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ORIGINAL PAPER

An Investigation of Petrol Metabolizing Bacteria Isolated from Contaminated Soil Samples Collected from Various Fuel Stations

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Abstract

The present study aimed to isolate the high-efficiency petrol metabolizing thermophilic bacteria from petrol contaminated soil samples. Isolation was carried out through enrichment culture, serial dilution and pour plate methods using the petrol supplemented minimal salt media. The isolated bacteria were analyzed to document growth behavior, petrol removal efficiencies, antibiotic resistance profile, and biochemical characteristics. The 16S rRNA based phylogenetic analysis helped to reveal the identity of isolated bacterial species and construct the phylogenetic trees. Total nine bacteria were isolated, out of which three (IUBP2, IUBP3, IUBP5) were identified as *Brevibacillus formosus*, one (IUBP1) was found similar to *Brevibacillus agri*, four (IUBP7, IUBP8, IUBP13, and IUBP14) shared homology with *Burkholderia lata*, and one (IUBP15) with *Burkholderia pyrrocinia*. All the isolates were fast growing and exhibited considerable petrol degradation potential. The highest petrol removal efficiency (69.5% ± 13.44/6 days) was recorded for the strain IUBP15 at a petrol concentration of 0.1% (v/v). All bacteria studied (100%) were positive for esculinase and phosphatase. Many strains exhibited positive responses for arginine dehydrolase (22%), *β*-naphthylamidase (11%), *β*-D-glucosaminide (33%), mannitol (55%), sorbitol (66%) and inulin (88%) fermentation test. While all were sensitive to the antibiotics, some of them were found resistant against chloramphenicol and oxacillin. The remarkable biochemical characteristics and considerable petrol removal potential (40–70%) highlights utilization of the bacteria isolated for petrol bioremediation, mineralization of organophosphates, dairy and food industry, and also as biofertilizers and biocontrol agents.

Key words: bioremediation, minimal salt media, green technology, gasoline and 16S rRNA profiling

Introduction

Petrol, also known as gasoline, is a mixture of alkanes (4–8%), alkenes (2–5%), isoalkanes (20–40%), cycloalkanes (3–7%), cycloalkenes (1–4%) and aromatics (20–50%). Aromatics include benzene, toluene, ethylbenzene, and xylene (BTEX). Some other substances like oxygen, sulfur, nitrogen, and metals are also present in low concentrations (Silva et al. 2018). Petrol is obtained during the distillation and refinement of petroleum. The hydrocarbon constituents of petrol due to their adverse impact on the environment and human health have been classified as the priority pollutants by the Environment Protection Agency (Varjani 2017; Yuniati 2018).

Petrol contains various volatile compounds like propane, butane, benzene, toluene, ethylbenzene, and xylene which are ultimately transferred to the atmosphere. The workers of the petroleum industry and petrol pumps are at high risk of exposure to these gasoline components (Rappaport et al. 1987; Cruz et al. 2017; Ekpenyong and Asuquo 2017). Petrol may also intrude indoor spaces from underground storage facilities and may lead to the explosion and serious health hazards after inhalation. Through oil spills, petrol enters the ecosystem and its use as fossil fuel also exerts an adverse impact on the biosphere. It is burned and oxidized in engines of motor vehicles to provide energy for transportation. The incomplete oxidation of petrol generates hydrocarbons which contribute to global warming.

Acute and chronic exposure to petrol hydrocarbons may occur through ingestion, inhalation as well as dermal route and result in various health hazards. Light-chain volatile compounds: toluene, ethylbenzene, and xylene, considered ototoxic compounds, are capable to damage the auditory system. Benzene has no safe

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exposure limit and it is a proved carcinogen (Silva et al. 2018). Gasoline hydrocarbons also affect the respiratory system (Sekkal et al. 2012). Other systemic health effects include the hematological, immunological, reproductive, dermatological, central nervous system, and renal pathologies (Ekpenyong and Asuquo 2017). The associated environmental hazards include contamination of soil and groundwater resources in addition to decreased agricultural productivity (Thapa et al. 2012; Perera 2017).

