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Original Article

Genotypic identification of oil-degrading bacterial isolates and physicochemical characterisation of produced water from the El-Faragh gas field in Libya

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Abstract

Produced water is generated from subsurface formations during crude oil production in oil and gas fields. Produced water samples from the El-Faragh gas field in the Al-Wahat district in northeastern Libya were collected to study its bacterial community structure. Since the physicochemical properties are crucial indicators for the level of pollution in the water, selective parameters of water samples such as pH, salinity, electrical conductivity, total dissolved solids (TDS), sulphate, phosphate, carbonate, bicarbonate, hardness, heavy metals (Ba, Ni, Cr, Cu, As, Fe, Pb, Cd, Se, Sr and Zn), total petroleum hydrocarbons (TPHs), Benzene, Toluene, Ethylbenzene and Xylene (BTEX) were analysed. Radioactive contamination was also evaluated using radiological scanning devices to measure the activity of alpha (α) and beta (β) particles and gamma (ɣ) rays. In this work, for the first time, the microbial community of the El-Faragh gas field was studied based on 16S rRNA analysis using a universal bacterial oligonucleotide primer set. The results showed that the produced water analysed in the current study contained high concentrations of salts (8443 ± 42.75 mg/L of TDS), TPHs (98.5 ± 2.18 mg/L), BTEX (97.03 ± 0.2 ppm) and heavy metals (Ba 2.42 ± 0.26 ppm, Fe 79.5 ± 0.95 ppm, Se 3.49± 0.39 ppm, Cd 0.02 ± 0.002 ppm and Sr 39 ± 0.36 ppm). Two petroleum-degrading bacterial strains were isolated and were found to be closely related to *Microbacterium ureisolvens* strain CFH S00084 with 98.96% gene sequence similarity and *Exiguobacterium aquaticum* strain IMTB-3094 with 99.36% gene sequence similarity. This study recommends that further culture-independent techniques, such as whole genome sequencing are still required.

Key words: produced water; El-Faragh gas field; *M. ureisolvens* strain CFH S00084; *E. aquaticum* strain IMTB-3094; Libya.

Introduction

A considerable amount of water is trapped in the reservoir rocks and comes to the surface during oil and gas production. This water is known as brine or produced water (Chikwe and Okwa, 2016, Lin *et al.*, 2020). Produced water has a very high salt concentration and it is up to

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four times higher in salinity than seawater. This is because produced water has a high amount of mineral salts up to 300,000 mg L^{-1}, and a high level of organic and inorganic compounds, in addition to the crude oil that can be partially soluble in water (Jiménez *et al.*, 2019). The physicochemical and biological properties of produced water greatly varies, depending on the geological formation of reservoirs, geographical location of the field and the type of product being extracted, whether it is gas or oil (Al-Ghouti *et al.*, 2019). Oil reservoirs are described as deep and extreme environments (Li *et al.*, 2007). These ecosystems are well known to have low oxygen content and distinguished from others by high salinity, pressure and temperature. This harsh and undesirable condition is not suitable for most life forms; however, petroleum reservoirs house a unique and complex ecosystem of microorganisms (Lenchi *et al.*, 2013). A broad spectrum of these organisms have been isolated from the produced water obtained during oil production (Kobayashi *et al.*, 2012, Elumalai *et al.*, 2021, Alshami *et al.*, 2022). Two bacterial identification approaches, culture-dependent and culture-independent are used to identify the bacterial species isolated from produced water that has been obtained from petroleum reservoirs worldwide. Examples of these places are Pakistan (Sheikh and Nazia, 2019) , Japan (Kobayashi *et al.*, 2012), USA and Canada (Kim *et al.*, 2018), Siberia (Bonch-Osmolovskaya *et al.*, 2003), China (Zhou *et al.*, 2020a) and Iraq (Al-Tamimi and Mahdi, 2015). However, due to the drawbacks and limitations of culture-dependent approaches, the use of molecular techniques, in particular, the 16S rRNA gene sequencing technique, has allowed to do a more comprehensive characterization of the microbial communities that inhabit petroleum reservoir ecosystems (Zhou *et al.*, 2020b). The isolated extremophiles from such by-products have improved our understanding of petroleum microbiology and environmental applications. These applications include but are not limited to oil spill treatments (Grossman et al., 1999). In addition, these microbial isolates play an active role in petroleum recovery (Banat, 1995, Banat *et al.*, 2000). However, our current knowledge of bacterial community diversity of produced water remains rather limited. Despite the role of the oil industry as the major or the only source of national income of the Libyan state, the available knowledge around the microbial diversity of petroleum products and especially the produced water is extremely limited. Therefore, further insight into the oil by-products microbial communities of the oil-rich region is much needed .

