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ORIGINAL PAPER

# Evaluation of bioremediation potential and biopolymer production of pseudomonads isolated from petroleum hydrocarbon-contaminated areas

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**Abstract** Bacteria are diverse and abundant in soils, but only a few bacteria have known to grow on hydrocarbon-contaminated areas and utilize complex carbon source such as crude oil for the synthesis of polyhydroxyalkanoate (bioremediation potential and the ability to produce important biopolymers). Among 32 samples collected from several sites of petroleum refinery soil and oily sludge of Iranian southwestern refineries, 45 oil-degrading pseudomonads were identified, and 33 % of the isolated *Pseudomonas* strains were able to produce polyhydroxyalkanoate using Gachsaran crude oil (2 % v/v) as carbon source. The repeated monomer composition of the copolymer produced from Gachsaran crude oil was determined by gas chromatography/mass spectrometry. The produced monomers composites contained: $C_8$ (3-hydroxyoctanoate), $C_{10}$ (3-hydroxydecanoate), $C_{12}$ (2-hydroxydodecanoate), $C_{14}$ (3-hydroxytetradecanoate), and $C_{16}$ (3-hydroxydecahexanoate), which are known as biopolymers. This study indicates oil-contaminated areas can be important sources for polyhydroxyalkanoate producers which can be used for the bioremediation of crude-oil-polluted sites; also polyhydroxyalkanoate has a functional role in bacterial survival and stress tolerance in the toxic environments and poor nutrient availability.

**Keywords** Biopolymers · Bioremediation · Polyhydroxyalkanoate · Pseudomonads

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## Introduction

Soil contamination with petroleum and petroleum-based hydrocarbons has caused critical environmental and health defects, and increasing attention has been paid for developing and implementing innovative technology for cleaning up this contaminant. Bioremediation methods are currently receiving favorable publicity as promising environmental-friendly, efficient, and cheap treatment technologies for the remediation of hydrocarbons and can be described as the conversion of chemical compounds by living organisms, especially microorganisms, into energy, cell mass, and biological waste products (Rahman et al. 2002; Minai-Tehrani et al. 2015; Barin et al. 2014). Some of the most important of these bioremediation products are polyhydroxyalkanoic acids (PHAs), which are family of biopolymers formed by the biological condensation of hydroxyalkanoic acids, produced by bacteria and archaea. PHAs are deposited as water-insoluble inclusions in the cells (Rehm and Steinbuchel 1999; Anderson and Dawes 1990). These natural polyesters are considered for several applications in the packaging, medical, pharmaceutical, agricultural, and food industries or as raw materials for the synthesis of enantiomerically pure chemicals and the production of paints due to its biodegradability (Rehm and Steinbuchel 1999; Zinn et al. 2001; Luengo et al. 2003; Reddy et al. 2008). PHAs are produced by bacteria under

unbalanced growth conditions when a carbon source is available in excess, and other nutrients are growth-limiting and act as a mechanism to store excess carbon and energy (Zinn et al. 2001; Rehm 2003; Ivanov et al. 2015). Polyester synthases are the key enzymes of polyester biosynthesis and catalyze the conversion of (*R*)-hydroxyacyl-CoA thioesters to polyesters with the concomitant release of CoA (Zinn et al. 2001; Rehm 2003).

PHAs are produced by many different bacterial cultures, the first report of PHA formation by a *Pseudomonas* species; *P. oleovorans*, described the production of a polymer containing 3-OH-octanoate by assimilation of medium- and long-chain length fatty acids (Desmet et al. 1983) and later reported that variety of pseudomonads, such as *P. putida*, *P. oleovorans*, and *P. aeruginosa* can produce PHAs with many different structures as energy and carbon storage materials when the cells are cultivated in the presence of various carbon sources (Huisman et al. 1989).

Most of the carbon substrates supplied for PHA production are pure alkanes, fatty acids or carbohydrates (Kim et al. 1997; Ashby et al. 2002), complex substrates, such as castor or euphorbia oil, resulted in PHA of ($C_6$–$C_{14}$) with *P. aeruginosa* (Eggink et al. 1995); however, the use of subproducts or wastes has hardly been explored at all, which may well be due to the complexity of their composition. *P. resinovorans* accumulated 15 % of the cellular dry weight of PHA from tallow (Cromwick et al. 1996).