In order to minimize health-related hazards, the decontamination of the environmental sources from petrol hydrocarbons is indispensable. For this purpose, various physicochemical approaches like thermal desorption, incineration, landfilling, and solvent extraction have been employed (Jain et al. 2011). These techniques suffer from certain limitations like high cost, labor-intensive, incomplete removal of pollutants, land disturbance, and inherent hazard of aggravating the prevailing situation etc. So, the physicochemical methods are being replaced by green technologies, which employ biological means for the purpose of decontamination. Use of bacteria for the removal of petrol is the most cost-effective technique providing required efficacy. The hydrocarbon decomposing bacteria, which are available commercially in freeze-dried forms, propagate to at least 2×10^8 colony forming unit per milliliter (CFU/ml) and they are considered suitable for bioremediation (Thapa et al. 2012). Some of these bacteria with capability of degrading petrol components are: *Flavobacterium* spp., *Rhodococcus* spp., *Serratia* spp., *Pseudomonas putida* HM346961, *Dietzia* spp., *Alcaligenes* spp., *Nocardia* spp., *Micrococcus* spp., *Burkholderia* spp., *Pseudomonas aeruginosa*, and *Bacillus pumilus* MVSV3 (Ridgway et al. 1990; Lu et al. 2006a; Avanzi et al. 2015; Mujahid et al. 2015; Morlett-Chávez et al. 2017; Chen et al. 2017; Surendra et al. 2017; Satyam et al. 2018).

Although the literature reports isolation and characterization of several bacteria capable of metabolizing different gasoline constituents, however, the bacteria reported so far are capable of metabolizing the limited number of gasoline constituents, which proves them less efficient for gasoline remediation. These bacteria metabolize different hydrocarbons of petroleum and oil but very few efficiently degrade multiple petrol constituents simultaneously. Moreover, the bacteria which can degrade some aromatic compounds fail to metabolize the aliphatic compounds and *vice-versa* (Fida et al. 2017; Zhao et al. 2017). A few earlier reported bacterial species capable of degrading multiple hydrocarbons have been studied only in the presence of a particular hydrocarbon and not in the petrol saturated environment (Guermouche M'rassi et al. 2015). It limits their potential of being used as petrol bioremediating agents. Such bacteria can only be effective in the form of consortium (Gurav et al. 2017; Sarkar et al. 2017; Bacosa et al. 2018). In order to achieve optimal degradation, the use of bacterial consortium consisting of bacteria capable of metabolizing different fractions/ constituents of petrol has been recommended (Patowary et al. 2016). This phenomenon is referred to as co-metabolism and is highly advantageous for effective degradation of mixtures, like petrol (Battikhi 2014). Bioremediation is not an omnipotent technology and is affected by several environmental factors. Moreover, the large-scale application of consortium, containing a wide range of bacterial species, for the purpose of bioremediation may encounter the problem of providing favorable conditions for optimum growth of diverse bacteria found in a consortium (Xu et al. 2018). In contrast to this, use of bacteria having the potential of degrading multiple constituents of petrol simultaneously in petrol saturated environment may result in increased bacterial access to the numerous carbon sources and more efficient bioremediation (Vignesh et al. 2016). The study presented in this manuscript was initiated to isolate bacteria capable of surviving in high gasoline environments and efficiently removing gasoline by the simultaneous breakdown of multiple gasoline components.

We tried to explore the highly efficient eco-friendly (i.e., lacking drug resistance and pathogenicity) petrol metabolizing bacteria, exhibiting fast growth rate, and capable of metabolizing multiple constituents of petrol.

Bioremediation is an eco-friendly technology which requires the selection of environmentally friendly bacteria lacking pathogenicity and drug resistance (Händel et al. 2013). Up to our knowledge, the literature already published does not contain any information regarding virulence status of petrol metabolizing bacteria (Lu et al. 2006b; Asiedu et al. 2014). During the present study to justify the future application as a bioremediating agent, we documented the antibiotics resistance potential of the isolated petrol metabolizing bacteria.

Constant spilling of petrol at the gas stations contaminates the surrounding soil which thus could be the rich reservoir of the petrol utilizing bacteria. Although various studies have reported the isolation of petrol metabolizing bacteria from the petrol-contaminated soil of petrol pumps, no one has explored our native sources. Moreover, under different habitats bacteria may employ unique degradative pathways and enzymes for remediation (Copley 2009; Bagga et al. 2015; Mujahid et al. 2015; Abou-Shanab et al. 2016). We, therefore, hypothesized that we might have found some unusual petrol metabolizing bacteria expressing novel petrol degradative enzymes and associated metabolic pathways in petrol contaminated soil of indigenous fuel stations. It was further speculated that the accomplishment of the proposed study may serve as a road map to revive petrol contaminated environmental resources.

Experimental

Materials and Methods

Screening of petrol metabolizing bacteria. For initial screening of petrol metabolizing bacteria, minimal salt media (MSM) was used. This medium contained; KH_2PO_4, 1 g; K_2HPO_4, 1 g; NH_4NO3, 1 g; $MgSO_4$, 0.2 g; $CaCl_2$, 0.02 g; $FeCl_3$ (0.5 M), 2 drops per 1000 ml of distilled water, and 100 µl petrol in 100 ml of medium (Ozyurek and Bilkay 2017). Initially, 1 g of soil sample was added to the medium (50 ml) and incubated for one week in shaker adjusted at 50°C and at 250 rpm. After a week, this culture was used as inoculum and added to a freshly prepared minimal salt medium supplemented with petrol. The culture was incubated in incubator shaker under the same conditions. The enrichment process was repeated for six weeks. After enrichment, the serial dilutions were prepared and each dilution (100 µl) was spread on petrol supplemented MSM agar medium solidified in Petri plates. Followed by spreading, the Petri plates were incubated at 50°C and the isolates exhibiting optimum growth were stored in the form of glycerol stock for future use.