The objectives of this study were to provide a physicochemical characterization of produced water from the El-Faragh Gas Field in Libya and to identify the bacterial isolates obtained from the produced water using the molecular tool of 16S rRNA gene sequence analysis.

Materials and Methods

Site description and samples collection:

This study was conducted on produced water from the El Faragh gas field, one of the master

gas fields of the Waha Oil Company (WOC), a branch of Libya's National Oil Corporation (NOC). The gas field is located 60 km southwest of the Jalu oil field in the Al-Wahat district in northeastern Libya. Produced water sample from the separator was collected on Nov 24th 2021 in Sterile Pyrex bottles in triplicates (N = 3). Sample replicates were stored at 4°C in the dark until use. Field parameters were measured before the samples were transported to the laboratory of the Libyan Petroleum Institute (LPI). Aliquots of Water samples for heavy metals (Ba, Ni, Cr, Cu, As, Fe, Pb, Cd, Se, Sr and Zn) analysis were acidified using concentrated nitric acid.

Physicochemical properties of produced water:

Physicochemical parameters of produced water including pH, electrical conductivity (EC), dissolved oxygen (DO) and carbon dioxide (CO2) were measured in the field laboratory according to standard methods (Table 1). The rest of parameters such as Bicarbonate, Carbonate, Chloride, salinity, total Hardness, Calcium Hardness, Magnesium Hardness, Calcium, Magnesium, Sodium, Sulphate, TDS, TPHs, BTEX and heavy metals, were analyzed once the samples arrived to the LPI Laboratories according to the standard methods mentioned in Table 1.

Table 1: Physicochemical analytical techniques used to analyse produced water.

Parameter	Method
EC	ASTM D-1125
pH – value @ 25 °C	ASTM D-1293
Bicarbonate	ASTM D-1067
Carbonate	ASTM D-1067
Chloride	ASTM D-516
Total Hardness	ASTM D-1126
Calcium Hardness	ASTM D-1126
Magnesium Hardness	ASTM D-1126
Calcium	ASTM D-511
Magnesium	ASTM D-511
Sodium	ASTM D-2791
Potassium	ASTM D-2791
Sulphate	DR/2500 Spectrophotometer
TDS	Calculated according to ASTM D 5907-96 a
TPHs	USA EPA 413.2
Salinity	(Calculated as NaCl (mg/L) (Cations + Anions)
DO	HI9146-04 Portable Dissolved Oxygen Meter
BTEX	5021A, analysis was performed by GC
Heavy Metals	EPA 200.7, analysis was performed by AAS ICP-OES

During the sampling, the survey for Naturally Occurring Radioactive Material (NORM), including α, β particles and ɣ rays, was conducted using the Digilert 100 Handheld Radiation Detector (Keison Products, UK) for α, β particles measurements (GREGORY and OGHENEVOVWERO, 2015) and TracercoTM T202 (Johnson Matthey Public Limited Company, UK) for ɣ rays measurements. The steps were carried out according to the manufacturer's instructions.

