"MINES" method for genomic DNA extraction from deep biosphere biofilms

Tanvi Govil[a], Wageesha Sharma[b], Neeraj K. Chauhan[c], Sudhir Kumar[b], David R. Salem[a,d,e,f,*], Rajesh K. Sani[a,d,g,*]

[a] Department of Chemical and Biological Engineering, South Dakota School of Mines and Technology, Rapid City, SD 57701, USA
[b] Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Solan, Himachal Pradesh, India
[c] Tuberculosis Research Laboratory, Vaccine and Infectious Disease Research Centre, Translational Health Science and Technology Institute, Faridabad, India
[d] Composite and Nanocomposite Advanced Manufacturing Centre – Biomaterials (CNAM-Bio), Rapid City, SD 57701, USA
[e] Department of Materials and Metallurgical Engineering, South Dakota School of Mines and Technology, Rapid City, SD 57701, USA
[f] Composites and Polymer Engineering Laboratory, South Dakota School of Mines and Technology, Rapid City, SD 57701, USA
[g] BuG ReMeDEE Consortium, South Dakota School of Mines and Technology, Rapid City, SD 57701, USA

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ABSTRACT

Successful and efficient extraction of high quality, high molecular weight genomic DNA from the environmental samples is an essential primary step to understand the genetic, metabolic and evolutionary characteristics of the microbial communities. Deep mine biofilm samples that contain high amounts of mucoid exopolysaccharide often pose difficulties to obtaining refined community DNA. To circumvent this hindrance, we report our "MINES" method which we developed for optimal biofilm DNA recovery suitable for all types of high-resolution downstream applications. The method is also suitable for samples collected from landfill compost, kitchen digest (KD), and for Gram-positive *Geobacillus* sp. strain WSUCF1 and Gram-negative *E. coli* DH5α strains. In one form of the method, use of a gentle preprocessing technique to loosen the mucoid layer, combined with a multi-lytic polyzyme treatment to maximize yields from all cell types in the biofilm sample, yielded > 1 μg of high molecular weight DNA (16–20 kb) per gram of the biofilm sample, with an A260/280 and A260/230 ratio of about 2. Furthermore, amplification of 16S rRNA genes as well as restriction digestion with BamHI and HindIII suggest that the newly developed method can minimize any inhibitory effects of contaminants. Results indicate that it is an appropriate methodology for the extraction of total genomic DNA for functional metagenomic studies and may be applicable to other environmental samples from which DNA extraction is challenging.

Importance: Our present knowledge of microorganisms and their enzymes from deep mine subsurfaces is based largely on laboratory studies of pure microbial cultures. These methods tend only to hit nearly 1% of the entire microbial community. In this regard, metagenomics, has emerged as a strategic approach to explore unculturable microbes through the sequencing and analysis of DNA extracted from the environmental samples. This research paper discusses our "MINES" method for genomic DNA extraction from deep biosphere biofilm samples.

1. Introduction

The present century has seen rapid, revolutionizing progress and improvement in a diverse range of molecular approaches, varying from traditional real-time polymerase chain reaction (RT-PCR) to the more complex systems such as metagenomic analysis, and sequencing of phylogenetic markers, including the 16S rRNA gene using next-generation sequencing (NGS). These foundational techniques have become standard tools to gain new insights into the microbial world in its indigenous community with high levels of resolution and extraordinary details. Biofilms, defined as a structured community of microbial cells enclosed in a self-produced polymeric matrix, represents one such syntrophic world of microbes. Many discoveries concerning the physiology, genetics, and ecology of biofilm organisms, as well as biofilm community structures, are being made owing to the application of transcriptomic approaches (Franklin, 2015). Unfortunately, the experimental procedures for DNA isolation from the microbial community members of the biofilm, which constitute the first crucial step for effective implementation of these cutting-edge functional genomics applications, have not received the attention they deserve, especially with respect to the lytic processes needed for DNA isolation. Plenty of aspects related to physiochemical characteristics of the biofilm can hinder the efficiency of the direct DNA extraction procedure.

