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ISOLATION AND IDENTIFICATION OF BROWN COAL SOLUBILIZING MICROORGANISMS IN COAL MINING SOIL

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ABSTRACT

Relatively few microbiologist, and perhaps other fewer scientist in the allied fields have seriously considered that microorganisms might be able to modify the physico-chemical structure of coal. The reason behind this is that, microbiologists usually prefer simple sugars, organic acids and the like as substrates for microbial activity, and they try to avoid the use of too complex substrate such as coal. The coal humic substance importance in crop yield improvement is understood and accepted. But the coal mine landscape that remain after mining process is devastated with infertile soil. Present study is directed towards isolating organisms that could replenish the fertility and condition coal mining soil for agriculture. During this study eight bacterial and four fungal isolates have shown significant ability to carry out solubilization of weathered coal, these cultures were identified as Serretia marcescens, Providencia rettgeri, Pseudomonas sp, Lysinibacillus macroides, Acinetobacter sp, Stenotrophomonas maltophilia among bacteria and Coprinus scerotigenis, Penicillium sp, Trametes versicolor, Cunninghamella sp among fungus.

Keywords; *Bacterial Isolates, Fungal Isolates, Environmental degradation, fertilizer, Humic substances.*

INTRODUCTION:

The worldwide coal deposits are considerably larger than those of oil and therefore, coal could become again the main resource of raw materials (feed stock) for the small and medium enterprise (SME). In this context the conversion technologies for coal are urgently needed to reduce environmental damages caused by the classic carbon-chemistry process. [5, 6]. Another reason to study the microbial conversion of coal is attributed to environmental problem of the coal mining areas (e.g., the huge open-cast mines in coal mining cities, Nigeria). When coal mining finishes in a region, the landscape that remains is usually devastated with infertile soil

which has to be re-cultivated [3]. The degradative activities of microbes towards the residual coal may be of significance if fertility is to be improved and intact soils recreated by mobilizing the humic substances in coal. However, even though coal is indeed comparatively resistant to microbial attack [4,2,1], but we hoped that there could be some microorganisms that could be capable of modifying the coal structure by different mechanism.

Usually, the microorganism grows slowly on coal particles but the growth is noticeably stimulated when naturally weathered or chemically pre-oxidized coals are used [7,10]. Residual cellulose and hemicelluloses that are found in brown coals might be an additional carbon source for microorganisms. The addition of mineral solutions in medium enriched with coal particles stimulates the microbial growth, indicating limitations of essential elements in native coal [7]. The utilization of brown coal accompanied by its solubilization by many aerobic microorganisms resulting to its derived products used as fertilizers and soil conditioners in agriculture cannot be over emphasized.

The present study focuses on the isolation and identification of microorganisms capable of solubilizing weathered coal particles in devasted infertile soils in coal mining zones for agricultural cultivation.

Materials and method

Collection of Soil Samples:

Soils samples were collected from 2 coal mining areas of Eastern region, Nigeria, with history of coal mining activities for more than 50 years. Surface soil from 0-15cm were collected, placed in plastic bags, transported on ice to the laboratory and stored at 4^0C until analysis. Soil samples were air-dried and sieved through a 10mm mesh prior to bacterial screening.

Screening and isolation of weathered coal Solubilizing bacteria.

Ten grams of soil samples were added to 100ml MSYM and enriched with an addition of 10mg ground coal particles. Samples were incubated on rotary shaker (150 rpm) at 30^0C for 7 days and then transferred to a fresh medium and incubated at the same conditions, after which, the cultures were regularly transferred every 3-4 days or until increased turbidity were evidence. After 3-4 times of repeated sub-culturing, 0.1ml culture broth was pipette and spread on MSYM+coal particle agar. Single colonies were selected and streaked on nutrient agar (NA) supplemented with 25,50 and 100ppm of the coal particles. Cultures were incubated at 30^oC for 3 days. Coal solubilizing isolates were selected from isolates which develop clear zone surrounding their colonies when grown on NA supplemented with 25,50 and 100ppm of the coal particles [8].

