iMedPub Journals www.imedpub.com

DOI: 10.21767/2471-8084.100048

Biochemistry & Molecular Biology Journal ISSN 2471-8084 **2017** Vol.3 No.3:20

# Identification and Mining of Hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) Degradation Bacteria through Molecular Techniques

### Abstract

Biodegradation of hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) explosives in soil consider a promising mechanism for these excessive applied compounds. One of most sophisticated challenges to identified bacterial explosive degrading is understands enzymatic and metabolic fundamentals of RDX degradation. RDX-contaminated soils were collected from four explosives contaminated sites at Riyadh province, Saudi Arabia. 16S rRNA was amplified through specific bacterial genes, phylogenetic tree was constructed. Among thirty eight clones which successfully sequence, *Proteobacterium, Burkholderia* and *Rhodococcus* were the major dominant identified genera in four location libraries with dedicated activity for RDX biodegradation. XpIA and XpIB (RDX degrading genes) were amplified and detected as molecular marker for evaluating RDX biodegradation from each four location isolates. Furthermore, XpIA and XpIB genes expression comparisons of bacterial isolates from varied locations indicated superiority of bacterial isolates from first location for XpIA and XpIB genes expression level. Meanwhile, second, third and fourth bacterial isolates were arranged discerningly.

Keywords: Hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX); 16S rRNA; XpIA; XpIB

### Fahad A Al-Dhabaan<sup>1\*</sup>

Department of Biology, Science and Humanities College, Alquwayiyah, Shaqra University, Saudi Arabia

#### \*Corresponding author: Fahad A. Al-Dhabaan

fahad.aldhabaan@gmail.com

Department of Biology, Science and Humanities College, Alquwayiyah, Shaqra University, Saudi Arabia.

Tel: +966 50 489 3691

**Citation:** Al-Dhabaan FA (2017) Identification and Mining of Hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) Degradation Bacteria through Molecular Techniques. Biochem Mol Biol J. Vol.3 No.3:20

Received: November 30, 2017; Accepted: December 12, 2017; Published: December 18, 2017

## Introduction

The explosive hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) is commonly applied to both military and industrial applications. Nowadays, RDX is considering a powerful environmental pollutant as a results of accumulation mainly from weapons manufactures and trainings [1,2]. Also, RDX was listed as possible human carcinogen by U.S. Environmental Protection Agency. Thus, applied some ranges of RDX have been restricted as a result of soil and groundwater pollution (U.S. Environmental Protection Agency, 2017). Bacterial degradation for RDX which consider a xenobiotic compound has been detected. RDX biodegradation mechanism may be understated in the light of comprising and fusing unusual cytochrome P450 system of flavodoxin domain to the N terminus of a cytochrome P450 (CYP177A1, XpIA). XpIB system played a key role as RDX biodegradation through flavodoxin reduction which dedicated to RDX degradation in Rhodococcus rhodochrous strain 11Y and catalyzes the reductive denotation of RDX, in final, under aerobic and anaerobic condition, RDX ring was cleavage [3]. 16S rRNA gene-based specific primers consider powerful genetic biomarkers tools that used as indicators for potential contaminant biodegradation. Regarding to previous findings, applied biomarkers targeting conserved regions of 16S rRNA gene to identify microbial organisms on taxonomic and phylogenetic levels are effective when biodegradation is dependent on a specific microbial strain and has been widely accepted as a sensitive and reliable technique to identify intestinal micro biota [4-6]. This investigation was carried out to achieve two main goals. Firstly, identify bacterial genera with capability of RDX components biodegradation. Secondly, evaluate biodegradation activity for RDX components.

## **Material and Methods**

#### Locations and sampling of soil

Soil samples were collected from four explosives contaminated sites at Riyadh province, Saudi Arabia (22.7554° N, 46.2092° E). Contaminated soil with explosives xenobiotic compounds had an average pH of 6.4, moisture content of 62.8%, conductivity of 290  $\mu$ S per cm by contrary, few meters away from the TNT-

contaminated location, non-contaminated soil had an average pH of 7.2, moisture content of 31%, conductivity of 378  $\mu S$  per cm.

EPA-method 8510 (Nitroaromatics, Nitramines, and nitrate esters by High Performance Liquid Chromatography, HPLC) was applied for extraction of explosives from soil and Agilent 1100 system (Agilent, Santa Clara, CA) with Chromosphere C18 reverse phase column (5 µm, 250 × 4.6 mm) was applied. The method detection limit was approximately 2  $\mu$ g/L for RDX. The detection limit was 5 µg/L for the RDX breakdown products hexahydro-3,5-dinitro-1-nitroso-1,3,5-triazine (MNX), hexahydro-5-nitro-1, 3-dinitroso-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5triazine (TNX), TNT or related nitroaromatics were undetected in the non-contaminated soil (<0.01 ppm detection limit). Upper 5-10 cm of tested soil was applied via taken 50 grams. Pooling of soil samples was compounded from thee replicating to make one sample and kept at 4°C during transportation. Sieves (2 mm) were employed to homogenize the samples. Obtaining rhizosphere soil was achievement via shaking for 10 min at max. Speed in sterile P-buffer (per l: 2.36 g Na, HPO,; 1.80 g NaH, PO,, 85.0 g NaCl and 200 µl Tween 80; pH 6.8). The tubes were subjected to centrifugation (2,500 g, 20 min), resulting pellet was kept. All soil samples were stored at -80°C till DNA extrication.

#### Amplification, cloning and sequence of 16S rDNA

16S rRNA gene diversity analysis was employed to identify five bacterial which isolate from five explosive contaminated sites at Riyadh province, Saudi Arabia. Total genomic DNA was extracted and purified using E.Z.N.A.®Soil DNA Kit (D5625-01, OMEGA bio-tek, USA) according to manufacturer protocol. 16S rDNA fragments were amplified by PCR technique using 27F and 1492R primers (Table 1). Biobasics, Canada) according to DeLong [7]. PCR amplification was carried out in a themal cycler (peqSTAR 96X Universal Gradient, Germany) under conditions as follows: 5-min initial denaturation at 95°C, 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by 10 min at 72°C and incubation at 4°C [8]. Gel documentation system (Geldocit, UVP, England), was applied for data analysis using Total lab analysis software. The PCR amplicons were blunt-ended with Klenow polymerase and ligated to an Eco RV-digested pBluescript II plasmid (Stratagene) and transformed into Escherichia coli JM 109 electro competent cells according to the manufacturer's instruction. Transformed ligated cells were cultured on LB agar plates containing 100 mg/ml Ampicillin, 100 mg/ml IPTG, and 100 mg/ml X-Gal. Genomics DNA of transformed white colonies were sequenced by Applied Biosystems<sup>®</sup> 3730 DNA Analyzer. GenBank database was compared with 16S rRNA nucleotide sequences [9] through the BLASTn program [10] and identified with Ribosomal Database Project (RDP) [11] and Alignments sequences were analysed on NCBI website using BLAST to confirm their identity. The Genetic distances and Multi Alignments were computed by Pairwise Distance method using ClusteralW software analysis.