Recently PHA productions by bacteria have been studied and their PHAs properties well-characterized. However, our finding about PHA-producing bacteria in hydrocarbon-contaminated regions and properties of these PHA remains limited. So far two PHA producers *Pseudomonas* spp. from hydrocarbon-contaminated area have been known: *P. stutzeri* 1317 (He et al. 1998) and *P. pseudoalcaligenes* strain YS1 isolated from oil-contaminated soils (Hang et al. 2002).

The main objective of this study was to investigate bioremediation potential and biopolymer production of pseudomonads isolated from petroleum hydrocarbon-contaminated areas of different regions of Iranian southwestern refineries. This study was carried out between October 2012 and February 2014.

## Materials and methods

### Reference strains

In this study, the following reference strains were used: *P. aeruginosa* ATCC 15442, *P. aeruginosa* ATCC 9027, *P. putida* ATCC 12633, *P. putida* ATCC 47054, *Alcaligenes eutrophus* ATCC 17699, and *Escherichia coli* ATCC 25922.

### Isolation and identification of *Pseudomonas* strains

*Pseudomonas* strains were isolated from contaminated soil and oily sludge samples collected from Iranian southwestern refineries (Table 1). A total of 32 samples were collected during the sampling period. Samples were serially diluted using sterile normal saline ($10^{-1}$–$10^{-5}$) and enriched by culturing aerobically at 30 °C in tryptic soy broth (TSB) (Merck, Germany) overnight. The enriched samples were inoculated on cetrimide agar (Merck, Germany) containing: Peptone from gelatin 20.0 (g/l); magnesium chloride 1.4 (g/l); potassium sulfate 10.0 (g/l); *N*-cetyl-*N*,*N*,*N*-trimethylammoniumbromide (cetrimide) 0.3 (g/l); agar–agar 13.6 (g/l); and 10 ml/l glycerol (Merck, Germany) and then incubated at 30 °C, overnight. Single colonies were achieved by repeated streaking method on cetrimide agar plates. After gram staining and determination of catalase and oxidase activities, isolates were identified by physiological and biochemical tests including: methyl red (MR), Voges–Proskauer (VP) test, gelatin hydrolysis, motility test, MacConkey growth, pigment production, urease test, indole test, nitrate reduction test, and lactose, glucose, and maltose utilization tests. These tests were selected to provide high discrimination among *Pseudomonas* spp., based on a high probability of a positive or negative result. Different phenotypic characteristics were evaluated as outlined in Bergey's manual of systematic bacteriology (Breed et al. 1975).

### Screening of PHA-producing microorganism

The pseudomonad-isolated strains from contaminated area were assayed for PHA production by using an initial Sudan Black B staining (Burdon 1946). Positively stained isolates were induced to accumulate PHA. These strains were incubated in 100 ml PHA production medium (6.0 g Na2HPO4, 3.0 g KH2PO4, 0.5 g NH4Cl, 0.5 g NaCl, 1.0 mM MgSO4, and 0.1 mM CaCl2 per liter of medium) (Goh and Tan 2012) containing 2 % (v/v) sterilized Gachsaran crude oil (with identified compounds; Table 2) as sole carbon source and then checked for PHA production by specific Nile red A dye staining method (Spiekermann et al. 1999). The PHA-accumulating colonies, after Nile red A staining, showed bright orange fluorescence on irradiation with UV light, and their fluorescence intensity increased with increase in PHA content of the bacterial cells.

### Quantitative analysis of PHAs

The isolates showing bright orange fluorescence on irradiation with UV light after Nile red staining were selected as PHA accumulators. These isolates were first grown in PHA production medium in 100-ml flasks and incubated at

**Table 1** Geographic location and type of contamination prevalent in different sampling sites

| Isolation sites | Geographic location | Type of sample | |
|---|---|---|---|
| | | Hydrocarbon-contaminated soil | Oily sludge |
| Gachsaran refinery | 3559287N 479844.88E | 5 | 2 |
| Shiraz Oil Refining Company | 3290352N 660341.84E | 2 | 2 |
| Farrashband gas refinery | 3192389N 607274E | 2 | 3 |
| Asalouyeh refineries | 3043170N 660111E | 6 | 4 |
| Shiraz petrochemical company | 3307721.23N 668001E | 2 | – |
| Khonj gasoline-contaminated soil | 3087454N 739653.26E | 2 | – |
| Shiraz (control-negative samples) | 3290352N 660341.84E | 2 | – |