Particularly, while working with biofilm samples, the two significant factors that hinder DNA extraction are i) binding of DNA to the

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EPS matrix (Wu and Xi, 2009; Steinberger and Holden, 2005; Corcoll, 2017) and ii) presence of microbial diversity in a biofilm community with different types of cell walls and cell membranes (Corcoll, 2017; Davey and O'Toole, 2000). It is believed that extracellular polymeric substances (EPS), which provide stability to the biofilm, comprise polysaccharides, proteins, humic substances and lipids. All these compounds can bind to nucleic acids during the DNA extraction process, making the overall process of DNA extraction technically challenging, necessitating use of harsh lysis steps. Harsh extractions increase the DNA yield but at the cost of DNA integrity, resulting in smaller DNA fragments that might be less suitable for further downstream analyses. This hypothesis was recently verified by Corcoll et al. who evaluated four commonly used DNA extraction kits for marine periphyton biofilms. They reported DNA yields higher than 10 ng/μl, with A260/280 absorbance ratio above 1.7 for all the four kits. However, extracted DNA had very low A260/230 ratios (0.4–0.5) and low DNA integrity, indicating the presence of a high concentration of contaminants (e.g., polysaccharides and salts), which can inhibit subsequent downstream analyses, including PCR amplification and DNA library preparation (Corcoll, 2017).

The recovery of DNA representative of all microbial species with the same efficiency also represents a major obstacle, as each prokaryotic species can have different susceptibilities to lytic enzymes and chemicals. Since harsh treatments affect DNA integrity and mild ones cause poor lysis, particularly for the classes of bacteria carrying thick layers of peptidoglycan, it is important to optimize the cell lysis conditions to obtain unfragmented genomic DNA in high amounts from abundant as well as rare representatives of each taxonomic group possessing a different thickness of cell wall (Bag et al., 2016). Otherwise, the analyses may impart biases on the ability to correctly describe the composition and biodiversity of a microbial community.

In the present study, we developed a highly sensitive method by combining enzymatic and mechanical lysis to extract DNA from the biofilms. Massive and often largely-unexplored microbial biofilm growths frequently occur in corridors and passages in deep mines (Govil et al., 2019). Thus, for this study, samples were collected from the deep biosphere (4850 ft. deep) of a mine known as the Sanford Underground Research Facility (SURF) located in Lead, South Dakota. The mine environment might be used as a model system to gain better insights to the diversity and distribution of microbes that have come to inhabit areas of this subsurface through time. This environment also provides clues to the nature of life that survives in carbon-poor extreme environments when it is shielded from cosmic radiation. Because samples from deep-mine environments are generally present in limited amounts, our developed method also maximizes biofilm DNA recovery from small amounts of samples with high DNA extraction yield and integrity, in a cost-efficient manner.

2. Materials and methods

2.1. Sample collection

The biofilm samples were collected over the course of an expedition to SURF in Lead, South Dakota (USA) in September 2017, from the Ross Campus (4850 ft. deep level). The underground mine ventilation system made the galleries dimly lit and aerated. Biofilm samples were collected from the walls of the mine corridors (WMC), from biofilms associated with water flows (WF) coming out through the fractures, and from the sites rife with slime deposits (SD) (as shown in Fig. 1). Samples were collected aseptically into sterile 50 ml Falcon tubes, which were filled to the brim with samples to avoid air entrapment, and surface contamination of samples was avoided as much as possible. The pH (4.23) and temperature (42 °C) were measured on the site at the time of biofilm samples collection. Samples were stored on wet ice in ice boxes and transported to the laboratory (1 h drive) where they were stored at −20 °C until processing for biofilm DNA (bDNA) extraction. In this study, extracellular DNA and intracellular DNA were not separated during DNA extraction.

2.2. DNA Extraction

Biofilm DNA (bDNA) from all the three samples (SD, WF and WMC) was extracted using two different variations of the "MINES" method, M1 and M2. In M1 - Extraction without Preprocessing- DNA extraction was carried out using the procedure outlined in Table 2. In M2 - Preprocessing plus Extraction- the biofilm samples were preprocessed as outlined in Table 1 to loosen the EPS/mucoid layer and then DNA was extracted as outlined in Table 2. These two versions of the method were compared and evaluated in this study with respect to the quality and purity of extracted DNA. The consideration of introducing a pre-lysis preprocessing step is based on the idea that the washing should promote removal of covalent cations and the easily dissolving organic compounds from the mine samples, promote sample dispersion and homogeneity, and decrease bacterial and DNA adsorption by EPS components (e.g. humic acids). Once extracted, the bDNA was stored at −20 °C until PCR amplification. During the entire procedure, bDNA was treated gently avoiding severe vortexing and repetitive harsh pipetting to prevent shearing.