Bacterial isolation, purification and morphological characterization:

Bacterial cells of the produced water were collected and filtered (0.22 µm pore size) under aseptic conditions. The pure cultures of bacteria were obtained using a dilution plate technique (Sun *et al.*, 2014, Liu *et al.*, 2021). Bacterial cells on the filters were resuspended in sterile saline solution and ten-fold serially diluted up to 10-7. 100 µL of the suspension was spread onto two different media, Bushnell Haas (BH) (SKU: H05-M349-500G, Himedia, Malaysia) and Nutrient Agar medium (Oxoid CM0003B nutrient agar, Thermo Fisher Scientific, UK) and then incubated at 37°C for 48 h. Thereafter, colonies were picked and purified twice. Stock cultures of each isolate were prepared from an individual colony from the second round of purification. The gram stain technique was carried out to confirm the purity of the isolated cells. Finally, the isolated colonies were then morphologically characterized based on their colour, size, shape, and texture using microscopy analysis (BRESSER Researcher Bino 40-1000x Microscope, Germany), following the methods described by Brown and Smith (2014).

Genetic identification of bacterial isolates:

Genomic DNA extraction:

The isolated and purified colonies were carefully collected and inoculated in to 5 mL of liquid medium prepared using Accumix Nutrient Broth (Tulip Diagnostics (P) Ltd, India), then incubated over-night at 37°C under 250 rpm agitation. 1 mL of the liquid culture was used for DNA extraction using the EasyPure® Bacteria Genomic DNA Kit (TransGen Biotech, Beijing), the kit was used according to the manufacturer's instructions.

PCR amplification of 16S rRNA gene:

The 16S rRNA gene was used to identify the isolated bacteria. The target gene was amplified by polymerase chain reaction (PCR) using a universal primer set (16S-27F and 16S-1492R) (CarthaGenomics Advanced Technologies, Tunisia) as shown in Table 2. The reaction mixture for the PCR amplification contains a green master mix (Solis BioDyne, Tartu, Estonia) of 25 µL mixed with 2 µL of each forward and reverse primer (10 pmol/ µL) and 5 µL of template DNA and supplemented to a final volume of 50 µL with nuclease-free water. The PCR cycles were performed using a thermocycler (Applied Biosystems™ 2720 Thermal Cycler, UK) as follows: initial denaturation at 94°C for 2 min, denaturation, 35 cycles at 94°C for the 40s, annealing at 55°C for 30s, and 1 min of extension at 72°C. Then the cycling was completed with a final extension step at 72 °C for 10 min. Thereafter, PCR

products were checked on an agarose gel.

Table 2: Primer sequences used for PCR amplification and sequencing

Primer	Primer sequence
1492R	5'-CGGCTACCTTGTTACGACTT-3'
27F	5-'AGAGTTTGATCCTGGCTCAG-3'

Sequence of PCR products**:**

PCR products were purified using the ExoSap-It kit (Applied Biosystems, USA). Sequencing analysis was carried out on the purified PCR products using the Big Dye Terminator V3.1 mix (Applied Biosystems, USA). Sequencing reaction products were purified using the BigDyeXteminator kit (Applied Biosystems USA). Traces were then produced by capillary electrophoresis using the 3730xl DNA Analyzer (Applied Biosystems, USA). The obtained 16S rDNA sequence data was then aligned with known 16S rDNA sequences in the Gene bank database to identify the isolated bacteria. The basic local alignment search tool (BLAST), at the National Center for Biotechnology Information (NCBI) was used for alignments (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The percentage of homology scores was used to identify the bacterial isolates (Blast, 2015).

Results and discussion

Physicochemical and radioactivity analysis of produced water:

The results of the physicochemical analysis (Table 3) indicated that most parameters have higher values than the recommended values for water quality standards of United States Environmental Protection Agency regulations (USEPA, 2002) and World Health Organization (WHO) guidelines for drinking water quality (Cotruvo, 2017). The high value of EC (12990 ± 160.9 µS/cm) may be explained by the fact that it is a reflection of the concentration of the TDS (8443 ± 42.75 mg/L) in the samples. The TDS concentration was 8.4-fold higher than the permissible concentration for drinking water quality according to WHO regulations (Cotruvo, 2017). A higher concentration of TDS increases the EC. This finding is consistent with that of Joel *et al.*, (2010) who suggested that the high value of TDS could be due to suspended and dissolved solids inherent in the formation of the water samples. An observation worth mentioning from the values in Table 3 is that the pH value (4.19 ± 0.02) is considered to be lower than the accepted limits of water quality (pH 6.5-8.5) based on USEPA regulations (https://www.epa.gov). This may be due to the reaction of hydrogen ions, produced through the ionization of water under acidic conditions, with bicarbonate ions to produce carbon dioxide which in turn reacts with water to form carbonic acid, leading to a decrease in the pH value (Raven *et al.*, 2020). The reduction in pH level can affect the oil-water separation process, which could lead to its obstruction (Chikwe and Okwa, 2016). This effect was evident in the high