Morphology. The colony morphology on agar medium and response to Gram staining and colony forming units (CFUs) of each bacterial isolate were documented.

Growth characteristics. The growth of bacteria was monitored turbidometrically (OD_{600}) by measuring absorbance at regular time intervals using UV-visible spectrophotometer. To better reflect the growth behavior, optical density was plotted versus time.

Biochemical characterization. The Remel RapID STR System (Thermo Scientific) was used for biochemical characterization of the isolated bacteria. This system included various qualitative biochemical tests like arginine dehydrolase test, esculinase test, sorbitol fermentation test, mannitol fermentation test, inulin fermentation test, raffinose fermentation test, ρ-nitrophenyl-α,D-galactosidase test, ρ-Nitrophenyl-α,D-glucosidase test, tyrosine β-naphthylamidase test, ρ-nitrophenyl-n-acetyl-β,D-glucosaminidase test, pyrrolidonyl peptidase test and lysine β-naphthylamidase test.

Quantitative study of petrol degradation by isolates. For the assessment of petrol degrading potential of the bacteria isolated, petrol supplemented MSM (50 ml) was added in 250 ml Erlenmeyer flask containing 1% (v/v) of the aqueous solution of 2, 6-dichlorophenolindophenol (DCPIP), a redox indicator. Overnight grown bacterial cultures (1 ml) were added as inoculum in the culture flask and incubated at 50°C and 250 rpm for seven days. The culture was collected after different time intervals and centrifuged (6000 rpm) at 4°C. The pelleted cells were discarded, and an absorbance of the supernatant was measured at 600 nm to assess the color change against a blank (Ozyurek and Bilkay 2017). DCPIP serves as a redox indicator and its decolorization or color change in petrol supplemented medium is a measure of petrol metabolizing capability of bacteria (Marchand et al. 2017).

Antibiotic sensitivity profiling. All isolated bacteria were subjected to disc diffusion antibiotic sensitivity assay (Bauer et al. 1966). Bacteria were tested against antibiotics teicoplanin (30 µg/disc), linezolid (10 µg/disc), linezolid (30 µg/disc), oxacillin (1 µg/disc) and chloramphenicol (30 µg/disc).

Molecular analysis. For molecular analysis, bacterial genomic DNA was extracted using organic method (Maniatis et al. 1982). For PCR (Polymerase Chain Reaction) amplification of 16S rDNA gene, specific primers F1 and R1 were designed (Table SI). The PCR (50 µl) reaction mixture contained; 5 µl of 10 × PCR buffer (Mg^{2+} free), 5 µl of $MgCl_2$, 1 µl of 10 mM dNTPs, 2 µl of 10 pM forward primer, 2 µl of 10 pM reverse primer, 0.25 µl of Taq DNA polymerase, 50 ng of template DNA and 29.75 µl of nuclease-free water. The conditions used for PCR amplification were as follows: initial denaturation (95°C, 5 min), 38 cycles consisting of denaturation (94°C, 40 seconds), annealing (58°C, 40 seconds) and extension (72°C, 30 seconds), followed by a final extension of 10 min at 72°C. Amplification of target DNA was confirmed by agarose gel electrophoresis and PCR amplicons purified using Monarch DNA Gel Extraction Kit (Cat# T1020S) were sent to Macrogen, Korea for the DNA sequencing. The obtained FASTA sequences were subjected to BLASTN analysis (http://blast.ncbi.nlm.nih.gov/blast/Blast.cgi) which aligned study sequences with the sequences available in the non-redundant nucleotide database of NCBI (National Center for Biotechnology Information). Once the taxonomic affiliation of bacterial sequences was revealed, 16S rRNA gene sequences were submitted to NCBI Genbank database. Accession numbers were assigned (Table I). Multiple sequence alignments of 16S rRNA gene sequences of isolated bacteria and the closely related bacterial sequences available in the NCBI database were carried out using Clustal omega multiple sequence alignment algorithms available at http://www.clustal.org/mbed.tgz. Results of multiple sequence alignment analysis were used to construct bootstrapped maximum composite likelihood neighbor-joining trees using Mega 7 software.

Sequences of nine isolates have been submitted to NCBI and accession numbers assigned are: IUBP1 (MH368051), IUBP2 (MH023312), IUBP3 (MH023313), IUBP5 (MH023314), IUBP7 (MH368052), IUBP8 (MH368053), IUBP13 (MH368054), IUBP14 (MH368055) and IUBP15 (MH368057) (Table I).