The M1 variation of the newly developed method was also tested for metagenomic DNA extraction from other environmental samples, namely Landfill Compost (LC) and Kitchen Digest (KD), as well as for genomic DNA extraction from a Gram-positive *Geobacillus* sp. strain WSUCF1 and Gram-negative *E. coli* DH5α, in order to confirm the suitability of this approach for other metagenomic samples and for cultivable organisms. Each individual extraction was performed on ~200 mg of cells. Other sources of contamination were controlled by adopting adequate laboratory sterile techniques.

2.3. Gel electrophoresis

The integrity of bDNA extracted by both variations of the method was assessed by gel electrophoresis. Specifically, 5 μl of each DNA extract from all M1 and M2 samples were loaded on an 0.8% agarose gel containing 0.5% ethidium bromide along with 3 μl of 10 kb 2log-DNA ladder (1000 μg/ml, New England Biolabs, USA). Electrophoresis was performed at 60 V for 60 min using TAE as electrophoresis buffer and the bands were visualized using gel documentation system (Bio-Rad, USA).

2.4. Quality and concentration of the DNA

The DNA concentration of the biofilm samples was measured by examining the absorbance of the sample at 260 nm and the amount of DNA was calculated (1.0 A_{260} unit = 50 μg/ml of DNA). Further, DNA quality was analyzed by measuring A260/280 ratio (DNA/protein) and A260/230 ratio (DNA/humic acid) using a Nanodrop spectrophotometer (Thermo Scientific) to check contamination by protein and humic acid substances, respectively. The data were presented as mean ± standard error of the mean. The response value given in these experiments is an average of the triplicate measurements and the results were analyzed and interpreted using the Design Expert Software package version 11.00 (Stat-Ease Inc., Minneapolis, Minnesota). The significant value was set as $p < .05$ and Values of Prob > |t| was set as < 0.05.

2.5. Determination of purity of DNA by restriction digestion

For all samples, 1 μg of bDNA was fragmented with Hind III (sequence 5'-AAGCTT-3′) and Bam HI (sequence 5'-GGATCC-3′), 1 U enzyme each; 20-μl reaction mixture volume in Cut Smart Buffer (New England Biolabs, USA); 2 h of digestion at 37 °C. The digested samples were run on an 0.8% agarose gel using 25 × 25 cm Horizontal high-throughput electrophoresis system (Sub-Cell Model 192 Cell, BioRad, USA) and then Spectroline Ultraviolet Transilluminator was used to capture the image.
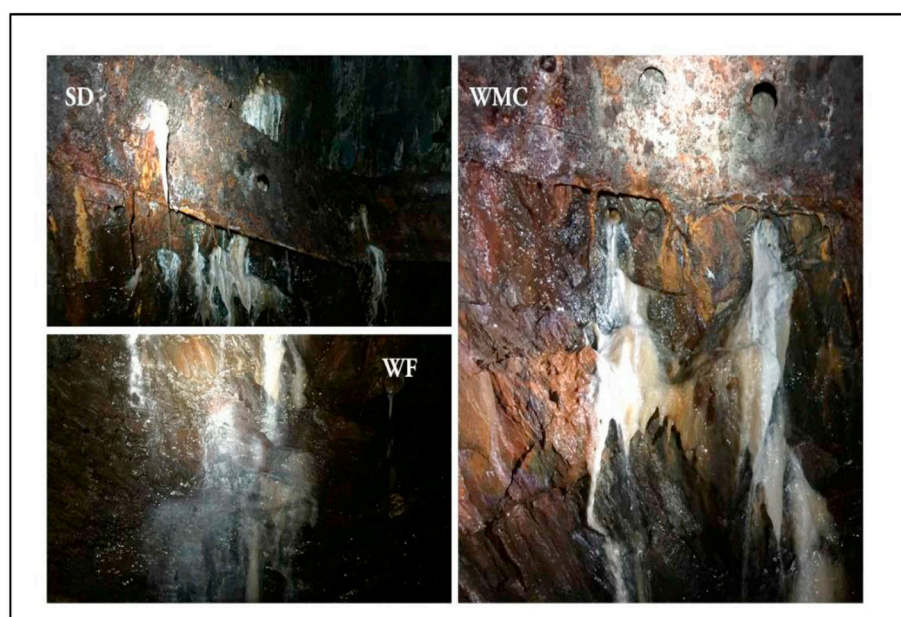
Fig. 1. Sampling sites in SURF mine from the Ross Shaft and #6 Winze at 4850 ft deep level. WMC: Biofilm sample from walls of mine corridor; WF: Biofilm sample associated with water flows coming out through the fractures; SD: Biofilm sample from the sites rife with slime deposits.

