Identification of Acid Mine Drainage Microorganisms from a Coal Mine in South Africa

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Abstract: Acid mine drainage (AMD) generated from mining processes is an on-going environmental concern. This study aimed to identify microorganisms (MOs) from a coal mine water source to determine organism diversity for its use as a bioremediation method of AMD contaminated sources. For the purpose of this study, a culture-dependent sample, previously collected from an open-mine-water-pit was used to assess the microbial community by 18S rRNA gene amplification and BLAST analysis. The analysis revealed the presence of 94 MOs, with 3 dominant MOs namely, Cercozoan; an uncultured bacterium and *Chorella vulgaris*. The use of this culture is not promising as the presence of Cercozoan, a protist type body will target other species for its own survival, hence inhibiting any positive effect the algae or bacteria may have on bioremediation potential of harmful AMD elements.

Keywords: Acid mine drainage, Gene amplification, Cercozoan, Bacteria, Algae.

1. INTRODUCTION

Acid mine drainage (AMD) is a term used to describe water or soil with an altered chemical composition due to drainage/run-off by mining processes. In Brazil, the United States and United Kingdom approximately 20,209km; 23,000km and 12,000km, respectively of rivers and other water sources are affected by AMD. In South Africa, coal-mining is one of the largest industrial processes as ~90% of electricity is sourced from coal [1] and due to excessive coal mining, 25% of land and rivers are contaminated by AMD [2]. AMD contaminated sources diminish vegetation and shorten the life-span of aquatic organisms [3-6]. The generation of AMD, unfortunately cannot be entirely prevented, however it can be treated in order to minimize and control the harmful effects of the AMD metals. In order to treat AMD contaminants physical, chemical and biological methods are applied. In recent times, with sustainable technologies in the limelight, research studies have begun to focus on a biological perspective to AMD treatment. Iron and sulphur-oxidizing bacteria and algae are the types of MOs that are used for AMD biological treatment. The advantages of using MOs are: (i) they are natural inhabitants of water sources and AMD contaminated water which means they grow well in an acidic environment with high metal concentrations; (ii) changes in pH and nutrients from natural to laboratory conditions will not directly affect the growth of MOs as they are adaptable to their environment; (iii) isolation and culturing under laboratory conditions to enhance microbial biomass concentration for effective commercial applications is simple [3-4,7-10].

The current aim of this research is to investigate the microbial diversity of an AMD-environmental sample from a South African coal mine in order to determine if the organisms of the culture is capable of AMD bioremediation.

2. MATERIALS AND METHODS

2.1. Sample

During the period of 2008/2009 a sample was collected from an open water pit from a coal mining area in Mpumalanga, South Africa. This sample was maintained as a culture-dependent sample at an independent site close to the mining area in Mpumalanga, South Africa. From the point of collection, the sample was maintained on site by continuous sub-culturing for its usage in various research aspects and molecular analysis was not conducted. In the year 2014, a collaboration between the 'site' and the University of the Witwatersrand lead to a request to have this sample analyzed. From the site culture vessel, 500mL of sample was collected from the top of the open vessel using a measuring beaker and dispensed into 50mL NUNC tubes. This sample was transported on ice to the University and stored at 4°C.

2.2. Analysis

The liquid culture (50mL) was supplied to Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa for the identification of all possible microbial species present in this culture. The DNA from the sample was

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amplified, using 18sRNA gene specific primers to generate clusters within a flow cell using Illumina. The generated clusters were subjected to sequencing using sequence-specific primers. Data generated from sequencing were trimmed and q30 or high quality reads were used for BLAST (website) analysis. The q30 reads from each cluster was BLAST and the top 'BLAST' hit was recorded as a hit number for each genus/species. This BLAST hit or read count was the number of times the sequenced cluster matched to a specific genus/species query sequence within the GENBANK database.

3. RESULTS AND DISCUSSION

Gene amplification of the 18S rRNA region revealed a complex community. A total of 94 organisms, identified to genus or species level is shown in Table **1**.

Table 1: Organisms Present in the Coal-AMD Sample

Name of Organism	Cluster Size	BLAST Hit
Microsporidium sp.	17	2
Blastocystis sp.	61	4
Acanthostichus kirbyi	1	1
uncultured *Myxobacterium*	1	1
uncultured *Actinobacterium*	2	1
Paraphysomonas sp.	171	3
Oocystis marssonii	1	1
Coccoid green	2	2
Allomyces macrogynus	1	1
Lachlania saskatchewanensis	1	1
Mycobacterium sp.	1	1
Chlorella sp.	1	1
Rhodotorula mucilaginosa	35	3
Paratrechina sp.	1	1
sulfur-oxidizing bacterium	1	1
uncultured *Planctomycete*	4	3
Astreptonema gammari	1	1
Hecalus viridis	1	1
Apis mellifera	2	1
Lusius sp.	2	2
Alternaria alternata	1	1
Acremonium-like *hyphomycete*	1	1
Vittaforma corneae	5	2
Orthosomella operophterae	39	2
Hybrizon buccata	1	1

(Table 1). Continued.

