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Research Article

Degradation of Polycyclic Aromatic Hydrocarbons by Bacteria

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ABSTRACT

Biodegradation or biological degradation is the rate of biological conversion of organic and inorganic compounds by living organisms, particularly the microorganisms. It involves conversion of complex compounds to simple non-toxic which acts as nutrients to microorganism, degrades organic compounds by secreting biosurfactants. Biotransformation is a process made by an organism to modify any chemical compound which ends with mineral compounds like H_2O, CO_2, NH^{4+}, etc. It is also referred as mineralization. It is used for partial biodegradation of organic complexes involving few reactions. Bioremediation is a process of utilizing microorganisms to remove the environmental pollutants i.e. toxic wastes, hydrocarbons, etc. The removal of organic wastes by microbes for environmental clean-up is the basic principle of bioremediation. Other terminology used for bioremediation is bio-treatment, bio-reclamation and bio-restoration.

Keywords: Biodegradation; Biotransformation; Mineralization; Biosurfactants

INTRODUCTION

Factors affecting biodegradation

There are several factors which can influence biodegradation of environmental pollutants. These include chemical nature of xenobiotic, capability of microorganism, oxygen supply, nutrients, salinity, temperature, pH, etc. [1,2]. Chemical nature of substrate is important which has to be degraded. In the recent developments there are two more factors which enhance the biodegradation by microorganisms [2-4].

Polycyclic aromatic hydrocarbons (pahs): Xenobiotics

Xenobiotic refers to the unusual, external and synthetic compounds such as insecticides, herbicides, refrigerants and other carbon-based complexes. It provides an effective and economic means of eliminating toxic chemicals, particularly the environmental pollutant

Polycyclic Aromatic Hydrocarbons (PAHs) are classified as multiple fused aromatic compounds which contain minimum two benzene rings and they are unsusceptible to degradation. They contain only carbon and hydrogen. They are ubiquitously as environmental pollutants and contain large class of unsaturated aromatic compounds. Hydrocarbons with more aromatic ring structures are linked with greater toxicity and possess greater threat to human life [5].

Aromatic compounds are lipophilic and non-polar in nature, which limits their mobility in environment [5]. They are used as solvents for other nonpolar compounds.

PAHs are abundant in universe, and recent studies have confirmed that they were formed possibly couple of billion years after big bang [5].

There are more than 80 compounds categorized as PAHs having 2 to 7 rings. U.S. Environmental Protection Agency (EPA) has enlisted 16 priority PAHs [5,6] due to their properties of carcinogenicity, toxicity, mutagenicity, genotoxicity and teratogenicity.

Aim and objectives

1. Enrichment, isolation and purification of polycyclic aromatic hydrocarbons degrading bacteria from contaminated soil samples.
2. Screening the isolates that are capable of utilizing PAHs as sole carbon source.
3. Identification of screened isolates based on 16s rRNA gene sequence analysis.

METHODS

Collection of samples

The contaminated soil samples were collected into a zip locks

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polythene bags from places like petrol pump and garage area site in Hyderabad (Moosapet and Mallepally). Samples were collected at a depth of 4-5 cm from the surface of soil. They were carefully brought to laboratory and transferred to sterile petri plate for further analysis [7,8].

Enrichment, determination, isolation and purification of polycyclic aromatic degrading bacteria Enrichments

1 gm of contaminated soil samples were inoculated in separate sterile conical flasks (100 ml) under both aerobic and anaerobic conditions containing modified mineral salt medium (broth), replacing pyruvate with 0.78 mM of Naphthalene and 0.56 mM of Anthracene as sole carbon and energy which were pre-dissolved in acetone solution (10 ml). PAHs were sterilized by filtration though a 0.2 µm PTFE filter membrane. The flasks were incubated in orbital shaker with shaking speed at 150 rpm for 2-3 days at 35 ± 2°C [8-15].