Table 1
Preprocessing of the mine biofilm sample: separation of the mucoid/EPS layer (M2 only).

Step	Procedure
1.	Weigh 200 mg of the biofilm sample in a 50 ml falcon tube.
2.	Add 3 ml of phosphate buffer (2 mM Na_3PO_4, 4 mM NaH_2PO_4, 9 mM NaCl, 1 mM KC1, pH 7.0) and incubate for 1 h at 65 °C with occasional stirring. This is done to loosen the interaction between microbial cells and the mucoid layer.
3.	Add 2 ml of the 35% formaldehyde to the suspension, mix well using a magnetic stirrer for 15 min, and keep the solution at 4 °C for 1 h.
4.	Add 10 ml of 1 N NaOH, mix well using a magnetic stirrer for 15 min, and keep at 4 °C for 3 h.
5.	Finally, add 3 volumes of 95% alcohol and incubate at 4 °C overnight.
6.	Centrifuge the solution at 15000 $\times g$ at 4 °C for 20 min. Separate out the supernatant in another falcon tube. This now contains the the EPS/mucoid material along with any extracellular environmental (eDNA) (Table 2), while the pellet contains the microbial cells that need to be lysed.

Table 2
Extraction of the biofilm DNA (bDNA) (M1 and M2).

Step	Procedure
1.	For M2: Weigh 200 mg of the unprocessed biofilm sample in a 50 ml falcon tube. For M1: Take the microbial cell fraction obtained after preprocessing of the biofilm samples (Table 1).
2.	Suspend the sample in 1 ml extraction buffer [100 mM Tris–HCl (pH 8), 100 mM EDTA, (pH 8.0), 1.5 M NaCl] supplemented with 1% tween 20, and 100 μl of MetaPolyZyme (Sigma-Aldrich, USA) (10 mg/ml) at 37 °C (with rotation) for 2 h.
3.	Add 100 μl volume of 10% SDS (w/v) and 250 mg autoclaved glass beads (Sigma, USA). Gentle vortex for 5 min.
4.	Add 25 μl volume of proteinase K (20 mg/ml) and incubate for 1 h (with rotation) at 37 °C.
5.	To remove the beads, spin down the suspension for 20 min, 6000 $\times g$ at room temperature. Distribute the supernatant into 2 ml Eppendorf tubes (500 μl each), avoiding the white surface layer. Pour the liquid slowly into the new tube; the surface layer will remain in the tube - if the surface layer breaks apart, use a pipette.
6.	Add 500 μl chloroform: isoamyl alcohol mix (24:1). Mix by inverting the Eppendorf tubes gently. Microfuge the sample 10 min at 12000 $\times g$. Take the upper aqueous phase into a fresh tube without disturbing the middle phase (or use cut tips to aspirate without disturbing the layers). Repeat this step twice.
7.	To the aqueous phase, add equal volume of isopropanol to precipitate the DNA, uniformly mix by gently inverting the tube and let it stand at room temperature for at least 30 min to 1 h. Then centrifuge the sample at 12000 $\times g$ for 10 min.
8.	Decant the supernatant and be careful not to lose the pellet (pellet may be difficult to see). Wash the pellet with 70% ethanol by centrifuging at 12000 rpm for 15 min. Dry the pellet to eliminate the ethanol with lids open by keeping it at 37 °C (or keep pellet overnight for drying at room temperature).
9.	Add 50–100 μl of Nuclease Free Water (or TE for long term-storage) to the pellet and incubate it for 10 min at 37 °C to dissolve the bDNA.
10.	To purify the extracted DNA obtained, QIAquick silica membrane based commercial Spin Columns (Qiagen, USA) were used. Load bDNA from step 10 onto the spin column. Microcentrifuge at 8000 $\times g$ for 1 min and discard the flow through. Keep the column at room temperature for 15 min, and again elute the purified bDNA using 50–100 μl nuclease free water (New England Biolabs, USA). Then store the bDNA at -20 °C.

Note: Steps 1 to 5 are for lysing the microbial cells and release of the bDNA into the solution.

