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Isolation and Characterization of Hydrocarbon and Petroleum Degrading Bacteria from Polluted Soil with Petroleum and Derivatives by MALDI-TOF MS Method

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ABSTRACT

Petroleum hydrocarbons are one of the most well-known environmental pollutants not only for plants, and animals but also for humans because of their carcinogenic mutagenic, toxic, and teratogenic effects. Hence, various remediation technologies have been used to overcome these environmental pollutants. Among them, bioremediation is novel promising sustainable approach for treating petroleum and petroleum derivative raw material deleterious components with their effective, low-cost eco-friendly features. In this perspective, the evaluation of soil samples was collected from areas containing petroleum and petroleum derivatives from Kazanlı and Karaduvar refinery regions in Mersin-Turkey. Hence, potential hydrocarbon and petroleum degrading bacteria were identified and characterized by the MALDI-TOF MS method as well as biochemical, physiological, and morphological tests. According to the MALDI-TOF MS analysis; members of *Bacillus*, *Stenotrophomonas*, *Enterobacter*, *Pseudomonas*, *Cupriavidus*, *Acinetobacter*, and *Escherichia* were successfully identified as hydrocarbon and petroleum degrading bacteria.

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Petroleum degrading bacteria; bioremediation; hydrocarbon; MALDI-TOF-MS

Introduction

There has been a significant increase in petroleum and petroleum derivative wastes with the growing human population and industrial revolution all over the world (Anis et al. 2013). Petroleum is an amalgam convolute of hydrocarbons, and compounds including oxygen, nitrogen, and sulfur (Angolini et al. 2015; Lješević et al. 2019; Aqeel et al. 2021).

Petroleum and petroleum derivative are raw materials that contain hazardous components, such as *n*-alkane, cycloalkane, and polycyclic aromatic hydrocarbons (PAHs) components (Lima et al. 2020). PAHs are the most well-known pollutants. So, 16 PAH has been classified as pollutants by the US Environmental Protection Agency (EPA) due to its carcinogenic, toxicity, and mutagenic effects on living things (Silva-Jiménez et al. 2018). PAHs are commonly situated worldwide and are known considerably detrimental to organic pollutants because of their carcinogenic, mutagenic, toxic, and teratogenic effects (Lješević et al. 2019, 2020; Sakshi et al. 2020). Furthermore, PAHs contaminations are potentially occurred by transport, mining, processing, storage, maintenance, leakage of storage tanks, accidental spills during oil extractions, etc. as anthropogenic based reasons which cause soil and groundwater contaminations (Abdel-Shafy and Mansour 2016; Lješevıć et al. 2019; Shi et al. 2019; Patel et al. 2020; Aqeel et al. 2021; Lahiri et al. 2021; Koolivand et al. 2022). As a result of these kinds of hazardous contaminations have not only negative impacts on the environment but also economic loss. Therefore, petroleum hydrocarbons lead to a considerable decrease in soil and water quality and health with their toxic pollutants (Sekkour et al. 2019). Thus, the polluted soils are required to be remove from PAHs contaminations because it is a critical concern urgently solve for the ecosystem.

PAHs pollution in the soil is a global environmental problem and needs to be resolved immediately. Hence, it is necessary to choose a suitable cleaning method to remove such contaminants from soil and water. For this reason, the increase in the frequency and risk of oil pollution has led to extensive studies in this area in the past decades (Dwivedi et al. 2019). Therefore, many remediation methods are currently exploited to remove petroleum pollutants in soil and water. Numerous physical, chemical, and biological applications like soil washing, encapsulation, solidification air sparging, adsorption, volatilization, photo-oxidation, chemical oxidation, emulsion breakers, photocatalytic degradation, chemical dispersants, compositing, and land farming have been utilized for PAH remediation from past decades (Dwivedi et al. 2019; Hu et al. 2020; Lima et al. 2020; Aqeel et al. 2021; Bekele et al. 2022; Koolivand et al. 2022). Among these remediation techniques, physical and chemical

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methods played a pivotal role and beneficial alteration in the environment. However, physical and chemical approaches demonstrated some restrictions because of their high-cost usage and have not shown precise destruction of contamination. Moreover, with these chemical and physical methods, PAH-based pollutants can transfer pollutants from one phase to another.

As alternative approaches, biostimulation, bioaugmentation, biodegradation, and bioremediation-based technologies have been the center of attraction by researchers due to their eco-friendly, and low-cost effective usage (Dwivedi et al. 2019; Hu et al. 2020; Aqeel et al. 2021; Bhatt et al. 2021; Gangola et al. 2022; Lin et al. 2022 Qamar et al. 2022). Among these technologies, bioremediation is a novel technique for the usage of oil-contaminated regions to understand microorganisms and how to degrade and remove petroleum organic pollutants from soil and water. Therefore, it is known as a profound and environmentally friendly technique.