Determination of bacterial growth using colorimeter

a. 0.1 ml Enrichment culture was taken and inoculated in 10 ml of Biebl and Pfennig broth media with respective aromatic hydrocarbon compounds as the sole source of carbon and energy, incubated at 35 ± 2°C for 3-4 days [16-20].

b. They were checked for the bacterial growth by measuring optical density in the colorimeter.

c. The reading were observed and recorded.

Isolation and purification

Enrichment cultures of PAHs degrading bacteria were purified by repeated streaking on nutrient agar plates. Single well isolated colonies were sub-cultured serially onto freshly prepared agar plates till all colonies growing were similar on two successive plates [21]. The isolated strains were re-inoculated in the modified mineral salt medium with PAHs as carbon source excluding yeast extract in order to determine that they have the capacity to utilize PAHs as sole source of carbon and energy.

DNA extraction from colony

A pure isolated and well grown colony of the desired culture was picked from the agar plates using sterile loop. The culture was suspended in 50 µl of sterile milli-Q-water and bacterial suspension was then lysed in a thermal cycler by performing denaturation at 96°C for 20 minutes [22].

Amplification of 16s rRNA Gene

Smaller sub-unit of rRNA varies across phylogenetic lines containing segments that are conserved at species, genus or kingdom level. Amplification of 16s rRNA sequences was done with polymerase chain reaction [23].

Amplification was routinely performed by pipetting 50 µl volumes in 0.2 ml microfuge vials using a DNA thermal cycler. All plastic ware were sterilized by autoclaved and ultraviolet irradiated. The primes used for the amplification of the 16s rRNA genes are F27 and R1492 [24].

RESULTS

Enrichment, isolation and purification of PAHS degrading bacteria

Two petroleum samples which were kept for enrichment in modified mineral salt medium with Naphthalene and Anthracene as sole carbon and energy source gave positive enrichment. 13 cultures were obtained from aerobic and anaerobic enrichments and purification was done by repeated streaking. The anaerobic strains could not grow when repeated sub culturing was done. So among them 5 aerobic strains were further chosen for degradation studies as these strains are growing well after repeated sub culturing [25].

Colony morphology

Bacterial cultures grown on modified mineral salt medium with Naphthalene and Anthracene as sole carbon source showed different colony morphological observation on nutrient agar plates.

Obtained result shows that strain 1 was found to utilize both anthracene and naphthalene. However, others strains could not show any degradation of the PAHs compounds (Naphthalene and Anthracene).

Agarose gel electrophoresis

Agarose Gel Electrophoresis showed single fragment of isolated nucleic acid with respect to strain JC 592. Other isolated samples were not amplified properly. It might be due to various reasons like low concentration of DNA, primers did not bind with the amplified product at the time of PCR, error in handling experimental protocols [26].

Genetic characterization: 16s rRNA gene sequence analysis

Amplified product of strain 11 was sent for 16s rRNA gene sequencing analysis at SciGenom, Kerala along with primers F16 and R1991. But the sample couldn't be sequenced due to low concentration of DNA.

DISCUSSION

Polycyclic Aromatic Hydrocarbons (PAHs) are environmental organic pollutants present in crude oil, incomplete combustion and fossil fuels. Use of bacterial strains to eradicate aromatic hydrocarbons from contaminated area has a great potential [27-30].

Achromobacter sp. and Pseudomonas aeruginosa were able to degrade hydrocarbons (petrol and diesel). There experiment was carried out for 20 days and degrading ability was determined every 5 days of time interval using gravimetric analysis. Pseudomonas aeruginosa have high degradation ability on diesel (34.4%) at the 10th day of incubation period whereas Achromobacter sp. was able to degrade (31.9%) of petrol at the end of 20 days of time interval [31-33].

CONCLUSION

In the present experiment, strain 1 was able to utilize and degrade both naphthalene and anthracene when experiment was carried for incubation period of 20 days by determining optical density every consecutive day. However, the degradation capabilities and pathways can be much more understood by using analytical

techniques like HPLC, GC- MS spectrometry which can be done in future along with 16s rRNA gene sequencing analysis.

