RESEARCH ARTICLE

Molecular characterization of a polycyclic aromatic hydrocarbons (PAHs) degrader, *Burkholderia contaminans* strain P14, isolated from aged oil-contaminated soil in Kuwait

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Abstract: Soil contamination by polycyclic aromatic hydrocarbons (PAHs) is a major environmental issue affecting soil quality and health. Bioremediation is an efficient approach to decontaminate these pollutants while posing the lowest risk to the environment. In this study, the physiochemical properties of oil-contaminated soils in Kuwait were investigated, selective screening and genetic identification of PAH degraders were performed, and the PAHs degradation pathway was predicted. High concentration of PAHs was detected in the soil samples, in particular for pyrene, phenanthrene, and fluorine. In total, 21 strains belonging to the genera *Pseudomonas* (9), *Burkholderia* (6), *Bacillus* (2), *Bordetella* (1), *Microbacterium* (1), *Micrococcus* (1), and *Kocuria* (1) showed ability to degrade PAHs. *Burkholderia* sp. P14 demonstrated a stable growth rate (5 to 8 days) in the presence of phenanthrene and fluorene. The genome sequence of the *Burkholderia contaminans* P14 strain comprises 80 genes involved in the degradation of the benzoate, and PAHs. The genes encoding for PAHs degradation were clustered into four distinct groups, including *pcaHG*, *pcaB*, *pcaIJ*, and *pcaKFR*. KEGG analysis suggested that PAHs were degraded in P14 via the protocatechuate and catechol branches of the β-ketoadipate protocatechuate degradation pathway. The genomic island regions in P14 differed from those in the reference genome of *B. contaminans* M14, indicating the novelty and genomic recombination of the strain. In conclusion, *B. contaminans* P14 strain has great potential to be used as bio-degrader for the restoration of oil-contaminated soils.
Keywords: Polycyclic aromatic hydrocarbons (PAHs), bioremediation, oil-contaminated soil, *Burkholderia contaminans*

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1 Introduction

Over the last several decades, numerous significant oil spills have severely impacted marine ecology. Due to oil leaks from industrial complexes and petrol stations, soil and groundwater contamination are considered a severe environmental and social hazard (Zhang et al., 2006). Among these contaminants are the ubiquitous aromatic compounds such as polycyclic aromatic hydrocarbons (PAHs) that contaminate the environment because of the incomplete combustion of organic materials in addition to agricultural, domestic, and industrial processes (Cheng et al., 2016; Lahkar and Deka, 2016). PAHs encompass some of the most persistent natural pollutants belongs to PAHs, which have severe biological effects such as carcinogenicity, mutagenicity, and genotoxicity (Ledra, 2011; Cauduro et al., 2020). To remove pollutants, many bacterial and fungal microorganisms are used in bioremediation as an eco-friendly method (Isaac et al., 2017). The harsh environments in contaminated sites allow the indigenous bacteria to develop extraordinary catabolic machines for survival (Moriya et al., 2007). In some soils, like that of Kuwait, the indigenous oil-degrading organisms are abundant. Oil-utilizing bacterial genera such as *Bacillus*, *Pseudomonas*, *Streptomyces*, and *Rhodococcus* predominate in Kuwaiti soil samples (Rabani et al., 2020; AL-Saleh and Akbar, 2015). This could be due to the high PAH level in Kuwait soil, which contains twice the acceptable PAH level (2 mg/kg) (Al-Yakoob et al., 1994). Several microbial groups have grown faster in Kuwait crude oil contaminated soil, including *Proteobacteria*, *Alphaproteobacteria*, *Chloroflexi*, *Chlorobi*, and *Acidobacteria* (Radwan et al., 1997). To effectively reduce the toxic constituents of crude oil, bioremediation of oil-contaminated soil in Kuwait is needed.

The PAH compounds can have two or seven benzene rings depending on their aromatic ring structure. They are excellent lubricants and intermediates for thermosetting polymers because of their unique physiochemical characteristics (Mackay and Callcott, 1998; Ausuri et al., 2021). Despite these characteristics, several bacteria can metabolize these molecules as a carbon and energy source through their biosyn-

thetic processes, playing a significant role in recycling the carbon of aromatic rings and decomposing such contaminants (Cauduro et al., 2020). However, the limited bioavailability of contaminants to the microorganisms responsible for degradation hampers the biodegradation of PAHs. The bioavailability of PAHs might be increased by using commercially available surfactants in bioremediation procedures (Obi et al., 2016). The potential for microbe bioremediation is greater than chemical stabilization, soil washing, electrokinetic extraction, and phytoremediation methods (Liu et al., 2018).

The adaptation of bacteria to such an ecosystem occurs in three ways, including genetic alterations that lead to novel metabolic pathways, inhibition of some enzymes, and stimulation of other microbes capable of transforming hydrocarbons. The development of molecular biology tools has recently made it possible to monitor this process directly. Catechol-based intermediates are formed during PAHs degradation through dioxygenases, leading to intermediates in the TCA cycle (Nogales et al., 2017; Ausuri et al., 2021). A prototypical model for biodegradation investigations is phenanthrene, which shares its structural backbone with other PAHs and is often used to identify PAH contamination (Mallick et al., 2010). Phenanthrene breakdown has been monitored in a wide variety of microbes, which includes the bacterial genera from *Pseudomonas*, *Bacillus*, *Arthobacter*, *Serratia*, *Stenotrophomonas*, *Shewanella*, *Spingobium*, *Acidovorax*, *Rhodococcus*, *Ralsonia*, *Brevibacterium*, and *Burkholderia* (Seemann, 2014; Alegbeleye et al., 2017; Morya et al., 2020; Ausuri et al., 2021).

Genome annotation of PAHs degraders provides in-depth knowledge of aromatic degrading genes and their catabolic pathways (Elufisan et al., 2020). The aromatic catabolic bacteria of the genus *Burkholderia* were perceived as a possible contender for bioremediation applications. However, there is insufficient research available on PAH degraders of *Burkholderia* species (Morya et al., 2020). Despite the global presence of *Burkholderia*, this genus is still relatively underexplored compared to other bacterial genera. Therefore, this study investigates the genomic characteristics of PAHs degradation of *Burkholderia contaminans* strain P14.

2 Materials and Methods

2.1 Sample collection and characterization

The oil-contaminated soil was collected from the Kuwait Oil Company (KOC), a Burgan oil field near the southern part of Kuwait (in May 2019). The upper 10 cm of the contaminated site was monitored for black coloration and oily smell. In a 1L screw-capped sterile container, soil samples were collected and transported on ice to the laboratory for immediate analysis. The sample was divided into three sections for chemical, microbial and molecular analysis and stored at 4, 15-24, and -20°C, respectively. Chemical and microbiological analyses of all samples were completed within 48 h after collection. The soil was sieved prior to use.

2.2 Analysis of physicochemical properties

The oil-contaminated soil was further analyzed for its physicochemical properties. The pH, conductivity, salinity, total organic compounds, total petroleum compounds and PAHs, content was determined according to USEPA 150.1, USEPA 120.1, SM 2510B, Llyod Khan (EPA9060), USEPA 3546, and USEPA 8270D, protocols, respectively (Kimbrough and Wakakuwa, 1989; EPA/600/4-79/020).