Zobell presented the first study on the interaction of microorganisms with hydrocarbon in the middle of the 18th century. He described that these microorganisms, which are widely distributed in nature, use carbon as their sole energy source. He also identified that microbial carbon consumption is highly dependent on the compounds in the oil mixture and its surrounding determinants (Zobell 1946; Ronald 1981). Kline Rumpton stated the presence of microbial effects between the soil on the asphalt road in 1956, and in 1959 determined the presence of bacteria living on the Harris pipeline. It has been determined that *Pseudomonas*, *Chromobacterium*, and *Bacillus* bacteria have the capability to biodegrade asphalt. It has been observed that these microorganisms have the ability to degrade 3–25% within a week (Traxler 1962). Hence, numerous research has been led to using a wide range of microorganisms that have abilities to degrade petroleum hydrocarbons. Especially, bacteria have a huge potential to degrade, resist, and remediate pollutants like heavy metals, radioactive substances, and hydrocarbons (Ruiz et al. 2016; Horváthová et al. 2018; Sezen et al. 2020; Ruiz et al. 2021). For instance, the phylum of Proteobacteria is widely situated inside the soil and water hydrocarbon polluted regions.

On the other hand, *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes* are well-known and crucial hydrocarbon degraders. Inside these phyla, *Acinetobacter*, *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Bacillus*, *Corynebacterium*, *Flavobacterium*, *Methylomonas*, *Micrococcus*, *Nocardia*, *Pseudomonas*, etc. have known as hydrocarbon-degrading bacteria from previous studies (Yudono et al. 2010; Dwivedi et al. 2019; Ruiz et al. 2021). Plenty of bacteria exploited in this kind of research are not strains of petroleum-contaminated areas. So far; it is supposed that isolation and characterization of native bacteria from the petroleum and hydrocarbon contaminant soils could be more effective usage because of their well-adapted formation to environmental conditions (Dwivedi et al. 2019).

The mechanisms of the biodegradation treatment are complicated and determining the role of each member of a microbial population during petroleum depletion is crucial. Identification of microorganisms is an important requirement to make specific bacterial classifications (Angolini et al. 2015). With matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), the spectral fingerprint of unknown bacterial isolates is compared with a spectral database of reference strains, and identification is based on the highest rate of similarity with species or genera reference spectral proteins (Alatoom et al. 2011). Al-Kaabi et al. (2018) were reported that *Bacillus cereus* dominates the bacterial population in their study in three oil-contaminated industrial areas where air and soil conditions are extreme in terms of salinity temperature, and UV radiation; however, they reported that a small part of the population consisted of *Bacillus sonorensis* and *Pseudomonas stutzeri* species. Moreover, it was also stated that six isolates were identified by MALDI-TOF mass spectrometry and *Bacillus sonorensis* was verified using ribotyping molecular techniques. It has been noted that the results are of crucial importance in the context of application in the biodegradation of petroleum hydrocarbons (use of native isolates), especially in the Arabian Gulf.

It is clearly demonstrated that petroleum and petroleum derivatives are caused serious environmental problems all over the world. To overcome these problems, the isolation and identification of novel hydrocarbon-degrading bacteria are required as an alternative approach instead to physical and chemical methods because of their eco-friendly, reliable and effective usage.

In this regard, the current study aimed to isolate and identification of local hydrocarbon-degrading bacterial strains from petroleum contaminated soils in Kazanlı and Karaduvar refinery regions in Mersin-Turkey. Totally; twenty-three bacteria were isolated and identified from the soil samples by using the MALDI-TOF MS method and supported by biochemical, physiological, and morphological tests.

Materials and methods

Collection of soil samples

Petroleum contaminated soil samples were collected from different areas of discharge, fill, and storage regions from Kazanlı and Karaduvar refinery region in Mersin-Turkey. The samples were stored in the sterile plastic zip bag and aseptically transferred to the Microbiology Laboratory of Kı rşehir Ahi Evran University and kept at 4 °C in a refrigerator until further use.

Crude oil and reference strain

The crude oil used in the study was provided from Aliağa Tüpraş Petroleum Refinery in İzmir-Turkey. Also, *Pseudomonas aeruginosa* ATCC 27853 strain was used as a control strain in the study.

Enrichment and isolation of hydrocarbon-degrading bacteria

The enrichment and isolation of hydrocarbon-degrading bacteria were carried out by using the following procedure.

The enrichment medium contains, per liter of 1 g KNO_3, 0.2 g $MgSO_4$, 0.1 g NaCl, 0.1 g $CaCl_2$,1 g K_2HPO_4 with 10 g petroleum contaminated soil. After autoclaving, the medium was added with 1% (v/v) crude oil as the sole carbon source. Then, soil samples were incubated in the prepared 100 ml mediums for 3 days at 28 °C at 180 rpm in a shaker incubator (MAXQ 4450). Then, 10 ml of these media were taken and transferred to fresh media again, and this process was repeated twice. 0.1 ml of the last incubation samples were taken and inoculated into Petri dishes containing nutrient agar and allowed to incubate. At the end of the incubation, the growing plaques were examined with a binocular microscope (Novex P-20), and bacterial isolates exhibiting different colony structures were selected and purified.