content of TPHs in the produced water of the El-Faragh gas field. The analysis shows that the TPHs concentration (98.5 ± 2.18 mg/L) was higher than the acceptable limit (20 mg/L) of the USEPA guidelines (USEPA, 2002).

The radioactivity survey was performed during the sampling. The screening of the total radioactivity presented in the form of α, β particles and ɣ radiation revealed that the concentrations were lower than the recommended activity concentrations of WHO (Cotruvo, 2017). Therefore, no further radioisotope-specific analysis was required.

BTEX and the low molecular weight hydrocarbons are the most common petroleum hydrocarbons abundant in produced water

Table 3: Physicochemical and radioactivity analysis of produced water (N = 3), data expressed as a mean ± SD.

Parameter	Results (Mean ± SD)	Range
EC	12990 ± 160.9 µS/cm@25ºC	13140 - 12820
pH – value @ 25 ºC	4.19 ± 0.02	4.21- 4.16
Bicarbonate	286 ± 9 mg/L	295 - 277
Carbonate	0 mg/L	-
Chloride	7232 ± 20.66 mg/L	7251 - 7210
Total Hardness	4120 ± 34.7 mg/L	4160 - 4098
Calcium Hardness	2040 ± 32.79 mg/L	2075- 2010
Magnesium Hardness	2080 ± 27.40 mg/L	2101- 2049
Calcium	816 ± 36.29 mg/L	857 -788
Magnesium	505 ± 13.23 mg/L	515 - 490
Sodium	2900 ±101.50 mg/L	3001 -2798
Potassium	39 ± 2.43 mg/L	41.8 - 37.5
Sulphate	2 ± 0.2 mg/L	2.2 – 1.8
TDS	8443 ± 42.75 mg/L	8477 - 8395
TPHs	98.5 ± 2.18 mg/L	101 - 97
Salinity	8400 ± 55.68 mg/L	8460 - 8350
DO	0.08 ± 0.008 mg/L	0.09 – 0.06
α, β Particles	0.18 ± 0.01 µSv/hr	0.19 – 0.15
ɣ rays	0 Bq/cm^2	-
Total radiation	0.1 ± 0.008 µSv/hr	0.11- 0.095

(Sheikholeslami *et al.*, 2019). Results relating to the volatile aromatic hydrocarbons of BTEX concentrations are shown in Table 4.

Five volatile organic compounds, namely benzene, toluene, ethylbenzenes, p,m-Xylene and o-Xylene exceeded the Permissible limits of

the WHO for drinking water quality (Cotruvo, 2017). These aromatic hydrocarbons significantly have an adverse effect on all biotic components of the ecosystem. For instance, Benzene has been classified as a carcinogenic agent by the USEPA (Akmirza *et al.*, 2017). Here, the benzene concentration (9.98 ± 0.24 ppm) exceeded the permissible limit for water quality (0.005 ppm) by USEPA (2002). In addition, the total BTEX content exceeded the previously recorded values (0.73 - 24.1 ppm) (Jiménez *et al.*, 2018). As regards the significant matter of environmental concern and based on the USEPA regulations, the permitted daily maximum oil and grease (O&G) for treated produced water discharge is 42 mg/L, and the monthly average limit is 29 mg/L. Thus, to meet environmental regulations as well as reuse and recycle the produced water of the El-Faragh gas field, the results of the current study place great emphasis on treating such oily-salty produced water through updating and/or maintaining the used physical, chemical, and biological techniques before discharging the water into the environment.

Table 4: Concentrations of volatile hydrocarbons (BTEX) in produced water, N = 3, data expressed as a mean ± SD.