#### Mining xpl RDX degrading genes

XpIA and XpIB (RDX degrading genes) encoding a novel flavodoxin

fused cytochrome P450 were mining, amplifying and sequence through xplA-HindIIIF and xplB-KpnF1 specific primers **(Table 1)** from transformed bacterial cells, for xplA and xplB respectively. Thermo Scientific<sup>™</sup> DreamTaq<sup>™</sup> Hot Start Master Mix (Thermo Scientific, USA, K9011) was applied according to manufacturer protocol. PCR started with an initial denaturation step of 95°C for 5 minutes, followed by 30 cycles of denaturation step of 95°C (30 s), annealing step (varied temperature determined from primers melting temperature) and extension step of 72°C (1-3 minutes). A final extension cycle at 72°C for 10 minutes was performed [12].

#### Western analysis for xpl RDX degrading genes

Selected transformed colonies were cultured and total soluble proteins were purified via TriFast (Peqlab, VWR company, isolation of RNA, DNA and Protein simultaneously). Then, SDS Polyacrylamide gel electrophoresis (SDS-PAGE) with 12% T was performed to distinguish and fragment total soluble proteins. Nitrocellulose membrane (Schleicher and Shuell BioScience, Protran BA 83, cellulosenitrate (E), 0.2  $\mu$ M, 200 × 200 mm, Dassel Germany) and filter paper (Whatman, 3 mm chromatography paper) were applied for performing western blotting technique. After protein transfer, membrane was incubated with anti-rabbit polyclonal antibodies were prepared by using purified XpIA and XpIB proteins, respectively, in rabbit [13] and XpIB [14] for one hour and reaction was stopped by washing the membrane with TBS, 0.1% Tween 20, TBS and water according to Chong [12]. Data analysis was performed via ChemiDoc-It<sup>®</sup> Imager (UVP, England).

 Table 1 Primers for identification of 16S rRNA and RDX degrading genes.

RDX Degrad- ing genes	PRUN518R	5'-ACTCCTACGGGAGGCAG- CAG-3';	La Para [8]
	PRBA338F	5'-ATTACCGCGGCTGCTGG-3'	
Bacterial 16S rRNA	xplB-KpnF1	5'-GGTACCATGGACATCAT- GAGT-3'	Chun Shiong Chong [12]
	xplA-HindIIIF	5'-AAGCTTATGACCGACGTA- ACTGTC-3'	



## **Results and Discussion**

This investigation aimed to identify and evaluate biodegradation capability of RDX components for different bacterial isolates. To achieve these goals, 16 rRNA specific genes were applied for identified thirty nine isolates from different four explosive contaminated sites at Riyadh province, Saudi Arabia. As shown in **Figure 1** all crude bacterial which were extracted and purified form four locations reflected clear 16 rRNA specific fragments of distinguishing ~ 950-base-pair. Then, the amplified 16S rDNA fragments were cloned and sequence. Thirty eight clones were successfully sequence. Sequence results indicated that, *Proteobacterium, Burkholderia* and *Rhodococcus* were the major dominant identified genera sequences among partial-sequence

analyses of 38 clones from each four location libraries from four explosive contaminated sites at Riyadh province, Saudi Arabia. **Table 2** showed identified bacterial to isolate from each location with the highest assigned homologous matches based on 16S rDNA sequences. Distinguish acceptance for 16S rDNA-based analysis has been founded for bacterial taxonomy and phylogenetic a number of 16S ribosomal data are being integrated into the Ribosomal Database Project [11].

Majority of bacterial genera for the first location was identified as  $\gamma$  proteobacterium, Burkholderia gladioli and Burkholderia metallica. Second location isolates was identified as different clones of  $\beta$  proteobacterium, Rhodococcus sp. KSM-B-3 and Rhodococcus sp. JFJ-Iso-Bact41. Different species of Burkholderia

 Table 2 Bacterial isolates identification with assigned homologous matches.

