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Short communication

Composition of bacterial community in enrichment cultures of shale by-products from Irati Formation, Brazil

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ABSTRACT

We examined microbial communities from enriched fine and retorted shale particles using sequencing of V4 variable region of 16S rRNA. High number of microbial genera was found in both enriched shale by-products that were dominate by Actinobacteria, Firmicutes and Proteobacteria, showing differences due to microbial colonization after the pyrolysis process.

Oil shale is defined as fine-grained sedimentary rock containing high amounts of organic matter that is predominantly kerogen, which will yield oil and combustible gas upon destructive distillation.[1] Oil shale resources are abundant and distributed in several countries around the world. The most important Brazilian oil shale reserve, the Permian Irati Formation located in São Mateus do Sul, Paraná, has the greatest potential for economic development because of its accessibility, grade, and wide-spread distribution.[1] The mining and processing of shale generate solid by-products that occur in two distinct stages the fine shale particles (FS) and retorted shale (RS). The FS are small fragments of shale rock measuring less than 0.25 in. with an oil content of approximately 15% and are 20% of mined rock; thus, FS are not usable in oil and gas processing.[2] Retorted shale (RS) is a waste by-product obtained from pyrolysis (550 °C) of oil shale processing.[3]

Although the interaction of oil shale with the microbial community is an interesting topic, until now, this subject has not been well examined. Due to the lack of information regarding the occurrence of microorganisms in the shale by-products from the Irati Formation, knowledge of the microbial community composition can generate important data and suggests how bacteria can act in providing complex nutrients, thus adding value to by-products as agricultural inputs. In this perspective, the purpose of this study was to examine the composition of microbial communities from enriched FS and RS using sequencing of V4 variable region of the 16S rRNA gene.

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Aiming to evaluate if there would be differences in the bacterial community over time sampling was carried out in 2008 (FSI and RSI) and 2011 (FSII and RSII) at Schist Industrialization Business Unit (Six) of Petrobras, in São Mateus, Paraná, Brazil (25°52′26″ S, 50° 22′58″ W) in storage piles of shale by-products as described by Goes et al.[4] An aliquot of 10 g from four samples of shale by-product were enriched in Luria Bertani (LB),[5] J. E. medium[6] and dispersed sterile water for 30 days prior DNA extraction using the MoBio Powersoil DNA isolation kit (Geneworks, Australia). Extracting high-purity DNA directly from environmental samples is essential for the study of microbial community composition. Direct DNA extraction was performed from by-products at least three times without success. To allow the proliferation of microorganisms that could grow on the medium provided and then make available extractable DNA; we used an enrichment method.[7] The total amount of DNA extracted was quantified and checked for purity with a Qubit™ Fluorometer using Quant-iT™ dsDNA BR assay kit (Invitrogen™). Bacterial DNA was amplified using primers F515 (5′-GTGCCAGCMGCCGCGGTAA-3′) and R806 (5′-GGACTACHVGGGTWTCTAAT-3′) targeting the V4 region of the SSU rRNA gene.[8] Barcoded libraries were sequenced on Ion 314™ Chip (10 Mb.p.) using the Ion Torrent Personal Genome Machine – PGM (Life Technologies, USA). Reads were filtered within the Ion Torrent server to remove low quality and polyclonal sequences. The resulting sequencing data sets were uploaded to the Metagenome Rapid Annotation using Subsystem Technology (MG-RAST) (http://metagenomics.nmpdr.org/), checked for low-quality reads prior to dereplication, assigned phylogenetic identification of phylum level using the Ribosomal Database Project (RDP) with 95% identity and the alpha diversity index calculation (http://rdp.cme.msu.edu/index.jsp).[9]

Differences between the taxonomical distribution were analyzed in the Statistical Analysis of Metagenomic Profiles program (STAMP).[10] The data corresponding to phylum distribution were submitted to ANOVA statistical and a multiple groups comparison test by Tukey-Kramer test ($p \leq 0.05$) to construct a principal component analysis (PCA) plot and a similarity dendrogram (heatmap) using the UPGMA algorithm (Unweight Pair Group Method with Arithmetic mean). The mean proportion between the phylum level samples was analyzed by Fisher's statistical test, with Newcombe-Wilson confidence intervals ($p \leq 0.05$).

