IDENTIFICATION OF ISOLATED ORGANISMS FROM PETROFIELDS BY GENE SEQUENCING

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ABSTRACT:

Hydrocarbon degradation can occur with diverse varieties of microbes in nature. Different chemical compounds have shown their susceptibility to the microbial degradation. . Fungal species generally forms carcinogenic trans – diols while bacterial species generally forms cis – diols. Contaminated soil contained significantly higher amount (50 – 75 %) of gram negative bacteria having genotypes enclosing genes compared to pristine soil (0 – 12.5 %). Application of bioinformatics tools like BLAST has identified microorganism1 as Bacillus cereus, microorganism2 as Pseudoxanthomonas mexicana, microorganism3 as Halomonas daqingensis and microorganism4 as Parapusillimonas granuli. These microbes were given names accordingly from PS11 to PS14 respectively. They were assigned unique identification numbers starting from KM192258 to KM192261

KEY WORDS : **IDENTIFICATION, GENE SEQUENCING, *Bacillus cereus Pseudoxanthomonas mexicana, Halomonas daqingensis, Parapusillimonas granuli.***

INTRODUCTION

. Few microbes show versatility for degradation of various compounds while some microbes can degrade only one or two components. 40 – 80 % of degradation of oil spills is managed by biodegradation process. Marine sediments, soil, estuaries, sea, etc. forms the various habitats for isolation of microbes having hydrocarbon degradation capability. Along with bacteria being the most significant agent for breakdown of hydrocarbons, few of the fungal species like *Candida*, *Fusarium*, *Trichoderma*,

Aspergillus are also known to degrade hydrocarbons. (Adibarata & Achibana 2009; Omotayo *et al*. 2011; Kafilzadeh *et al*. 2010) Amongst bacterial species, few species known for biodegradation of hydrocarbons are *Pseudomonas*, *Acinetobacter*, *Bacillus*, *Alcaligens*, *Micrococcus*. β – oxidation process is involved in biodegradation of alkanes. (Ting *et al*. 2009; Joo & Kim 2013) Aromatic hydrocarbon rings are generally hydroxylated to form diols which form cathecols and subsequently give intermediates of the TCA cycle.

Total degradation of aromatic hydrocarbons produces harmless end products like CO_2 and water. Earlier exposure of bacteria to hydrocarbons leads to increased degradation capacity along with the raised population of degrading bacteria at the site of contamination. Bacteria isolated from contaminated sites have greater chances to have plasmid which codes gene responsible for the degradation of hydrocarbon. Few well studies plasmids of *Pseudomonas* are TOL plasmid for toluene degradation, XYL for xylene degradation, CAM for camphor and SAL for salicylate.

MATERIALS AND METHODS

There are several methods are available for identification of microbes. Primary method includes study of colony morphology and gram staining. Both these method will give rough idea about the characteristic of microbes. Various Biochemical test as mentioned in the Bergey's manual and MIDI analysis can provide more information about the microbes but ribosomal small subunit sequencing is one of the most efficient and accurate method for identification of living organism. Since sequences of these subunit is highly conserved hence can be used widely for identification of microbes also.

As compare to the whole genome these region is very small and unique which makes it more potential for identification. However there are other conserved genes also but rarely used for identification. (Thenmozhi et al. 2011; Kumar et al. 2006; Shukla et al. 2010) Here, 16s rRNA sequencing was done as all the microbes were prokaryotes and the sequences obtained were compare with the database available on NCBI.(Singh & Fulekar 2010; Mittal & Singh 2009) All the sequences were submitted to NCBI and given universal identification numbers.

RESULTS

As mentioned earlier ribosomal RNA gene sequencing was used for the identification of microbes. 16s ribosomal gene sequencing is one of the most reliable method for accurate identification of microbes. (Okoh 2006) The following nucleotide sequences were obtained which were further analyzed using bioinformatics tools.