Morphological and biochemical characterization of bacterial strains

Bacterial isolates were inoculated into Nutrient Agar (NA) medium. The bacterial colonies growing on NA medium; colony dimension, colony margin shapes, colony color and their top view, etc. were examined under a light microscope. Furthermore, Gram staining, motility, catalase, and oxidase tests were used to determine morphological, physiological, and biochemical characterizations of bacterial isolates by using conventional methods according to the Harley and Prescott (2002) method.

Identification of bacterial strains with MALDI-TOF-MS method

Identification of 23 strains was performed via their matchless peptide and protein profiles (mass spectra) which are formed by the MALDI-TOF MS device (Bruker Microflex LT, Germany). The MALDI Biotyping was performed with two separate bioinformatics software combined with the instrument, one is MALDI Biotyper (version 3.1) and the other is FlexAnalysis software (version 3.4), which is used for mass spectra studies. For microbial biomass analysis, a single colony was selected from each strain and a fine was spotted by smearing on a ground steel MSP 96-dot target (Bruker Daltonics) with the aid of a sterile wooden applicator stick. Then, all the spots were led to air dry at room temperature. Later on, 1.0 µL of a solution of the α-cyano-4-hydroxycinnamic acid matrix (HCCA matrix, Bruker) in 50% acetonitrile, 47.5% water, and 2.5% trifluoroacetic acid (Sigma-Aldrich Inc., Germany) was added at all the spots and air-dried at room temperature. Thereafter, for spectral analysis, the MSP 96-spot target was located in the MALDI-TOF MS device. The system was run using an optimized procedure for identifying microorganisms in linear positive ion mode within a mass range of 2000 to 20,000 Da. A mass spectrum exemplified by *m/z* in this range is exploited for the determination of bacterial strains based on individual mass peaks corresponding to the specific ribosomal proteins of each microorganism. To acquire each spectrum; 240 laser shots were obtained in 40-step steps from distinct fields of the sample spot and analyzed using default settings. A 60 Hz nitrogen laser at 337 nm was used as the ion source. Each sample was examined in triplicate and the highest readings were contained in the analysis. The log scale from 0.000 to 3,000 describes the level of database-to-identity matching. The microbial identification of scores was explicated according to the manufacturer's suggestions as follows: scores between 2,300 and 3,000 were 'possible species identification,' scores between 2,000 and 2,299 were 'possible species identification,' scores between 1,700 and 1,999 were 'probable genus identification,' scores below 1,699 were 'unreliable' reported as 'genus identification.' The nearest matches were ranked according to these score values, with the highest showing the highest resemblance in the mass spectra (Cheng et al. 2016; Cameron et al. 2017).

Internal quality control for MALDI-TOF MS was carried out with seven peaks (m/z, 5095.39312; 5381.28950; 6254.88327; 7273.94901; 10,297.9928; 13,681.32001; and 16,952.88117 Da) determined with a standard deviation of 58.65 and maximum peak error of 77.20 ppm.

The principle component analysis of bacteria

PCA allows generating clustered groups of spectra with similar variational properties and visualizing the differences between them. The data can be demonstrated in a 2D or 3D coordinate system. On the other hand, it is mostly sufficient to utilize 2D plotting PC1 against PC2, as it usually presents more than 80% of the whole variance between samples. In the current study, the MALDI Biotyper Compass Explorer software was used for better visualization of between reference strain and isolates. Besides, according to the manufacturer's procedure, dendogram clusters were formed for understanding the hierarchical relationship with strains by using this software. All analyzes (PCA and dendrograms) were implemented according to the standard operating procedure for the device and firmware (Samad et al. 2020).

Results

In this study, petroleum and petroleum derivative contaminated soil samples were provided from petroleum filling, discharge, and storage regions from Kazanlı and Karaduvar refinery regions in Mersin-Turkey. Following isolation steps; twenty-three bacteria were isolated successfully with obtained single colonies from petroleum contaminated soils in eight different sampling regions in Kazanlı and Karaduvar refinery. Afterward, morphological, physiological, and biochemical properties of the isolates were examined with related tests, such as cell morphologies, motility, Gram properties, oxidase, and catalase activities. Then; according to the results of morphological and biochemical tests demonstrated that 10 of 23 were shaped with smooth (S) type of colonies and 12 of 23 were rough (R) type of colonies and 1 of 23 were mucoid (M) type of colony. Thirteen of 23 the bacteria were Gram (+) and 10 of 23 were Gram (−). Moreover, the 3% KOH test was analyzed for supporting Gram properties, this test was used to confirm the Gram properties of bacteria and thus gave the same results as the Gram test. According to the

Table 1. Morphological, biochemical characteristics of bacterial isolates from polluted soil with petroleum.