1         Uncultured gamma proteobacterium isolate ST8-6666         EU3309318.1           2         Uncultured gamma proteobacterium isolate ST8-666E         EU330989.1           3         Uncultured gamma proteobacterium isolate ST8-47E         EU330989.1           4         Uncultured gamma proteobacterium isolate ST8-47E         EU330989.1           5         Uncultured gamma proteobacterium isolate ST8-47E         EU33098.1           6         Uncultured gamma proteobacterium clone Ros102         AM23724.1           7         Uncultured gamma proteobacterium clone Ros30         AM23724.1           8         Burkholderia gladioli         A8023647.1           9         Burkholderia gladioli         A8023647.1           9         Burkholderia gladioli         A8023647.1           1         Burkholderia gladioli         A8023647.1           2         Uncultured beta proteobacterium clone Ros17         AM237273.1           3         Uncultured beta proteobacterium clone Ros51         AM237273.1           4         Uncultured beta proteobacterium clone Ros28         AM237268.1           7         Uncultured beta proteobacterium clone Ros78         AM237272.1           8         Uncultured beta proteobacterium clone Ros78         AM237272.1           10         Rhodococcus Sp. KSM-8	Location	Strain	Match of highest homology	Accession
2         Uncultured gamma proteobacterium isolate ST-847E         EU33098.1           3         Uncultured gamma proteobacterium isolate ST-847E         EU33098.1           4         Uncultured gamma proteobacterium isolate ST-847E         EU33098.1           5         Uncultured gamma proteobacterium isolate ST-847E         EU33098.1           6         Uncultured gamma proteobacterium isolate ST-847E         EU33098.1           7         Uncultured gamma proteobacterium clone Ros119         AM23724.1.1           6         Uncultured gamma proteobacterium clone Ros102         AM23724.1.1           7         Uncultured gamma proteobacterium clone Ros10         AM23724.1           8         Burkholderia gladioli         AB023646.1           9         Burkholderia metallico strain R-16017         AM23727.1           2         Uncultured beta proteobacterium clone Ros10         AM23727.1           4         Uncultured beta proteobacterium clone Ros51         AM23726.1           7         Uncultured beta proteobacterium clone Ros58         AM23726.1           8         Uncultured beta proteobacterium clone Ros78         AM23726.1           9         Rhodococcus sp. SKM-8-3         AB03235.1           10         Rhodococcus sp. SKM-8-3         AB03235.1           1         Burkholderia apina s		1	Uncultured gamma proteobacterium isolate STR-666E	FU330018 1
3         Uncultured gamma proteobacterium isolate 5T8-666E         EU330936.1           4         Uncultured gamma proteobacterium isolate 5T8-666E         EU330988.1           5         Uncultured gamma proteobacterium isolate 5T8-667E         EU330988.1           6         Uncultured gamma proteobacterium isolate 5T8-667E         EU330988.1           7         Uncultured gamma proteobacterium clone Ros102         AM237240.1           8         Burkholderia gladioli         A8023647.1           9         Burkholderia gladioli         A8023646.1           1         Burkholderia gladioli         A8023646.1           2         Uncultured beta proteobacterium clone Ros17         AM237273.1           3         Uncultured beta proteobacterium clone Ros14         AM237273.1           4         Uncultured beta proteobacterium clone Ros15         AM237263.1           5         Uncultured beta proteobacterium clone Ros28         AM237263.1           6         Uncultured beta proteobacterium clone Ros38         AM237263.1           9         Rhodococcus sp. JF.1so-Bact41         AM57727.2           8         Uncultured beta proteobacterium clone Ros38         AM237263.1           9         Rhodococcus sp. JF.1so-Bact41         AM57727.2           10         Rhodococcus sp. JF.1so-Bact41		2	Uncultured gamma protoobacterium isolate STB-000L	EU330910.1
3         Uncultured gamma proteobacterium isolate STS-847E         EU330898.1           1         5         Uncultured gamma proteobacterium clone Ros119         AM237241.1           6         Uncultured gamma proteobacterium clone Ros102         AM237240.1           7         Uncultured gamma proteobacterium clone Ros102         AM237240.1           8         Burkholderia gladioli         AB023647.1           9         Burkholderia gladioli         AB023647.1           9         Burkholderia gladioli         AB023647.1           9         Burkholderia roteobacterium clone Ros107         AM237275.1           1         Burkholderia roteobacterium clone Ros11         AM237275.1           3         Uncultured beta proteobacterium clone Ros12         AM237275.1           4         Uncultured beta proteobacterium clone Ros12         AM237276.1           5         Uncultured beta proteobacterium clone Ros28         AM237264.1           7         Uncultured beta proteobacterium clone Ros38         AM237264.1           9         Rhodococcus sp. IFI-Ioo-Bact1         AJ867727.2           10         Rhodococcus sp. IFI-Ioo-Bact1         AJ867727.2           11         Burkholderia alpina strain P0-04-17-38         JF 763852.1           3         Burkholderia ingina strain P0-04-17-38		2	Uncultured gamma protochacterium isolate STS-647E	EU330090.1
4         Uncultured gamma proteobacterium clone Ros119         AM237241.1           1         6         Uncultured gamma proteobacterium clone Ros102         AM237241.1           7         Uncultured gamma proteobacterium clone Ros102         AM237241.1           7         Uncultured gamma proteobacterium clone Ros102         AM237241.1           9         Burkholderia gladioli         AB023647.1           9         Burkholderia gladioli         AB023645.1           1         Burkholderia gladioli         AB023645.1           2         Uncultured beta proteobacterium clone Ros107         AM237275.1           3         Uncultured beta proteobacterium clone Ros12         AM237274.1           4         Uncultured beta proteobacterium clone Ros15         AM237275.1           5         Uncultured beta proteobacterium clone Ros51         AM237276.1           6         Uncultured beta proteobacterium clone Ros58         AM237266.1           7         Uncultured beta proteobacterium clone Ros78         AM237261.1           8         Uncultured beta proteobacterium clone Ros78         AM237261.1           9         Rhodococcus sp. KSM-B-3         AB03265.1           10         Rhodococcus sp. KSM-B-3         AB03255.1           12         Burkholderia alpina strain PO-04-17-38	1	3	Uncultured gamma proteobacterium isolate STB-666E	EU330918.1
15Uncultured gamma proteobacterium clone Ros119AMU237241.16Uncultured gamma proteobacterium clone Ros102AMU237240.18Burkholderia gladioliAB023647.19Burkholderia gladioliAB023646.19Burkholderia gladioliAB023646.11Burkholderia gladioliAB023646.12Uncultured beta proteobacterium clone Ros107AMU23727.13Uncultured beta proteobacterium clone Ros107AM23727.14Uncultured beta proteobacterium clone Ros51AM23727.15Uncultured beta proteobacterium clone Ros51AM23727.16Uncultured beta proteobacterium clone Ros54AM23726.17Uncultured beta proteobacterium clone Ros58AM23726.18Uncultured beta proteobacterium clone Ros78AM23726.19Rhodococcus sp. IFI-Iso-Bact41AB6371.210Rhodococcus sp. IFI-So-Bact41AB6371.211Burkholderia diffus strain R-15930NR_042633.12Burkholderia diffus strain R-15930NR_042631.13GBurkholderia diffus strain R-1544NR_027587.13GBurkholderia diffus strain R-1545NR_042635.13GBurkholderia diffus strain R-15930NR_042633.14Burkholderia diffus strain R-1545NR_042635.13Burkholderia diffus strain R-1545NR_042635.14Burkholderia semailis strain R-2419NR_042635.14Burkholderia semailis strain R-15930NR_14271.15Burkholde		4	Uncultured gamma proteobacterium Isolate STS-847E	EU330898.1
6         Uncultured gamma proteobacterium clone Ros102         AM237242.1           7         Uncultured gamma proteobacterium clone Ros100         AM237240.1           8         Burkholderia gladioli         AB023647.1           9         Burkholderia gladioli         AB023646.1           1         Burkholderia gladioli         AB023646.1           2         Uncultured beta proteobacterium clone Ros107         AM237275.1           3         Uncultured beta proteobacterium clone Ros12         AM237273.1           4         Uncultured beta proteobacterium clone Ros14         AM23727.1           4         Uncultured beta proteobacterium clone Ros15         AM23727.1           4         Uncultured beta proteobacterium clone Ros16         AM237268.1           7         Uncultured beta proteobacterium clone Ros78         AM237264.1           8         Uncultured beta proteobacterium clone Ros78         AM237261.1           9         Rhodococcus sp. IKSN-B-3         AB032365.1           10         Rhodococcus sp. IKSN-B-3         AB032365.1           11         Burkholderia alpina strain P0-04-17-38         NR_042633.1           12         Burkholderia alpina strain P0-03-17-38         NR_042635.1           13         Burkholderia alpina strain P0-04-17-38         NR_042635.1		5	Uncultured gamma proteobacterium clone Ros119	AM237241.1
A         Uncultured gamma proteobacterium clone Ros30         AM237240.1           8         Burkholderia gladioli         AB023647.1           9         Burkholderia gladioli         AB023647.1           9         Burkholderia gladioli         AB023647.1           1         Burkholderia gladioli         AB023646.1           2         Uncultured beta proteobacterium clone Ros107         AM237275.1           3         Uncultured beta proteobacterium clone Ros12         AM237273.1           4         Uncultured beta proteobacterium clone Ros51         AM237276.1           5         Uncultured beta proteobacterium clone Ros54         AM237264.1           6         Uncultured beta proteobacterium clone Ros78         AM237264.1           7         Uncultured beta proteobacterium clone Ros78         AM237264.1           8         Uncultured beta proteobacterium clone Ros78         AM237264.1           9         Rhadacoccus sp. KSM-B-3         AB032365.1           10         Rhadacoccus sp. KSM-B-3         AB032365.1           11         Burkholderia alpina strain PO-041-738         NR_146371.1           8         Burkholderia alpina strain PO-041-738         NR_146371.1           13         Burkholderia ubanesis strain R-219         NR_042635.1           7 <td>6</td> <td>Uncultured gamma proteobacterium clone Ros102</td> <td>AM237242.1</td>		6	Uncultured gamma proteobacterium clone Ros102	AM237242.1
