Biotransformation of aromatic hydrocarbon: Naphthalene to Aliphatic Hydrocarbons through *Staphylococcus pasteurii* RD2

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**Abstract:** Aromatic hydrocarbons like naphthalene are common environmental pollutants of petrochemical waste. Microorganisms have been exploited since long back to clean up such pollutants by converting them into either non-toxic or less toxic aromatic or aliphatic compounds. A bacterial strain have been isolated from oil sludge of Guwahati Refinery, Assam and was identified as *Staphylococcus pasteurii* RD2 (NCBI accession number MG680735) through 16s rDNA sequence analysis and molecular phylogeny. The bacterial strain transforms Naphthalene, a common hazardous aromatic hydrocarbon found in petrochemical waste, into a number of less hazardous aliphatic hydrocarbons. Detection of compounds such as Decane, Dodecane, Tetradecane, Hexadecane, Eicosane, and heptane by GC-MS analysis of naphthalene enrichment culture broth suggested that the bacterial strain was able to transform naphthalene into different aliphatic hydrocarbons with less toxicity and having chain length of C7 to C20. It has also been depicted a pathway to obtain aliphatic hydrocarbons with higher caloric value from aromatic hydrocarbon waste.

**Key words:** Biotransformation, *Staphylococcus pasteurii*, Aromatic hydrocarbon, Naphthalene.

**Introduction**

Hydrocarbons are a group of organic compounds composed exclusively of carbon and hydrogen and are either monocyclic- or polycyclic aromatic in structure. Monocyclic aromatic hydrocarbons like benzene, ethylbenzene, toluene and xylene (BTEX) having a single benzene ring, are commonly found in gasoline and are highly volatile substances (Coates *et al.*, 2002). Polycyclic aromatic hydrocarbons (PAHs) contain two or more benzene rings and are relatively less aqueous solubility than monocyclic aromatic hydrocarbons. Aromatic hydrocarbons are considered as highly toxic, and carcinogenic to human health. These pollutants are released from combustion of fossil fuels and hydrocarbons that enter the ecosystem due to their lipophilic property and polluted the environment. The aromatic hydrocarbons like Naphthalene, Benzene, toluene, ethylbenzene, and xylene are commonly found in crude petroleum and petroleum products are considered as one of the major causes of environmental pollution (Farhadian *et al.*, 2008). Among the PAHs naphthalene is the simplest one and has been extensively used as a model for biodegradation. Microbial degradation of aromatic compounds have been reported by a number of authors (Seo *et al.*, 2009, Meckenstock *et al.*, 2004, Lin and Chen, 2010, Mrozik *et al.*, 2003, Annweiler, 2000, Weelink *et al.*, 2002, Doley *et al.*, 2017). Biotransformation of aromatic hydrocarbon into short and medium chain length polymers (polyhydroxylaconate) have been reported by a number of authors. Hori *et al.*, (2009) and Trautwein *et al.*, (2008) reported the biosynthesis of short-chain length poly (3-hydroxyalkanoates) by *Rhodococcus aetherivorans* and *Aromatobacter aromaticum* from toluene and other volatile aromatic compounds. Ni *et al.*, (2010) reported the biodegradation of BTEX compounds along with the biosynthesis of valuable biopolymers from the aromatic compounds. *Pseudomonas putida* F1 strain was able to transformed aromatic compounds; benzene, toluene and ethyl benzene (BTE) in to medium chain length hydrocarbons (MCL-PHAs). They further reported the production of elastomeric MCL-PHAs containing 3-hydroxydocendecenoate unit from BTE compounds by *P. jutha* TY16, using a novel continuous feeding system of gaseous substrates. The gamma ray mutant strain *Pseudomonas* species EBN8 synthesized medium chain length PHA copolyester as reported by Abid *et al.*, (2016). They identified the metabolite as polyhydroxybutyrate by LCMS and FTIR spectroscopy and exhibited the molecular mass of m/z 448.5 through ESI-MS analysis. The biocconversion of mono and polycyclicaromatic hydrocarbons in to different chain length polyhydroxalkanoates have been reported by a number of authors, however, biotransformation of aromatic compounds in to medium or long chain aliphatic compounds have not been reported.

The aim of this study is to convert hazardous aromatic hydrocarbon such as Naphthalene that are common pollutant of petrochemical waste to less hazardous aliphatic hydrocarbons and also to explore a pathway to obtain hydrocarbons with high caloric value from aromatic hydrocarbon waste.

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Materials and Methods

Chemicals: All chemicals are of analytical grade, purchased from Sisco Research Laboratory Pvt. Ltd., Mumbai.