Bacteria code	Colony shape morphology	Cell	Gram characteristic		Biochemical tests		Motility
			Gram reaction	%3 KOH	Catalase	Oxidase	
FH 3-5	S	Rod	+	−	+	−	+
FH 3-6	S	Rod	+	−	+	−	+
FH 5-1	S	Rod	−	+	+	+	+
FH 5-4	R	Rod	−	+	+	+	+
FH 5-5	S	Rod	−	+	−	+	+
FH 6-1	S	Rod	−	+	+	+	+
FH 7-1	R	Coccobacilli	−	+	+	+	+
FH 7-2	R	Coccobacilli	−	+	+	+	+
FH 7-3	S	Rod	−	+	−	+	+
FH 8-2	S	Rod	+	−	+	+	+
FH 8-5	R	Rod	+	−	+	+	+
FH 8-6	R	Rod	+	−	+	+	+
FH 8-7	R	Rod	+	−	+	+	+
FH 8-8	R	Rod	+	−	+	+	+
FH 8-9	R	Rod	+	−	+	+	+
FH 8-10	R	Rod	+	−	+	+	+
FH 8-11	R	Rod	+	−	+	+	+
FH 8-12	R	Coccobacilli	−	+	+	+	+
FH 17-2	S	Cocci	+	−	+	+	−
FH 18-1	S	Coccobacilli	−	+	+	+	+
FH 18-7	S	Coccobacilli	−	+	+	+	+
FH 18-8	M	Rod	+	−	+	+	+
FH 18-9	R	Rod	+	−	+	+	+

Table 2. Identified bacteria isolated from petroleum polluted soil samples by MALDI TOF MS.

PCA number	Microorganism	Score
1	*Bacillus pumilus* DSM 354 DSM	1.534
2	*Acinetobacter calcoaceticus* LMG 10518 LMG	1.722
3	*Cupriavidus necator* DSM 531 HAM	1.94
4	*Bacillus pumilus* DSM 354 DSM	1.673
5	*Pseudomonas aeruginosa* ATCC 27853 THL	2.276
6	*Cupriavidus necator* DSM 531 HAM	1.999
7	*Cupriavidus necator* DSM 531 HAM	2.085
8	*Bacillus subtilis* DSM 5552 DSM	1.928
9	*Bacillus pumilus* DSM 27T DSM	1.846
10	*Bacillus pumilus* DSM 354 DSM	1.554
11	*Escherichia coli* DH5alpha BRL	2.332
12	*Stenotrophomonas maltophilia (Pseudomonas hibiscicola)* LMG 980T HAM	2.082
13	*Enterobacter cloacae* 13159_1 CHB	1.987
14	*Bacillus subtilis* DSM 5552 DSM	1.895
15	*Stenotrophomonas maltophilia (Pseudomonas hibiscicola)* LMG 980T HAM	1.965
16	*Enterobacter cloacae* MB11506_1 CHB	2.054
17	*Bacillus pumilus* DSM 354 DSM	1.78
18	Bacillus *sonorensis* DSM 13779T DSM	1.539
19	*Bacillus pumilus* DSM 27T DSM	1.433
20	*Bacillus altitudinis* CS 809_1 BRB	1.651
21	*Bacillus subtilis* DSM 5552 DSM	1.962
22	*Bacillus pumilus* DSM 27T DSM	1.709
23	*Escherichia coli* MB11464_1 CHB	2.225

catalase and oxidase activities of 23 strains were shown only two of the strains were negative for both catalase and oxidase and all the other strains were positive in both tests. The detailed information about morphological and biochemical tests were given in Table 1. Moreover, besides morphological, and biochemical tests, one of the well-known molecular-based methods MALDI-TOF MS test system was exploited for the identification of bacterial strains from petroleum contaminated soils.

Hence; 23 strains were identified from petroleum contaminated soils by using the MALDI-TOF MS method. The results of this test system demonstrated that the potential petroleum degrading bacteria grouped in *Bacillus*, *Stenotrophomonas*, *Enterobacter*, *Pseudomonas*, *Cupriavidus*, *Acinetobacter*, and *Escherichia* as genus level.

In this study, Principal Component Analysis (PCA) was performed for twenty-three identified bacteria (Table 2). In addition, the MALDI-TOF MS spectra of 3 species of bacteria (*Bacillus subtilis*, *Cupriavidus necator*, and *Stenotrophomonas maltophilia*) with a very high capacity to break down petroleum and its derivatives were evaluated in detail. Lastly, baseline $(M+H^+)^+$ peptide peaks were evaluated for these bacteria.

A two-dimensional scattering profile was created by performing a principal component analysis of twenty-three bacteria detected at the species level (Figure 1(A)). Accordingly, *Bacillus sonorensis* (PCA no: 18; yellow spot) was separated from the other twenty-two bacteria with the highest variance (26%) (Figure 1(A)). In addition, the variance value of *Cupriavidus necator* (CN) (PCA no: 3, 6, 7) bacteria was determined as 12% compared to other bacteria (*Bacillus subtilis*