**Table 2** Gachsaran oil analysis using GC/MS

| Compound | % | Compound | % | Compound | % |
|---|---|---|---|---|---|
| 2-Methylpropane | 0.233 | 1,2-Dimethylcyclohexane, trans | 0.449 | Undecane | 3.040 |
| Butane | 1.076 | Octane | 3.028 | 2,5-Dimethylstyrene | 0.229 |
| 2-Methylbutane | 1.430 | 2,4-Dimethylheptane | 0.283 | 1-Methyl-2-propenylbenzene | 0.169 |
| Pentane | 2.009 | Ethylcyclohexane | 0.867 | Tetralin | 0.202 |
| 2,2-Dimethylbutane | 0.042 | 2,6-Dimethylheptane | 0.498 | Dodecane | 2.747 |
| 2-Methylpentane | 1.762 | 1,1,3-Trimethylcyclohexane | 0.897 | 2,6-Dimethylbutane | 0.913 |
| 3-Methylpentane | 1.001 | 2,5-Dimethylheptane | 0.320 | 5-Methyltetralin | 0.392 |
| Hexane | 2.452 | Ethylbenzene | 0.869 | 6-Methyltetralin | 0.373 |
| Methyl cyclopentane | 1.392 | 1,2,4-Trimethylcyclohexane | 0.389 | Tridecane | 2.74 |
| Benzene | 0.388 | p-Xylene | 1.625 | Tetradecane | 2.989 |
| Cyclohexane | 0.747 | 4-Methyloctane | 0.380 | Pentadecane | 2.830 |
| 2-Methylhexane | 1.251 | 2-Methyloctane | 0.688 | Hexadecane | 2.701 |
| 3-Heptane | 0.151 | 3-Methyloctane | 0.889 | Heptadecane | 2.640 |
| 3-Methylhexane | 1.172 | m-Xylene | 0.840 | Pristane | 1.715 |
| 1,2-Dimethylcyclopentane | 0.454 | Nonane | 3.020 | Phytan | 1.845 |
| 1,3-Dimethylcyclopentane | 0.505 | Propylcyclohexane | 0.542 | Nonadecane | 2.091 |
| Isopropylcyclobutane | 0.879 | 2,6-Dimethyloctane | 0.984 | Eicosane | 2.187 |
| Heptane | 2.792 | Propylbenzene | 0.348 | Heneicosane | 1.779 |
| Methylcyclohexane | 2.175 | 1-Ethyl-3-methylbenzene | 0.624 | Docosane | 1.729 |
| 1,1,3-Trimethylcyclopentane | 0.234 | 1-Ethyl-4-methylbenzene | 0.335 | Tetracosane | 1.377 |
| Ethylcyclopentane | 0.453 | 1,2,3-Trimethylbenzene | 0.431 | Pentacosane | 1.085 |
| 2,4-Dimethylhexane | 0.212 | 4-Methylnonane | 0.615 | Hexacosane | 1.038 |
| 1,2,4-Trimethylcyclopentane | 0.377 | 2-Methylnonane | 0.621 | Heptacosane | 0.887 |
| 1,2,3- Trimethylcyclopentane | 0.408 | 1,2,4-Trimethylbenzene | 1.124 | Octacosane | 0.851 |
| 1,2,3- Trimethylpentane | 0.058 | Decane | 3.077 | Nonacosane | 0.847 |
| Toluene | 1.529 | 4-Methyldecane | 0.889 | Tricosane | 0.625 |
| 2-Methylheptane | 1.723 | 1-Methyl-3-propylbenzene | 0.533 | Hentriacontane | 0.587 |
| 1,2-Dimethylcyclohexane | 1.803 | 4-Ethyl-1,2-dimethylbenzene | 0.325 | Triacontane | 0.604 |
| 1,4-Dimethylcyclohexane | 0.323 | 1-Ethyl-2,3-dimethylbenzene | 0.264 | 3,7-Dimethylnonane | 0.425 |
| 1-Ethyl-2-methylcyclopentane | 0.551 | 2-Methyldecane | 0.782 | Octadecane | 2.260 |