8Burkholderia gladioliAB023647.19Burkholderia gladioliAB023647.11Burkholderia gladioliAB023646.11Burkholderia gladioliAB023646.12Uncultured beta proteobacterium clone Ros107AM237275.13Uncultured beta proteobacterium clone Ros107AM237273.14Uncultured beta proteobacterium clone Ros1AM237273.15Uncultured beta proteobacterium clone Ros51AM237268.17Uncultured beta proteobacterium clone Ros28AM237268.18Uncultured beta proteobacterium clone Ros78AM237268.19Rhodococcus sp. KSM-B-3AB032365.110Rhodococcus sp. JFI-Iso-Bact411AJ867727.21Burkholderia alfina strain R-15930NR_Q42633.12Burkholderia alpina strain R-15930NR_Q42633.13Burkholderia alpina strain P0-03-17-38JF_763852.14Burkholderia alpina strain R-2419NR_Q42633.14Burkholderia alpina strain R-2419NR_Q42633.15Burkholderia alpina strain R-2419NR_Q42633.16Burkholderia ubonensis strain GC4NR_Q4253.19Enterobacter cloace strain R130KC990813.19Enterobacter cloace strain R130KC990813.19Enterobacter cloace strain R120KC990813.19Enterobacter cloace strain N201NR_149271.110Enterobacter cloace strain R120KC990813.111Rhodococcus shim NUC3NR_149271.112Rhodococcus shim NUC3<		7	Uncultured gamma proteobacterium clone Ros30	AM237240.1
9Burkholderia gtadioliAB023646.11Burkholderia metallica strain R-16017NR_042636.12Uncultured beta proteobacterium clone Ros107AM237275.13Uncultured beta proteobacterium clone Ros12AM237273.14Uncultured beta proteobacterium clone Ros51AM237273.14Uncultured beta proteobacterium clone Ros59AM237273.15Uncultured beta proteobacterium clone Ros59AM23726.16Uncultured beta proteobacterium clone Ros59AM23726.17Uncultured beta proteobacterium clone Ros78AM23726.18Uncultured beta proteobacterium clone Ros78AM23726.19Rhodococcus sp. ISH-iso-Bact41AJ867727.210Rhodococcus sp. ISH-iso-Bact41AJ867727.211Burkholderia djina strain P0-03-17-38NR_042633.122Burkholderia djina strain P0-03-17-38NR_146371.13Burkholderia dpina strain P0-04-17-38NR_146371.14Burkholderia najina strain R-2419NR_042635.15Burkholderia ubonensis strain E264NR_042635.16Burkholderia najina strain R-2419NR_042635.17Enterobacter cloacae strain R15AV01713.18Enterobacter cloacae strain R15AV01713.19Enterobacter cloacae strain R15AV01713.110Enterobacter cloacae strain R15AV01713.111Rhodococcus soli strain DSD51WNR_143271.112Rhodococcus soli strain DSD51WNR_143799.213GBurkholderia sp.		8	Burkholderia gladioli	AB023647.1
1Burkholderia metallica strain R-16017NR_04256.12Uncultured beta proteobacterium clone Ros107AM237275.13Uncultured beta proteobacterium clone Ros12AM237274.14Uncultured beta proteobacterium clone Ros51AM237273.15Uncultured beta proteobacterium clone Ros59AM237274.16Uncultured beta proteobacterium clone Ros58AM237268.17Uncultured beta proteobacterium clone Ros78AM237269.18Uncultured beta proteobacterium clone Ros78AM237269.19Rhodococcus sp. ISH-BB-3AB032365.110Rhodococcus sp. ISH-B-3AB032365.110Rhodococcus sp. ISH-B-B-3AB032365.111Burkholderia diffusa strain R-15930NR_042633.12Burkholderia diffusa strain R-15930NR_04263.13Burkholderia diffusa strain R-15930NR_04263.14Burkholderia diffusa strain R-15930NR_04263.15Burkholderia alpina strain P0-03-17-38IF_763852.14Burkholderia alpina strain R120KC990813.15Burkholderia abinansis strain GTC-P3-415NR_040830.17Enterobacter cloacae strain R130KC990813.18Enterobacter cloacae strain R102KC990813.19Enterobacter cloacae strain R10KC990813.14Enterobacter cloacae strain R10KC990813.19Enterobacter cloacae strain SEA01KY625189.14Enterobacter cloacae strain R10KY625189.14Enterobacter cloacae strain SEA01<		9	Burkholderia gladioli	AB023646.1
2Uncultured beta proteobacterium clone Ros107AM237275.13Uncultured beta proteobacterium clone Ros12AM237274.14Uncultured beta proteobacterium clone Ros51AM237273.15Uncultured beta proteobacterium clone Ros59AM237273.16Uncultured beta proteobacterium clone Ros58AM23726.17Uncultured beta proteobacterium clone Ros78AM237264.19Rhodococcus sp. KSM-B-3AB032365.110Rhodococcus sp. KSM-B-3AB032365.111Burkholderia diffusa strain R-15930NR_042633.122Burkholderia diffusa strain R-0-03-17-38JF_763852.13Burkholderia alpina strain P0-04-17-38NR_042633.14Burkholderia alpina strain P0-04-17-38NR_042635.13Burkholderia alpina strain R-2419NR_042635.14Burkholderia ubnesis strain GC-P3-415NR_042635.15Burkholderia ubnesis strain R-2419NR_042635.16Burkholderia ubnesis strain R-2419NR_042635.17Enterobacter cloacae strain R130KC990813.18Enterobacter cloacae strain R130KC990813.19Enterobacter cloacae strain R120KC990813.110Enterobacter cloacae strain R120KC990813.111Rhodococcus soli strain DSDS1WNR_1134799.23Uncultured gamma proteobacteriumAM237251.14Enterobacter cloacae strain SEA01KY655189.14Enterobacter cloacae strain SEA01KY655189.14Enterobacter cloacae strain	2	1	Burkholderia metallica strain R-16017	NR_042636.1
3Uncultured beta proteobacterium clone Ros12AM237274.14Uncultured beta proteobacterium clone Ros1AM237273.15Uncultured beta proteobacterium clone Ros59AM237268.17Uncultured beta proteobacterium clone Ros78AM237269.18Uncultured beta proteobacterium clone Ros38AM237269.19Rhodococcus sp. KSM-B-3AB032365.110Rhodococcus sp. KSM-B-3AB032365.120Rhodococcus sp. JF-Iso-Bact41AB67727.211Burkholderia diffusa strain R-15930NR_042633.122Burkholderia dipina strain P0-03-17-38JF_763852.13Burkholderia alpina strain P0-04-17-38NR_042635.14Burkholderia ulpina strain R-15930NR_042635.15Burkholderia ulpina strain R-2419NR_042635.16Burkholderia ubonensis strain R-2419NR_042635.17Enterobacter cloacae strain R130KC990813.18Enterobacter cloacae strain R130KC990813.19Enterobacter cloacae strain R15AV017123.110Enterobacter cloacae strain R15NR_149271.112Rhodococcus shi strain DSDSIVNR_143273.14Enterobacter cloacae strain S201KY625189.14SBurkholderia apina proteobacteriumAM23723.14Enterobacter cloacae strain S201KY625189.15Burkholderia sp. MSMB122KF378609.16Uncultured beta proteobacterium clone R035AM237265.17Rhodococcus sp. MSMB122KF378609.1<		2	Uncultured beta proteobacterium clone Ros107	AM237275.1
4Uncultured beta proteobacterium clone Ros51AM237273.15Uncultured beta proteobacterium clone Ros59AM237272.16Uncultured beta proteobacterium clone Ros28AM237268.17Uncultured beta proteobacterium clone Ros78AM237269.18Uncultured beta proteobacterium clone Ros78AM237269.19Rhodococcus sp. KSN-8-3AB032365.110Rhodococcus sp. KSN-8-3AB032365.110Rhodococcus sp. KSN-8-3AB032365.111Burkholderia difusa strain R-15930NR_042633.12Burkholderia difusa strain PO-03-17-38JF_763852.13Burkholderia alpina strain PO-04-17-38NR_146371.14Burkholderia trailnonesis strain R-2419NR_042635.15Burkholderia trailnonesis strain R-2419NR_042635.16Burkholderia train GTC-P3-415NR_040830.17Enterobacter cloacae strain RJ30KC990813.18Enterobacter cloacae strain RJ30KC990813.19Enterobacter cloacae strain RJ30KC990813.110Enterobacter cloacae strain RJ30NR_149271.111Rhodococcus sol strain DD551WNR_134799.23Uncultured gamma proteobacteriumAM237233.14SBurkholderia sp. MSMB122KF378609.14Enterobacter cloacae strain DS51WNR_04237261.17Rhodococcus hogii strain DSM 20307NR_049101.14Burkholderia sp. MSMB122KF378609.16Uncultured beta proteobacterium clone Ro35AM237265.		3	Uncultured beta proteobacterium clone Ros12	AM237274.1
25Uncultured beta proteobacterium clone Ros59AM23727.16Uncultured beta proteobacterium clone Ros28AM237268.17Uncultured beta proteobacterium clone Ros78AM237261.18Uncultured beta proteobacterium clone Ros78AM237261.19Rhodococcus sp. KSM-B-3AB032365.110Rhodococcus sp. KSM-B-3AB032365.112Burkholderia diffuos strain R-15930NR_042633.12Burkholderia dipina strain P0-03-17-38JF_763852.13Burkholderia dipina strain P0-04-17-38NR_146371.14Burkholderia bina strain P0-04-17-38NR_042635.15Burkholderia ubina strain CP-04-17-38NR_042635.16Burkholderia ubina strain CP-04-17-38NR_042635.17Enterobacter cloacae strain R130KC990813.17Enterobacter cloacae strain R130KC990813.19Enterobacter cloacae strain R130KC990813.19Enterobacter cloacae strain N20KC990813.110Enterobacter cloacae strain R130NR_14271.111Rhodococcus soli strain UC33NR_14271.112Rhodococcus soli strain DSD51WNR_13479.213Uncultured gamma proteobacteriumKY3278.14Enterobacter cloacae strain SEA01KY625189.14Enterobacter cloacae strain SEA01KY625189.14Enterobacter cloacae strain SEA01KY625189.14Enterobacter cloacae strain SEA01KY625189.14GUncultured beta proteobacterium clone Ro77<		4	Uncultured beta proteobacterium clone Ros51	AM237273.1
6Uncultured beta proteobacterium clone Ros28AM237268.17Uncultured beta proteobacterium clone Ros78AM237269.18Uncultured beta proteobacterium clone Ros78AM237264.19Rhodococcus sp. KSM-B-3AB032365.110Rhodococcus sp. JFJ-Iso-Bact41AB67727.211Burkholderia diffusa strain R-15930NR_042633.12Burkholderia dipina strain P0-03-17-38JF_763852.133Burkholderia dipina strain P0-03-17-38NR_146371.14Burkholderia dipina strain P0-04-17-38NR_042635.136Burkholderia ubina strain R-2419NR_042635.14Burkholderia ubonensis strain R-2419NR_042635.15Burkholderia ubonensis strain GTC-P3-415NR_040830.17Enterobacter cloacae strain RJ30KC990813.18Enterobacter cloacae strain RJ30KC990813.19Enterobacter cloacae strain S15AV017123.110Enterobacter cloacae strain NJ20KC990811.19Rhodococcus soli strain DSD51WNR_143799.24Enterobacter cloacae strain S051WNR_134799.24Enterobacter cloacae strain SEA01KY625189.14Enterobacter cloacae strain SEA01KY625189.14Enterobacter cloacae strain SEA01KY625189.15Burkholderia sp. MSMB122KF378609.16Uncultured beta proteobacterium clone Ro77AM237265.17Rhodococcus sp. MSIB SI20NR_041910.17Rhodococcus sp. MSIB SI20NR_0419		5	Uncultured beta proteobacterium clone Ros59	AM237272.1
7Uncultured beta proteobacterium clone Ros78AM237269.18Uncultured beta proteobacterium clone Ros38AM237264.19Rhodococcus sp. KSM-B-3AB032365.110Rhodococcus sp. JFI-Iso-Bact41AB67727.211Burkholderia diffusa strain R-15930NR_042633.12Burkholderia alpina strain P0-03-17-38JF_763852.13Burkholderia alpina strain P0-04-17-38NR_146371.14Burkholderia alpina strain P0-04-17-38NR_042633.14Burkholderia alpina strain P2419NR_042635.15Burkholderia thailandensis strain E264NR_027587.16Burkholderia ubonensis strain GC-P3-415NR_042635.17Enterobacter cloacae strain R130KC990813.18Enterobacter cloacae strain R130KC990813.19Enterobacter cloacae strain R15AY017123.110Enterobacter cloacae strain N115AY017123.111Rhodococcus humicola strain D5D51WNR_134799.24Enterobacter cloacae strain SEA01KY625189.14Enterobacter cloacae strain SEA01KY625189.14Enterobacter cloacae strain SEA01KY625189.14Enterobacter cloacae strain SEA01KY625189.15Burkholderia proteobacterium clone R077AM237265.17Rhodococcus hoagii strain D5D51WNR_041910.17Rhodococcus hoagii strain D5D51WNR_041910.16Uncultured beta proteobacterium clone R077AM237265.17Rhodococcus hoagii strain D5D51WNR_		6	Uncultured beta proteobacterium clone Ros28	AM237268.1