Table I
Phylogenetic analysis of petrol metabolizing bacteria.

Group	Isolate	Accession No.	Closest phylogenetic relative	Similarity	URLs
Group I	IUBP2	MH023312	*Brevibacillus formosus* strain NBRC 15716	100%	https://www.ncbi.nlm.nih.gov/nuccore/MH023312.1
	IUBP3	MH023313	*Brevibacillus formosus* strain NBRC 15716	100%	https://www.ncbi.nlm.nih.gov/nuccore/MH023313.1
	IUBP5	MH023314	*Brevibacillus formosus* strain NBRC 15716	100%	https://www.ncbi.nlm.nih.gov/nuccore/MH023314.1
Group II	IUBP1	MH368051	*Brevibacillus agri* strain NBRC 15538	100%	https://www.ncbi.nlm.nih.gov/nuccore/MH368051
Group III	IUBP7	MH368052	*Burkholderia lata strain 383*	99%	https://www.ncbi.nlm.nih.gov/nuccore/MH368052.1
	IUBP8	MH368053	*Burkholderia lata strain 383*	99%	https://www.ncbi.nlm.nih.gov/nuccore/MH368053.1
	IUBP13	MH368054	*Burkholderia lata strain 383*	99%	https://www.ncbi.nlm.nih.gov/nuccore/MH368054.1
	IUBP14	MH368055	*Burkholderia lata strain 383*	99%	https://www.ncbi.nlm.nih.gov/nuccore/MH368055.1
Group IV	IUBP15	MH368057	*Burkholderia pyrrocinia* strain LMG 14191	98%	https://www.ncbi.nlm.nih.gov/nuccore/MH368057

Results

Morphology. Nine petrol metabolizing bacteria were isolated from the soil sample of a petrol pump through enrichment culture technique. All isolated bacteria were found to be Gram-positive bacilli. The number of the bacteria was enumerated (Table SII).

Molecular analysis. The molecular study used as the first level of screening classified nine petrol metabolizing bacteria into four groups (Fig. 1). Group I included three isolates IUBP2, IUBP3 and IUBP5 exhibiting similarity to *Brevibacillus formosus*. Group II consisted of only one isolate IUBP1 homologous to *Brevibacillus agri*. Four isolates sharing homology with *Burkholderia lata* were included in Group III. While Group IV, a group of *Burkholderia pyrrocinia* comprised of one isolate IUBP15 (Table I).

Phylogenetic analysis. To trace the phylogenetic history of the isolated bacteria, the phylogenetic trees were constructed. The isolates IUBP7, 8, 13, 14 and 5 were assigned to genus *Burkholderia*. Study of the evolutionary relationship revealed that IUBP15 shared common ancestry with *B. pyrrocinia* (Accession number NR 118075.1) and *Burkholderia ambifaria* (Accession number NR 118051.1). Moreover, they shared the same clade with a strong (93) bootstrap value. While IUBP8,