Volatile hydrocarbon	Conc (ppm)	Range	WHO drinking water limits (ppm)
Benzene	9.98 ± 0.24	10.17 - 9.71	0.01
Toluene	18.87 ± 0.16	19 - 18.69	0.7
Ethylbenzenes	32.5 ± 0.36	32.81 - 32.11	0.3
p,m-Xylene	5.74 ± 0.39	5.98 - 5.29	-
o-Xylene	29.95 ± 0.54	30.31 - 29.33	-
Total xylenes	35.69 ± 0.39	36.1 – 35.88	0.5
Total BTEX	97.03 ± 0.2	97.22 - 96.81	1.5

Several heavy metals were detected in the produced water sample, with the findings shown in Table 5. The results of the heavy metal analysis in the produced water showed a variation in heavy metal concentrations. The results revealed a high concentration of Ba 2.42 ± 0.26 ppm, Fe 79.5 ± 0.95 ppm, Se 3.49± 0.39 ppm, Cd 0.02 ± 0.002 ppm and Sr 39 ± 0.36 ppm , which exceeded the permissible limits under the provisions of the Libyan standard specification No. 10 for the year 2008 (Ali *et al.*, 2020). The concentrations of the metals Cr 0.01 ± 0.003, Ni > 0.01, Pb < 0.03, As < 0.02 and Zn 4.45 ± 0.12 ppm were within the permissible limits of the Libyan specifications No. 10 for the year 2008 (Ali *et al.*, 2020). The concentrations of Cu (> 0.002 ppm) was lower than the detention limit of the ICP-OES instrument. This

variation in the heavy metals in the produced water can be explained by the geological formation and the age of the oil well (Igunnu and Chen, 2014).

Table 5: Heavy metals concentration in produced water (ppm) N = 3, data expressed as a mean ± SD.

Element	Conc (ppm)	Range	Libyan standards (2008) (ppm)
Ba	2.42 ± 0.26	2.64 - 2.13	1.3
Ni	< 0.01	-	0.07
Cr	0.01 ± 0.003	0.014 – 0.008	0.05
Cu	< 0.002	-	1
As	< 0.02	-	0.01
Fe	79.5 ± 0.95	80.1 - 78.4	0.3
Pb	< 0.03	-	0.01
Cd	0.02 ± 0.002	0.023 - 0.018	0.003
Se	3.49± 0.39	3.94 - 3.23	0,04
Sr	39.± 0.36	39.4 - 38.7	7
Zn	4.45 ± 0.12	4.57 - 4.33	5

Isolation and genetic identification of bacterial communities of produced water:

Although the BH medium is recommended for the enrichment of hydrocarbon-degrading microorganisms that inhabit the oil-contaminated environments, no bacterial colonies were observed using this medium after one week of incubation. A possible explanation for this might be that the use of BH medium as an isolating medium depends on the nutritional requirements and additives that bacteria prefer. In addition, the temperature, pH and incubation time can be critical for successful bacterial isolation, which means using the modified BH medium or a general-purpose nutrient medium such as nutrient agar. Two different bacterial strains were isolated from produced water (Figure 1) using the dilution plate technique. The colonies grew on Nutrient Agar plates after 24 hours of incubation under aerobic conditions. The first isolated bacterial strain is aerobic and appeared to have a convex colony morphology, non-motile, appearing smooth texture, and pale yellow to bright yellow (Figure. 1A). The colonies of the second isolate appeared motile, smooth in texture, and orange-coloured (Figure. 1B). Both isolated genera were Gram-positive and short rod-shaped bacteria. The abundance of Gram-positive bacteria in produced water ecosystem should not be surprising. This is because they have a thicker cell membrane compared to Gram-negative bacteria. The cell envelope allows Gram-positive bacteria to grow successfully in the highly variable-produced water environment, where salinity levels and TPHs are high (Zhuang *et al.,* 2003).