30 °C on orbital shaker at 150 rpm. After 48-h incubation, cells were centrifuged at 3000×*g* for 10 min, followed by washing the pellet with distilled water and freeze-drying the retained cells. PHA quantification was performed according to Reddy et al. (2008); in this method, 20 mg of dried bacterial cells were suspended in 5 ml of 6 % sodium

hypochlorite and incubated for 1 h at 37 °C. Digested suspension was centrifuged at 12,500×*g* for 3 min. Sediment was washed with distilled water, acetone, and 96 % ethanol, respectively, and the last sediment (PHA granules) was dissolved in chloroform. Chloroform evaporated at room temperature, and 5 ml of concentrated sulfuric acid (95–97 %) was poured on residual sediment and heated at 80 °C for an hour in a water bath. After cooling, absorbance was read at 235 nm. Sulfuric acid was used as blank (Reddy et al. 2008). Analysis was performed in triplicate for all cases. Crotonic acid standard curve was plotted in 1–10 μg/ml sulfuric acid.

### Molecular identification

Total DNA was extracted from the bacterial strains using the boiling method (Franzetti and Scarpellini 2007). The 16S rRNA gene was amplified from genomic DNA using standard polymerase chain reaction (PCR) protocols. The bacterial 16S rRNA loci were amplified using the species-specific forward primer: PA-GS-F (5′-GACGGGTGAGTAATGCCTA-3′) and the reveres primer PA-GS-R (5′-CACTGGTGTTCCTTCCTATA-3′) (Spilker et al. 2004).

PCR amplifications were performed in a final volume of 25 μl. The reaction mixtures consisted of 2 μl of the DNA template, 2.5 μl 10×PCR buffer (75 mM Tris–HCl, pH 9.0, 2 mM $MgCl_2$, 50 mM KCl, 20 mM $(NH_4)_2SO_4$), 1 μl dNTPs (50 μM), 1 μl DNA polymerase (1 U Ampli Taq DNA polymerase), and 1 μl (25 pmol) of PA-GS-F and PA-GS-R (All reagents used were analytical grade and were purchased from CinnaGen, Iran), and the volume of the reaction mixture was brought up to 25 μl using distilled deionized water. PCR program for amplification of PA-GS primers consisted of initial denaturation at 95 °C for 2 min, 25 cycles of amplification with denaturation at 94 °C for 20 s, annealing at 54 °C for 20 s, extension at 72 °C for 40 s, and final extension of the incompletely synthesized DNA at 72 °C for 1 min in the BioRad thermal cycler (MJ Mini, BioRad, USA). The PCR products were analyzed in 1.0 % agarose gel containing 0.5 μg/ml of ethidium bromide and subjected to electrophoresis in a 1X TAE buffer. Gels were visualized under UV light and documented using Uvitec System DOC-008.XD (*UVItec* Ltd, Cambridge, UK). A molecular weight marker with 100-bp increments (100 bp plus ladder, Vivantis, Malaysia) was used as a DNA standard.

### Sequencing of the 16S rRNA gene

After amplification of the 16S rRNA gene from extracted DNA, the PCR product was purified according to the instructions of a commercial kit (Qiagen, Germany), and the PCR products were sequenced with the 16S forward primer and 16S reverse primer in a difficult sequencing model DNA sequencer (Genefanavaran, Tehran, Iran). The sequences obtained were elaborated by the software Chromas 2.13 (Technelysium Pty Ltd. Helensvale, Queensland, Australia), and the results were compared with sequences found in the gene bank www.ncib.nlm.nih.gov.

### PHA characterization

Gas chromatography/Mass spectrometry (GC–MS) analyses of PHA were run on Agilent 7000 mass spectrometer coupled to Agilent 7890A series gas chromatograph in electron impact (EI) mode. GC–MS instrument was equipped with DB 1 capillary column (30 m, 0.32 mm diameter, 0.25 mm film thickness). Methyl esters of extracted PHA were prepared based on this method; purified polymer was hydrolyzed at 100 °C for 2 h in chloroform (1 ml), methanol (1 ml), and 97 % sulfuric acid (0.15 ml). For phase separation after allowing the samples to be cooled, 1 ml of distilled water was added and the mixture was vortexed for 1 min (Lee and Choi 1997; Goh and Tan 2012). The organic phase which was located at the bottom of conic tube was collected and analyzed by GC–MS. Organic phase (0.5 μl) was injected (split ratio 1:25) with helium (He) as carrier gas. The injection port temperature was 240 °C, and the detector temperature was 230 °C. The initial column temperature was 80 °C (maintained for 4 min), with an increase of 8 °C $min^{-1}$ to temperature of 160 °C (maintained for 6 min). In final step, temperature was increased from 160 up to 240 °C and held for 5 min more. The flow rate of the He carrier gas was 1.2 ml $min^{-1}$.