Microorganism Sequence 1

gaaaccgggg ctaataccgg ataacatttt gaacccgcat ggttcgaaat tgaaaggcgg cttcggctgt cacttatgga tggacccgcg tcgcattagc tagttggtga ggtaacggct caccaaggca acgatgcgta gccgacctga gagggtgatc ggccacactg ggactgagac acggcccaga ctcctacggg aggcagcagt agggaatctt ccgcaatgga cgaaagtctg acggagcaac gccgcgtgag tgatgaaggc tttcgggtcg taaaactctg ttgttaggga agaacaagtg ctagttgaat aagctggcac cttgacggta cctaaccaga aagccacggc taactacgtg ccagcagccg cggtaatacg taggtggcaa gcgttatccg gaattattgg gcgtaaagcg cgcgcaggtg gtttcttaag tctgatgtga aagcccacgg ctcaaccgtg gagggtcatt ggaaactggg agacttgagt gcagaagagg aaagtggaat tccatgtgta gcggtgaaat gcgtagagat atggaggaac accagtggcg aaggcgactt tctggtctgt aactgacact gaggcgcgaa agcgtgggga gcaaacagga ttagataccc tggtagtcca cgccgtaaac gatgagtgct aagtgttaga gggtttccgc cctttagtgc tgaagttaac gcattaagca ctccgcctgg ggagtacggc cgcaaggctg aaactcaaag gaattgacgg gggcccgcac aagcggtgga gcatgtggtt taattcgaag caacgcgaag aaccttacca ggtcttgaca tcctctgaaa accctagaga tagggcttct ccttcgggag cagagtgaca ggtggtgcat ggttgtcgtc agctcgtgtc gtgagatgtt gggttaagtc ccgcaacgag cgcaaccctt gatcttagtt gccatcatta agttgggcac tctaaggtga ctgccggtga caaaccggag gaaggtgggg atgacgtcaa atcatcatgc cccttatgac ctgggctaca cacgtgctac aatggacggt acaaagagct gcaagaccgc gaggtggagc taatctcata aaaccgttct cagttcggat tgtaggctgc aactcgccta catgaagctg gaatcgctag taatcgcgga tcagcatgcc gcggtgaata cgttcccggg ccttgtacac accgcccgtc acaccacgag agtttgtaac acccgaagtc ggtgggggta accttttttg ggagccagcc c

Microorganism 2

agtgcggggt aatggcccac caaggcgacg atcggtagct ggtctgagag gatgatcagc cacactggaa ctgagacacg gtccagactc ctacgggagg cagcagtggg gaatattgga caatgggcgc aagcctgatc cagccatacc gcgtgggtga agaaggcctt cgggttgtaa agcccttttg ttgggaaaga aatcctgtcg attaatactc ggtggggatg acggtaccca aagaataagc accggctaac ttcgtgccag cagccgcggt aatacgaagg gtgcaagcgt tactcggaat tactgggcgt aaagcgtgcg taggtggtgg tttaagtctg ctgtgaaagc cctgggctca acctgggaat tgcagtggat actggatcac tagagtgtgg tagagggatg cggaatttct ggtgtagcag tgaaatgcgt agagatcaga aggaacatcc gtggcgaagg cggcatcctg ggccaacact gacactgagg cacgaaagcg tggggagcaa acaggattag ataccctggt agtccacgcc ctaaacgatg cgaactggat gttgggtgca acttggcacc cagtatcgaa gctaacgcgt taagttcgcc gcctggggag tacggtcgca agactgaaac tcaaaggaat tgacgggggc ccgcacaagc ggtggagtat gtggtttaat tcgatgcaac gcgaagaacc ttacctggtc ttgacatcca cggaactttc cagagatgga ttggtgcctt cgggaaccgt gagacaggtg ctgcatggct gtcgtcagct cgtgtcgtga gatgttgggt taagtcccgc aacgagcgca acccttgtcc ttagttgcca gcacgtaatg gtgggaactc taaggagacc gccggtgaca aaccggagga aggtggggat gacgtcaagt catcatggcc cttacgacca gggctacaca cgtactacaa tggtggggac agagggctgc aaacccgcga gggtgagcca atcccagaaa ccctatctca gtccggattg gagtctgcaa ctcgactcca tgaagtcgga atcgctagta atcgcagatc agcattgctg cggtgaatac gttcccgggc cttgtacaca ccgcccgtca caccatggga gtttgttgca ccagaagcag gtagctt