2.3 Microbial culturing and molecular identification

Microorganisms capable of degrading PAHs were cultured using a standard spread technique. A 15 g soil sample was suspended in 40 ml of 50 mM phosphate buffer (pH 7.2) and then vigorously shaken for 15 min at low speed. Serial dilutions were performed, and 0.1 ml of sample was spread on nutrient agar and Bushnell-Haas minimal agar (source) plates (Cohen-Bazire et al., 1957) and incubated at 30°C for three weeks. Furthermore, Bushnell-Haas minimal agar plates were incubated in the presence of phenanthrene and fluorene (source) (250 mg /ml) for 21 days. The pure cultures were stored in 15% Luria-Bertani (source) broth-glycerol at -70°C for molecular identification.

Genomic DNA was extracted from isolated pure bacterial cultures using the Wizard Genomic DNA purification kit as recommended by the manufacturer (Promega, USA). The 16S rDNA gene was amplified from extracted bacterial genomic DNA by polymerase chain reaction (PCR) (source) using 27F and 1492R primers, and the standard procedure was followed as suggested by Kuske et al. (1997). The amplified products were analyzed on 1% agarose gel and documented using Gel Doc (Bio-rad). Following mplification amplification, the PCR products were eluted for DNA sequencing. The purified 16S rDNA sequences from different microbial cultures were subjected to sequencing, and a basic alignment search tool (BLAST) analysis was used to identify their taxonomy. Sequences were assigned to recognized representatives of the main bacteria based on scores of 97% or higher.

2.4 Optimizing microbial growth on PAH

Bacterial growth on different PAHs was analyzed using phenanthrene and fluorene as the sole carbon source. Microorganism growth was monitored using Bushnell-Haas minimal agar media supplemented with 250 mg/ml PAH. Their growth curve pattern was determined using the viable cell count and optical density reading at OD_{600} (spectrophotometer). Overnight cultures grown in Bushnell-Haas minimal broth medium supplemented with PAH were centrifuged for 5 min at 7,000 rpm and washed twice with 25 mM phosphate buffer. The bacterial cultures were then diluted 1: 1000 in fresh Bushnell-Haas minimal broth medium with sterile phenanthrene or fluorine (250 mg/ml) as the only carbon

sources. Cultures were grown at 30°C with agitation for 7 days. Every 24 h, samples were taken directly to measure OD_{600} or diluted serially and seeded to determine a viable colony-forming unit (CFU) count. Based on the results obtained from the biochemical and microbial analysis, the P14 culture was selected for complete genome sequencing.

2.5 Genome sequence of *Burkholderia contaminans* P14 strain

Total genomic DNA was sequenced and analyzed for P 14 grown in phenanthrene. The genomic libraries were performed at Omic to view facility (Germany). Short insert libraries were prepared: Genomic DNA was sheared by sonication. DNA fragments with an average size of 200-400 bp were purified with Agencourt Ampure XP beads. Fragments were end-repaired and 3'-adenylated. The adapters were ligated to the ends of 3'-adenylated fragments. Fragments with adapters were PCR-amplified, followed by purification with Agencourt AMPure XP beads. Double-stranded PCR products were heat denatured and circularized by the splint oligo sequence, thus forming single-stranded circular DNA (ssCir DNA). DNA nanoballs (DNBs) consisting of 300+ copies were formed from ssCir DNA molecules by rolling-cycle replication. Sequencing of DNB libraries was performed on MGI DNBSEQ-G400 in 2 $\times$ 150 bp mode.

The quality of the reads was checked with FastQC v0.11.7 (Andrews, 2018), and the read quality trimming was performed with the BBTools package v38.45 (Bushnell, 2019). This included the removal of duplicate reads, human sequences, adapter sequences, low entropy reads, and trimming of bases with quality scores $<$ 20. Reads with invalid or ambiguous bases and reads with a length $<$ 50 base pairs (bp) were discarded. Only the results read survive quality trimming as the pairs entered downstream analysis. The functional annotation and circular plots of the draft genomes were performed with Prokka v1.14.5 (Seemann, 2014). The average nucleotide identity (ANI) was calculated using the EzGenome website, as described earlier by Yadva et al. (2021). A phylogenetic tree for the selected strain was performed using MEGA X software, where Neighbor-joining with kimura-2-parameter with 500 bootstrap replicates was used to generate the dendrogram (Kumar et al., 2018).

2.6 PAH operon and pathway analysis

The reference genomes *Burkholderia contaminans* M14 and FL-1-2-30-S1-D0 with accession numbers CP009743.1 and CP013390.1, respectively, were selected and used to compare and characterize the PAH operon in our native strain of *B. contaminans* P14. PAH degradation pathway analysis was performed using the KEGG website (Moriya et al., 2007).

2.7 Nucleotide sequence accession numbers

The 16S rDNA sequences of the PAH degrading bacterial cultures were deposited in the GenBank database under the accession numbers OP278994 to OP279013 for P2, F2, P11, 3, P12, F4, P4, P13, F5, P5, P14, F6, P15, F8, P8, F9, F10, P10, and P17. In contrast, the isolate F1 16S rDNA sequence was deposited under the accession number OP279015. The annotated genome sequence was submitted JANGYH0000000001 to JANGYH00000000068 accession number was assigned under SUB11831399 project ID.

3 Results

3.1 Physiochemical properties of oil-contaminated soil

Standard protocols were used to examine the contaminated soil sample for its physiochemical characteristics, and the results are shown in Table 1. The pH of the sample was alkaline (8.36) with 0.08% salinity. The total concentration of organic compounds was 54259 mg/kg containing the following PAHs: naphthalene, acenaphthylene, acenaphthene, benzo (b) fluoranthene fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo (a)anthracene, chrysene, benzo (k) fluoranthene, benzo (a) pyrene, indeno (1,2,3-cd) pyrene, dibenz(a,h) anthracene and benzo (g,h,i) perylene). A high concentration of pyrene was observed (1212.07 mg/kg), while a lower concentration was observed for dibenz (a,h) anthracene (40.406 mg/kg).

Table 1. Physicochemical properties of aged oil-contaminated soil

Parameter	Result
Salinity (%)	0.08
pH	8.36
Conductivity µS/m	2.39
Total organic carbon (mg/kg)	54259
TPHs (mg/kg)	45700
Naphthalene	162.993
Acenaphthylene	222.91
Acenaphthene	140.284
Fluorene	326.644
Phenanthrene	710.685
Anthracene	165.764
Fluoranathene	412.711
Pyrene	1212.07
Benzo (a)anthracene	246.074
Chrysene	433.038
Benzo (b) fluoranthene	183.145
Benzo (k) fluoranthene	45.002
Benzo (a) pyrene	302.939
Indeno (1,2,3-cd) pyrene	205.373
Dibenz(a,h) anthracene	40.406
Benzo (g,h,i)perylene	264.025

3.2 Isolation and characterization of bacteria from the oil-contaminated soil

After the enrichment cultivation, 43 different pure bacterial isolates were identified, which could grow in the presence of Phenanthrene (PHE) and fluorene (FLU) for 21 days.