2.6. Determination of purity of DNA by PCR

The DNA samples were subjected to PCR amplification to check the intactness and the presence of any inhibitory material that can interfere with the amplification. The region encoding 16S rRNA gene (1465 bp) was amplified using universal eubacterial primers 27F (5′-AGAGTTTG

ATCCTGGCTCAG-3′) and 1492R (5′-CGGTTACCTTGTTACGACTT-3′) to determine whether PCR inhibitors were present in the isolated DNA. The reaction mix consisted of 0.5 μl of the metagenomic DNA as the template, 1 μmole of each primer, 12.5 μl of 2× GoTaq Master Mix (Promega) (supplied in 2× Colorless GoTaq Reaction Buffer (pH 8.5), 400 μM dATP, 400 μM dGTP, 400 μM dCTP, 400 μM dTTP and 3 mM

MgCl$_2$), with final volume make up to 25 µl with nuclease free water (New England Biolabs, USA). Thermal cycling was conducted in an Eppendorf Mastercycler Gradient (Marshall Scientific, USA) with the following PCR program: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 1.5 min with a final extension for 10 min at 72 °C. Visual comparison was done under UV light after electrophoresis of 3 µl each of the amplicons on an 0.8% agarose using a gel documentation system (Bio-Rad, USA).

3. Results and discussion

The last decade has been marked by several studies focused on understanding the distribution of microorganisms on the surface and in the subsurface of the Earth (Govil et al., 2019; Rastogi et al., 2013). The deep terrestrial subsurface, such as those exemplified by ultra-deep mines, represents an emerging area in which a bewildering array of metabolic capabilities developed, to cope with an environment characterized by limited nutrient availability, high temperature and pressure (Rastogi et al., 2009). Over a number of years, we have been focusing on extremophiles isolated from the deep biosphere of the Sanford Underground Research Facility (SURF, 4850 ft. deep), formerly known as Homestake Mine, to develop unique extremophilic bioprocesses for various industrial applications including production of biofuels, biopolymers, and other value-added products. A variety of biofilms grow in this mine's corridor walls, where water seeps from intersections or from fractures, which provide an excellent deep-subsurface environment accessible for study. Direct extraction of good quality inhibitor-free environmental DNA (eDNA) from such biofilm samples for subsequent community fingerprinting, has high significance. Fig. 2 and Table 3 show the results of the biofilm DNA (bDNA) extracted using the "MINES" methodology discussed in this study.

In version M2 of the newly developed method, the biofilm samples were preprocessed to loosen the outer mucoid/exopolysaccharide layer and release the bacterial cells free before adding lysis reagents. The main EPS extraction methods that have been widely used by researchers previously include, in the decreasing order of efficiency, use of formaldehyde + NaOH > formaldehyde + heating > EDTA > heating > centrifugation (D'Abzac et al., 2010; Zhang et al., 2012). In the present study, based on these results, the outer EPS layer was loosened by sequential washing with phosphate buffer, formaldehyde and NaOH.

Next, because the biofilm sample collected from the mines contains large numbers of microbial cells belonging to different phyla (Blanco et al., 2014) and are reasonably heterogeneous in terms of their genomic contents, morphology and architecture of their cell wall, it is necessary to disrupt them unbiased in order to investigate the whole microbial community rigorously. Chemical lysis of most of pathogens can be achieved by using agents such as detergents, chaotropic salts or by enzymatic treatment. However, lysis is a significant challenge for thick-walled organisms and high-energy mechanical disruption methods, such as sonication and bead beating, are commonly used (Vandeventer et al., 2011). In 2017, under the Extreme Microbiome Project (XMP) launched by the Association of Biomolecular Resource Facilities Metagenomics Research Group (ABRF MGRG), a novel multienzyme extraction blend of six enzymes (Achromopeptidase, Chitinase, Lyticase, Lysostaphin, Lysozyme and Mutanolysin) called PolyZyme or MetaPolyZyme, was designed to extract total metagenomic DNA from the cell wall of bacteria, yeast, and fungi (Tighe et al., 2017). Therefore, for unbiased lysis of the entire representative microbial community, we employed a combination of PolyZyme and mechanical (bead beating) techniques to lyse cells. Direct DNA extraction methodology was followed to lyse the cells *in-situ,* i.e., while within the sample.