8Uncultured beta proteobacterium clone Ros38AM237264.19Rhodococcus sp. KSM-B-3AB032365.110Rhodococcus sp. KSM-B-3AB032365.110Burkholderia dijina strain R-15930NR_042633.12Burkholderia alpina strain P0-03-17-38JF_763852.13GBurkholderia alpina strain P0-04-17-38NR_146371.14Burkholderia talpina strain P0-04-17-38NR_042635.15Burkholderia semnalis strain R-2419NR_042635.16Burkholderia ubonensis strain GTC-P3-415NR_040830.17Enterobacter cloacae strain RJ30KC990813.18Enterobacter cloacae strain RJ20KC990813.19Enterobacter cloacae strain N15AY017123.19Enterobacter cloacae strain N15AY017123.110Enterobacter cloacae strain N15NR_149271.112Rhodococcus soli strain DSD51WNR_134799.23Uncultured gamma proteobacteriumAM237233.14Enterobacter cloacae strain SEA01KY625189.14SBurkholderia sp. MSMB122KF378609.16Uncultured bat proteobacterium clone Ro77AM237261.17Rhodococcus hoagii strain DSM 20307NR_041910.18Uncultured bat proteobacterium clone Ro35AM237265.19Rhodococcus sp. NClB 9646AY017136.1		7	Uncultured beta proteobacterium clone Ros78	AM237269.1
9Rhodococcus sp. KSM-B-3AB032365.110Rhodococcus sp. JFI-Iso-Bact41AJ867727.21Burkholderia diffusa strain R-15930NR_042633.12Burkholderia alpina strain P0-03-17-38JF_763852.13Burkholderia alpina strain P0-04-17-38NR_042637.14Burkholderia alpina strain P0-04-17-38NR_027587.14Burkholderia thailandensis strain E264NR_027587.15Burkholderia ubonensis strain GTC-P3-415NR_040830.17Enterobacter cloacae strain RJ30KC990813.18Enterobacter cloacae strain RJ30KC990811.19Enterobacter cloacae strain N15AY017123.110Enterobacter cloacae strain N151NR_0427387.111Rhodococcus soli strain DSD51WNR_14379.23Uncultured gamma proteobacteriumAM237233.14Enterobacter cloacae strain SEA01KY625189.14Enterobacter cloacae strain SEA01KY625189.15Burkholderia sp. MSMB122KF378609.16Uncultured beta proteobacterium clone Ro77AM237261.17Rhodococcus hoagi strain DSN 20307NR_041910.18Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		8	Uncultured beta proteobacterium clone Ros38	AM237264.1
10Rhodococcus sp. JFJ-Iso-Bact41AJ867727.211Burkholderia diffusa strain R-15930NR_042633.12Burkholderia alpina strain P0-03-17-38JF_763852.133Burkholderia alpina strain P0-04-17-38NR_146371.14Burkholderia alpina strain P0-04-17-38NR_042635.14Burkholderia athalandensis strain E264NR_027587.15Burkholderia ubonensis strain GTC-P3-415NR_040830.16Burkholderia ubonensis strain GTC-P3-415NR_040830.17Enterobacter cloacae strain RJ30KC990813.18Enterobacter cloacae strain RJ30KC990811.19Enterobacter cloacae strain NJ15AY017123.110Enterobacter cloacae strain Vivi171 MF661959.111Rhodococcus soli strain DC53NR_149271.123MR_149271.1MA237233.144Enterobacter cloacae strain SEA01KY625189.145Burkholderia sp. MSMB122KF378609.166Uncultured garma proteobacterium clone Ro77AM237261.17Rhodococcus hoagii strain DSN 20307NR_04191.017Rhodococcus hoagii strain DSN 20307NR_04191.018Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		9	Rhodococcus sp. KSM-B-3	AB032365.1
1Burkholderia diffusa strain R-15930NR_042633.12Burkholderia alpina strain P0-03-17-38JF_763852.13GBurkholderia alpina strain P0-04-17-38NR_146371.14Burkholderia thailandensis strain E264NR_027587.15Burkholderia ubonensis strain GTC-P3-419NR_042635.16Burkholderia ubonensis strain GTC-P3-415NR_040830.17Enterobacter cloacae strain RJ30KC990813.18Enterobacter cloacae strain RJ20KC990813.19Enterobacter cloacae strain RJ20KC99081.1.19Enterobacter cloacae strain RJ20NR_149271.110Enterobacter cloacae strain NJ20NR_149271.111Rhodococcus shumicola strain UC33NR_149271.112Rhodococcus soli strain DSD51WNR_149271.14Enterobacter cloacae strain SEA01KY625189.14Enterobacter cloacae strain SEA01KY625189.15Burkholderia sp. MSMB122KF37869.16Uncultured beta proteobacterium clone R077AM237261.17Rhodococcus hoagii strain DSM 20307NR_041910.18Uncultured beta proteobacterium clone R035AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		10	Rhodococcus sp. JFJ-Iso-Bact41	AJ867727.2
2Burkholderia alpina strain P0-03-17-38JF_763852.133Burkholderia alpina strain P0-04-17-38NR_146371.143Burkholderia thailandensis strain E264NR_027587.153Burkholderia semnalis strain R-2419NR_042635.163Burkholderia ubonensis strain GTC-P3-415NR_040830.176Burkholderia choace strain RJ30KC990813.188Enterobacter cloacae strain RJ20KC990811.199Enterobacter cloacae strain RJ20KC99081.1101MF661959.1NR_149271.1101MF661959.1NR_149271.12Rhodococcus shain DC33NR_149271.121AN237233.1AM237233.13Uncultured gama proteobacteriumAM237233.145Burkholderia sp. MSMB122KF378609.145Burkholderia sp. MSMB122KF378609.171Rhodococcus hoagii strain DSM 20307NR_041910.18Uncultured beta proteobacterium clone Ro35AM237265.198Nncultured beta proteobacterium clone Ro35AM237265.1	3	1	Burkholderia diffusa strain R-15930	NR_042633.1
3Burkholderia alpina strain P0-04-17-38NR_146371.14Burkholderia thailandensis strain E264NR_027587.15Burkholderia semnalis strain R-2419NR_042635.16Burkholderia ubonensis strain GTC-P3-415NR_040830.17Enterobacter cloacae strain RJ30KC990813.18Enterobacter cloacae strain RJ20KC990811.19Enterobacter cloacae strain RJ20KC99081.110Enterobacter cloacae strain Vivi171 MF661959.1110Enterobacter cloacae strain Vivi171 MF661959.12Rhodococcus humicola strain UC33NR_149271.12Rhodococcus soli strain DSD51WNR_134799.23Uncultured gamma proteobacteriumAM237233.14Enterobacter cloacae strain SEA01KY625189.15Burkholderia sp. MSMB122KF378609.16Uncultured beta proteobacterium clone Ro77AM237261.17Rhodococcus hoagii strain DSM 20307NR_041910.18Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		2	Burkholderia alpina strain P0-03-17-38	JF_763852.1
ABurkholderia thailandensis strain E264NR_027587.13SBurkholderia semnalis strain R-2419NR_042635.16Burkholderia ubonensis strain GTC-P3-415NR_040830.17CEnterobacter cloacae strain RJ30KC990813.18CEnterobacter cloacae strain RJ20KC990811.19CEnterobacter cloacae strain RJ20AY017123.110Enterobacter cloacae strain Vivi171 MF661959.111Rhodococcus humicola strain UC33NR_149271.12Rhodococcus soli strain DSD51WNR_134799.23Uncultured gamma proteobacteriumAM237233.14Enterobacter cloacae strain SEA01KY625189.14GUncultured beta proteobacterium clone Ro77AM237261.17Rhodococcus hoagii strain DSM 20307NR_041910.18Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		3	Burkholderia alpina strain P0-04-17-38	NR_146371.1
3SNR_042635.116Burkholderia ubonensis strain GTC-P3-415NR_040830.17CKC990813.117KC990813.1KC990813.118CEnterobacter cloacae strain RJ30KC990811.19CEnterobacter cloacae strain RJ20KC99081.119IAY017123.1AY017123.110Enterobacter cloacae strain Vivi171 MF661959.111Rhodococcus humicola strain UC33NR_149271.12Rhodococcus soli strain DSD51WNR_134799.23Uncultured gamma proteobacteriumAM237233.14SBurkholderia sp. MSMB122KF378609.14GUncultured beta proteobacterium clone Ro77AM237261.17Rhodococcus hoagii strain DSM 20307NR_041910.18Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		4	Burkholderia thailandensis strain E264	NR_027587.1
S6Burkholderia ubonensis strain GTC-P3-415NR_040830.117CCC		S	Burkholderia semnalis strain R-2419	NR_042635.1
11KC990813.1866KC99081.1966AY017123.19101AY017123.11071MF661959.1171MF661959.1278NR_149271.127NR_134799.2300NR_134799.2300NR_134799.2458NK25189.1458NK25189.1450NR23723.1460NR23726.171NR_040coccus hoagii strain DSM 20307NR_041910.180Nncultured beta proteobacterium clone Ro35AM237265.198Nncultured beta proteobacterium clone Ro35AM237265.1		6	Burkholderia ubonensis strain GTC-P3-415	NR_040830.1
8Enterobacter cloacae strain RJ20KC99081.19Enterobacter cloacae swain RJ15AY017123.110Enterobacter cloacae strain Vivi171 MF661959.11Rhodococcus humicola strain UC33NR_149271.12Rhodococcus soli strain DSD51WNR_134799.23Uncultured gamma proteobacteriumAM237233.14Enterobacter cloacae strain SEA01KY625189.14SBurkholderia sp. MSMB122KF378609.17Rhodococcus hagii strain DSM 20307NR_041910.18Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		7	Enterobacter cloacae strain RJ30	KC990813.1
9Enterobacter cloacae swain RJ15AY017123.11010Enterobacter cloacae strain Vivi1711MF661959.111Rhodococcus humicola strain UC33NR_149271.122Rhodococcus soli strain DSD51WNR_134799.230Uncultured gamma proteobacteriumAM237233.145SKY625189.16Uncultured beta proteobacterium clone Ro77AM237261.17Rhodococcus hoagii strain DSM 20307NR_041910.18Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		8	Enterobacter cloacae strain RJ20	KC990811.1
10Enterobacter cloacae strain Vivi171 MF661959.111Rhodococcus humicola strain UC33NR_149271.122Rhodococcus soli strain DSD51WNR_134799.230Uncultured gamma proteobacteriumAM237233.145SKY625189.145Burkholderia sp. MSMB122KK5378609.16Uncultured beta proteobacterium clone Ro77AM237261.178Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		9	Enterobacter cloacae swain RJ15	AY017123.1
1Rhodococcus humicola strain UC33NR_149271.122NR_134799.230NR_134799.230AM237233.146KY625189.150SUrcultured gamma proteobacterium SEA01KY625189.1450SUrcultured beta proteobacterium clone Ro77AM237261.160NR_041910.1NR_041910.1780NR_0255.1AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		10	Enterobacter cloacae strain Vivi17	1 MF661959.1
2Rhodococcus soli strain DSD51WNR_134799.233Uncultured gamma proteobacteriumAM237233.145KY625189.1KY625189.145OBurkholderia sp. MSMB122KF378609.16Uncultured beta proteobacterium clone Ro77AM237261.17Rhodococcus hoagii strain DSM 20307NR_041910.18Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1	4	1	Rhodococcus humicola strain UC33	NR_149271.1
3Uncultured gamma proteobacteriumAM237233.1446KY625189.145Burkholderia sp. MSMB122KF378609.16Uncultured beta proteobacterium clone Ro77AM237261.17Rhodococcus hoagii strain DSM 20307NR_041910.18Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		2	Rhodococcus soli strain DSD51W	NR_134799.2
4Enterobacter cloacae strain SEA01KY625189.145Burkholderia sp. MSMB122KF378609.16Uncultured beta proteobacterium clone Ro77AM237261.17Rhodococcus hoagii strain DSM 20307NR_041910.18Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		3	Uncultured gamma proteobacterium	AM237233.1
45Burkholderia sp. MSMB122KF378609.16Uncultured beta proteobacterium clone Ro77AM237261.17Rhodococcus hoagii strain DSM 20307NR_041910.18Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		4	Enterobacter cloacae strain SEA01	KY625189.1
6Uncultured beta proteobacterium clone Ro77AM237261.17Rhodococcus hoagii strain DSM 20307NR_041910.18Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		5	Burkholderia sp. MSMB122	KF378609.1
7         Rhodococcus hoagii strain DSM 20307         NR_041910.1           8         Uncultured beta proteobacterium clone Ro35         AM237265.1           9         Rhodococcus sp. NCIB 9646         AY017136.1		6	Uncultured beta proteobacterium clone Ro77	AM237261.1
8Uncultured beta proteobacterium clone Ro35AM237265.19Rhodococcus sp. NCIB 9646AY017136.1		7	Rhodococcus hoagii strain DSM 20307	NR_041910.1
9 <i>Rhodococcus</i> sp. NCIB 9646 AY017136.1		8	Uncultured beta proteobacterium clone Ro35	AM237265.1
		9	Rhodococcus sp. NCIB 9646	AY017136.1