Table 2. Molecular identification of PAH degrading strains

No.	Bacterial isolates	16S rRNA sequence Accession number	BLAST identification	Accession number of most identical species	Homology (%)
1	P2	OP278994	*Pseudomonas stutzeri*	KY194762.1	100
2	P11	OP278996	*Pseudomonas songnenensis*	KX982786	99.83
3	F2	OP278995	*Burkholderia sp*	AB545639.1	98
4	P3	OP278997	*Bordetella petrii*	EU082174	100
5	P12	OP278998	*Pseudomonas stutzeri*	KY194762	100
6	F4	OP278999	*Bacillus cereus*	MK592620	100
7	P4	OP279000	*Microbacterium aerolatum*	MT525272	100
8	P13	OP279001	*Kocuria sediminis*	MH178354.1	99.64
9	F5	OP279002	*Pseudomonas stutzeri*	CP073105	99.86
10	P5	OP279003	*Pseudomonas stutzeri strair*	CP073105.1	99.64
11	P14	OP279004	*Burkholderia sp.*	FJ392830.1	99.86
12	F6	OP279005	*Pseudomonas sp.*	MK737162.1	99.86
13	P6	OP279006	*Burkholderia sp.*	MT626032.1	99.68
14	P15	OP279007	*Burkholderia cenocepacia*	CP054820.1	100
15	F8	OP279008	*Burkholderia cenocepacia*	CP054817.1	100
16	P8	OP279009	*Pseudomonas stutzeri*	CP046902.1	99.71
17	F9	OP279010	*Pseudomonas stutzeri*	AJ312165.1	99.71
18	P17	OP279013	*Bacillus cereus*	KX783593.1	100
19	F10	OP279011	*Burkholderia cenocepacia*	CP054820.1	100
20	P10	OP279012	*Micrococcus aloeverae*	KX607117.1	99.93
21	F1	OP279015	*Pseudomonas stutzeri*	JF461537.1	99.43

Among the 43 cultures, 21 isolates were subjected to 16S rDNA sequencing for culture identification. On BLAST analysis, it was noticed that 7 isolates belong to *Pseudomonas stutzeri*, 3 isolates of *Burkholderia cenocepacia*, 3 isolates of *Burkholderia* sp., 2 isolates of *Bacillus cereus*, and one isolate each of *Pseudomonas songnenensis*, *Bordetella petrii*, *Microbacterium aerolatum*, *Micrococcus aloeverae*, *Pseudomonas* sp. and *Kocuria sediminis*. These species can grow solely on phenanthrene or fluorene from the Kuwait crude oil soil sample. Strains *Pseudomonas stutzeri* P2 and P12, *Bordetella petrii* P3, *Microbacterium aerolatum* P4, *Micrococcus aloeverae* P10, *Pseudomonas songnenensis* P11, *Kocuria sediminis* P13, *Burkholderia* sp. P14 and F2, *Bacillus cereus* P17 and F4, and *Pseudomonas* sp. F6 were selected for further analysis (Table 2).

3.3 Microbial growth in the presence of phenanthrene and fluorene

We first characterized the growth kinetics of the selected cultures using PHE and FLU as the sole carbon source. The *Burkholderia* sp. P14 and *B. cenocepacia* P15 cultures exhibited a long lag phase of up to 4 days and maintained a stationary phase until the 8^{th} day. The isolates P2, P3 and F2, indicated a decline in their turbidity on 3^{rd} day and later maintained a stationary phase till 7^{th} day. The *Micrococcus aloeverae* P10 displayed low optical density initially and obtained maximum growth after the 3^{rd} day of incubation. Most cultures entered the log phase on day 7 of incubation when phenanthrene was used as the sole energy source (Figure 1). Sterile broth without the substrate and cell inoculum served as a negative control.

Subsequently, the bacterial count was estimated in the presence of substrates (Figure 2). It was observed that the isolates *Burkholderia* sp. F2, *Pseudomonas* sp. F6, *Kocuria sediminis* P13, and *Burkholderia cenocepacia* P15 showed more bacterial count in the presence of fluorene and maintained a stationary phase until the 7^{th} day of incubation. Although the growth of the *Bacillus cereus* F4 isolates declined on days 3 and 4 in the presence of PHE and FLU, respectively. The bacterial isolates *Bordetella petrii* P3, *Microbacterium aerolatum* P4, *Pseudomonas songnenensis* P11, *Pseudomonas stutzeri* P12, and *Bacillus cereus* P17 showed more growth in the presence of phenanthrene than fluorene. The growth for *Burkholderia* sp. P14, in the presence of phenanthrene and fluorene, has maintained a stationary phase from day 5 to 8. Each bacterial culture growth count was estimated and noticed that a decline in log CFU/ml was achieved as the number of days of incubation increased.

3.4 Genomic features of the *Burkholderia contaminans* strain P14

Due to the physiochemical and microbiological properties, isolate P14 was selected for complete genome sequencing to discover the genes involved in the PAHs degradation pathway. On sequencing, it was perceived that the genome of the P14 strain draft comprises 68 contigs, with an estimated genome size of 8,584,157 bp. The longest contig that was sequenced possessed a 978,010 bp size. ANI analysis indicated 99.95%

