Identification of Isolated Organisms from Petro Fields 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 microorganism asBacillus cereus, microorganism2 as Pseudoxanthomonasmexicana, microorganism3 as Halomonasdaqingensis and microorganism4 as Parapusillimonasgranuli. These microbes were given names accordingly from PS11 to PS14 respectively. They were assigned unique identification numbers starting from KM192258 to KM192261

Keywords: IDENTIFICATION, GENE SEQUENCING, *Bacillus cereus Pseudoxanthomonasmexicana, Halomonasdaqingensis, Parapusillimonasgranuli.*

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

gaaaccggggctaataccggataacatttttgaacccgcatggttcgaaattgaaaggcggcttcggctgtcacttatggatggacccgcgtcgcattagctagttggtgaggtaacggctcaccaaggcaacgatgcgtagccgacctgagagggtgatcggccacactgggactgagacacggcccagactcctacgggaggcagcagtagggaatcttccgcaatggacgaaagtctgacggagcaacgccgcgtgagtgatgaaggctttcgggtcgtaaaactctgttgttagggaagaacaagtgctagttgaataagctggcaccttgacggtacctaaccagaaagccacggctaactacgtgccagcagccgcggtaatacgtaggtggcaagcgttatccggaattattgggcgtaaagcgcgcgcaggtggtttcttaagtctgatgtgaaagcccacggctcaaccgtggagggtcattggaaactgggagacttgagtgcagaagaggaaagtggaattccatgtgtagcggtgaaatgcgtagagatatggaggaacaccagtggcgaaggcgactttctggtctgtaactgacactgaggcgcgaaagcgtggggagcaaacaggattagataccctggtagtccacgccgtaaacgatgagtgctaagtgttagagggtttccgccctttagtgctgaagttaacgcattaagcactccgcctggggagtacggccgcaaggctgaaactcaaaggaattgacggggggccccgcacaagcggtggagcatgtggtttaattcgaagcaacgcgaagaaccttaccaggtcttgacatcctctgaaaaccctagagataggccttctccttcgggagcagagtgacaggtggtgcatggttgtcgtcagctcgtgtcgtgagatgttgggttaagtcccgcaacgagcgcaacccttgatcttagttgccatcattaagttgggcactctaaggtgactgccggtgacaaaccggaggaaggtggggatgacgtcaaatcatcatgccccttatgacctgggctacacacgtgctacaatggacggtacaaagagctgcaagaccgcgaggtggagctaatctcataaaaccgttctcagttcggattgtaggctgcaactcgcctacatgaagctggaatcgctagtaatcgcggatcagcatgccgcggtgaatacgttcccgggccttgtacacaccgcccgtcacaccacgagagtttgtaacacccgaagtcggtggggtaaccttttttgggagccagcc c

Microorganism 2

agtgcggggtaatggcccaccaaggcgacgatcggtagctggtctgagaggatgatcagccacactggaactgagacacggtccagactcctacgggaggcagcagtggggaatattggacaatgggcgcaagcctgatccagccataccgcgtgggtgaagaaggcctttcgggttgtaaagcccttttgttgggaaagaaatcctgtcgattaatactcggtggggatgacggtacccaaagaataagcaccggctaacttcgtgccagcagccgcggtaatacgaagggtgcaagcgttactcggaattactgggcgtaaagcgtgcgtaggtggtggtttaagtctgctgtgaaagccctgggctcaacctgggaattgcagtggatactggatcactagagtgtggtagagggatgcggaatttctggtgtagcagtgaaatgcgtagagatcagaaggaacatccgtggcgaaggcggcatcctgggccaacactgacactgaggcacgaaagcgtggggagcaaacaggattagataccctggtagtccacgccctaaacgatgcgaactggatgttgggtgcaacttggcacccagtatcgaagctaacgcgttaagttcgccgcctggggagtacggtcgcaagactgaaactcaaaggaattgacggggggcccgcacaagcggtggagtatgtggtttaattcgatgcaacgcgaagaaccttacctggtcttgacatccacggaactttccagagatggattggtgccttcgggaaccgtgagacaggtgctgcatggctgtcgtcagctcgtgtcgtgagatgttgggttaagtcccgcaacgagcgcaacccttgtccttagttgccagcacgtaatggtgggaactctaaggagaccgccggtgacaaaccggaggaaggtggggatgacgtcaagtcatcatggcccttacgaccagggctacacacgtactacaatggtggggacagagggctgcaaacccgcgagggtgagccaatcccagaaaccctatctcagtccggattggagtctgcaactcgactccatgaagtcggaatcgctagtaatcgcagatcagcattgctgcggtgaatacgttcccggggccttgtacacaccgcccgtcacaccatgggagtttgttgcaccagaagcaggtagctt

Microorganism 3

ggggaaacccaggctaataccgcatacgtcctacgggagaaagcaggggacctttcgggccttgcgctatcggatgagcctatgtcggattagctggttggtgaggtaatggctcaccaaggcgacgatccgtagctggtctgagaggatgatcagccacatcgggactgagacacggcccgaactcctacgggaggcagcagtggggaatattggacaatgggcgcaagcctgatccagccatgccgcgtgtgtgaagaaggccctcgggttgtaaagcactttcagtggggaagaaagccttccggttaatacccgggaggagggacatcacccacagaaga

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cagaaagctgatctcagtccggatcggagtctgcaactcgactccgtgaagtcggaatcgctagtaatcgtgaatcagaatgtcacggt
gaatacgttcccgggccttgtacacaccgcccgtcacaccatgggagtggactgcaccagaagtggttagcctaaccttcgggaggg
cgatcaccacgg

Microorganism 4

ggggggataactacgcgaaagcgtggctaataccgcatacgccctacgggggaaagggggggattcttcggaacctctcactattg
gagcggccgatatcggattagctagttggtggggtaaaggcctaccaaggcgacgatccgtagctggtttgagaggacgaccagcca
cactgggactgagacacggcccagactcctacgggaggcagcagtggggaattttggacaatgggggcaaccctgatccagccatc
ccgcgtgtgcgatgaaggccttcgggttgtaaagcacttttggcaggggaagaaacaggtctggcgaatacctggactgaatgacggta
cctgcagaataagcaccggctaactacgtgccagcagccgcggtaatacgtagggtgcaagcgttaatcggaattactgggcgtaaa
gcgtgcgcaggcggttcggaaagaagggtgtgaaatcccagggcttaaccttggaatggcattcttaactaccgggctagagtatgtc
agagggggggtagaattccacgtgtagcagtgaaatgcgtagagatgtggaggaataccgatggcgaaggcagcccctgggataat
actgacgctcatgcacgaaagcgtggggagcaaacaggattagataccctggtagtccacgccctaaacgatgtcaactagctgttgg
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cgtcaagtcctcatggcccttatgggtagggcttcacacgtcatacaatggtcgggacagagggtcgccaagccgcgaggcggagcc
aatcccagaaacccgatcgtagtccggattgcagtctgcaactcgactgcatgaagtcggaatcgctagtaatcgcggatcagcatgtc
gcgtgatacgt

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