and Enterobacter were dominant for third location isolates. Fourth isolates location were identified generally as *Rhodococcus*, *Burkholderia*, *b* proteobacterium. Genetic similarity between thirty nine isolates and within location isolates were estimated and phyllogenetic tree was constructed for all studied locations as shown in **Figure 2**. More light was added to our findings through using 16S rRNA gene sequence similarity in identified bacterial genera was added [15]. They identified genus *Comamonas* based on 16S rRNA gene sequence search in Ribosomal Database Project, small subunit rRNA and large subunit rRNA databases together with the phyllogenetic tree analysis. Also, our finding was in agreements on Chong et al. [11]. They constructed Phyllogenetic analysis of the 16S rRNA gene sequences to compare the four RDX degrading *Rhodococcus* isolates. Also, for



identified monooxygenase-associated genes in *Pseudonocardia dioxanivorans* CB1190 used as biomarkers to biodegrade 1,4-dioxane in pure cultures and environmental samples [6]. Likewise of our results of applied bacteria-specific primers to amplify the 16S rRNA genes to construct phylogenetic analysis for bacterial biodegradation of explosives in groundwater [16] *Clostridium and Pseudomonas* known explosive-degrading multiple sequences strains were identified and grouped. With agreement on dominance of  $\alpha$ - and  $\beta$ -*proteobacteria* which isolated and identified from RDX-contaminated saturated soils samples [17] identified the same bacterial phyla using terminal restriction fragment length polymorphism (tRFLP) analysis of 16S rRNA genes.

