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Isolation And Characterization Of Oil Degrading Bacteria From Industrial Environments

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ABSTRACT:

In order to improve bioremediation applications, the objective of this study was to isolate and characterize oil-degrading bacteria in industrial environments. Samples were collected from contaminated soils in oil refineries and terminals, and negative bacteria were isolated and sequenced from the sample collection. through the use of column purification techniques, PCR (polymerase chain reaction), gel electrophoresis, Gram stain, and Sanger sequencing. data interpretation. 16s rRNA (ribosomal ribonucleic acid) was used for their identification. Find bacteria in the sample to grow them to break down hydrocarbons and oil refinery sludge. It is very useful to find and grow negative bacteria to eliminate oil residues, because it reduces air, soil and water pollution. Therefore, it can be concluded that the introduced bacterial isolates. These introduced bacteria function primarily to break down long-chain hydrocarbons, which are broken down by oil-degrading bacteria found in marine sedimentary environments. Bacteria for bioremediation were detected in the microbial culture based on sequence homology and phylogenetic analysis.

KEY WORDS: biodegradation, bioremediation

INTRODUTION:

Oil is the most sought-after energy source and plays a major role in the energy needs of industrialized countries. So it was necessary to do this, but oil sludge is one of many pollutants. Most of the sludge is released into the environment untreated, which can lead to a number of environmental complications. The hydrocarbons in the sludge can slowly seep into the soil and contaminate source rocks and groundwater. On the other hand, the light hydrocarbons evaporate and form a layer of oil-contaminated dust, which can lead to air pollution. One of the main problems in oil refineries is the safe disposal of the oil sludge that is generated during the refining process. Although burning oil sludge is a quick and easy way to get rid of it, it causes more pollution. It has an economic impact as it is expensive to treat and dispose of oil sludge safely. Companies have to invest in specialized technologies, and improper disposal of oil sludge can result in fines or legal action for environmental violations. In addition, oil sludge contains residual hydrocarbons, so if it is not properly treated to recover usable oil, energy is wasted. The health hazard is Hazardous materials found in oil sludge include benzene, lead, arsenic, and other carcinogens. Workers who handle oil sludge without appropriate safety equipment risk inhaling or inhaling these toxins. Workers and surrounding populations can suffer from respiratory problems, skin irritations and other health complications after exposure to oil sludge. Significant environmental damage, health hazards, operational inefficiencies and high costs are all associated with oil sludge. In fact, the objective of the preliminary study was the separation and characterization of the microorganisms present in the oil sludge.

MATERIALS AND METHODS

Serial dilution

Take a test tube of 11 test tubes and calculate 1 gram of sample with the balanced weight, the 10 test tubes filled with 9 ml of distilled water in each 11 test tubes marked. 1 is 100 tubes filled with 10 ml, then another tube 10-1 to 10-10, 1 gram of sample is distributed in the first tube, then To ensure uniform dilution, mix well the contents of the first dilution tube. Transfer a known volume from the first dilution tube to the second dilution tube containing the diluent with a clean pipette tip. Each subsequent dilution tube should be prepared using the same dilution procedure. When the desired dilution series is reached, repeat steps 6-7 for the other dilutions. then 100 will pull 10 more test tubes in the incubator in 24 hours.

Media preparation

Prepare the medium for the growth of bacteria or other organisms, the medium is also a source of energy for the growth of bacteria, take a conical flask, wash and rinse with ethanol, then dry after calculating the nutrient agar-agar according to the weight of the balanced machine, then mix the nutrient broth (0.65) and agar-agar (0.75) in distilled water (50 ml), autoclave the medium preparation, including the empty Petri dish, cover with newspaper, also autoclave the medium and petri. then put in laminar airflow.

Pour plate

Take a 3 liter petri dish and prepare the medium. The inoculated agar is poured into a sterile petri dish and allowed to solidify in a laminar airflow.

Spread plate

3 pour plates are labeled, one for 10-3, one for 10-7 and one for control. Pipette 50 µl of the desired dilution series onto the center of the surface of a combustion plate. Spread the sample uniformly over the surface of the medium using the sterile glass spreader while carefully turning the Petri dish upside down. Incubate the plate at 37°C for 24 hours.