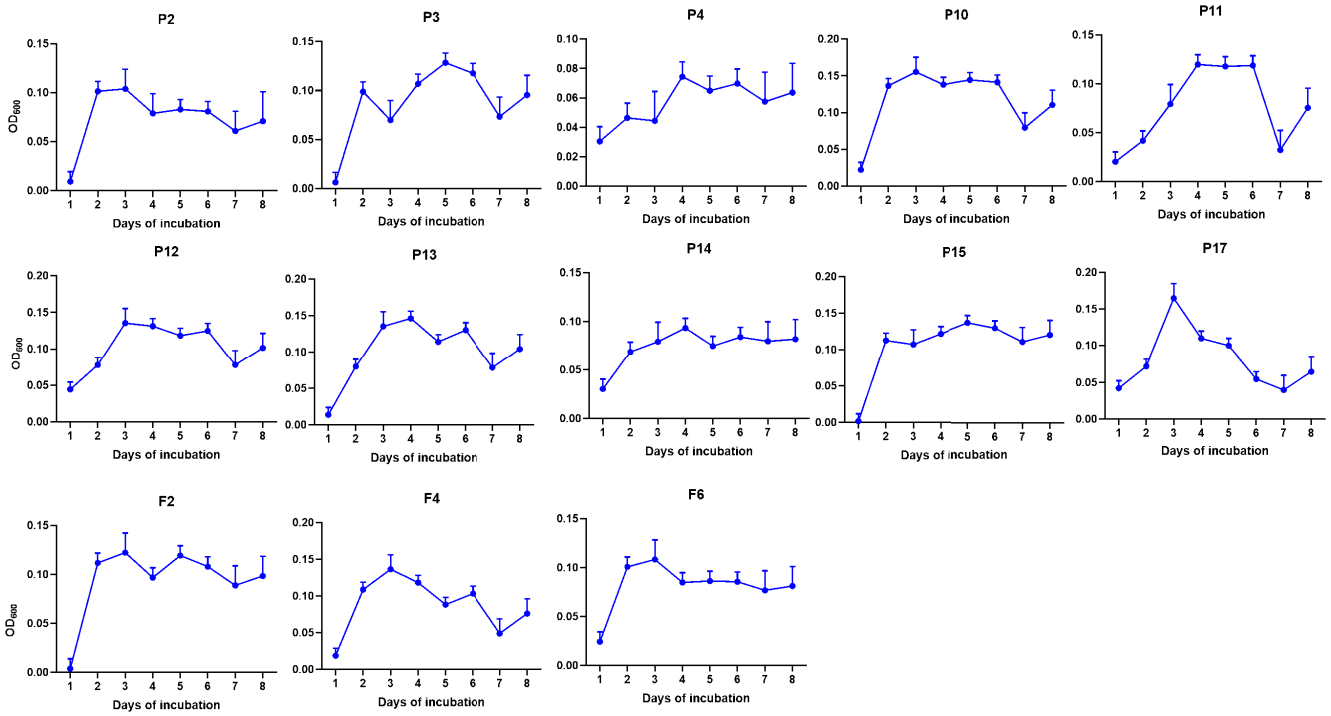

Figure 1. Growth curve pattern of PAH degraders in the presence of phenanthrene (A) and fluorene (B).

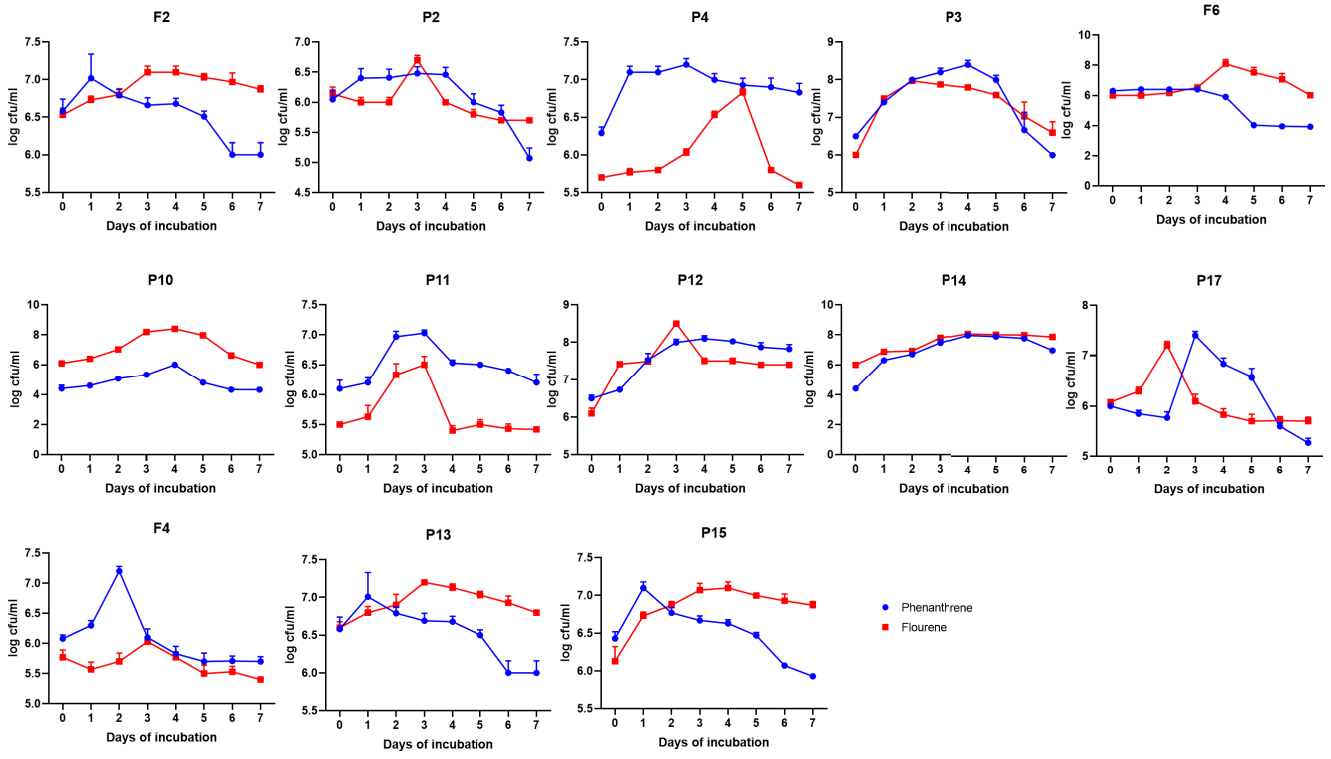

Figure 2. Bacterial count of different PAH degrading strains in the presence of phenanthrene and fluorene.

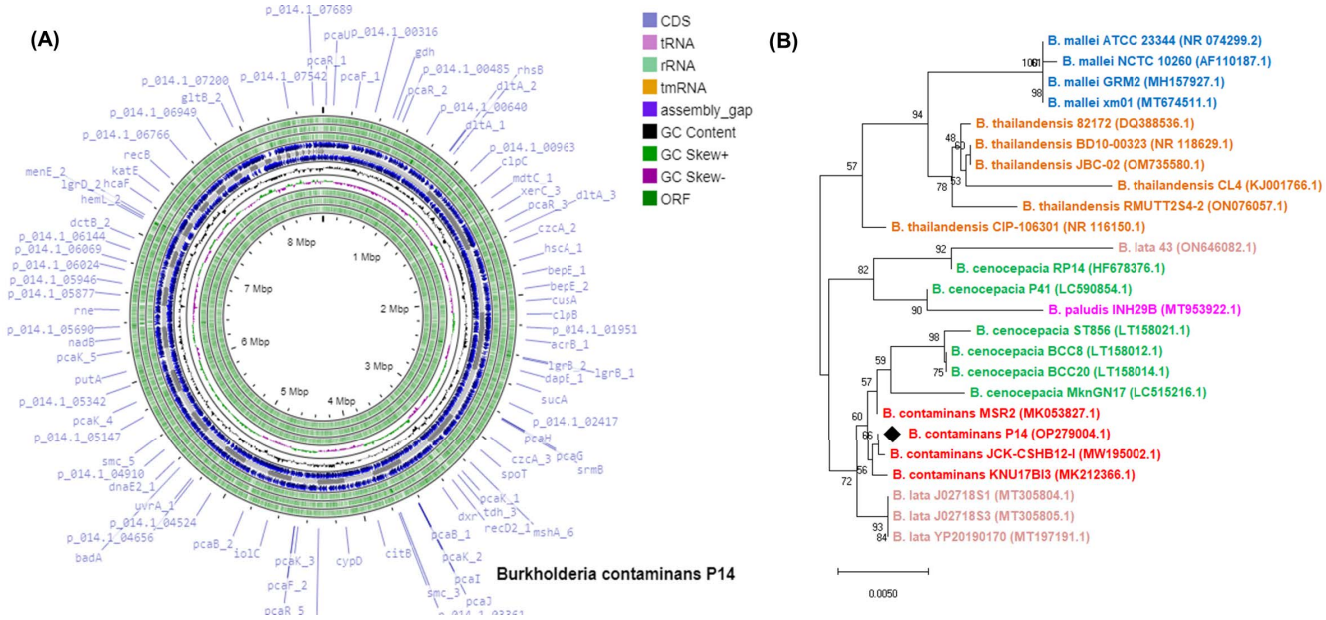

Figure 3. Circular genome view of *B. contaminans* P14 strain and the phylogenetic view.