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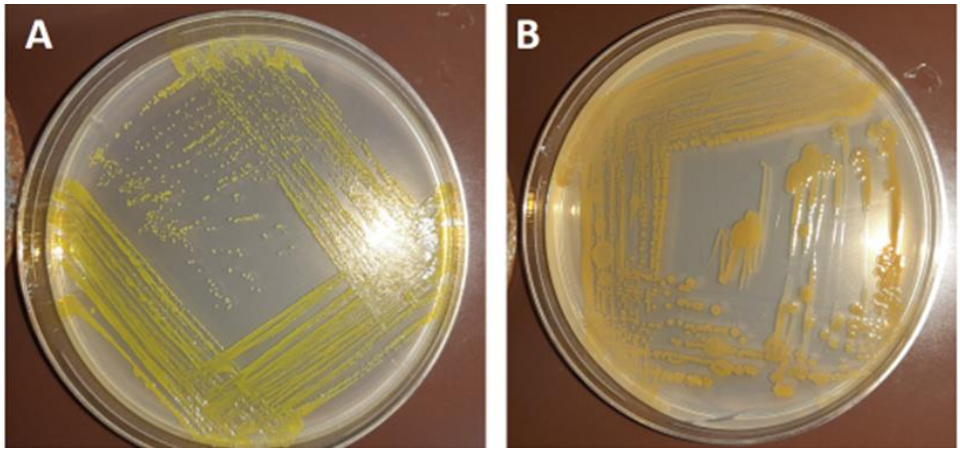

Figure 1: Appearance of the isolated colonies from produced water on solidified Nutrient Agar medium. The first and second bacterial isolates were labeled as A and B respectively.

Despite phenotypic characterisation and biochemical assays to identify the bacterial species still being applied in practice, genome analysis has become imperative and widely used for bacterial identification.

A 16S rRNA gene sequence analysis was used in this study to overcome the drawbacks of using traditional methods. The bacterial isolates were identified and characterised to the strain level. The 16S rRNA gene sequences quality shown in the Figures 2 and 3. The BLASTn search tool revealed that the bacterial isolate (A) from produced water of the El-Faragh gas field shared sequences identical with the *Microbacterium* genus, with a high degree of gene sequence identity (98.96%) to *Microbacterium ureisolvens* strain CFH S00084 (GenBank accession number NR_171452.1) (Table 6).

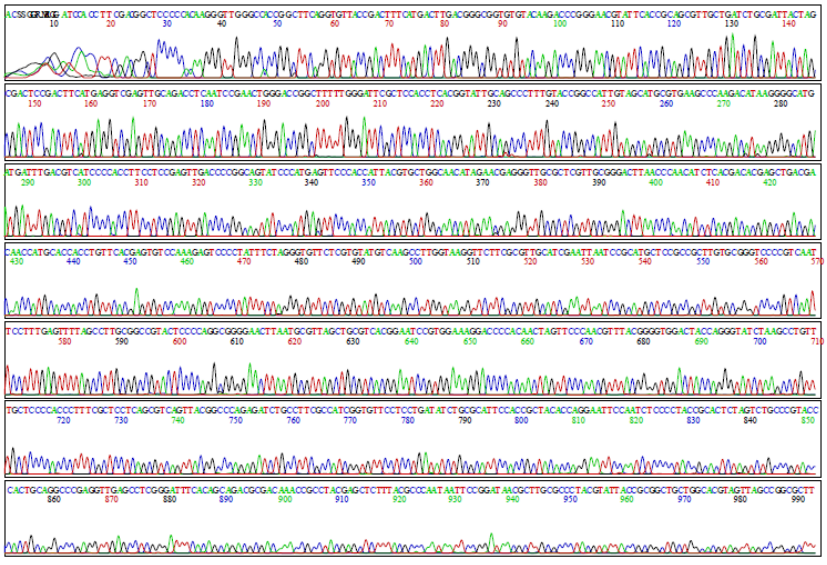

Figure 2: Chromatograms depicting 16S rRNA gene sequence quality of *M. ureisolvens*