Steak plating

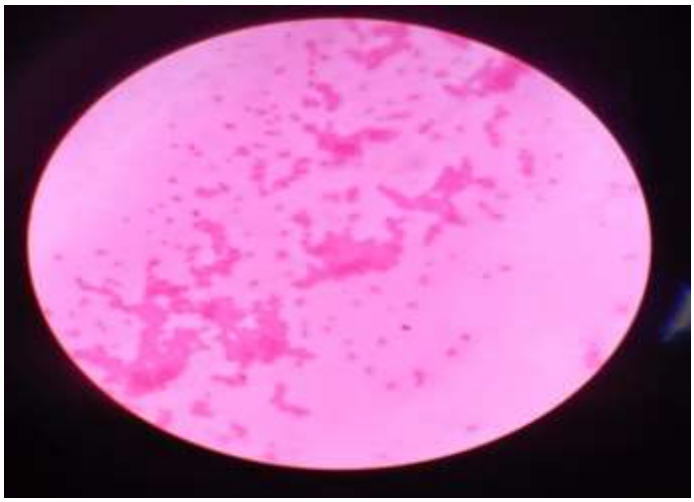
This is also a subculture of taking broth (0.52) and agar-agar (0.6 g) mixed in 40 ml of distilled water, then squeeze the cotton and autoclave, then remove everything and pour into a Petri container dish, then solidify in laminar. lamp, the inoculation cycle is taken, the lamp is on, the cycle is very hot and we wait for the cycle to return to normal temperature, the bacteria are taken from the spreading plate with the inoculation cycle, the boiling is done by five. methods, but we use the continuous steak method, the inoculum plates are incubated at 37 ° C for 24 hours.

Inoculation

Two test tubes are taken, the liquid (0.13) is mixed with distilled water (10 ml), then placed in a laminar, 10 ml is divided into two test tubes of 5 ml each, then the flask is turned, the cycle is nice and warm, wait until the flask returns to normal temperature and the bacteria from the plate spread into the mixed solution of the test tube, also incubate at 37 ° C for 24 hours.

Gram staining

The Gram stain test is used to identify bacteria and classify them as Gram positive or Gram negative. Take a slide in a drop of distilled water and bacteria taken from a steak. Peel them on the blade, then add a drop of purple crystals. Wait a minute later, wash with distilled water and add grams of iodine. Wait a minute later, wash with distilled water and add a drop of bleach or ethanol. Wait a minute later, wash with distilled water and finally add a drop of saffron. Wait a minute, then wash with distilled water. Gram stain is a Gram negative with two colours. red to pink and Gram positive is a thick layer of peptidoglycan, so purple or purple. Our bacteria are gram negative.



DNA isolation

The isolation process involves the identification of DNA bands that separate DNA from other cellulosic compounds. DNA isolation should involve several steps. This involves a 1 ml culture sample collected in an Eppendorf tube, centrifuged at 4000 rpm for 5 minutes. Decant the supernatant, add 100 µl of PBS, 400 µl of cell lysis buffer and 5 µl of proteinase to the pellet tube, incubate at 55 °C for 1 to 2 hours, add 500 µl of saturated phenol to the sample and mix well for 5 minutes, centrifuge. at 12,000 rpm for 10 minutes, transfer the supernatant to a new tube, add 25:24:1 phenol:chloroform:isoamyl alcohol to a tube, mix for 5 min with inversion, centrifuge at 12,000 rpm for 10 min, transfer the supernatant and add 500 µl of chloroform, mix for 5 min with inversion, centrifuge at 12,000 rpm for 10 min, carefully transfer the water. layer in a new tube and add an equal volume of 100% ethanol or isopropanol, Store at -20°C for 1 to 2 hours, Centrifuge at 14,000 rpm for 20 minutes, Decant the supernatant, add 1 ml of 70% ethanol, Centrifuge at 14,000 rpm for 10 minutes to the supernatant, Dry the pellet at room temperature to remove droplets, add 50 to 60 µl of elution buffer.

Gel Electrophoresis and Quality check

used to identify DNA bands and quality control from an isolated sample that they use in gel electrophoresis, they only see under UV light. The quantity was measured with the NanoDrop spectrophotometer and the quality was determined with a 1.5% agarose gel. A single high molecular weight DNA band was observed.

Result:

PCR Amplification

Microbial DNA was amplified with universal primers for the identification of bacteria by PCR from the DNA isolated above. A single discrete PCR amplicon band of 1523 bp was observed after agarose gel resolution. The PCR product was purified to remove contaminants.

Purification by Column

PCR products were purified by column purification kit method. PCR purification, or PCR purification, is a process that prepares purified DNA for downstream applications. Here are some steps for PCR purification: Add an enzyme mixture Add an enzyme mixture to the PCR reaction and let it stand at a specific temperature. One enzyme digests the excess primers, while another enzyme renders the nucleotides useless in the reaction. Heat the reaction mixture Heat the reaction mixture to 80 degrees to inactivate the enzymes. Using a column Insert a connecting column into a collection tube, add the solution to the column and centrifuge. Then transfer the PCR reaction mixture to the tube, add the binding solution and centrifuge again. Repeat these steps with the wash solution, then spin without adding the wash solution. Add the elution solution Add the elution solution to the column, centrifuge and discard the column. The PCR amplification products are found in the eluate.

Sequencing

The purified PCR amplicon was sequenced with one of the universal forward and reverse primers. Sequencing was performed using the BDT Cycle Sequencing Kit v3.1 on the ABI 3500 Genetic Analyzer.