REFERENCES

1. Jones DM, Douglas AG, Parkes RJ, Taylor J, Giger W, Schaffner C. The recognition of biodegraded petroleum-derived aromatic hydrocarbons in recent marine sediments. Marine Pollution Bulletin. 1983;14(3): 103–108.
2. Larkin MJ, Kulakov LA, Allen CC. Biodegradation and Rhodococcus: Masters of catabolic versatility. Curr Opin Biotechnol. 2005;16:282–290.
3. Basta T, Buerger S, Stolz A. Structural and replicative diversity of large plasmids from sphingomonads that degrade polycyclic aromatic compounds and xenobiotics. Microbiol. 2005;151: 2025–2037.
4. Adebusoye SA, Ilori MO, Amund OO, Teniola OD, Olatope SO. Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. World J Microbiol Biotech. 2007;23(8):1149–1159.
5. Bartha R, Bossert I, The Treatment and Disposal of Petroleum Wastes. In: Petroleum Microbiol. R. M. Atlas, (ed) 1984;553–578.
6. Qin W, Fan F, Zhu Y. Anaerobic biodegradation of benzo(a)pyrene by a novel Cellulosimicrobium cellulans CWS2 isolated from polycyclic aromatic hydrocarbon-contaminated soil. Brazilian Journal of Microbiology. 2017;49(2):258-268.
7. Sibi G, Simaria C, Pant G. Characterization and Evaluation of Polycyclic Aromatic Hydrocarbon (PAH) Degrading Bacteria Isolated from Oil Contaminated Soil. Appli Microbiol. 2015;
8. Wongsa P, Tanaka M, Ueno A, Hasanuzzaman M, Yumoto I. Isolation and characterization of novel strains of Pseudomonas aeruginosa and Serratia marcescens possessing high efficiency to degrade gasoline, kerosene, diesel oil, and lubricating oil. Curr Microbiol. 2004; 49(1):415-422.
9. Kulshreshtha S. Genetically engineered microorganisms: A Problem solving approach for bioremediation. J Bioremed Biodeg. 2013; 4:e133.
10. Jong-Su Seo, Young-Soo Keum, Qing X. Li. Bacterial degradation of aromatic compounds. Int J Environ Res Public Health. 2009; 6(1): 278–309.
11. Deng MC, Li J, FR Liang, M Yi, XM X. Isolation and characterization of a novel hydrocarbon degrading bacterium Achromobacter sp HZ01 from the crude oil contaminated seawater at the Daya Bay, Southern China. Mar Pollut Bull. 2014; 83:79-86.
12. Ueno A, Hasanuzzaman M, Yumoto I, Okuvama H. Verification of degradation of n-alkanes in diesel oil by Pseudomonas aeruginosa strain WatG in soil microcosms. Curr Microbiol. 2006;52:182-185.
13. Guo W, Li D, Tao Y, Gao P, Hu J. Isolation and description of a stable carbazole- degrading microbial consortium consisting of Chryseobacterium sp. NCY and Achromobacter sp. NCW Curr Microbiol. 2008;57:251–257.
14. Weissenfels WD, Beyer M, Klein J. Degradation of phenanthrene, fluorene, and fluoranthene by pure bacterial cultures. Appl Microbiol Biotechnol. 1990;32:479–484.
15. Seo JS, Keum YS, Hu Y, Lee SE, Li QX. Phenanthrene degradation in Arthrobacter sp. P1-1: Initial 1, 2-, 3, 4- and 9, 10-dioxygenation, and meta- and ortho-cleavages of naphthalene-1, 2-diol after its formation from naphthalene-1, 2-dicarboxylic acid and hydroxyl naphthoic acids. Chemosphere. 2006; 65:2388–2394.
16. Samanta SK, Chakraborti AK, Jain RK. Degradation of phenanthrene by different bacteria: Evidence for novel transformation sequences involving the formation of 1- naphthol. Appl Microbiol Biotechnol. 1999; 53:98–107.