## Results and discussion

### Isolated bacteria

Pseudomonads were isolated from refinery-contaminated soil and oily sludge samples and the morphological, physiological, and genomic properties of this isolates were investigated. Out of 32 samples, 45 *Pseudomonas* spp. were isolated; from these isolates, 16 isolates were identified as *P. aeruginosa*, 13 isolate as *P. stutzeri,* 11 isolates as *P. putida*, and five isolates as *P. fluorescenc* via microbiological methods and 16S rRNA sequencing assay. All isolates showed compatible results between phenotypic and genotypic characteristic. Of the 45 isolates, 15 bacterial strains were tested positive with the Sudan Black B and Nile red A colony staining. The PHA-accumulating pseudomonads were one-third of the total 45 *Pseudomonas* isolates. Bacteria are diverse and abundant in soils, but only a few reports have focused on the bacterial growth on

hydrocarbon-contaminated areas and using of complex compound such as crude oil for PHA production (bioremediation ability and biopolymer production). Present findings are in accordance with other studies; several *Pseudomonas* Spp. from hydrocarbon-contaminated soil had been identified (Wongsa et al. 2004; Mulet et al. 2011) and biosurfactant and rhamnolipid-producing native *Pseudomonas* has been isolated from crude-oil-contaminated soil (Saikia et al. 2012; Di Martino et al. 2014). However, the PHA production in these pseudomonads had not been extensively assessed. Only two *Pseudomonas* species, *P. stutzeri* 1317 (He et al. 1998) and *P. pseudoalcaligenes* strain YS1 isolated from oil-contaminated soil (Hang et al. 2002), was found to be able to synthesis PHA from glucose, soybean oil, and octanoate but not from crude oil. Fluorescent pseudomonads are well studied for their ability to produce PHA from various carbon sources (Huisman et al. 1989; Witholt and Kessler 1999; Sun et al. 2007).

## Quantification assay and characterization of the PHA produced by *Pseudomonas* isolates

These 15 PHA-positive isolates were first grown in PHA production medium in 100-ml flasks and were employed to extract PHA after 7 days of incubation on orbital shaker. Of these, four isolates were found to grow and produce significant amount of PHA using Gachsaran crude oil (2 % v/v) as sole carbon source. The following strains were divided into four different *Pseudomonas* strains based on their partial 16S rRNA gene sequences. Table 3 shows the details of these bacteria and PHA yields using crude oil (2 % v/v) as sole carbon source (Table 3). The repeated monomer composition of the copolymer produced from Gachsaran crude oil was determined by GC–MS of the hydroxymethyl esters obtained by acid hydrolysis of the polymer, consisting of repeated units. When petroleum was used as carbon source for *Pseudomonas* spp., the produced monomers composites contained: $C_8$ (3-hydroxyoctanoate), $C_{10}$ (3-hydroxydecanoate), $C_{12}$ (2-hydroxydodecanoate), $C_{14}$ (3-hydroxytetradecanoate), and $C_{16}$ (3-hydroxydecahexanoate), which are known as biopolymers (Table 4; Fig. 1). In this study, it has been shown that crude oil is suitable substrate for PHA production and the new *Pseudomonas* strains had high growth ability in medium containing petroleum (crude oil) and accumulated PHA. The monomer composition of the polymer varies with the substrate supplied. This is the first time $C_8$, $C_{10}$, $C_{12}$, $C_{14}$, and $C_{16}$ monomers are described in a PHA produced by *Pseudomonas* spp., when using petroleum as carbon source. Importance of petroleum as a carbon source for PHA production is its availability and low cost in some regions such as Middle East countries. Also, petroleum hydrocarbons of crude and refined fossil fuel are the most widespread contaminants in the environment. Although *n*-alkanes are easily biodegradable, very long chain *n*-alkanes, branched-chain hydrocarbons, and polycyclic aromatic hydrocarbons are difficult to degrade. Therefore, PHA production from crude oil is economically significant and environmentally remedial. The concentration variations of crude oil as carbon source from 0.5 to 3 % v/v showed that with 2 % v/v crude oil, the highest biomass occurs and the greatest production of PHA was obtained, but when the concentration of crude oil rose above 2 % v/v, there was an inhibitory effect on bacterial growth and PHA production, and this inhibitory effect was ascribed to problems linked to the toxicity of crude oil (compounds such as sulfur compounds) and the difficulty to access to the nutrients in the culture medium. He et al. (1998) described that *P. stutzeri* 1317 isolated from oil-contaminated soil was found to grow well in glucose and soybean oil as a sole carbon

