport in to bacterial cells in aerobic conditions. This leads to lower diversity of aerobic organisms in mine area. However, as we go deeper the soil becomes more anaerobic with lower pH that facilitates mineral transport hence; microbial diversity of anaerobes is mostly observable. Microbes belonging to the classes or super oxidizing capacity of minerals mostly predominate in mine areas. This is the reason why sulphur oxidizers, users of inorganic energy sources such as Iron oxidizing bacteria, certain photosynthetic bacteria and obligate lithotrophs are clearly observable. Percolation of water through mine areas therefore results in more bioleaching activities. Boon *et.al.* in 1995 has shown that in pyrite ore areas bacterial iron oxidation occurs with higher ease. *Thiobacillus ferroxdians* has been shown to oxidize a zinc sulphide almost in the single fashion. Chaudhary and co-workers have reported sulphurite concentrates to be very rich in zinc sulphide oxidation by *Thiobacillus*.

The role of Iron bacteria in acid mine drainage of Bituminous coal mines is well documented. Iron bacteria belong to two most common species *Sphaerotilus* and *Leptothrix*. Both are them are sheathed bacteria and are chemoheterotrophs. Other iron oxidizers include *Gallionella* spp. and *Thiobacillus ferroxidans*. Stringent growth conditions and their fastidious nature make them difficult to isolate. Biominerlization procedures are becoming very common which are primarily utilizing iron-oxidizing bacteria.

Diversity of microbes in soil area needs more exotic research as more and more fastidious organisms may be present in that areas. The difficulty of isolation results from the heterogeneous physiochemical conditions required by most of the fastidious organisms. It is mostly observed that a very high material containing up to 10^9 per ml can be obtained from mine areas. Majority of these cells are unicellular microbes associated with oxides. They were only visible when stained with DNA binding fluorescent dye, acridine orange and viewed by Epi fluorescent microscopy. This explains why cursory examination techniques fail to isolate most of the organisms. This also explains the fastidious nature of these organisms as they can be isolated under specific conditions. E. G. Hanert, H.H. has shown that stalk forming aerobic nutralophilic organisms can be either purified on enrichment or pure culture in laboratory where it can grow only at very low oxygen tension in opposing gradients of oxygen and ferrous ions. Lithotrophically growing CO_2 fixers grow to very limited extend as meastrophic growth.

Methods and Materials

The study was divided into 3 major experiments namely,

- Isolation of microbes from soil, coal and water sample.
- To study the Morphological and Biochemical characteristics.
- To screen the isolates for Iron Bacteria.

Sample Collection and Purification of strains

Soil samples were collected from different surface areas of Gondegaon Open Cast Mine, WCL, Nagpur Area, Called FS II(northen site sliding area), FS III(Eastern Sight) and FS I (western site). 3 samples of coal, water and soil were collected from the above mentioned sites. The collected samples, were then diluted in 1:10 w/v in sterile distilled water for making suspension. Further 1ml of the suspension was inoculated and separated with sterile spreader on the Nutrient Agar plate. Then it was incubated for 24-48 hours at 37°C in an incubator. Colonies formed after 48 hours incubation period, were further inoculated on nutrient agar slants to get isolated colonies.

Morphological, Biochemical & Cultural Characteristics

Two major microscopic examination namely Gram staning and motility were carried out. Biochemical characterisation of all the isolates is required to be done for complete identification of these strains, IMViC test, and sugar fermentation, Triple sugar iron agar test, cytrochrome oxidize test, and catalase test and lysine decarboxylase test were carried out for all the strains.

This work is original and first time presented by the author in this edition.

Our aim was to study the microbial diversity of mine area soil and water. Therefore it was necessary to study each isolate. We were also check the presence of Iron bacteria. Two Medias namely "Isolation Medium for Iron bacteria", "Iron Oxidizing Medium" (Twin Pack) were used. As they are specially designed for the isolation of Iron Bacteria only. (As per recommended in HIMEDIA manual).

Results

Isolation of the primary isolates from the soil water and coal. 23 distinct isolates were identified on a nutrient Agar Plate based on their colony characteristics. These isolates were marked SA, SB, SC, WA, WB, WC, CA, CB and CC Stains with Subscripts 10^{-1}, 10^{-2} based on the selected site. All the 23 isolates were also morphologically characterized on the basis of Gram's staining and Motility.

Colony Characterization of the Iron Bacteria.

The colony characterization with respect to Nutrient Agar and Isolation Medium for Iron Bacteria was performed.

Albert's Stain for screening of Iron Bacteria

The strains showing distinct positive growth in Isolation Medium for iron Bacteria were subjected to Albert's Stain reaction and the granules were observed microscopically.

Conclusion

The present study revealed the microbial diversity of Gondegaon Open Cast Coal Mine, WCL, Nagpur area. More than 75% (18 out of 23) isolates are belonging to Iron Bacteria. Out of 18, 6 isolates were slow growers and needs more suitable conditions for growth. All the Iron bacteria showed distinct inclusion bodies of iron quenched within them. Other bacteria could not be isolated due to their fastidious nature.

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