The three biofilm samples examined were heterogenous and differences in DNA yield from similar amounts of different samples were observed in both versions of the method. Nevertheless, we successfully extracted a sufficient amount of quality DNA from all the tested samples
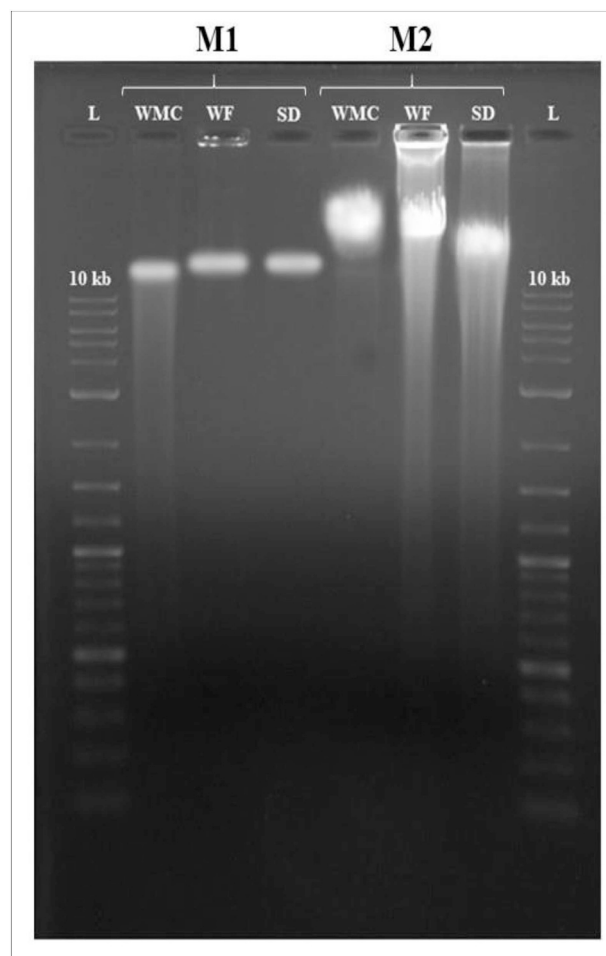

Fig. 2. Agarose gel electrophoresis (0.8%) of microbial genomic DNA isolated from biofilm samples. WMC; Biofilm sample from walls of mine corridor; WF: Biofilm sample associated to water flows coming out through the fractures; SD: Biofilm sample from the sites rife with slime deposits; M1 = Extraction without preprocessing; M2 = Extraction with preprocessing; L = 10 kb 2-Log-DNA ladder (NEB).

(Fig. 2, and Table 3). The total yield of bDNA, irrespective of the sample types, was always higher, typically 971–1715 ng/g in the M2 procedure compared to the extraction performed without preprocessing (471–533 ng/µl) in the M1 procedure (Table 3). The introduction of preprocessing to the extraction procedure (in M2) increased the concentration of DNA extracted from mucoid biofilm samples by almost 3-times. This may be because detergents present in the lysis buffers resulted in the dissolution of the gelatinous mucus created during the preprocessing step, which released even the extracellular DNA present in the collected sample, and thus led to a high quantity of DNA recovered from the biofilm samples. Besides which, the use of a combination of bead beating along with polyZyme pre-treatment with lysing enzymes, likely ensured full lysis of recalcitrant microbes. Correspondingly, in terms of quality of DNA, all the isolated DNA samples (M1 and M2) had A260/280 between 1.65 and 2.0, and A260/230 between 1.8 and 2.5 (Table 3). Also, the "MINES" methodology enabled recovery of a larger amount of High Molecular Weight bDNA (maximum size, 16 to 20 kb), indicating a smaller amount of DNA shearing (Fig. 2).

DNA isolated after M2 (extraction with preprocessing) was digested easily, while DNA isolated using M1 (without preprocessing) was not (Fig. 3.1). This indicates that the EPS layer hindered the restriction digestion of DNA, which was digested easily after the layer was partially removed or loosened following the entire M2 procedure. Complete digestion of bDNA indicates absence of inhibitory compounds in the isolated DNA samples (Fig. 3).

Table 3

Average concentration and total recovery of nucleic acids isolated from different biofilm samples from SURF mine.

Sample	Method	Nucleic acid concentration (ng/μl)	Nucleic acid concentration (ng/g sample)	A260/280	A260/230
WMC	M1	100.33 ± 7.38	501.66 ± 2.38	1.74 ± 0.016	1.86 ± 0.016
WF	M1	94.33 ± 11.00	471.65 ± 55.00	1.85 ± 0.023	1.66 ± 0.065
SD	M1	106.67 ± 8.98	533.35 ± 41.49	1.64 ± 0.036	1.60 ± 0.076
WMC	M2	394.33 ± 7.29	1971.65 ± 2.28	1.89 ± 0.014	1.89 ± 0.016
WF	M2	374.67 ± 5.00	1873.35 ± 25.00	1.99 ± 0.023	2.48 ± 0.065
SD	M2	243 ± 2.98	1215 ± 10.38	1.90 ± 0.036	2.10 ± 0.0766