**Table 3** Details of the four bacterial strains isolated from oil-contaminated areas and PHA yields using crude oil (2 % v/v) as sole carbon source

| Isolation sites | Closest match >99 % homology (Accession number) | CDW (g/l) | PHA content (% w/w) |
|---|---|---|---|
| Shiraz Oil Refining Company (oily sludge) | *P. aeruginosa* strain $SDS_3$(HQ 230975) | 0.67 ± 0 | 23.13 |
| Shiraz Oil Refining Company (contaminated soil) | *P. aeruginosa* strain XB7(KF 44738) | 0.64 ± 0.01 | 21.87 |
| Farrashband gas refinery (oily sludge) | *P. stutzeri* strain PS-SRU-ICU (JF 264901) | 0.52 ± 0.0 | 23.26 |
| Farrashband gas refinery (contaminated soil) | *P. aeruginosa* strain H1 (JX 100389) | 0.65 ± 0.02 | 20 |

**Table 4** Monomer composition of the copolymer produced from crude oil (2 % v/v) as sole carbon source

| Strain | Monomer [mol (%)] | | | | |
|---|---|---|---|---|---|
| | $C_8$ | $C_{10}$ | $C_{12}$ | $C_{14}$ | $C_{16}$ |
| *P. aeruginosa* strain $SDS_3$ | 14.2 | 70.5 | 2.7 | 11.8 | 0.8 |
| *P. aeruginosa* strain $XB_7$ | 4.2 | 59 | 6.3 | 29.4 | 1.1 |
| *P. stutzeri* strain PS- SRU-ICU | ND | 53.4 | ND | 46.6 | ND |
| *P. aeruginosa* strain $H_1$ | ND | ND | ND | ND | 64 |

*ND* not detected, $C_8$: 3-hydroxyoctanoate, $C_{10}$: 3-hydroxydecanoate, $C_{12}$: 3-hydroxydodecanoate, $C_{14}$: 3-hydroxytetradecanoate, $C_{16}$: 3-hydroxyhexadecanoate