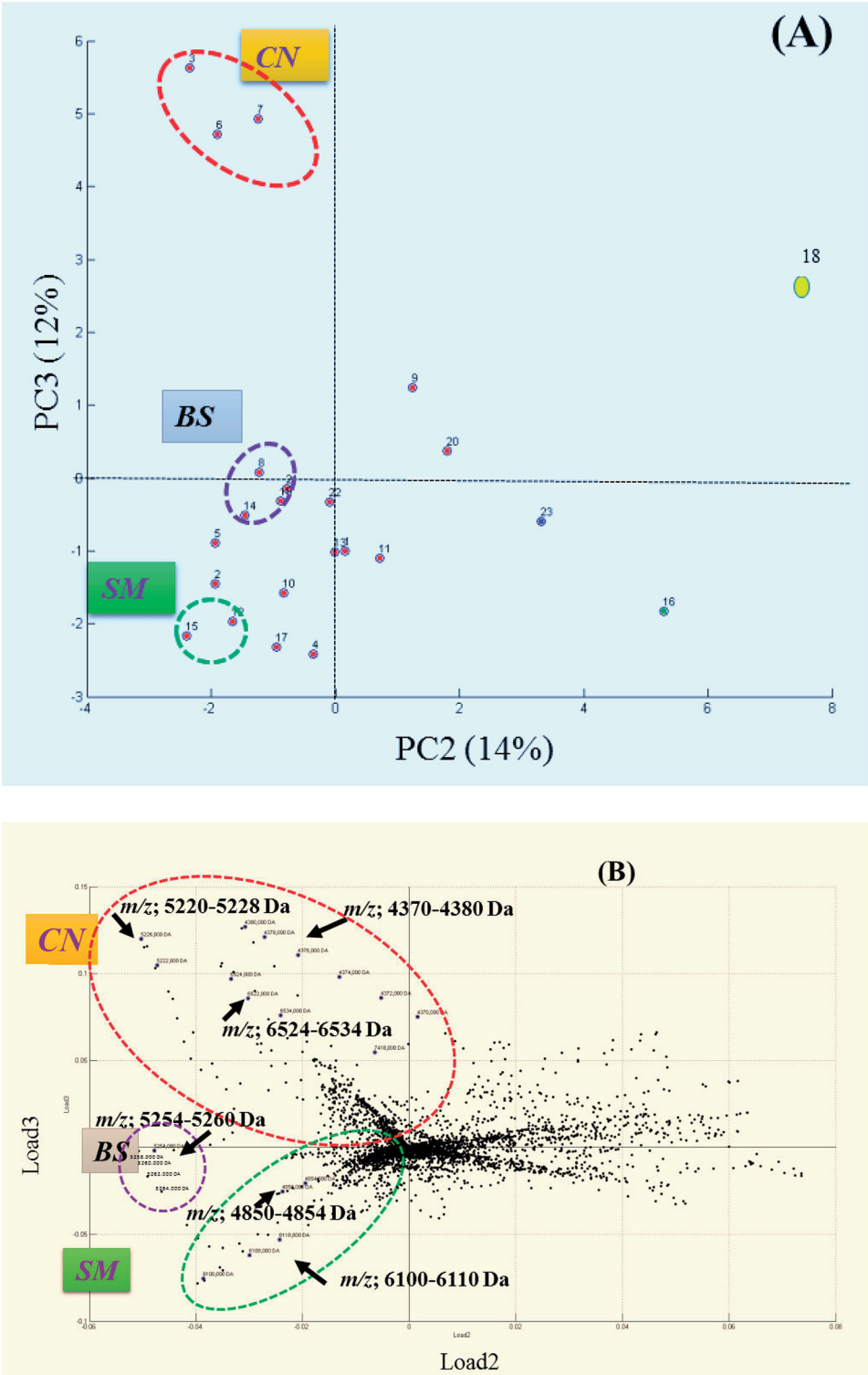

Figure 1. 2D Scatter profile (A) and spectral mass loading projections of twenty three bacteria isolated from petroleum contaminated soil. Each spot represents one spectrum in the scatter profile. The black plot represents $(M+H^+)^+$ peptide or protein peak. Both profiles were generated by PCA Figure (B).

(BS) and *Stenotrophomonas maltophilia* (SM) and the rest). The projections of $(M+H^+)^+$ mass values are seen in the 2D spectral mass loading graph (Figure 1(B)) according to the positions of these three species (*BS*, *CN*, and *SM*), which have high petroleum bio-cracking capacity, in the 2D scattering graph (Figure 1(A)). Each black dot in the graph corresponds to a $(M+H^+)^+$ peptide or protein peak and the mass values of some are given above (Figure 1(B)). It is an important graphic, especially in determining the mass values at very high intensity, namely biomarker $(M+H^+)^+$ masses.