homology to the reference genome of *Burkholderia contaminans* GCF_000987075.1. The total genome of P14 comprises 66% GC content, with 42,212 CDS, 4 rRNA, and 75 tRNA. The GC content, GC skew +/- and comprehensive antibiotic resistance database (CARD) are represented in black, green, purple and red color in Figure 3A. Further, the phylogenetic tree obtained for P14 strain suggests that, this strain belongs to *B. contaminans* as it clustered with other strains of that species (Figure 3B). It can be observed that the *B. contaminans* species is closely related to other strains of *B. lata* and *B. cenocephacia*. However, these three species of *Burkholderia* were quite divergent from the strains of *B. mallei* and *B. thailandensis*.

Whole-genome analysis coupled with phenanthrene degradation tests provided insight into the underlying bacterial processes in the P14 strain. The GC content of the strain obtained in this investigation was compared to other strains of the same species. Compared to the reference genome of *B. contaminans* M14, our native isolate was observed to have more genome size, whereas the M14 strain had 8,509,249 bp genome size, 66.4% GC content, 5 rRNAs and 65 tRNAs.

3.5 Genomic islands (GIs) prediction in *B. contaminans* P14 strain

The gene clusters that are gained through the horizontal gene transfer (HGT) mechanism in the P14 strain were identified by Island Viewer 4, where a total of 72 genomic islands were predicted (Figure 4). The GI comprised 734 genes ranging from 4 to 85 Kbp in the regions. The genes encoding carbohydrate transporter, protein metabolism, DNA replication, transcription regulators, Type IV secretion system, DNA-binding proteins and Endo and exoribonuclease were mainly involved. Also, many insertion elements, transposons, toxin/antitoxin system, and recombinase genes were present in various GI regions and represent the HGT phenomenon. The examined GI regions did not include the genes required for the degradation of aromatic compounds. It was reported that in M14 strain there are 49 GIs that haven't been found in any of the other *B. contaminans* genomes.

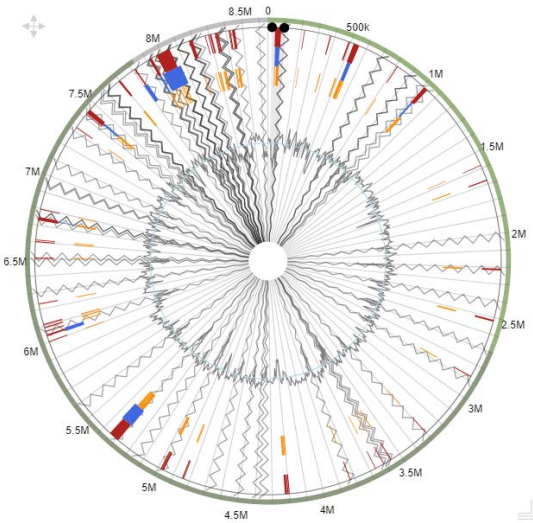

Figure 4. Genomic island regions in P14 strain predicted by integrated (red), IslandPath-DIMOB (blue), and SIGI-HMM (orange).

3.6 Detection of PAH degrading genes in the genome of the *B. contaminans* P14 strain

To identify potential genes involved in the PAH and benzoate pathway, we relied on the open reading frames (ORFs) or proteins that were projected to produce. Eighty genes are involved in the degradation of the benzoate, naphthalene and PAHs pathways in the genome of the P14 strain (Figure