Name of Organism	Cluster Size	BLAST Hit
uncultured soil	3	3
Albizzia julibrissin	1	1
Frontopsylla spadix	1	1
P.marinus small	107	1
uncultured *Nitrospira*	3	2
Myrmecia croslandi	4	3
Rhodobacteraceae bacterium	1	1
Geobacillus thermoleovorans	1	1
Rickettsia conorii	1	1
Chlamydaster sterni	432	4
Mycobacterium chelonae	1	1
uncultured *Bacteroidetes*	1	1
Fusarium cerealis	1	1
Claviceps purpurea	1	1
Triticum aestivum	20	1
Bacillus sp.	17	5
Reticulitermes flavipes	5	5
Bacillus cereus	1	1
Wolbachia endosymbiont	2	2
Rickettsia prowazekii	2	2
uncultured bacterium	13	11
Micronuclearia podoventralis	6	2
uncultured candidate	1	1
Spirochaeta sp.	1	1
uncultured delta	1	1
Pseudocolus fusiformis	13	2
Colpodella edax	1	1
Septata intestinalis	330	4
Arthrinium sp.	1	1
Erinaceus europaeus	49	3
Encephalitozoon cuniculi	188	5
Cyclotella meneghiniana	1	1
uncultured *Acidobacteriaceae*	2	1
Schizoprymnus sp.	2	1
uncultured *Actinomycete*	1	1
Desmodesmus pirkollei	1054	4
Pseudomonas syringae	1	1
M.bisecta gene	95	1
A.erinacea dna	1	1
uncultured alpha	1	1
Anurofeca richardsi	1	1

(Table 1). Continued.

Name of Organism	Cluster Size	BLAST Hit
A.erinacei 28s	7	4
unidentified bacterium	1	1
Epicoccum sp.	2	1
Sakaguchia dacryoidea	45	1
Fusarium sp.	83	2
Cantharis rustica	1	1
Nucleospora salmonis	39	3
Joinvillea ascendens	9031	4
Chyphotes mellipes	3	3
Nosema apis	3	1
S.communis gene	252	1
Mirawara sp.	2	1
Ctenocephalides felis	1	1
Castellaniella caeni	14	1
Caloscypha fulgens	2	1
uncultured *Chloroflexi*	1	1
Leitoscoloplos pugettensis	1	1
Thioalkalivibrio thiocyanodenitrificans	11	1
Chlorella vulgaris	43213	8
Ichthyophonida sp.	2	2
uncultured Gamma	2	2
Mus musculus	1	1
uncultured *Cercozoan*	1410	15
uncultured beta	1	1
Enterocytozoon bieneusi	14	1
Phyllidia coelestis	1	1
uncultured *Pseudomonas*	2	1
Zea mays	15	3

The 94 organisms are distributed as 7:protist, 7:algae, 20:fungi, 31:bacteria and 29:other (ants, insects, maize, trees, flowers). The initial observation, based on cluster size, showed the organisms constituting the major fraction of the culture are bacteria and the minor fractions to be the protist and algae (Table **1**). BLAST analysis narrowed this diverse range to three dominant organisms (Table **1**). The greater the number of hits, the greater the number of times the genus/species appeared as a top hot, therefore the greater is the occurrence of that particular organism. From these results, the culture is a mixed species culture dominated by Cercozoan-type species, uncultured bacteria, and *Chorella* (*C.*) *vulgaris* (Table **1**). The main organism, Cercozoan, is a protist-amoeba-type water body commonly associated with open-water or stagnant streams [11-14]. The presence of the uncultured bacterium is not unexpected as freshwater organisms such as the protists/Cercozoan identified serves as host species for the growth of bacterial organisms [13] and the large number of BLAST hits (Table **1**) shows that the bacterial growth is supported within the culture. The bacterium is referred to as "uncultured" as there is an unknown comparison to the GENBANK database and narrowing it down to a particular species requires 16S rRNA amplification. *C. vulgaris* is a freshwater microalgae present in open water bodies [15] and its occurrence is not unusual based on the origin of the culture. In an investigation by Pucciarelli and co-authors [16] a protist 'bloom' [16] or rapid growth of the species was caused by metal accumulation. In this study, the culture medium used on site may be a contributing factor for the stimulated growth of Cercozoan and the continuous sub-culturing allowed it to dominate. Pucciarelli and co-authors [16] also showed that Chorella species can exist as "endosymbiotic algae" [16] with protist bodies. The algae in this study could be associated with the Cercozoan.

It is possible that *C. vulgaris* could have been a dominant species when the culture was collected and harvested in 2008/2009 but over time sub-culturing and the outdoor environment caused the introduction of other organisms and contaminants resulting in this particular type of species being phased out or adaption of the culture to the method of culturing. Additionally, the dominance of Cercozoan may have resulted in the algae species being engulfed or fed on by the protist, as this phenomenon is common of protist-type species as a method to acquire nutrients [13,16]. In 2014, when the culture was collected for molecular analysis, the organisms documented were indicative of the culture at that particular time point. Furthermore, if the culture is re-examined at a later stage, will the organisms be the same or different? Based on the organisms identified, the use of the present culture is not promising for AMD bioremediation as a protist-dominant culture will target the algae, even the bacteria for its own survival, thus organisms that are capable of exerting a positive effect for AMD biotreatment instead serves as a source to sustain the growth of protist bodies.

4. CONCLUSION

A diverse community of 94 organisms, identified to the genus and species level was found growing in an

open-mine-water-pit. BLAST analysis narrowed this diverse range to three dominant organisms of Cercozoan, uncultured bacterium and *Chorella vulgaris*. The use of the present culture is not promising for AMD bioremediation as a protist-dominant culture will likely target the algae, even the bacteria for its own survival and it will be difficult to pin-point the organisms that are contributing to remediation of AMD. A more suitable approach will be to isolate organisms from this culture and test it against AMD.

CONFLICTS OF INTERESTS

It was requested by collaborators to omit: name of the coal mine and name of the site area from the paper.

There are no Conflicts of Interests from authors, ED Deenanath and R Falcon.

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