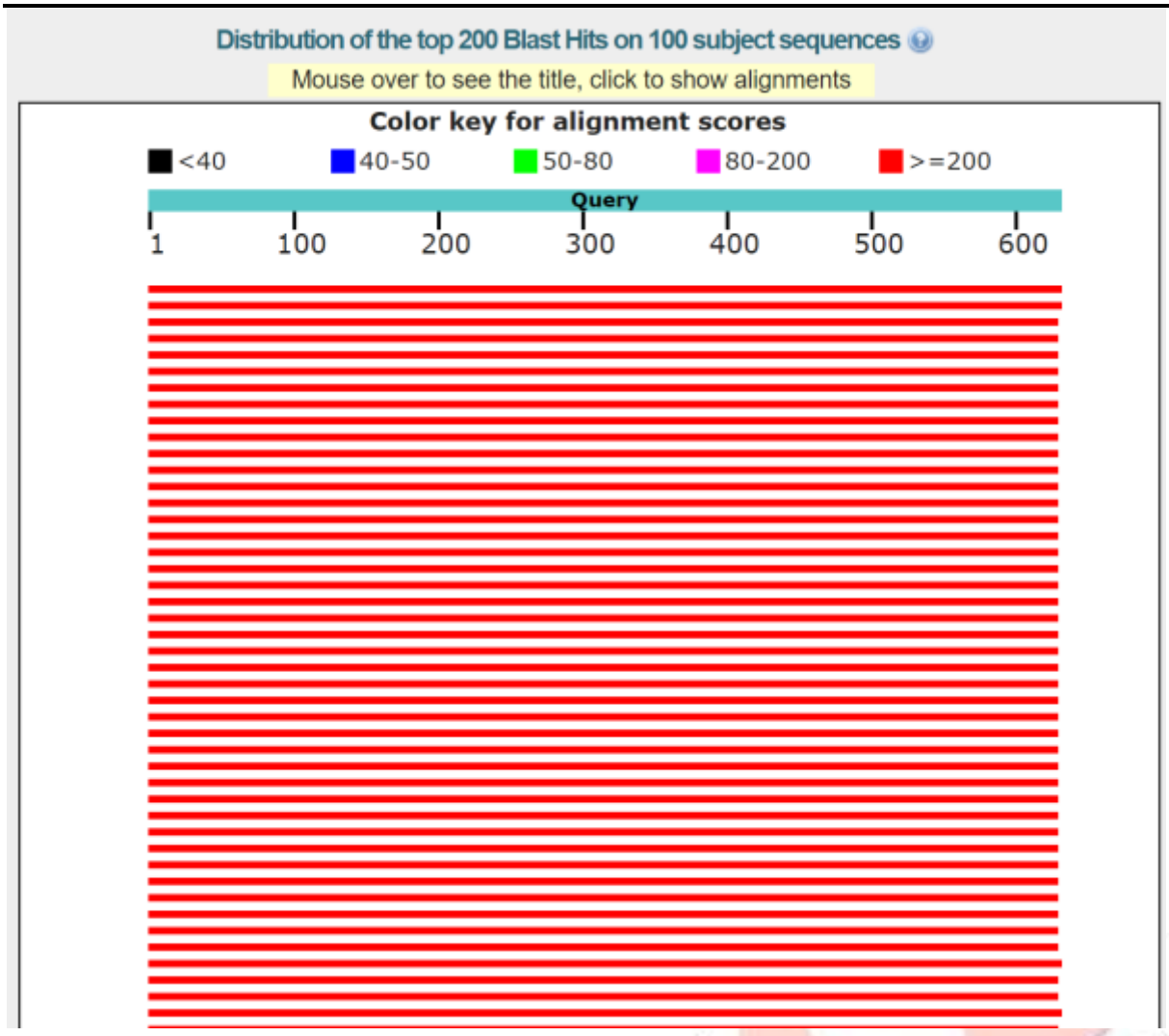
Data analysis & Interpretation

27F	AGAGTTTGATCCTGGCTCAG
1492R	CGGTTACCTTGTTACGACTT

>PCR_Bac_16SF_C04.ab1

CGGGCCTTACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCA
TTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTG
ACATCCTCTGACAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCAT
GGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGAT
CTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGT
GGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGG
TACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATT
GTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCG
GTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGA
AGTCGGTGGGGTAACCTTTTTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAG
TCGTAACAAGGTAACCGAA

The consensus sequence was aligned using BLAST tool



Based on maximum identity score first ten sequences were selected for further analysis.

Using NCBI software, the sequence distance was calculated and the phylogenetic tree was constructed.

Based on sequence homology and phylogenetic analysis, the Microbial culture, was found to be

Bacillus cereus strain CH1 16S ribosomal RNA gene, partial sequence submission in NCBI our gen bank id: PQ427146.1

https://www.ncbi.nlm.nih.gov/nuccore/PQ427146.1

Conclusion

The conclusion of the isolation and characterization oil-degrading bacteria from industrial environments for bioremediation they DNA isolate, run the gel electrophoresis and PCR amplifying , sequencing was done using BDT v3.1 Cycle sequencing kit on ABI 3500 Genetic Analyzer, the finally find the **Bacillus cereus strain CH1 16S ribosomal RNA gene, partial sequence** submission in NCBI our gen bank id: PQ427146.1

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