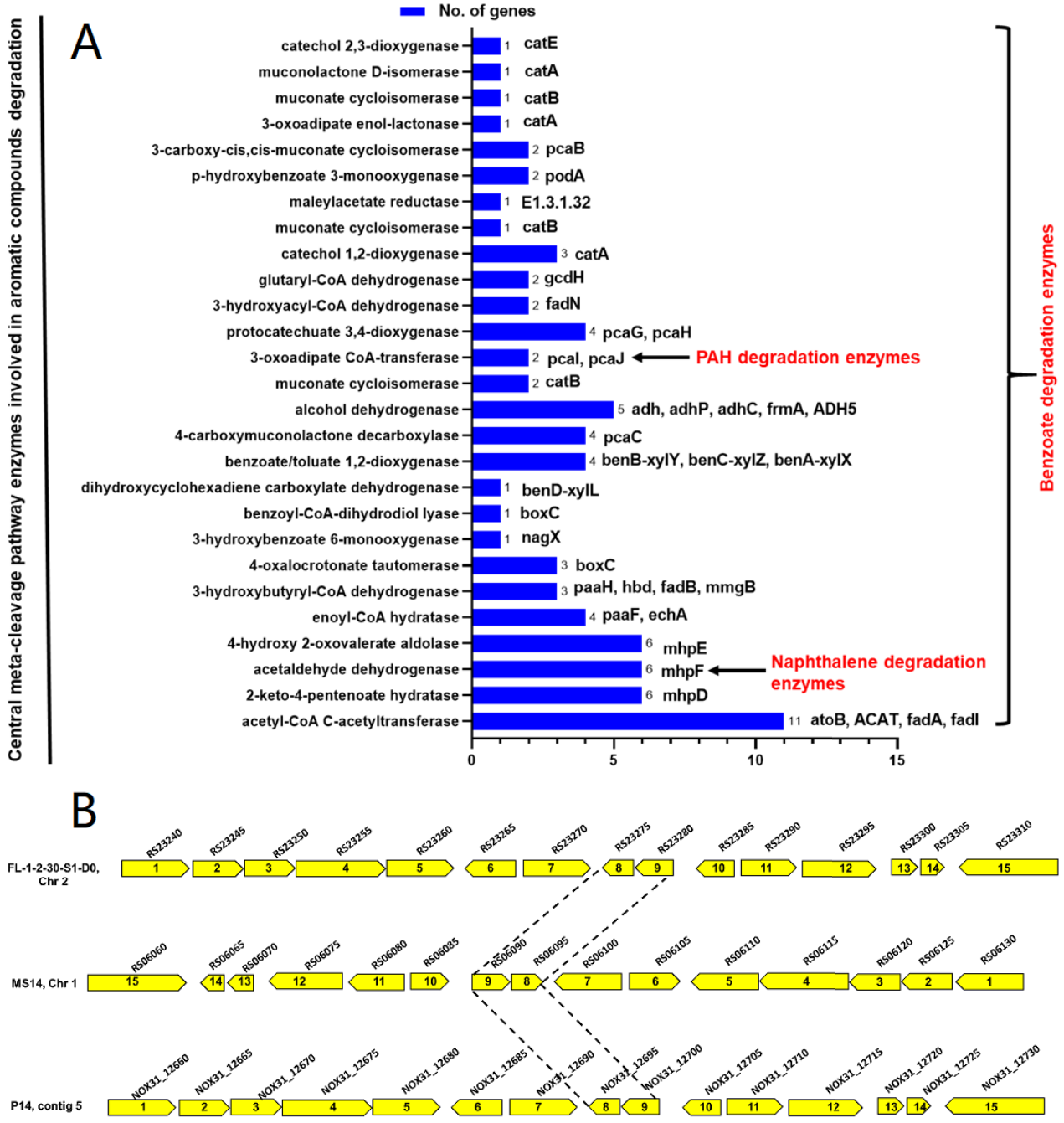

Figure 5. Central meta-cleavage pathway enzymes involved in the degradation of aromatic compounds (A) and comparative analysis of the PAH operon between strains of *B. contaminans* FL1-2-30-S1-D0, M14, and P14 (B). Codes 1-15 represent different open reading frames (ORFs) encoding as follows ORF1- glutathione ABC transporter substrate binding protein (gsiB), ORF2- glutathione ABC transporter permease (gsiC), ORF3- glutathione ABC transporter permease (gsiD), ORF4- peptidase of the P1 family, ORF5- M55 family metallopeptidase, ORF6- alpha/beta hydrolase, ORF7- transcriptional regulator helix turn helix, ORF8- protocatechuate 3,4-dioxygenase alpha subunit (*pcaG*), ORF9- protocatechuate 3,4-dioxygenase beta subunit (*pcaH*), ORF10- Yix/YebB-like N1pC/P60 family cysteine hydrolase, ORF11- transcriptional regulator helix turn helix, ORF12- pca operon transcription factor, ORF13- Type II toxin The dotted lines indicate the main genes responsible for PAH degradation among *B. contaminans* strains. Chr represents the chromosome.

5A). The PAHs operon was annotated and compared with other reference genomes of *B. contaminans* M14 and FL-1-2-30-S1-D0. A total of 15 genes involved in the PAH operon and the enzymes protocatechuate 3,4-dioxygenase, alpha and a beta subunit (EC: 1.13.11.3) encoded the genes *pcaG* and *pcaH* respectively were found responsible for the PAHs degradation (Figure 5B, Table S1). The strain FL1-2-30-S1-D0 had 99% homology with P14 strain, while the orientation of PAH genes in M14 was different compared to P14 strain. The genes *pcaG* and *pcaH* were present in contig 5, while they are present on chromosome 1 and 2 of M14 and FL1-2-30-S1-D0, respectively.

3.7 Protocatechuate degradation pathway in P14 strain

The KEGG pathway analysis suggested that the protocatechuate 3,4-dioxygenase enzyme is required to break the aromatic rings by ortho-cleavage. In degrading aromatic compounds, this enzyme catalyzes the intradiol addition of both oxygen atoms from molecular oxygen. Mineralization occurs through the intermediate 3-oxoadipate during this sort of cleavage. This pathway is called as β-ketoadipate protocatechuate degradation pathway or the ortho cleavage pathway. A schematic representation of the PAH degradation pathway in *B. contaminans* P14 is illustrated in Figure 6. Aromatic chemicals such as 4-hydroxybenzoate and benzoate are transported to the protocatechuate and catechol branches of this pathway. The intermediate product 3-oxadipate further gets converted to acetyl CoA and succinyl CoA, leading to the tricarboxylic acid (TCA) cycle. Genes encode the PAHs pathway are clustered into four distinct groups, including *pcaHG*, *pcaB*, *pcaIJ*, and *pcaKFR* and found to be localized in contigs 5, 11, 8 and 13, respectively, of *B. contaminans* P14.

4 Discussion

Bioremediation is now an essential method for cleaning up soil, sediment, and groundwater contaminated by PAHs. Pollutants with more remarkable persistence, such as PAHs, are seldom treated by bioremediation usually because the microorganisms native to polluted areas lack the degradative mechanisms (Vogel, 1996; Kim et al., 2003). Bioremediation through microbial metabolic processes at contaminated sites is among the most effective and environmentally acceptable options (Lee et al., 2019).

In reality, soil salts are essential for maintaining the osmotic balance of cells and a critical factor for microorganisms to perform their physiological and metabolic processes (Zhang et al., 2011). In this study, the low salinity (0.08%) and alkaline pH (8.36) in the oil-contaminated soil allowed the growth of PAH-degrading bacteria. Also, soil pH in light chestnut and soddy-podzolic soils in Kalmykia, Germany, and Egypt has increased from 7.87 to 9.2 due to oil pollution (Bragina and Bulacheva, 2011; Seo et al., 2006). In a study

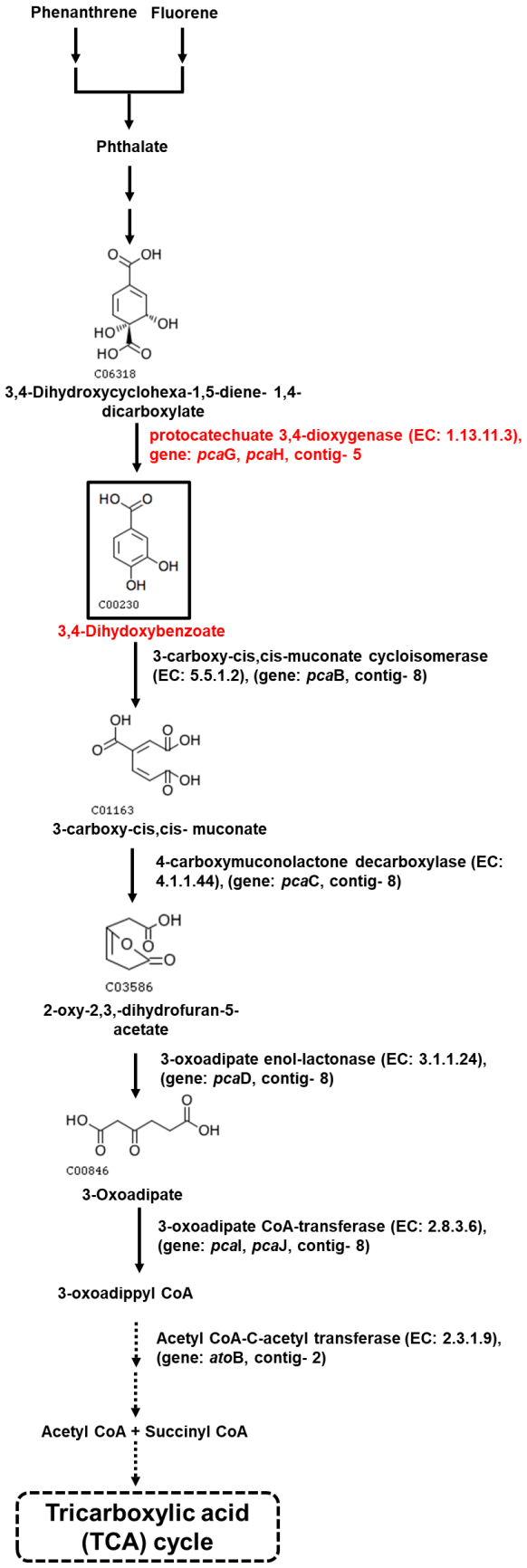

Figure 6. Proposed PAH degradation pathway from strain P14 of *B. contaminans*. The genes involved in the pathway are represented in brackets.