Characterization of Bacterial Isolates

Morphological Studies:

Bacterial isolates were grown in MSYM +coal particles and incubated at 30^0C until either turbidity or colony was observed. Gram's stain and cell morphology was investigated under microscope (1000 x magnification) [8]

16s DNA Analysis:

Genomic DNA was extracted by boiling method [8]. One ml of cell culture grown in nutrient broth at 30^0C for 22hrs was centrifuged at 10,000 rpm for 10 min then washed 2 times with buffer (pH.7.8). Washed cell was resuspended with 0.3 ml buffer (pH 7.8) then boiled at 100^0C for 10 min, followed immediately by cooling at 0^0C for 5min. The boiling and cooling step was repeated 3 times. The 16s DNA from the cell extract obtained with the above procedure was amplified by PCR using 63F (5-CAGGCCTAACACATGCAAGTC-3) and 1492R (5-ACGGCTACCTTGTTACGACTT-3-) primers. The reaction composition consisted of 0.2mM each dNTP, 0.2M of each primer, 5110XPCR buffer, 101 cell extract, 2.5 units Taq DNA polymerase and sterile deionized water to a final volume of 50litter. Following a hot start (94^0C for 3 min), 25 cycles of amplification were used (94^0C for 1 min, 50^0C for 45 sec, 72^0C for 2 min) followed by a final single extension of 72^0C for 10 mins. The PCR products were separted by electrophoresis on a 1% agarose gel and visualized under uv light after staining with ethidium bromide. The amplified PCR product was purified using QIA quick PCR purification kit (QIAGEN, Inc) according to the manufacturer's instruction. DNA sequencing was performed by Macrogen, Inc. (South korea) and 16s DNA sequences were BLAST search against Gen Bank database (http://www.ncbi.nim.nih.gov/).

Isolation, Identification and Screening of Fungus:

The collected soil samples were amended with 25ppm of the coal particles mixed thoroughly and this mixture is directly incubated at room temperature for about two weeks. The soil samples were washed with distilled water and allowed to stand still for 30 mins. After all the soil debris has settled down, the supernatant was decanted into a sterile test tube and serially diluted. Dilutions below 10^{-6} were plated in Potato Dextrose Aga (PDA)+ coal particle medium. After 6-7 days of incubation a number of fungal strains were observed on the plate [9]. The most prominent fungus was selected and identified as *penicillium sp*. The isolated fungal colonies were transferred on to the PDA slants and afterwards once again tested to grow in presence of coal particles by culturing them in PDA +coal partilces medium, cultures showing highest degree of solubilization were selected for further studies [8,10].

Bioremediation Assay:

To study the bioremediation of coal particle using *Penicillium* sp, two different culture media were prepared in triplicate – medium containing PDA and 0.5 percent (w/w) of coal particles and medium containing PDA, 0.5 percent [w/w] of coal particles with the spores of *Penicillium* sp. The plates were then maintained for about 8 days at 28^0C in an incubator [10].