WMC; Biofilm sample from walls of mine corridor; WF: Biofilm sample associated to water flows coming out through the fractures; SD: Biofilm sample from the sites rife with slime deposits; M1 = Extraction without preprocessing; M2 = Extraction with preprocessing; L = 10 kb 2-Log-DNA ladder (NEB).
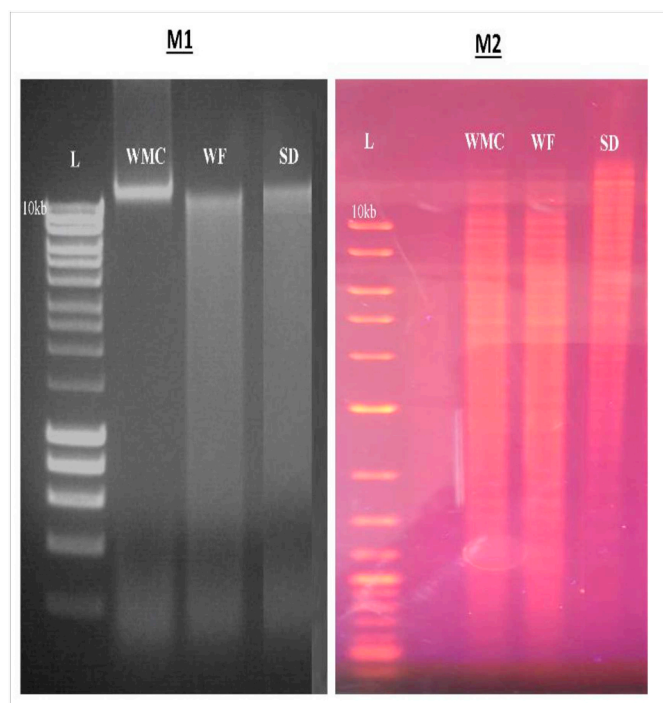

Fig. 3. Restriction fragment analysis of extracted DNA subjected to Hind III and Bam HI digestion, on an 0.8% agarose gel. WMC; Biofilm sample from walls of mine corridor; WF: Biofilm sample associated to water flows coming out through the fractures; SD: Biofilm sample from the sites rife with slime deposits; M1 = Extraction without preprocessing; M2 = Extraction with preprocessing; L = 10 kb 2-Log-DNA ladder (NEB).

Fig. 4. PCR amplification of complete 16S rRNA gene of biofilm samples from the mine. WMC: Biofilm sample from walls of mine corridor; WF: Biofilm sample associated to water flows coming out through the fractures; SD: Biofilm sample from the sites rife with slime deposits; M1 = Extraction without pre-processing; M2 = Extraction with preprocessing; L = 10 kb 2-Log-DNA ladder (NEB).

Additionally, the quality of the isolated nucleic acid, further assessed by PCR amplification of the 16S rRNA gene (Fig. 4), revealed that amplification was successfully carried out with all the tested bDNA templates extracted using the current methodology, with and without preprocessing. However, while an intense amplified band was observed at 1.5 kb from the three samples (WMC, WF, and SD) when pre-processing was performed, the 16S rRNA gene bands amplified from the extracted DNA without pre-processing were light in intensity. Hence, genomic DNA purified by the "MINES" procedure discussed in this paper was sufficiently clean and could be used successfully for restriction digestion and PCR amplification, which confirmed the suitability of the isolated DNA for NGS application.