by Darma et al. (2016), PHE and pyrene were detected at a concentration of 500 and 250 mg/l, respectively, in the oil-contaminated soil of Malaysia. Wang et al. (2020) revealed that microbial plasmolysis occurs in high salt concentrations, inhibiting further bacterial growth and reducing PAH degradation. These results are under previous studies, where the presence of various microbial communities indicated maximum degradation of PAH at low salinity and pH 6-8 (Zhang et al., 2006; 2020). Soils with lower salinities support a more diverse and robust microbial population, promoting collaboration between various bacteria, and ultimately leading to enhanced PAH reduction (Li et al., 2022). Due to their resistance and carcinogenicity to humans, High molecular weight (HMW) PAHs with 4-7 rings are substantially more dangerous than the low molecular weight (LMW) PAHs, which have 2-3 aromatic rings (Dai et al., 2022). Our study noticed that the pyrene (a 4-ringed PAH) is present at a high concentration and followed by PHE (a 2-ringed PAH) in the soil sample, isolating a more potent PAH degrader is essential to eliminate the pollutants at the contaminated site.

Enrichment using artificial synthetic basal media has often resulted in the enrichment of microbes that can only thrive well in laboratory settings, not in polluted areas (Jacques et al., 2009). It is worth mentioning that the isolated microbes may digest organic contaminants in the lab. However, they frequently have a poor degradation capacity in field conditions owing to several adverse circumstances. Several methods, including enrichment culture, metagenomics, stable isotope probe and fluorescence in situ hybridization (FISH), have been used to detect and isolate bacteria capable of efficiently collapsing organic contaminants at polluted sites (Li et al., 2022). In the current study, we have employed specific selective media to isolate PAH degraders in substrates like PHE and FLU, followed by taxonomical identification. Many articles reported the presence of PAH-degrading bacteria that were isolated from industrial waste, petroleum and oil-contaminated soil sites from different regions of the world, mainly genera of *Pseudomonas*, *Acinetobacter*, *Bacillus*, *Cronobacter*, and *Enterobacter* were observed (Podsiadło and Krzysko-upicka, 2013; 2016; Tirkey et al., 2021; Telesiński and Kiepas-Kokot, 2021).

Catabolic potentials for various aromatic compounds have been postulated for several members of the genera *Pseudomonas*, *Rhodococcus*, *Mycobacterium*, *Sphingomonas*, and *Burkholderia* (Afzal et al., 2013; Lee et al., 2019). According to our research, the oil-contaminated location in Kuwait possesses a wide variety of bacteria, including *Pseudomonas*, *Burkholderia*, *Bacillus*, *Mycobacterium*, and *Bordetella*. *Burkholderia* spp. has been shown to have the ability to survive and multiply in water-/soil-based habitats with low nutrition levels (Ahn et al., 2014; Ho et al., 2011). In certain studies, it was reported that in treated soils where the composition of the bacterial community was studied using Illumina sequencing, *Burkholderia* species emerged as the predominant (Li et al., 2017; Liu et al., 2019). Taxonomical identification of various bacterial strains with PAH degrading ability helped us to perform further in-depth analysis related to their genomic characteristics. Many other species, including the above mentioned bacterial strains, have been reported to have the potential for both hydrocarbon use and heavy metal resistance (Ali et al., 2011; Rabani et al., 2020). In a recent study by Nzila et al. (2021), *Staphylococcus haemoloticus* has been shown to reach maximum growth by 6-8 days in the presence of pyrene, PHE and anthracene with a maximum count of 10^7 to 10^8 cfu/ml. However, in our study, cultures such as P14, P12, F2, P13, and P15 maintained a bacterial count of 10^6 to 10^7 cfu/ml on days 6-7.

Due to the high growth and tolerance of PHE and FLU of the *Burkholderia contaminans* strain P14, further in-depth genome analysis was performed on this organism. It has been perceived that significant growth was seen in the degradation test for toluene, aniline, kerosene, and naphthalene by *Burkholderia* sp. demonstrating tolerance of the organism to the hydrocarbons. Multiple enzymes for the breakdown of aromatic hydrocarbons, as well as three-ring fission pathways, are synthesized by *Burkholderia* (Barriault and Sylvestre, 2004; Rashid et al., 2015). Although environments are often polluted with various organic chemicals, most biodegradation research has concentrated on isolating and characterizing bacteria capable of degrading a particular component (Oberoi et al., 2015). As a result, most bioremediation efforts require either a single microbe or a consortium of microorganisms capable of degrading mixed organic pollutants. Despite the widespread presence of pollutants such as PAH and BTEX, relatively few bacterial species have been documented to possess the ability to degrade these chemicals. Naphthalene, BTEX, and aliphatic hydrocarbons degrading *Rhodococcus* and *Burkholderia* sp. were reported to have been effectively isolated. However, the genomic and biochemical properties of these strains for biodegradation were not studied (Auffret et al., 2009; Rashid et al., 2015).

Microorganisms that have recently been isolated from polluted soils may have unique catabolic capabilities and the resilience of thriving in harsh environments with a dearth of nutrients (Oves et al., 2012; Rahmeh et al., 2021). These organisms, which can also fail PAHs, can provide a cost-effective and viable solution for remediation (Kuppusamy et al., 2016). In our current analysis, the draft genome of *B. contaminans* P14 showed a larger genome size and a slight variation compared to the reference genome of *B. contaminans* M14. Different species of *Burkholderia* have been reported to have approximately 62-68% GC content with two to three chromosomes (Deng et al., 2016). Compared to the reference genome *B. contaminans* M14, our native isolate was found to have more genome size, suggesting its novelty and genetic recombination events (Deng et al., 2016). These results demonstrate the adaptability and endurance of the P14 strain in extreme conditions.