17. Kazunga C, Aitken MD. Products from the incomplete metabolism of pyrene by polycyclic aromatic hydrocarbon-degrading bacteria. Appl Environ Microbiol. 2000; 66:1917–1922.
18. Yamazoe A, Yagi O, Oyaizu H. Degradation of polycyclic aromatic hydrocarbons by a newly isolated dibenzofuran-utilizing Janibacter sp. strain YY-1. Appl Microbiol Biotechnol. 2004;65:211–218.
19. Hedlund BP, Geiselbrecht AD, Staley JT. Marinobacter strain NCE312 has a Pseudomonas-like naphthalene dioxygenase. FEMS Microbiol Lett. 2001;201:47–51.
20. Schneider J, Grosser R, Jayasimhulu K, Xue W, Warshawsky D. Degradation of pyrene, benz[a]anthracene, and benzo[a]pyrene by Mycobacterium sp. Strain RJGII- 135, isolated from a former coal gasification site. Appl Environ Microbiol. 1996;62:13–19.
21. Rehmann K, Hertkorn N, Kettrup AA. Fluoranthene metabolism in Mycobacterium sp. Strain KR20: Identity of pathway intermediates during degradation and growth. Microbiology. 2001;147:2783–2794.
22. Kim YH, Freeman JP. Effects of pH on the degradation of phenanthrene and pyrene by Mycobacterium vanbaalenii PYR-1. Appl Microbiol Biotechnol. 2005;67:275–285.
23. Balashova NV, Kosheleva IA, Golovchenko NP, Boronin AM. Phenanthrene metabolism by Pseudomonas and Burkholderia strains. Process Biochem. 1999; 35:291–296.
24. Becher D, Specht M, Hammer E, Francke W, Schauer F. Cometabolic degradation of dibenzofuran by biphenyl-cultivated Ralstonia sp. strain SBUG 290. Appl Environ Microbiol. 2000; 6:4528–4531.
25. Zhou NY, Al-Dulayymi J, Baird MS, Williama PA. Salicylate 5-hydroxylase from Ralstonia sp. strain U2: A monooxygenase with close relationships to and shared electron transport proteins with naphthalene dioxygenase. J Bacteriol. 2002;184:1547–1555.
26. Kirimura K, Furuya T, Sato R, Ishii Y, Kino K, Usami S. Biodesulfurization of naphthothiophene and benzothiophene through selective cleavage of carbon-sulfur bonds by Rhodococcus sp. strain WU-K2R. Appl Environ Microbiol. 2002;8:3867–3872.
27. Boonchan S, Britz ML, Stanley GA. Surfactant-enhanced biodegradation of high molecular weight polycyclic aromatic hydrocarbons by Stenotrophomonas maltophilia. Biotechnol Bioeng. 1998; 59:482–494.
28. Juhasz AL, Stanley GA, Britz ML. Microbial degradation and detoxification of high molecular weight polycyclic aromatic hydrocarbons by Stenotrophomonas maltophilia strain VUN 10,003. Lett Appl Microbiol. 2000;30:396–401.
29. Habe H, Chung JS, Kato H, Ayabe Y, Kasuga K, Yoshida T, et al. Characterization of the upper pathway genes for fluorene metabolism in Terrabacter sp. strain DBF63. J Bacteriol. 2004;186:5938–5944.
30. Grosser RJ, Warshawsky D, Vestal JR. Indigenous and enhanced mineralization of pyrene, benzo[a]pyrene, and carbazole in soils. Appl Environ Microbiol. 1991;57:3462–3469.
31. Kanissery RG, Gerald K. Biostimulation for the enhanced degradation of herbicides in soil. Appli Environmental Soil Sci. 2011; pp.10.
32. Resnick SM, Gibson DT. Regio- and stereospecific oxidation of fluorene, dibenzofuran, and dibenzothiophene by naphthalene dioxygenase from Pseudomonas sp. strain NCIB 9816–4. Appl Environ Microbiol. 1996;62:4073–4080.
33. Sibi G, Simaria C, Pant G Characterization and evaluation of Polycyclic Aromatic Hydrocarbon (PAH) degrading bacteria isolated from oil contaminated soil. Appli Micro. 2015;