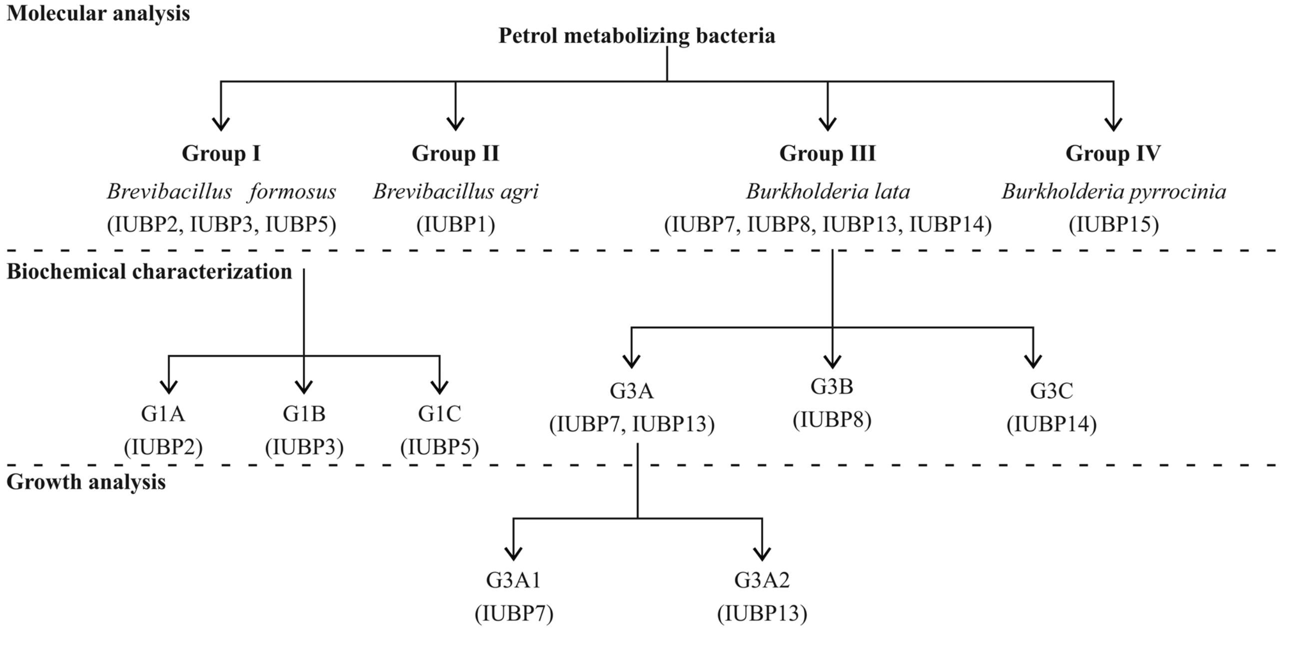
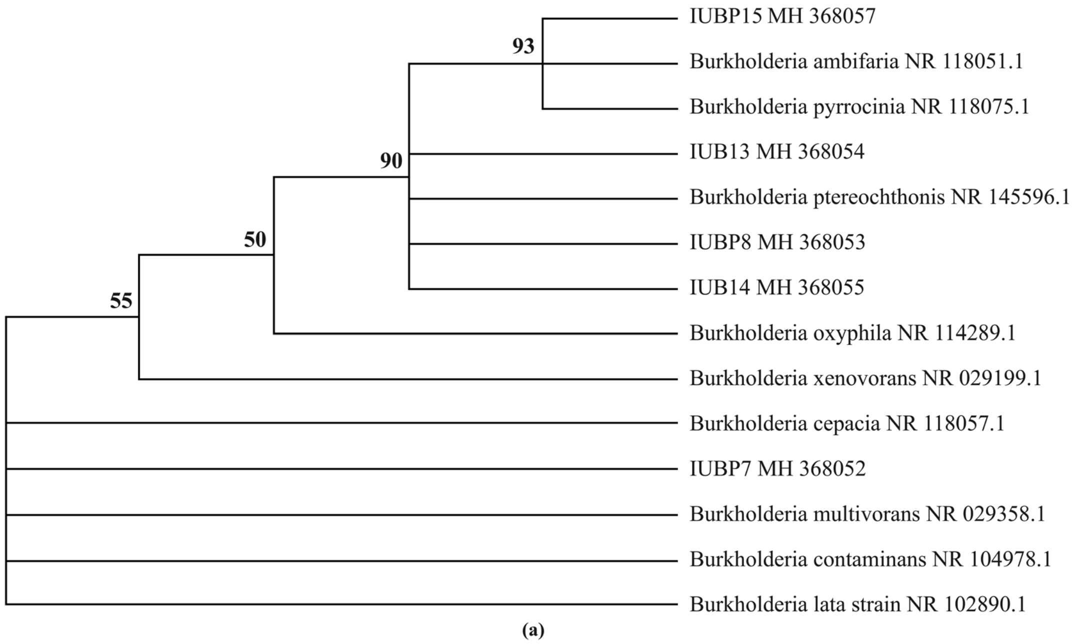


Fig. 1. Characteristics of petrol metabolizing bacteria based upon molecular, biochemical and growth curve analysis. The ribotyping, biochemical, growth behavior analysis helped to discriminate among the bacteria isolated.