Bacterial strain: Bacterial strain Staphylococcus pasteuri RD2 has been isolated from oil sludge of Guwahati Refinery, Assam and identified by 16srDNA sequence analysis and molecular phylogeny as reported earlier (Doley and Barthakur 2017).

Isolation, detection, and identification of metabolite

To identify naphthalene and its transformation products, the bacterial strain was inoculated (CFU 3x10^6) in 250 ml Erlenmeyer flasks containing 100 ml of naphthalene (60 ppm in n-hexane) enrichment nutrient medium and incubated at 37°C. Seven days after the incubation, the suspension was extracted three times with n-hexane. The combined hexane extract were concentrated in rotary evaporator to about 5 ml. Residues were examined for naphthalene and hexane extractable transformation products by GC-MS. Naphthalene enrichment culture without bacterial strain treated as control.

To determine the Naphthalene utilization by the bacterial strain in naphthalene enrichment culture broth was monitored periodically by measuring the optical density at 310 nm in UV-visible spectrophotometer (Agilent Carry-60 UV-visible spectrophotometer). Culture media inoculated with bacterial suspension without supplementation of naphthalene was treated as control.

Results

The bacterial strain isolated from oil sludge has already been identified as Staphylococcus pasteuri RD2 through 16srDNA sequence alignment (Clustal W) and molecular phylogeny (Distance Metrix, in MEGA7). The PCR amplicon generated a sequence having 1485 base pair (Fig. 1) have been submitted in Gene Bank of NCBI (Accession no. MG680735). Morphology of the bacterial strain was analyzed by Screening Electron Microscopy (Plate 1 A and B).

GATGAAACGTGGGGCGGCTGCTAAATACATG
CAAGTCCGACGAAAGATAAGGAGCTTGCC
TCTTTTAGCTTACGCGGACACGCGGTAGAG
TAAACGTCGATTAACCTTACCTATAAGACTG
GGATAACTCTGGGAACCCCGAGGCTAAATAC
CGGATAAGATTTTGAACGCTTGGAGGACTAC
AGTGAAAGATTTTGATACCCGTGGGTCCTTA
AGTGGATTCGCCCGGTATTTAGCTATTGAG
TAAGTGTTACAGGGCTTACCAAGGCAACGATA
CGTAGGCGGCTTGAGAGGGTGATCGGCCCA
CACTGGAACTTGAGAACAGGGTCACACCT
ACGGGAGACGACGATGAGGAACTCTTTCGCG
AATGGGCGAGCAGGGCCAACGACAGGCC
GGGTAGGTAGTAAGGGTTCTCTGAGATCGTA
AAAATCGTTATTACGAGGAAACAAACGTT
GTAAGTAACTGTGCAAGCTTTTACGACGGTAC
CTGATCAGAAAGCAGCCGGCTAATACAGTCG
CCAGCAGCGGGCTATAACTCGTAGGTCGGCA
AGCGTTATCGGGAATATTTTGGTTATTGAG
CCGCGTAGGTTAAGCTTCTGCTATCGTA
CTGACGCTGTATGTGAAAGCCTAGGGGAT
CATTGGAAATCCGAAACTCCATTAGTGAG
GGTGTTCCGCCCCCTATTGCTCGACAGTCAA
GCAAATACGCTCCTCGGGGAGTG
GCAGTTTAAATTGAAACGCAACGCGGAACG
CCGAACTACCTGGCAAGGGAGATCGA
GATGACGTCAAATCATCATTGCCCCCTTATAG
TTGGGCTACACAGCTGCTAACATTGCAAAAC
TCAAAAGGCGACTAAACGCGGGATCTAAG
GCAAATCCATAAAGTGTTTCAGTAGTG
ATGGATACTGCACTCCGACTACATGGAACG
TTGGAATCTGCGTAGAATCGTAGACATCAGAT
GCTACGGGTGAATACGTTCCCCGCTCTGT
ACACACCGCGCTCACAACAGAGATTTTG
TAAACCCGAGGCGGGTGGAGTAACCCCA
TATGGGCTACAGCGTGCTGCAAGGTTGACAA
ATGATTGGGTGGTGAAGGTAA

Fig. 1. Sanger Aligned rDNA sequence of RD2

Plate 1. Scanning Electron Microscopic images (magnification A=30.46 and B=100.68) of Staphylococcus pasteuri RD2

Biotransformation of Naphthalene by Staphylococcus pasteuri RD2:

The bacterial strain Staphylococcus pasteuri RD2 was able to grow on the culture media by utilizing naphthalene as carbon and energy source. Optical density (OD_{310}) of the naphthalene enrichment culture media of Staphylococcus pasteuri RD2 exhibited a gradual decrease from 48 hours onward (fig.2).