The two samples of RS and two of FS were enriched with two culture media, LB and J. E. media, with the intent of favoring different bacterial groups, however only LB medium allows DNA extraction and PCR amplification.

In total, 157,156 reads were generated with an average length of 214 bp, and 69% of the sequences were annotated with the MG-Rast server (E value $<10^{-5}$) after filtration (MG-Rast ID: mgm4560416.3, mgm4560417.3, mgm4560419.3, mgm4560420.3). We assessed the fraction of species sequenced from shale by-products by the rarefaction curves, which tended to approach the saturation plateau for FS samples, while RS samples showed that this by-product has not been exhaustively sampled (Fig. 1).

The results showed that 90% of the sequences were assigned to the domain bacteria. Of the 17 phyla detected in the shale by-products Firmicutes, Proteobacteria and Actinobacteria were the most abundant (Fig. 2). The FS had the lowest alpha diversity index with FSI at 6.26 and FSII at 6.86, while the α-diversity indices from RS (I and II) phylum were 24.34 and 15.87, respectively, showing that high bacterial diversity in the RS samples. The differences found between the two by-products could be partially explained by chemical composition or due to the microbial colonization after the pyrolysis process, since it involves high temperature treatment seems like this could be a sterilizing step with regards to the extant microorganisms. In areas surrounding the oil shale industry in the north-eastern part of Estonia were found that, even after 10 years, a few dominant populations and low diversity characterized the microbial community in this area.[11,12]

We employed STAMP software to examine the taxonomic profiles of enriched samples from the two RS and FS

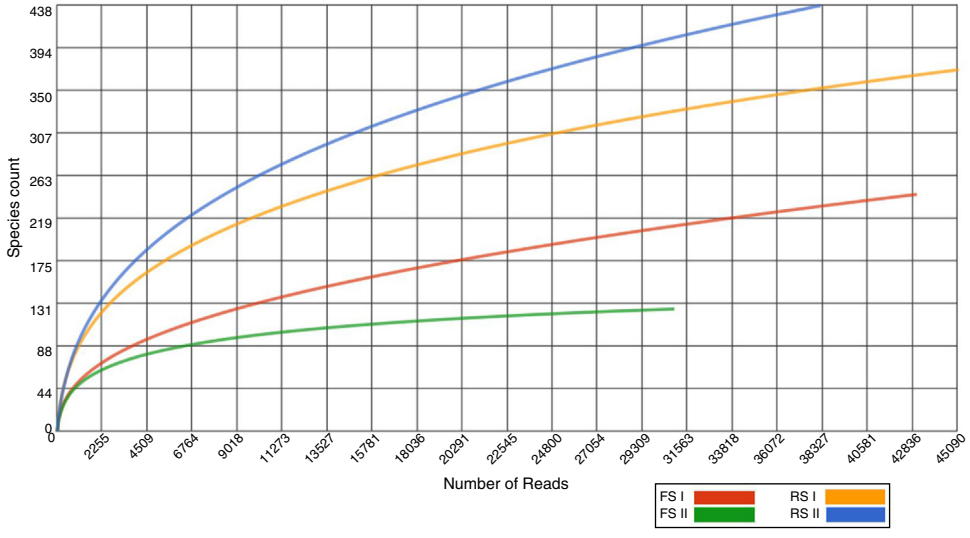

Fig. 1 – Rarefaction curves for bacterial libraries of the shale by-product samples from Irati formation. Phylotypes defined at 95% similarity of sequence and analyzed in MG-Rast.

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