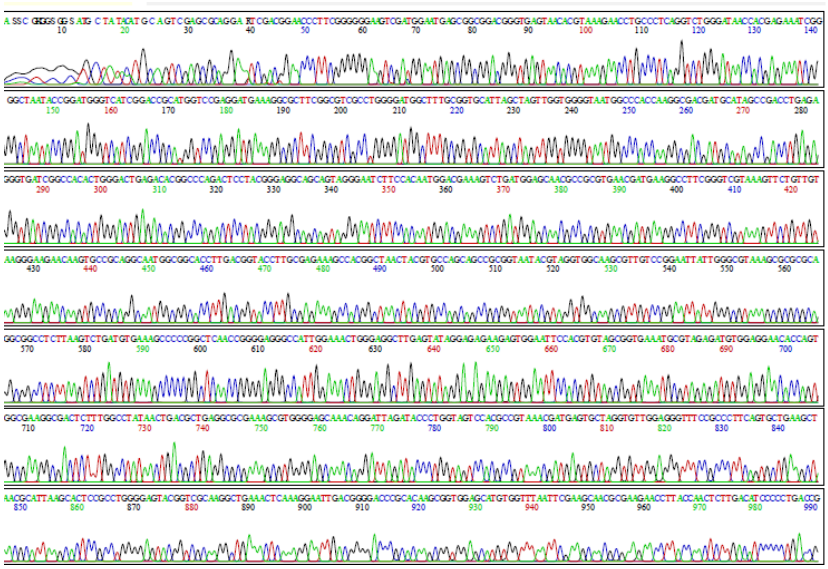

Figure 3: Chromatograms depicting 16S rRNA gene sequence quality of *E. aquaticum*

Table 6: BLASTn results of the 16 S rRNA gene sequences of the isolated bacteria

No.	Scientific Name	Max Score	Query Cover	E value	Identity %	Length bp	Accession number
A	*Microbacterium ureisolvens* strain CFH S00084	2398	100%	0	98.96%	1458	NR_171452.1
B	*Exiguobacterium aquaticum* strain IMTB-3094	2562	100%	0	99.36%	1480	NR_109413.1

The second isolate (B) was found to have 99.36 % gene sequence identity to *Exiguobacterium aquaticum* strain IMTB-3094 (GenBank accession number NR_109413.1) (Table 6). Therefore, based on 16S rRNA gene sequence analyses, the bacterial isolates can be classified into the genera of *Microbacterium* and *Exiguobacterium*. Contig cleaned sequences for both isolates are depicted in the supplementary data. Although species of the *Microbacterium* genus have not been previously isolated from produced water, they have been isolated for several times from oil-contaminated environments and characterised as crude oil-degrading bacteria using the 16S rRNA gene sequencing technique. For instance, *Mycobacterium vanbaalenii* sp. nov from contaminated soils at the Champion International Superfund Site in Libby, *Microbacterium oleivorans* sp. nov. from oil storage cavern 126 near Etzel (Germany), *Microbacterium hydrocarbonoxydans* from oil-contaminated soil in Germany (Schippers *et al.*, 2005), *Microbacterium aquimaris* from the Persian Gulf and the Caspian Sea (Hassanshahian *et al.*, 2012),

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and *Microbacterium esteraromaticum* from the Digboi oil refinery, India (Kumari *et al.*, 2018). Therefore, due to the scarcity of research relating to the microbial diversity of produced water, the results of the current study could shed some light on the microbial structure of such extreme environments. The current results with the isolation of the *E. aurantiacum* strain IMTB-3094 are consistent with Su *et al.,* (2021) which isolated *E. aurantiacum* strain SW-20 from produced water of the Changqing Oilfield, China. The study demonstrates that some genes in the *E. aurantiacum* strain SW-20 might be related to salt tolerance and oil hydrocarbon degradation, indicating that *E. aurantiacum* has doubtless potential in the bioremediation of oil pollutants. The extremophile *E. aurantiacum* that was isolated from the produced water of the El-Faragh gas field has been characterised by its ability to survive in high levels of salinity (8400 ± 55.68 mg/L), TPHs (98.5 ± 2.18 mg/L), and TBEX (97.03 ± 0.2 mg/L). It can also grow at acidic conditions (pH 4.19 ± 0.02) and is resistant to heavy metals stressors (including strontium). Therefore, these results provide further support for the hypothesis that strains of *E. aurantiacum* are petroleum-degrading bacteria.