It is seen that there are separate biomarkers $(M+H^+)^+$ masses in each of the three species (*B. subtilis, C. necator* and *S. maltophilia*). To examine this in more detail, we evaluated the spectra of all three species. Spectra of isolates identified as three *B. subtilis species* (PCA no: 8, 14, 21) are presented in Figure 2. Biomarker $(M+H^+)$ at 5254–5260 Da

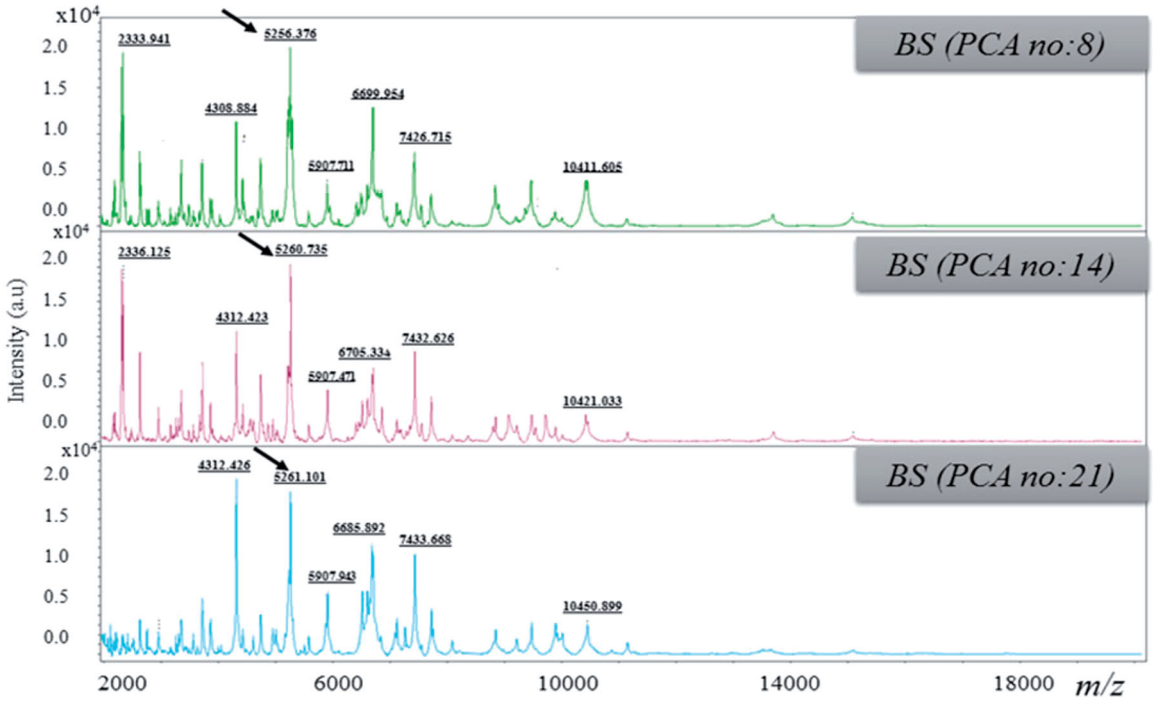

Figure 2. Representative MALDI-TOF MS spectrum of three *Bacillus subtilis* bacteria isolated from the petroleum contaminated soil.

is seen in the 2D spectral mass loading graph (Figure 1(B)). The peptide molecules appear to have the highest intensity. This is followed by the $(M+H^+)^+$ peptide molecule of 4308–4312 Da and a value common to almost all *Bacillus* sp. All three biomarker $(M+H^+)^+$ peptide molecules of *C. necator* bacteria are dominantly seen in the 2D spectral mass loading graph (Figure 1(B)). When the spectrum given in Figure 3 is examined, it is seen that these peptides (*m/z*; 5226 ± 2; 4382 ± 2; 6527 ± 3 Da) are consistently present in all three isolates (PCA no: 3, 6, 7). Both Figures 2(B) and 4 show that $(M+H^+)^+$ peptide molecules with 4856 ± 2 and 6103 ± 4 Da values are biomarker peptide molecules of *Stenotrophomonas maltophilia* bacteria.

Discussion

Microorganisms in the environment supply various roles in the ecosystem. Especially, they have a key role in the numerous biological processes, such as biodegrading various environmental contaminants, nutrient uptake of plants, biological decay in the soil, restriction to the growth of other harmful microorganisms, etc. Ribosomal proteins are the most well-known and reliable biomarkers for the identification of bacteria. The properties of ribosomal proteins include their abundance and moderate hydrophobicity to promote efficient ionization (Ashfaq et al. 2022). Promising innovations in the instrumentation facilitate fast and low-cost identification of bacteria related commonly to ribosomal MS fingerprints, leading to the ability to obtain better-resolved spectra of intact proteins in high efficiency (Cain et al. 1994; Sandrin et al. 2013). Thus, using this method, a sufficient amount of stable mass signal can be obtained for ribosomal protein peptides, typically 2000 to 20,000 Da. Mass signals are exploited to create profile spectra containing a series of conserved peaks at the genus, species, and even subspecies level (Ashfaq et al. 2022). Due to the expensive costs and time-consuming conventional methods, MALDI-TOF MS is of great interest for the routine identification of bacteria (Clark et al. 2018).

In establishing the phylogenetic identity of bacteria, MALDI-TOF MS-based principal component analysis (PCA) and hierarchical clustering of protein spectra, and identification of bacterial isolates by placing them into groups at acceptable genus and species levels will contribute to this field. For instance, Clark et al. (2018) demonstrated that hierarchical clustering of MALDI-TOF MS protein data was highly associated with 16S rRNA similarity from environmental bacteria grown in the freshwater sponge.

