KEROSENE DEGRADERS JUSTIFIED WITH GAS CHROMATOGRAPHIC AND GENETICAL ANALYSES

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OVERVIEW

Kerosene is a mixture of hydrocarbons that is deployed in aviation fuels and contributes to extremely noxious pollution during spills. Because of aromatic hydrocarbons found in its structure, kerosene is carcinogenic and a threat to the health of living organisms. This makes kerosene imperative to be removed from the environment before its significant spread over large habitat. Bioremediation is a safe, effective and economic method for the cleaning up of hydrocarbons from the contaminated areas. Bacteria, one of the best candidates for bioremediation, have variety of catabolic genes and enzymes responsible in degradation. Alkane monooxygenases, coded by *alk*B gene, are the key enzymes in aerobic degradation of alkanes, which is the major component of kerosene. In this study, 22 bacteria isolated from river water samples next to a petroleum refinery were tested for their growth ability in Bushnell Haas Medium containing 1% kerosene as a sole source of carbon. Kerosene degradation ability of the isolates were shown through gas chromatographic (GC) analyses and presence of *alkB* gene was investigated by polymerase chain reaction (PCR). Results of the present study revealed the potential of the selected isolates to degrade kerosene and their possible use in the bioremediation of kerosene-contaminated areas.

INTRODUCTION

Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products [1]. Kerosene, one of the most commonly spilled petroleum product, is a combustible hydrocarbon liquid widely used as a fuel in airfield, industry and households. Kerosene is a thin, clear liquid formed from hydrocarbons obtained from the fractional distillation of petroleum between 150°C and 275°C, resulting in a mixture with a density of 0.78–0.81 g/cm^3. It is composed of carbon chains that typically contain between 6 and 22 carbon atoms per molecule [2]. As shown in Figure 1, kerosene contains lower-molecular-weight compounds, that are toxic or mutagenic and can be hazardous to plants, animals, and humans. Therefore, remedial action is required to restrict environmental hazard [1]. Bioremediation is a technique in which biological systems such as microorganisms are used to remove or degrade harmful chemicals to less or non-toxic form. However, various microorganisms degrade these compounds in different rates. Therefore, it is necessary to evaluate their biodegradation capabilities. In order to select efficient bacteria for bioremediation, several methods like gas chromatography have been investigated to evaluate biodegradation ability of hydrocarbon degrading bacteria [3]. Alkanes, major components of kerosene are being degraded by *alkB* gene, which codes for the alkane monooxygenase enzymes. These enzymes hydroxylates alkanes to alcohols, further being oxidized to fatty acids and finally catabolized by the bacterial β-oxidation pathway (Figure 2). The presence of such catabolic genes has been widely used as functional biomarker for the characterization of aerobic alkane-degrading bacteria. Therefore, the aim of this study is to determine bacteria genetically capable of degrading kerosene to use in possible bioremediation efforts.

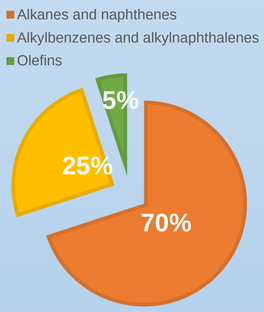


Figure 1. Major components are branched and straight chain alkanes and naphthenes (cycloalkanes), which normally account for at least 70% by volume. Aromatic hydrocarbons in this boiling range, such as alkylbenzenes (single ring) and alkylnaphthalenes (double ring), do not normally exceed 25% by volume of kerosene streams. Olefins are usually not present at more than 5% by volume [2].

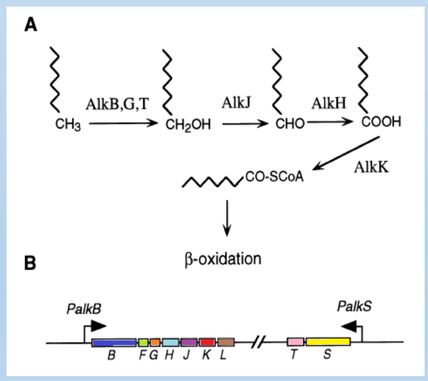


Figure 2. Metabolic pathway of alkane oxidation [4]

METHODS

Water samples collected from hydrocarbon contaminated river waters were used to isolate kerosene degrading bacteria. For selective isolation, Bushnell Haas Medium containing 1% kerosene as a sole source of carbon was used [5]. Kerosene degraders were grown for 21 days and analyzed by Agilent 6850 Gas Chromatograph for determination of kerosene degradation rates. The degradation percent was calculated by subtracting the kerosene content of the untreated sample from the residual kerosene after treatment and divided by the kerosene of the untreated sample multiplying by hundred.