XpIA and XpIB (RDX degrading genes) were applied as molecular marker for evaluating RDX components biodegradation capability of previous identified isolates. As shown in **Figure 3**, specific differentiation for XpIA and XpIB proteins level were detected for thirty eight identified bacterial genera which isolated from four different locations. XpIA and XpIB biodegradation genes were only expressed for all first location isolates. Second location expressed XpIA and XpIB biodegradation genes for all isolates except identified bacterial isolates as *Burkholderia metallica* strain R-16017 (NR\_042636.1) isolate. Interestingly, identified bacterial isolates as uncultured beta *proteobacterium* clone Ros107 (AM237275.1) was failed to express XpIB biodegradation gene. Third isolate location reflect clear to distinguish expression profile for XpIA and XpIB genes, only bacterial isolates which identified as *Burkholderia seminalis* strain R-2419 (NR\_042635.1) and *Enterobacter cloacae* strain Vivi17 (MF661959.1) suppressed XpIA and XpIB genes. Furthermore, XpIB biodegradation gene was suppressed for bacterial isolating which identified as *Burkholderia ubonensis* strain GTC-P3-415 (NR\_040830.1) and *Enterobacter cloacae* strain RJ30 (KC990813.1). Identify bacterial which isolates from fourth location reflected the lowest existence of XpIA and XpIB biodegradation genes comparing with other locations were *Rhodococcus humicola* strain UC33 (NR\_149271.1) and *Rhodococcus soli* strain DSD51W (NR\_134799.2) suppressed XpIA and XpIB genes. XpIA gene was suppressed for uncultured gamma *proteobacterium* (AM237233.1) and *Rhodococcus sp.* NCIB 9646 (AY017136.1) and *Burkholderia sp.* MSMB122 (KF378609.1) suppressed XpIB gene.