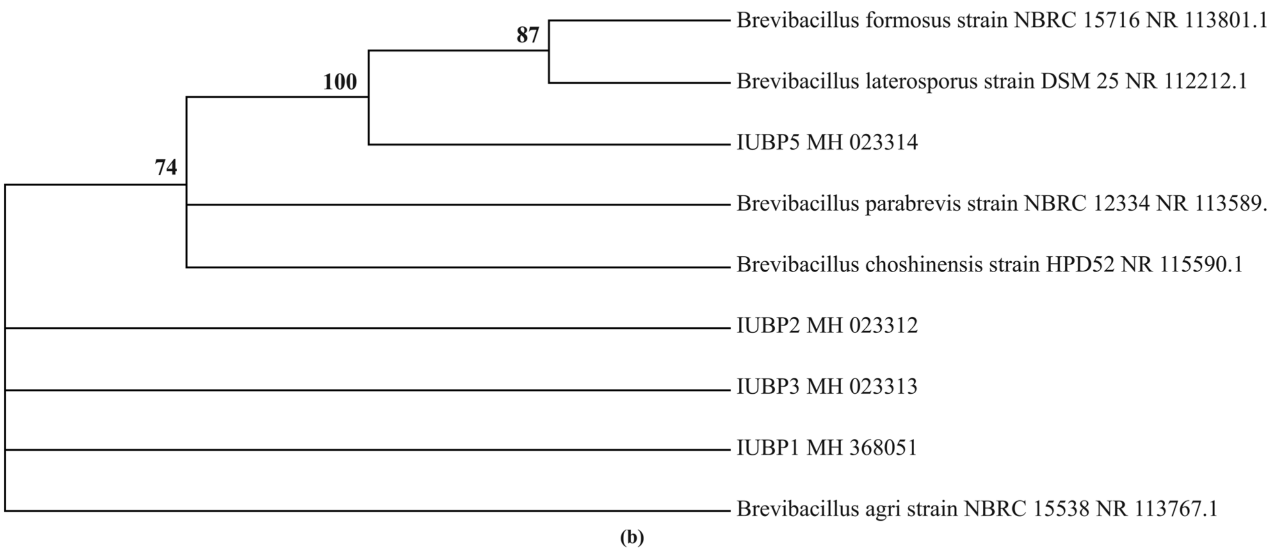


Fig. 2. A) Phylogenetic tree of petrol metabolizing bacteria exhibiting homology with *Burkholderia*.
B) Phylogenetic tree of petrol metabolizing bacteria exhibiting homology with *Brevibacillus*.

13 and 14 were found closely related to each other as they originated from the same lineage with strong bootstrap value (90). However, IUBP7 distantly related to the other isolates of genus *Burkholderia* (Fig. 2A).

Molecular analysis placed isolates IUBP1, 2, 3 and 5 in genus *Brevibacillus*. As per evolutionary study, IUBP5 shared clade (bootstrap value = 87) with *B. formosus* strain NBRC 15716 (Accession number NR 113801.1) and *Brevibacillus laterosporus* strain DSM (Accession number NR 112212.1). While IUBP1, IUBP2, and IUBP3 shared the same clade with each other and with *B. agri* strain NBRC 15538 (Accession number NR 113767.1) (Fig. 2B).

Biochemical characterization. The isolated bacteria were biochemically characterized through fourteen tests (Table SIII and Fig. S1) and all were found negative for raffinose fermentation (RAF) test, D-galactoside (GAL) test, tyrosine β-naphthylamide (TYR) test, D-glucoside (GLU) test, lysine β-naphthylamide (LYS) test and pyrrolidine β-naphthylamide (PYR) test. All bacteria were esculinase and phosphatase producers. While only two (IUBP3 and 14) were arginine dehydrolase producers. Five isolates (IUBP2, 3, 7, 13 and 15) were capable of fermenting mannitol and six (IUBP2, 3, 5, 7, 13 and 15) were noticed to be sorbitol fermenting and all except IUBP1 were inulin fermenting. Only one bacterium, IUBP1, was found positive for hydroxyproline β-naphthylamidase and three (IUBP2, 3 and 8) were examined to be glucosaminidase producers. The biochemical analysis helped to differentiate between the members of the group I and group III. All the members of the group I and two members of the group III (IUBP8 and 14) were distinctive from each other. However, IUBP7 and IUBP13 were biochemically similar and thus placed in group G3A.

Growth rate. On the basis of molecular and biochemical characteristics, all isolates except members of group G3A were found different. So, the growth rate of the molecularly and biochemically similar members of group G3A was compared to detect their distinctiveness (Table SIV). Both isolates (IUBP7 and 13) have different growth rate and, therefore, were considered to be different bacteria. Growth behavior of petrol degrading bacteria having similar molecular and biochemical profile was illustrated graphically (Fig. S2).

Petrol removal efficiency. Petrol removal efficiency for all nine bacteria was measured. Maximum degradation efficiency (69.5% ± 13.44) was observed for IUBP15, while isolate IUBP1 exhibited minimum petrol removal efficiency (41% ± 32.6) (Table SV).

Antibiotic resistance profile. All isolates tested were found sensitive to teicoplanin and linezolid and exhibited resistance to chloramphenicol and oxacillin. Zone of inhibition was recorded. Maximum zone of inhibition (37.5 mm ± 0.071) was observed for IUBP8 upon linezolid (30 μg) treatment. For linezolid (10 μg) a maximum zone of inhibition (32.5 mm ± 0.071) was recorded for IUBP14. In the case of teicoplanin, a maximum zone of inhibition (22.5 mm ± 0.2121) was shown for IUBP7.

Discussion

Screening for petrol metabolizing bacteria from contaminated soil of petrol pump resulted in the isolation of nine different bacterial species. All isolates were capable to grow in MSM and metabolizing petrol as a sole source of carbon.