In the last decade, there has been a rapid increase in the identification of various bacteria from soil, plants, water, and others with the MALDI-TOF MS method. However, studies on this promising method in the diagnosis of bacteria are still not sufficient. This method was initially used solely for confirmation but over time it has been exploited for the identification of bacteria. Despite the increased interest in identifying bacteria found in soil with MALDI-TOF MS in the last five years, there are few studies on its use for studies on bioremediation (Ashfaq et al. 2022). For instance, Santos et al. (2017) identified cultivable organic-degrading bacteria (*Pseudomonas stutzeri and Acinetobacter haemolyticus*) in groundwater near natural gas extraction using MALDI-TOF MS.

According to previous studies, it is well-known that anthropogenic based factors consume about five million tons of crude oil into the environment per year. This extreme usage of petroleum and derivatives have been caused serious damage not only to humans but also to all factors related to the environment (Gogoi et al. 2003;

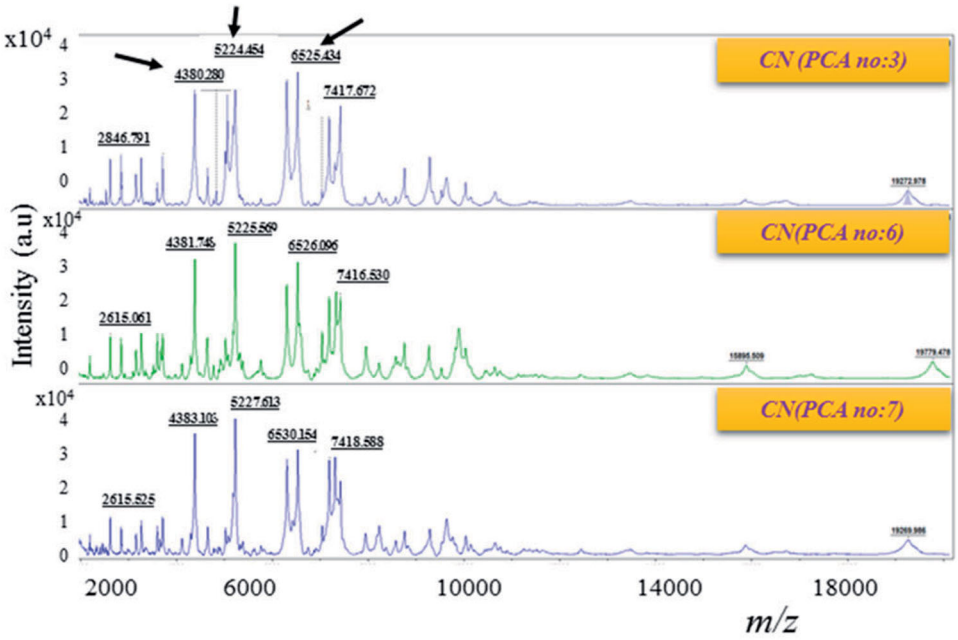

Figure 3. Representative MALDI-TOF MS spectrum of three of *Cupriavidus necator* bacteria isolated from the petroleum contaminated soil.

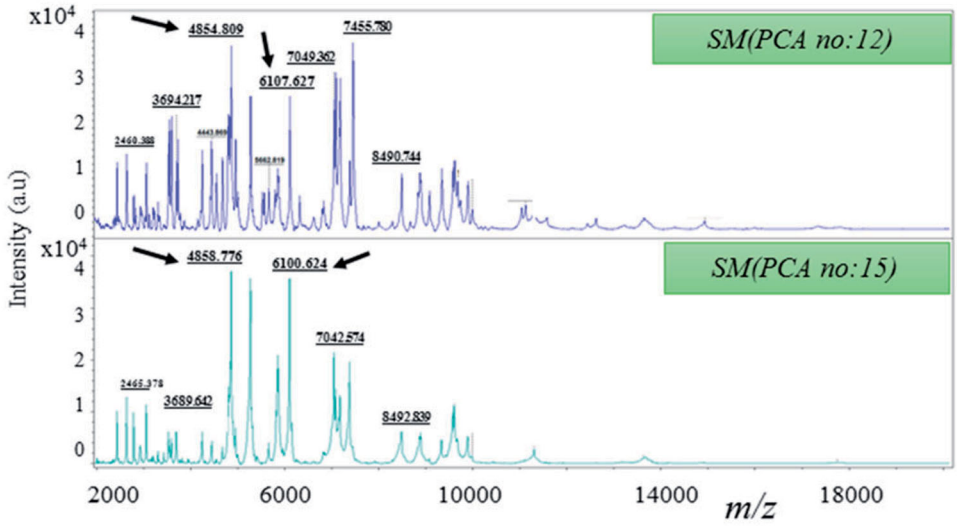

Figure 4. Representative MALDI TOF MS spectrum of two *Stenotrophomonas maltophilia* bacteria isolated from the petroleum contaminated soil.