Some strains of *Burkholderia* sp. have significant biotechnological promise due to their capacity to digest chemical

contaminants, and these organisms may exploit a wide variety of aromatic chemicals for energy and carbon (Shaheen et al., 2020). The *pcaH* gene sequence from *Paraburkholderia fungorum* strain ATCC BAA-463 was found to be 100% identical to *Burkholderia fungorum* strain FM-2, which was discovered during an investigation on PHA degradation (Liu et al., 2018). The effects of various minerals on the microbial degradation of PAHs and the underlying process were recently investigated in research in *Sphingomonas* sp. GY2B was attached to different minerals (kaolinite and quartz) that promoted PHE breakdown (Gong et al., 2016). Studies on the biodegradation process have also indicated the importance of aerobic and anaerobic environments for microorganisms (Himmelberg et al., 2018; Ni et al., 2018). Syntrophic breakdown of PHE by *Mycobacterium* and *Burkholderia* was detected in a soil sample contaminated with four aromatic chemicals in a metagenomic analysis (Ohtsubo et al., 2016). In our analysis, it was observed that the genes *pcaG* and *pcaH* are responsible for PAH degradation in the P14 strain. Central aromatic intermediates were dearomatized by ring cleavage, producing TCA cycle intermediates (Ledra, 2011). In the current data set analyzed, the proteins PcaR and PcaK are used to control the expression of the *pca* operon and in the cellular transport of benzoate, respectively (Gerischer et al., 1998). Thus, it is presumed that the genes arranged in *pcaIJBHGKFR* are induced by protocatechuate. Many microbial groups are presumably possessed by benzoate-degrading genes involved in central aromatic metabolic pathways, especially Firmicutes, Actinobacteria and *Proteobacteria* (2021). Therefore, the breakdown of LMW and HMW PAHs with *Burkholderia* sp. will be efficient and environmentally friendly, as suggested by Revathy et al. (2015). The P14 strain's potential use will become apparent when further investigation is conducted into its in situ bioremediation technologies.

5 Conclusions

Our results suggest that novel microorganisms with higher catabolic activity and greater dynamism can be discovered from oil-contaminated soil samples with high pH, low salt and high polycyclic hydrocarbon concentrations. It is hypothesized that oil pollution has facilitated the enrichment of oil-metabolizing genera and the emergence of community-supporting genera that are resistant to different PAHs. In our study, we employed an efficient method to detect and reduce oil pollution by screening different PAH-degrading bacteria from a polluted soil sample. The strains screened and selected in the current analysis have the potential to serve as the core microorganisms in bioremediation. The ability of isolated bacteria *Burkholderia contaminans* P14 to grow and mineralize hydrocarbon compounds was confirmed by the whole genome sequencing technique. The presence of novel genomic island regions in P14, when compared to the reference genome of *B. contaminans* M14, suggests the strain's novelty and genetic recombination events. The KEGG analysis revealed the involvement of PAH degrading genes in *pcaGH*. These results have profound implications for future bioremediation of oil-contaminated soils, both in the domains of academic investigation and engineering application.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data.

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Table S1. Genetic features of PAH operon among *B. contaminans* strains of M14, FL-1-2-20-S1-D0 and P14

Gene locus	Protein product	Gene	Positions	Orientation	Gene length (bp)	Protein length (aa)
MS14 (CP009743.1)						
RS06060	amino acid permease		1409670..1410944	+	1275	424
RS06065	XRE family transcriptional regulator		1411107..1411403	-	297	98
RS06070	Type II toxin-antitoxin system RelE/ParE family		1411476..1411836	-	361	pseudo
RS06075	pca operon transcription factor PcaQ	pcaQ	1411947..1412933	-	987	328
RS06080	helix-turn-helix domain-containing protein		1413011..1413748	-	738	245
RS06085	YiiX/YebB-like N1pC/P60 family cysteine hydrolase		1413869..1414444	+	576	191
RS06090	Protocatechuate 3,4-dioxygenase beta	pcaH	1414719..1415426	+	308	103
RS06095	Protocatechuate 3,4-dioxygenase alpha	pcaG	1415430..1416023	+	412	138
RS06100	helix-turn-helix transcriptional regulator		1416482..1417261	-	780	259
RS06105	alpha/beta hydrolase		1417404..1418183	+	294	98
RS06110	M55 family metallopeptidase		1418222..1419049	-	828	275
RS06115	P1 family peptidase		1419046..1420128	-	1083	360
RS06120	Glutathione ABC transporter permease	gsiD	1420128..1421021	-	894	297
RS06125	Glutathione ABC transporter protein	gsiC	1421029..1421949	-	921	306
RS06130	Glutathione ABC transporter substrate binding protein	gsiB	1422029..1423591	-	1563	520
FL-1-2-30-S1-D0 (CP013390.1)						
RS23240	Glutathione ABC transporter substrate binding protein	gsiB	1329175..1330737	+	1563	520
RS23245	Glutathione ABC transporter protein	gsiC	1330817..1331737	+	921	306
RS23250	Glutathione ABC transporter protein	gsiD	1331745..1332638	+	894	297
RS23255	P1 family peptidase		1332638..1333720	+	1083	360
RS23260	M55 family metallopepidase		1333717..1334544	+	828	275
RS23265	alpha/betahydrolase		1334583..1335362	-	780	259
RS23270	helix-turn-helix transcriptional regulator		1335505..1336284	+	780	259
RS23275	Protocatechuate 3,4-dioxygenase alpha	pcaG	1336744..1337337	-	594	197
RS23280	Protocatechuate 3,4-dioxygenase beta	pcaH	1337341..1338048	-	708	235
RS23285	YiiX/YebB-like N1pC/P60 family cysteine hydrolase		1338323..1338898	-	576	191
RS23290	helix-turn-helix transcriptional regulator		1339019..1339756	+	738	245
RS23295	pca operon transcription factor	pcaQ	1339834..1340820	+	987	328
RS23300	Type II toxin-antitoxin system		1340931..1341290	+	360	119
RS23305	XRE family transcriptional regulator		1341363..1341659	+	297	98
RS23310	amino acid permease		1341822..1343096	-	1275	424
P14 (JANGYH010000005.1)						
NOX31_12660	Glutathione-binding protein GsiB	gsiB	228168..229730	+	1563	520
NOX31_12665	glutathione ABC transporter permease GsiC	gsiC	229810..230730	+	921	306
NOX31_12670	glutathione ABC transporter permease GsiD	gsiD	230744..231637	+	894	297
NOX31_12675	P1 family peptidase		231637..232719	+	1083	360
NOX31_12680	M55 family metallopeptidase		232716..233543	+	828	275
NOX31_12685	alpha/beta hydrolase		233582..234361	-	780	259
NOX31_12690	helix-turn-helix transcriptional regulator		234504..235283	+	780	259
NOX31_12695	Protocatechuate 3,4-dioxygenase alpha chain	pcaG	235742..236335	-	594	197
NOX31_12700	Protocatechuate 3,4-dioxygenase beta chain	pcaH	236339..237046	-	708	235
NOX31_12705	Hypothetical protein		237319..237894	-	576	191
NOX31_12710	helix-turn-helix domain-containing protein		238015..238752	+	738	245
NOX31_12715	pca operon transcription factor PcaQ	pcaQ	238830..239816	+	987	328
NOX31_12720	Type II toxin-antitoxin system RelE/ParE family toxin		239927..240286	+	360	119
NOX31_12725	XRE family transcriptional regulator		240359..240655	+	297	98
NOX31_12730	amino acid permease		240818..242092	-	1275	424