Microorganism 3

ggggaaaccc aggctaatac cgcatacgtc ctacgggaga aagcagggga cctttcgggc cttgcgctat cggatgagcc tatgtcggat tagctggttg gtgaggtaat ggctcaccaa ggcgacgatc cgtagctggt ctgagaggat gatcagccac atcgggactg agacacggcc cgaactccta cgggaggcag cagtggggaa tattggacaa tgggcgcaag cctgatccag ccatgccgcg tgtgtgaaga aggccctcgg gttgtaaagc actttcagtg gggaagaaag ccttccggtt aatacccggg aggagggaca tcacccacag aagaagcacc ggctaactcc gtgccagcag ccgcggtaat acggagggtg cgagcgttaa tcggaattac tgggcgtaaa gcgcgcgtag gcggcttgat aagccggttg tgaaagcccc gggctcaacc tgggaacggc atccggaact gtcaggctag agtgcaggag aggaaggtag aattcccggt gtagcggtga aatgcgtaga gatcgggagg aataccagtg gcgaaggcgg ccttctggac tgacactgac gctgaggtgc gaaagcgtgg gtagcaaaca ggattagata ccctggtagt ccacgccgta aacgatgtcg actagccgtt gggtccttcg cggactttgt ggcgcagtta acgcgataag tcgaccgcct ggggagtacg gccgcaaggt taaaactcaa atgaattgac gggggcccgc acaagcggtg gagcatgtgg tttaattcga tgcaacgcga agaaccttac ctacccttga catcctgcga acccttcgga gacgaagggg tgccttcggg aacgcagaga caggtgctgc atggctgtcg tcagctcgtg ttgtgaaatg ttgggttaag tcccgtaacg agcgcaaccc ttgtccctat ttgccagcga ttcggtcggg aactctaggg agactgccgg tgacaaaccg gaggaaggtg gggacgacgt caagtcatca tggcccttac gggtagggct acacacgtgc tacaatggtc agtacaaagg gttgcgaact tgcgagagtg agccaatccc agaaagctga tctcagtccg gatcggagtc tgcaactcga ctccgtgaag tcggaatcgc tagtaatcgt gaatcagaat gtcacggtga atacgttccc gggccttgta cacaccgccc gtcacaccat gggagtggac tgcaccagaa gtggttagcc taaccttcgg gagggcgatc accacgg

Microorganism 4

gggggataac tacgcgaaag cgtggctaat accgcatacg ccctacgggg gaaagggggg gattcttcgg aacctctcac tattggagcg gccgatatcg gattagctag ttggtggggt aaaggcctac caaggcgacg atccgtagct ggtttgagag gacgaccagc cacactggga ctgagacacg gcccagactc ctacgggagg cagcagtggg gaattttgga caatgggggc aaccctgatc cagccatccc gcgtgtgcga tgaaggcctt cgggttgtaa agcacttttg gcagggaaga aacaggtctg gcgaatacct ggactgaatg acggtacctg cagaataagc accggctaac tacgtgccag cagccgcggt aatacgtagg gtgcaagcgt taatcggaat tactgggcgt aaagcgtgcg caggcggttc ggaaagaagg gtgtgaaatc ccagggctta accttggaat ggcattctta actaccgggc tagagtatgt cagagggggg tagaattcca cgtgtagcag tgaaatgcgt agagatgtgg aggaataccg atggcgaagg cagccccctg ggataatact gacgctcatg cacgaaagcg tggggagcaa acaggattag ataccctggt agtccacgcc ctaaacgatg tcaactagct gttggggcct tcgggcctta gtagcgcagc taacgcgtga agttgaccgc ctggggagta cggtcgcaag attaaaactc aaaggaattg acggggaccc gcacaagcgg tggatgatgt ggattaattc gatgcaacgc gaaaaacctt acctaccctt gacatgtctg gaatcccgaa gagatttggg agtgctcgca agagaaccgg aacacaggtg ctgcatggct gtcgtcagct cgtgtcgtga gatgttgggt taagtcccgc aacgagcgca acccttgtca ttagttgcta cgaaagggca ctctaatgag actgccggtg acaaaccgga ggaaggtggg gatgacgtca agtcctcatg gcccttatgg gtagggcttc acacgtcata caatggtcgg gacagagggt cgccaagccg cgaggcggag ccaatcccag aaacccgatc gtagtccgga ttgcagtctg caactcgact gcatgaagtc ggaatcgcta gtaatcgcgg atcagcatgt cgcgtgatac gt

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