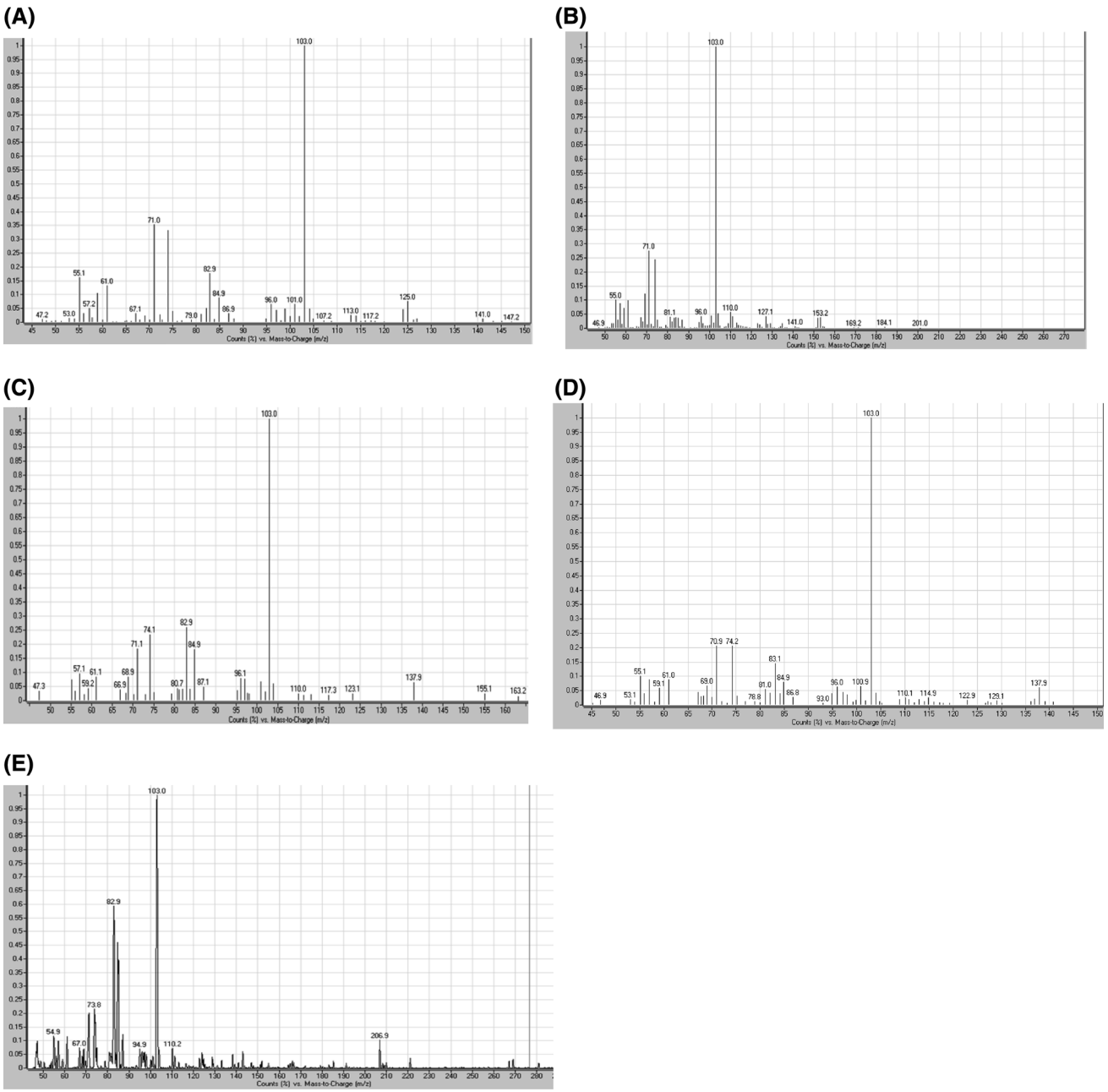


**Fig. 1** Mass spectra of (**a**) methyl 3-hydroxyoctanoate, (**b**) methyl 3-hydroxydecanoate, (**c**) methyl 3-hydroxy dodecanoate (**d**) methyl 3-hydroxytetradecanoate, and (**e**) methyl 3-hydroxyhexadecanoate

source, respectively, and synthesized PHA containing medium chain length monomers of hydroxyalkanoates ranging from $C_6$ to $C_{14}$ (He et al. 1998). Fernandez et al. (2005) reported that residual waste frying and other oily wastes are suitable substrates for PHA production by *P. aeruginosa* 42A2, and they found that monomer composition of the PHA polymers varies with the substrate supplied. $C_7$, $C_9$, $C_{14:2}$, and $C_{16:0}$ were described as a monomers of polymer (Fernandez et al. 2005), and these studies support present results.

Studies on other bacteria had shown that the accumulation of reserve polymers such as PHA could help bacteria to withstand starvation and hostile environmental conditions (Rehm and Steinbuchel 1999; Pham et al. 2004; Di Martino et al. 2014). Oil-contaminated soils contain about 84 % carbon, 14 % hydrogen, 1–3 % sulfur, and <1 % of nitrogen and other compounds (Atlas 1995). Excess carbon with <1 % nitrogen makes these sites a potential source for isolating PHA producers since the synthesis of PHA is favored by environmental stress (Anderson and Dawes

1990; Steinbuchel and Fuchtenbusch 1998; Pham et al. 2004). Therefore, the accumulation of PHA in hydrocarbon-contaminated soil bacteria might also increase the survival capabilities of these bacteria in the toxic environments and poor nutrient availability, which can be used for the bioremediation of crude oil polluted sites.

## Conclusion

Microorganisms from hydrocarbon-contaminated area are often viewed as an unexploited resource for biotechnological advances. In this study, PHA-producing pseudomonads were isolated and characterized from hydrocarbon-contaminated area and indicate a possible role for PHA in bacterial survival and stress tolerance in the toxic environments and poor nutrient availability. Also it was observed that crude oil is a suitable substrate for PHA production. These results suggest that oil-contaminated areas can be important sources for PHA producers, which can be used for the bioremediation of crude oil polluted sites. More studies about genetic characterization of these bacteria would be useful to demonstrate the possible association between bioremediation, PHA accumulation, and stress tolerance by these bacteria.

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