Results and Discussion

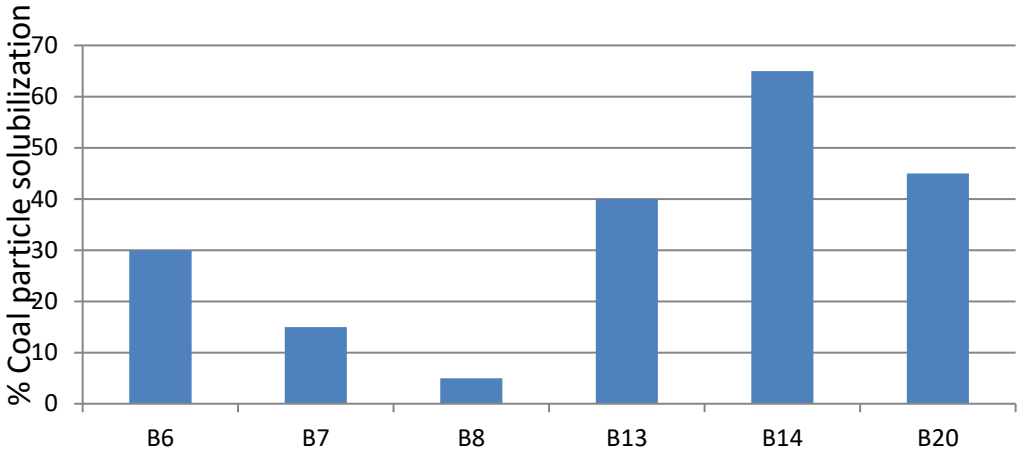
During this study eight (08) bacterial isolates (Table 1) were found to show ability to carry-out the solubilization of the coal particles, these cultures were identified as *Lysinidacillus macroides, Stenotrophomonas maltophilia, Acinetobacter sp, Pseudomonas sp, Previdencia rettgeri and Serretia marcescens.* Their percentage solubilization performances are shown in [Figure 1] with *Pseudomonas* sp showing the highest percentage (65%) while the least among the eight isolates, stenotrophomonas maltophilia had five percent (5%).

Fungi isolates (Table 2) were found to show significant ability to solubilize the coal particles in this study. The isolates and their percentage coal solubilization (Figure 2) shows that penicillium has the highest capacity with (82%), followed by *Cunninghamella* sp (78%), *Coprinus screotigenis* (70%) and *Trametes versicolor* (55%)

Table 1: Isolated bacteria from coal mining soil

S/NO	ISOLATES	IDENTIFIED CULTURES
1.	B13	*Serretia marcescens*
2.	B20	*Providencia rettgeri*
3.	B14	*Pseudomanas sp*
4.	B6	*Lysinibacillus macrolides*
5.	B7	*Acinetobacter sp*
6.	B8	*Stenotrophomonas maltophilia*

Figure 1: % solubilization of coal particles by the isolated culture

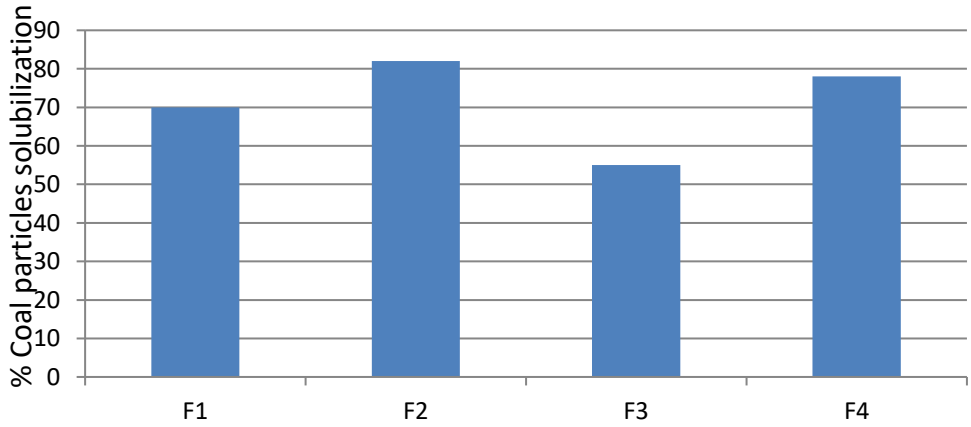


Identified culture isolates

Table 2: Isolated fungi from coal mining soil

S/NO	ISOLATES	IDENTIFIED CULTURES
1.	F1	*Coprinus scerotigenis*
2.	F2	*Penicillium sp*
3.	F3	*Trametes versicolor*
4.	F4	*Cunninghamella sp*

Figure 2: % Solubilization of coal particles by the isolated culture



Identified culture isolates

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