While earlier studies generally report that methods extracting the highest amounts of DNA are also the methods that produce the lowest DNA purity and integrity (Corcoll, 2017), our results showed that the M2 procedure used in this study (i.e. pre-extraction processing of the sample for washing EPS/humic substances), produced DNA with higher yield, purity and integrity. The most significant observation was that
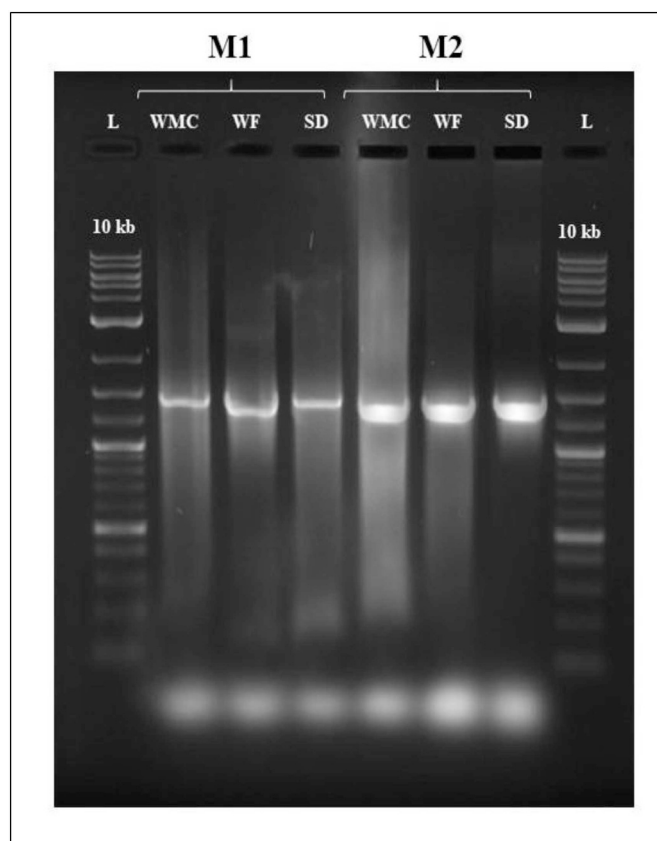
both procedures (M1 and M2) reduced shearing of the genomic DNA which is needed for the downstream molecular analyses, such as PCR and large insert genomic library construction, without further purification or selection steps.

Additionally, as an indicator of the applicability of our methodology to extract metagenomic DNA, M1 yielded good concentration and quality of DNA from other environmental samples as well as cultivatable organisms of both Gram-positive and Gram-Negative architecture. The concentration of DNA (ng/μl), A260/280 and A260/230 were respectively found to be 266 ± 0.25 ng/μl, 1.74 ± 0.048 and 1.86 ± 0.075 for the LC sample; 222.2 ± 0.72 ng/μl, 1.64 ± 0.36 and 1.66 ± 0.088 for the KD sample; 242.2 ± 0.22 ng/μl, 1.80 ± 0.086 and 2.06 ± 0.065 for *Geobacillus* sp. strain WSUCF1;
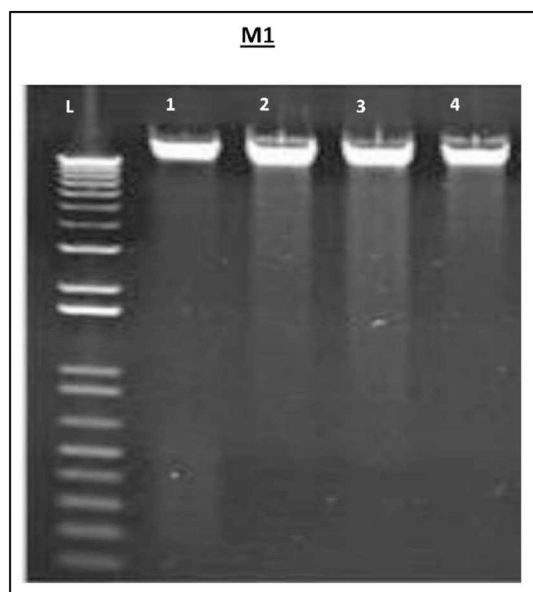
Fig. 5. Agarose gel electrophoresis of microbial genomic DNA isolated from various environmental and bacterial samples. 1: Landfill Compost (LC); 2: Kitchen Digest (KD); 3: *Geobacillus* sp. strain WSUCF1; and 4: *E. coli* DH5α; M1 = Extraction without preprocessing; L = 10 kb 2-Log-DNA ladder (NEB).

and 256.0 ± 0.27 ng/μl, 1.96 ± 0.096 and 2.16 ± 0.055 for *E. coli* DH5α (Fig. 5).

4. Conclusion

An improved method (the "MINES" method) was developed for purification and extraction of intact and good quality metagenomic DNA from a minimal amount of deep mine (SURF) biofilm samples, as well as extraction of cultivable microorganisms. It was evident that sample preprocessing, to loosen the outer mucoid/exopolysaccharide layer, increased DNA yield in the DNA extracts. Overall, the "MINES" biofilm extraction method overcomes two major problems: i) inappropriate rupturing of cells and ii) contamination with EPS/humic substances. The quality and quantity of extracted DNA are suitable for various downstream applications including restriction enzyme digestion and PCR amplification. Therefore, the methodology developed in this paper is appropriate for samples from difficult environments, such as those with high organic matter contents.

Declaration of competing interest

None.

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