Morphology. All these isolates were Gram-positive bacilli. The reason behind the dominant incidence of Gram-positive bacteria could be that in Southern Punjab day time temperatures are usually high and osmotic pressure may vary periodically over a daily cycle. A stronger cell envelope of Gram-positive bacteria enables them to proliferate more efficiently when compared to Gram-negative bacteria (Silhavy et al. 2010). This is parallel with the earliest studies in which petroleum hydrocarbons metabolizing bacteria like *Bacillus cereus*, *Proteus mirabilis*, *Bacillus subtilis*, *Enterococcus faecalis*, *Streptomyces* sp. ERI-CPDA-1, *Bacillus mojavensis* ATHE13 and *Bacillus licheniformis* ATHE9 have been reported (Balachandran et al. 2012; Eskandari et al. 2017; Ozyurek and Bilkay 2017).

Molecular characterization. Most of the petrol metabolizing bacteria (IUBP7, 8, 13, 14 and 15) isolated during the present study belonged to the genus *Burkholderia*. Our results are consistent with the earlier studies which have reported different species of *Burkholderia* capable of degrading variable aliphatic and aromatic hydrocarbons of petrol (Marin et al. 2001; Chakraborty et al. 2010; Mujahid et al. 2015). Moreover, petrol hydrocarbon metabolizing *Brevibacilli* have also been reported in the literature (Xue et al. 2006; Mnif et al. 2011; Zhan et al. 2017).

Biochemical characterization. In order to discriminate among different strains of petrol metabolizing bacteria belonging to the same species, their biochemical potential was investigated. In literature, biochemical characterization of different petrol decomposing bacteria has been reported (Lu et al. 2006b). However, this study is the first to report enzymes like arginine dehydrolase, esculinase, naphthylamidase, glucosaminidase, phosphatase enzymes and capabilities to ferment sorbitol, mannitol, and inulin.

Arginine dehydrolase (ARG) is the enzyme, which catalyzes the conversion of arginine to putrescine. ARG was detected in only two bacteria (IUBP3 and IUBP14). This enzyme has been identified as a potential anticancer agent for the treatment of hepatocellular melanomas and carcinomas. Hence, ARG positive bacteria can be further exploited for therapeutic purposes (Sharma et al. 2017).

Esculinase (ESC) test confirms the presence of esculinase, which catalyzes breakdown of esculin into esculetin and dextrose. In the present study, all the bacteria isolated were positive for esculinase and thus hold potential to be used in food industry, synthesis of *o*-alkyl glucoside, cosmetics and pesticides (Rani et al. 2014).

In our study, results of fermentation tests revealed 56% bacteria positive for mannitol, 66% positive for sorbitol and 88% positive for inulin. However, none of the bacteria was capable to ferment raffinose. Inulase-positive bacteria can be effectively used for the production of gluconic acid, mannitol, ethanol and fructose syrup (Singh et al. 2017).

Hydroxyproline β-naphthylamidase expedites catabolism of hydroxyproline β-naphthylamide and β-naphthylamine. In this study, only 11% of isolated bacteria were naphthylamidase-positive. The ρ-nitrophenyl-n-acetyl-β-D-glucosaminide (NAG) test was carried out to detect glucosaminidase enzyme, which hydrolyzes p-nitrophenyl substituted glycoside and releases ρ-nitrophenol. In our study, 33% of the isolates were found positive for glucosaminidase. Many studies have reported the use of glucosaminidase as biocontrol agents and in the production of important biological compounds (Scigelova and Crout 1999).

The ρ-nitrophenyl phosphate (PO_4) test is performed to detect the presence of phosphatase enzyme in bacteria. The phosphatase enzyme regulates the breakdown of ρ-nitrophenyl phosphate into ρ-nitrophenol. All isolated bacteria were positive for the phosphatase enzyme. Hence, it can be utilized for designing biosensors for environmental monitoring or as an indicator for sufficient pasteurization of milk, mineralizing organophosphates, assessment of heavy metals precipitation from effluents and in immunoassays (Nalini et al. 2015).

Growth rate. All the bacterial isolates were fast growing and exhibited exponential growth until 6 hours (IUBP14), 24 hours (IUBP5 and 13) and 30 hours (IUBP1, 2, 3, 7, 8 and 15). In our study, the maximum OD (0.27) was observed for IUBP3 while minimum OD (0.1) was observed in case of IUBP5, 7, 13 and 14. The growth rate of our bacteria is comparable with the earlier reported bacteria. Many of the isolates showed optimal growth at six hours (Table SIV). While contrary to our results, maximum OD (1.4) and minimum OD was considerably higher (0.2) in the previous reports (Vignesh et al. 2016).