As shown in **Figure 4**, XpIA and XpIB proteins level were compared between bacterial isolates from varied locations which performed via western blotting technique indicated that, bacterial isolates from first location reflected the highest XpIA and XpIB proteins level and second; third and fourth bacterial isolates were arranged distinctly for XpIA and XpIB proteins level. Among first location isolates which were superior levels comparing with other locations, *Burkholderia gladioli* isolates (AB023647.1 and AB023646.1) showed the highest proteins level. On the other hand, Rhodococcus sp. KSM-B-3 (AB032365.1) and Rhodococcus sp. JFJ-Iso-Bact41(AJ867727.2) expressed the highest XpIA and XpIB proteins level comparing with rest isolate from second location. Third isolates location reflected highly distinguishable proteins level for Burkholderia diffusa strain R-15930 (NR\_042633.1), Burkholderia alpina strain PO-





03-17-38 (JF\_763852.1) and Burkholderia alpina strain PO-04-17-38 (NR\_146371.1). All bacterial isolates from fourth location showed the lowest proteins level for XpIA and XpIB genes. By contrary of our findings, xpIA, hydA, onr, xenA, and xenB genes which associated with RDX degradation were disappeared in all of the groundwater RDX contaminant samples [16]. Our previous findings for quantifying XpIA protein level through western blotting were supported by presented results of Chong [12]. Who detected only a single band with 47 kDa with XpIA antibody cross-reactivity? One of important explanation for XpIA and XpIB expression level variation which detecting in our findings were cleared in the light of Chong [3] through applying Western blot analysis of crude extracts probed with XpIA and XpIB antibodies and indicated that that XpIA and XpIB protein levels were

## References

- Rylott EL, Jackson RG, Sabbadin F, Seth-Smith HM, Edwards J, et al. (2011) The explosive-degrading cytochrome P450 XpIA: biochemistry, structural features and prospects for bioremediation. Biochim Biophys Acta 1814: 230-236.
- 2 Seth-Smith HMB, Edwards J, Rosser SJ, Rathbone DA, Bruce NC (2008) The explosive-degrading cytochrome P450 system is highly conserved among strains of Rhodococcus spp. Appl Environ Microbiol 74: 4550–4552.
- 3 Chong CS, Sabir DK, Lorenz A, Bontemps C, Andeer P, et al. (2014). Analysis of the xplAB-containing gene cluster involved in the bacterial degradation of the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine. Appl Environ Microbiol 80: 6601-6610.
- 4 Matsuki T, Watanabe K, Fujimoto J, Takada T, Tanaka R (2004) Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. Appl Environ Microbiol 70: 7220-8
- 5 Layton BA, Walters SP, Lam LH, Boehm AB (2010) Enterococcus spe-

distinguishably decrease in. Also, open reading frame of xplB was not disrupted which cleared possibility for affection of mutations transcript stability or translational efficiency.

## Conclusion

Our findings for identification and mining for biodegradation of hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) summarized that different bacterial genera with certain biodegradable activity were identified via 16S rRNA molecular marker technique. Furthermore, specific biodegradation genes, XpIA and XpIB showed different expression levels consistent with locations of bacterial isolation. Further studies were requirement to understand biodegradation molecular metabolic interaction for isolating from different locations.

cies distribution among human and animal hosts using multiplex PCR. J Appl Microbiol 109: 539–547.

- 6 Gedalanga P, Pornwongthong P, Mora R, Chiang SD, Baldwin B, et al. (2014) Identification of biomarker genes to predict biodegradation of 1,4-dioxane. Appl Environ Microbiol 80: 3209-3218.
- 7 DeLong EF (1992) Archaea in coastal marine environments. Proc Natl Acad Sci USA 89: 5685–5689.
- 8 LaPara TM, Nakatsu CH, Pantea L, Alleman JE (2000) Phylogenetic analysis of bacterial communities in mesophilic and thermophilic bioreactors treating pharmaceutical wastewater. Appl Environ Microbiol 66: 3951–3959.
- 9 Benson DA, Boguski MS, Lipman DJ, Ostell J, Ouellette BFF, et al. (1999) GenBank. Nucleic Acids Res 27: 12–17.
- 10 Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402.
- 11 Cole JR, Wang Q, Fish JA, Chai A, McGarrell DM, et al. (2014) Ribosomal Database Project: Data and tools for high throughput rRNA analysis. Nucleic Acids Res 42: D633-D642.

- 12 Chong CS (2011) Biodegradation of RDX in *Rhodococcus* spp. University of York Department of Biology, USA.
- 13 Rylott EL, Jackson RG, Edwards J, Womack GL, Seth-Smith HM, et al. (2006) An explosive-degrading cytochrome P450 activity and its targeted application for the phytoremediation of RDX. Nat Biotechnol 24: 216–219.
- 14 Jackson RG, Rylott EL, Fournier D, Hawari J, Bruce NC (2007) Exploring the biochemical properties and remediation applications of the unusual explosive-degrading P450 system XpIA/B. Proc. Natl Acad Sci USA 104: 16822–16827.
- 15 Yadav V, Prakash S, Srivastava S, Verma PC, Gupta V, et al. (2009) Identification of Comamonas species using 16S rRNA gene sequence. Bioinformation 3: 381–383.
- 16 Fuller ME, McClay K, Higham MP, Hatzinger PB, Steffan RJ (2010) Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) Bioremediation in Groundwater: Are Known RDX-Degrading Bacteria the Dominant Players? Bioremediation J 14: 121–134.
- 17 Ringelberg DB, Reynolds CM, Walsh ME, Jenkins TF (2003) RDX loss in a surface soil under saturated and well drained conditions. J Environ Qual 32: 1244-1299.