To determine the prevalence of alkane monooxygenase enzyme in efficient kerosene degraders, total DNA from each culture were extracted by following phenol/chloroform extraction method starting with 1 mL culture grown in Nutrient Broth Medium [6]. Once total DNA was purely obtained, it was used as a template for PCR amplification of *alkB* gene. The used primer set and product length are described in Table 1. PCR amplification was carried out in 25 µL reaction mixture containing 100 ng DNA, 1.25 U Taq polymerase, 10pmol of forward and reverse primer, 200 µM of each dNTP and 2.5 µL of 10X Taq reaction buffer. An initial denaturation of 4 min at 95ºC followed by 30 cycle of denaturation for 1 min at 94ºC, annealing for 1 min at the optimum temperature and elongation for 2 min at 72ºC and final extension step for 10 min at 72ºC was performed.

Table 1. Primer used in this study

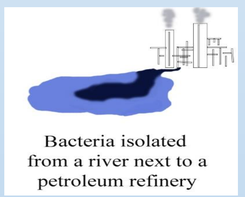
Primer name	Primer sequence (5'-3')	Amplicon size (bp)	Annealing temperature (ºC)	References
***alkB*-f** ***alkB*-r**	AACATAACCGTGGCCATCAC AACACCACGCTGTACATCCA	500	55	Dussán and Numpaque, 2012

RESULTS

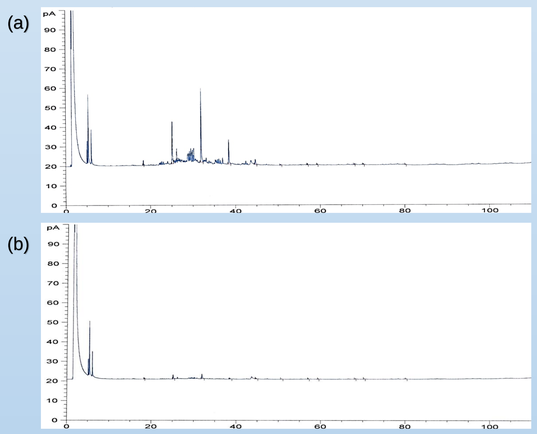
Kerosene degradation abilities of the isolates were determined by GC analysis. After 21 days of aerobic incubation, 7 isolates namely; *Pseudomonas plecoglossicida* Ag10, *Staphylococcus aureus* Ba01, *Stenotrophomons rhizophila* Ba11, *Delftia acidovorans* Cd11, *Acinetobacter calcoaceticus* Fe10, *Pseudomonas koreensis* Hg11 and *Acinetobacter johnsonii* Sb01 showed 69-84% kerosene degradation. *Acinetobacter johnsonii* Sb01 was found to be the most efficient kerosene degrader with 84% (Figure 3b). The gene *alkB* encoding alkane monooxygenases were detected by PCR amplification for all the isolates based on the amplicons with the expected sizes of 500, respectively. As shown in Figure 3c, *alkB* gene was detected in Ag10, Ba11, Cd11, Fe10, Hg11 and Sb01. Studies for Ba01 is still in progress.

CONCLUSIONS

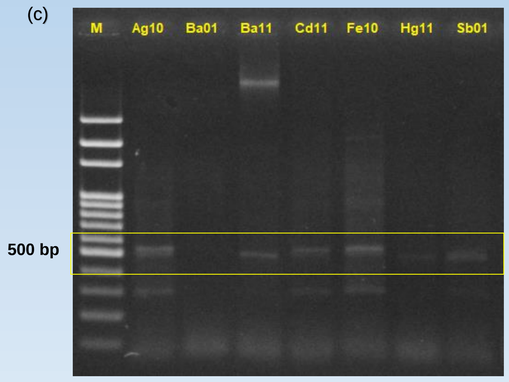
Hydrocarbon degradation by bacteria depends on their adaptive response to the hydrocarbons present in the environment and this adaptation contains both selective enrichment and genetic changes. This adaptation leads to enhanced hydrocarbon utilization and expression of the hydrocarbon-catabolizing genes. However, the current literature shows that the diversity of the *alkB* gene in environmental samples is still far from being well characterized. In this study, we have determined 7 different strain of bacteria as efficient kerosene degraders by GC analyses and showed the presence of their *alkB* genes through PCR. For the future studies, the degradation abilities of the selected isolates should be tested in bioremediation field studies.



Testing for their ability to degrade kerosene



GC profiles of kerosene oil extracted from the aqueous phase of the medium after 21 days of incubation without inoculant as a control (a) with the inoculant of Sb01 (b)



PCR-based analyses of *alkB* gene in the surface water isolates Ag10, Ba01, Ba11, Cd11, Fe10, Hg11 and Sb01. M marker gene ruler 100–1000 bp.

Figure 3. Schematic overview of the study

ACKNOWLEDGMENT

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