Petrol degrading efficiency. All the nine bacteria incubated in the presence of petrol (0.1% v/v) for seven days at 50°C showed different petrol degrading efficiencies. The highest petrol degradation efficiency (69.5% ± 13.44 / 6 days) was observed for IUBP15. While highest petrol removal efficiency reported in the literature is 30% per seven days for *Bacillus tequilensis* grown in the presence of crude oil (1% v/v) for seven days at 30°C (Ozyurek and Bilkay 2017). In the present study, the lowest petrol degradation rate (41% ± 32.6 / 4 days) was noticed for IUBP1. However, in the literature the lowest degradation efficiency has been reported to be 80% for 21 days for HCS2 bacterial strain, incubated in MSM containing 50 mg/l petrol (0.005%) for 30 days at 30°C (Avanzi et al. 2015). The petrol degrading efficiency of other isolates IUBP2 (62% ± 19.34 / 7 days), IUBP3 (66% ± 9.90 / 6 days) and IUBP14 (63% ± 1.41 / 5 days) was somehow comparable to the reported petrol degradation rate (60% / 21 days) of HCS1 grown in the presence of 50 mg/l petrol (0.005%) for 30 days at 30°C (Avanzi et al. 2015). Based on this comparison, in this study the bacterial strains could be a better choice than many previously reported bacteria due to considerable efficiency (up to 69.5% petrol removal efficiency achieved in 4–7 days), the capability to grow in petrol saturated environment (0.1%), and at a higher temperature (50°C). Most of the previously isolated bacteria are known to grow at 30°C in the presence of lower petrol concentration (0.005%) and exhibit delayed degradation (21 days). Variations in biodegradation potential reflect the presence of different enzyme systems and metabolic pathways responsible for petrol catabolism.

In order to explore the petrol degradation capabilities and pathways existing in our isolates, we performed a GC-MS based analysis of bacterial metabolites. Our results confirmed the metabolism of multiple constituents, like alkanes, cycloalkanes, and aromatics including benzene, toluene, naphthalene, and ethylbenzene of petrol (data not shown). Hence, the ability to metabolize both, aliphatic and aromatic components of petrol, makes the isolated bacteria better choice for effective petrol remediation than earlier known petrol remediating bacteria. Moreover, multi-potential bacteria of the present study do not highlight the need to exploit the phenomenon of co-metabolism and application of bacterial consortium.

Antibiotic sensitivity profiling. The application of bacteria for eco-friendly bioremediation is restricted by their antibiotics resistance potential. The antibiotic-resistant bacteria can adversely affect the environment through their virulence and cannot be used as a whole cell preparation for effective bioremediation. The petrol metabolizing bacteria decompose petrol due to the presence of genes encoding petrol metabolizing enzymes. In order to decide whether to use a whole cell, an enzyme or a gene for bioremediation the antibiotic resistance profile of isolated bacteria was investigated. All isolates were found resistant to teicoplanin and linezolid. Hence, the whole cell uses of study bacteria cannot be recommended but their enzymes and genes can be exploited in multiple ways.

In case the desired enzymes are extracellular then their supernatant will be used for synthesizing nanoparticles, while in case of intracellular enzymes, their cell lysate can be used for purification of enzymes and for synthesizing nanoparticles. The desired genes can be cloned into any environmentally friendly bacteria for their expression.

Nanoparticles due to their high surface area to volume ratio are highly reactive and can decontaminate effluents in lesser time (Guerra et al. 2018). Bacteria due to their capability to mobilize, immobilize and reduce the metal ions, can easily precipitate metals at nanoscale (Iravani 2014). The bacterial exopolysaccharides based silver nanoparticles have been previously reported as effective, eco-friendly and cheaper tools for remediation of textile dyes (Saravanan et al. 2017). The enzymes of present study isolates can be used to synthesize nanoparticles for remediation of the petrol hydrocarbons.

Conclusion

Petrol hydrocarbons-based pollution is a real-world issue (Perera 2017). The bacteria isolated and characterized during the present study can serve as the promising tools in future for reclamation of petrol contaminated environmental resources because of their fast growth rate in the presence of petrol as an only carbon source, the capability to remove a wide range of constituents of petrol simultaneously and without the need of growth within a consortium. The ability to produce a variety of enzymes highlights the future industrial significance of study isolates. Due to drug resistance potential a whole cell uses of the bacteria isolated cannot be recommended. However, the valuable genes and enzymes can be exploited through alternate ways like cloning of the genes into a non-virulent expression system or through the synthesis of the enzymes-based nanoparticles. Further study of factors influencing the growth and metabolism, exploitation of enzyme systems, metabolic pathways and associated genes will help to design the best system for achieving optimum removal of petrol hydrocarbons.

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Author's contributions

SE conceived the idea and designed study. FM performed all bench top work and wrote first draft of manuscript. All authors contributed to finalize the manuscript.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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