Dwivedi et al. 2019). There are numerous risk factors caused to crude oil pollution, such as mining, tank accidents, pipeline leakage, transport, storage, etc. These factors are among the main serious reasons for water, groundwater, and soil contamination which are damage to the environment and economic loss (Xia et al. 2015; Sekkour et al. 2019; Shi et al. 2019).

Especially the pollution of the soil environment caused by oil and petroleum derivatives is among one of the most important problems that need to be solved on a global scale today. Hence, many methods and strategies have been used to decrease pollution of petroleum contaminant soils, such as chemical, physical, and biological processes (Shi et al. 2019; Lima et al. 2020; Patel et al. 2020; Rodríguez-Salazar et al. 2021).

As an alternative method to physical and chemical applications, bioremediation of petroleum and derivatives in contaminated soils has been reported as a recent, eco-friendly approach with an affordable cost which is depending on the metabolic capabilities of microorganisms to degrade soil contaminants, such as hydrocarbon degradation (Watanabe 2001; Lješević et al. 2020; Patel et al. 2020). In this regard, microbial degradation is a profitable application for using hydrocarbon degradation environments instead of physical and chemical applications. When the literature data were evaluated, we compared our findings according to the literature, it has been shown that hydrocarbon-degrading potentials of some close species belonging to the bacteria species in our study were investigated previously. For instance, Mariano et al. (2007) reported that bioremediation of diesel

oil contaminated soil from a petrol station in Brazil and they have been noted that *Staphylococcus hominis* is the predominant species in their research. In another study, *Stenotrophomonas maltophilia* was successfully used in the biodegradation of methyl tert-butyl ether (Alfonso-Gordillo et al. 2016).

A study by Ilmiah et al. (2020) has been exploited *Bacillus subtilis* to overcome environmental problems caused by fluorene contamination, which is one of the PAH compounds and causes toxic, mutagenic, and permanent effects on the environment.

Larik et al. (2019) isolated the *Stenotrophomonas maltophilia* strain 5DMD from oil-contaminated mud extracted from the Suleman exploratory oil well in Pakistan. According to their results, they demonstrated worthy potential for biodegradation of petrochemical hydrocarbons. Moreover, Faiza et al. (2019) isolated *S. maltophilia* from contaminated marine sediments and seawater in the port of Oran in northwestern Algeria. They reported that this strain can be used as an excellent environmentally friendly degrader for the biological remediation of marine environments contaminated with petroleum and petroleum hydrocarbons, effectively using high concentrations of crude oil as the sole carbon and energy source. According to a report by Lima et al. (2020), they isolated and characterized hydrocarbon-degrading numerous *Bacillus* species from gas stations leaking-contaminated groundwater in the Southern Amazon in Brazil. Shaikha et al. (2021) performed MALDI-TOF MS and PCA analyzes of indigenous Qatari bacterial strains isolated from oil-contaminated areas and revealed that the diversity among *Bacillus* subspecies was clearly detected. Like the results we obtained in our study, PCA clustering in Shaikha et al.'s (2021) study revealed great biodiversity among the strains studied at the protein level. While the distances between clusters show variations at the group level, the distance between the strains within each cluster indicates differences in protein profiles at the strain level. The approach using protein profiles can be informative in distinguishing strains of the same species (Fernández-No et al. 2013). The resolving capacity of MALDI-TOF MS is higher than 16S rRNA sequencing as it covers a wider range of proteins than the 16S ribosomal subunit. Indeed, it has been suggested by some researchers that MALDI-TOF MS can be used to characterize isolates with much higher sensitivity than 16S rRNA sequencing (Fernández-No et al. 2013; Shaikha et al. 2021).

As a result, in this study, the diversity between twenty-three bacteria with MALDI-TOF MS analysis could be explained at the genus and species level, even if not at the level of ribotyping. With MATLAB-based PCA analysis, it has been very useful in estimating common biomarkers. The fact that 16S-ribosomal peptides and proteins in the 2000–20,000 Da range unique to each bacterium are associated with representatives of their expressed genes corresponding to these masses by PCA mass values analysis will provide an important clue for further analysis. In relation to this, PCA analysis makes important contributions to revealing the close or distant relationship of bacterial isolates with each other.

This study provides evidence of the concept that the MS profiling generated by MALDI-TOF-MS could be carried out rapidly to determine *Bacillus* spp. *Cupriavidus necator*, *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., etc. isolates from the petroleum contaminated soil samples. In addition, whole-cell MALDI-TOF MS has been shown to be a useful tool for the rapid identification and classification of various strains of microorganisms.

Conclusions

In this study, twenty-three strains were successfully isolated from petroleum contaminated soils in Kazanlı and Karaduvar refinery regions in Mersin-Turkey. The identification of these strains was carried out by MALDI-TOF MS analyses as sensitive, rapid, and economical in terms of both labor and costs involved. Furthermore, the results showed that precisely bioremediation treatments fulfill a reasonable solution for petrol contaminated environment. Hence, these strains can be used in the bioremediation of petroleum-contaminated soils. Therefore, it is clearly understood that bioremediation approaches with advanced microbial identification techniques are a forceful promising sustainable strategy in the petroleum contaminated soils for further studies.

Disclosure statement

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