GC-MS analysis (Fig. 3) of the biotransformation products of Naphthalene by the bacterial strain
Staphylococcus pasteuri RD2 reveal the presence of heptane (RT 5.357), decane (RT 11.224), Dodecane (RT 17.589), tetradecane (RT 23.938), Hexadecane (RT 29.300) and Eicosane (RT 33.978) as major products.

Figure 2. Utilization of naphthalene by Staphylococcus pasteuri RD2

Figure 3. GC-MS chromatogram of naphthalene biotransformation products

Discussion
The bacterial strain isolated from oil sludge of Guwahati Refinery and was identified as Staphylococcus pasteuri. The DNA sequence of 1485 base pair length (Doley and Barthakur 2017) have been submitted in the NCBI Gene Bank with accession number MG680735(https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=45972). Screening Electron Microscopic analysis suggested the coccoid nature of cells, arranged in packets (plate1A, 1B). Gradual decrease of optical density (O.D.310nm) of naphthalene in the culture broth suggested the utilization of naphthalene by the bacterial strain as carbon and energy source (fig.2).

Microbial degradation of aromatic hydrocarbons has been reported by a number of authors (Karimi et al., 2002, Heider et al., 1999, Abide et al, 2016, Doley and Barthakur 2017). We have reported earlier the degradation of naphthalene in to its derivatives by Staphylococcus pasteuri RD2 (Doley and Barthakur 2017). However, limited reports have been found on biotransformation of mono- and polycyclic aromatic compounds in to medium chain length hydrocarbons such as polyhydroxyalcanoate (PHA). Narancic et al., (2012) reported the conversion of a range of polyaromatic hydrocarbon compounds to medium chain length PHA. The bacterial strain Pseudomonas sp. TN301 was able to convert polycyclic aromatic hydrocarbons such as naphthalene, phenanthrene and chrysene in to mel-PHA. Srivastav and Tripathi (2013) reported the production of medium and short chain length polyhydroxyalkanoate (PHA) by Alcaligenes species MCIM 5085 when supplemented with fatty acid in basal media which could potentially serve as precursors for bioplastic production. Nikodinovic et al., (2008) demonstrated the conversion of monoaromatic compounds such as Benzene, Toluene, Ethylbenzene and Xylene (BTEX) to medium chain length polyhydroxyalkanoate by two Pseudomonas strains strain F1 and mt-2. Pseudomonas putida F1 accumulate mel-PHA from toluene, benzene and ethylbenzene whereas P. putida mt-2 accumulates mel-PHA from toluene and p-xylene. Ward et al., (2005) reported the conversion of aromatic hydrocarbon styrene and phenylacetic acid into polyhydroxyalkanoate by Pseudomonas putida CA-3. In this paper we have reported the biotransformation of polycyclic aromatic hydrocarbon naphthalene to aliphatic hydrocarbons by the bacterial strain Staphylococcus pasteuri RD2 isolated from oil sludge of Guwahati Refinery. GC-MS analysis reveals the presence of a number of aliphatic hydrocarbons in naphthalene enrichment culture medium of Staphylococcus pasteuri RD2. Compounds detected such as heptane (C7H16O), decane (C10H22), Dodecane (C12H26), tetradecane (C14H30), Hexadecane (C16H32) and Eicosane (C20H42) having a chain length of C2 to C20 carbon atom indicated the biotransformation of naphthalene to such aliphatic hydrocarbons. This study reveals that the bacterial strain Staphylococcus pasteuri RD2 have the ability to transform the common pollutant such as naphthalene in to less toxic aliphatic hydrocarbons along with the formation of different carbon length aliphatic hydrocarbons with higher caloric value from the aromatic waste.

Conclusions
The present studies strongly suggest the ability of Staphylococcus pasteuri RD2 to transform naphthalene into various aliphatic hydrocarbons as major product. The GC-MS analysis detected the probable compounds like Decane, Dodecane, Tetradecane, Hexadecane, Eicosane and Heptane. The bioconversion of Naphthalene in to aliphatic hydrocarbons, mediated by Staphylococcus pasteuri RD2 depict a pathway to manage toxic aromatic hydrocarbon waste released to our environment from different sources as well as it also depict a pathway to obtain aliphatic hydrocarbons with higher caloric value from aromatic hydrocarbon wastes.
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