Conclusion

According to the results of the current study, most physicochemical features of the produced water of El-Faragh gas field (low dissolved oxygen and pH, elevated concentrations of TPHs, BTEX, salinity, TDS and some heavy metals) may pose a hazard to the biota. Under this environmental stress, two oil-degrading bacteria, *E. aurantiacum* strain IMTB-3094 and *M. ureisolvens* strain CFH S00084 were isolated and identified using the 16S rRNA sequencing technique. Due to the scarcity of research on bacterial communities of the Libyan-produced water and the two isolated genera of the current study that have previously been proven as oil-degrading bacteria, they could be used as a biological technique for Libyan-produced water treatment. There is abundant room for further progress in screening the microbial diversity of produced water in Libyan oil reservoirs using more genotypic tools such as molecular typing and metagenomic analysis. These biotechnology techniques could contribute to generating the Libyan Genbank of oil systems in the future.

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Author contribution:

The authors confirm sole responsibility for the following: First and second authors study conception and design. First author, bacterial isolation and genetic work, interpretation of results, and manuscript preparation. Second author, physicochemical analysis of the samples.

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Supplementary data

Contig_clean of *M. ureisolvens* strain CFH S00084

CTTGCTGGGTGGATCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTGGACTCTGGGATAAGCGCTGGAAACGGCGTCT
AATACTGGATATGAACCACGAAGGCATCTTCAGTGGTTGGAAAGATTTTTCGGTCTGGGATGGGCTCGCGGCCTATCAGCTTGTTGGT
GAGGTAATGGCTCACCAAGGCGTCGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTC
CTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCAACGCCGCGTGAGGGATGACGGCCTTCGGGT
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CAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTGGGGAGCAAACAGGCTTAGA
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ACGCATGCTACAATGGCCGGTACAAAGGGCTGCAATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCCCAGTTCGGATTGAGG
TCTGCAACTCGACCTCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGTCTTGTACACAC
CGCCCGTCAAGTCATGAAAGTCGGTAACACCTGAAGCCGG

Contig_clean sequence of *E. aquaticum* strain IMTB-3094

TCGACGGAACCCTTCGGGGGGAAGTCGATGGAATGAGCGGCGGACGGGTGAGTAACACGTAAAGAACCTGCCCTCAGGTCTGGGAT
AACCACGAGAAATCGGGGCTAATACCGGATGGGTCATCGGACCGCATGGTCCGAGGATGAAAGGCGCTTCGGCGTCGCCTGGGGAT
GGCTTTGCGGTGCATTAGCTAGTTGGTGGGGTAATGGCCCACCAAGGCGACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACA
CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCC
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CCTTGCGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAA
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GCAGGTCAGCATACTGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGG
TGAGGTAACCTTAGGGAGCCAGCCGCCGA

التعريف الجيني للعزلات البكتيرية المحللة للنفط والتوصيف الكيميائي والفيزيائي للمياه المصاحبة لحقل غاز الفارغ في ليبيا

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المستخلص

المياه المصاحبة هى المياه التي يتم إنتاجها من التكوينات الجوفية أثناء إنتاج النفط الخام في حقول النفط والغاز. في هذه الدراسة تم جمع عينات مياه منتجة من حقل غاز الفارغ في منطقة الواحات شمال شرق ليبيا، وذلك لدراسة المجتمع البكتيري لهذه الخزانات . نظرًا لأن الخصائص الفيزيائية والكيميائية للمياه هي مؤشرات مهمة للدلالة على مستوى تلوثها ، فإن خصائص مختارة لعينات المياه تم قياسها وهي تتضمن الأس الهيدروجيني، الملوحة، التوصيل الكهربائي ، المواد الصلبة الذائبة الكلية ، الكبريتات ، الفوسفات ، الكربونات ، البيكربونات ،عسر الماء ، المعادن الثقيلة ، التركيز الكلي للهيدروكربونات البترولية، الهيدروكربونات المتطايرة، وهي البنزين ، التولوين ، بنزين والزيلين (بتكس). تم أيضًا تم تقييم التلوث الإشعاعي باستخدام أجهزة المسح الإشعاعي لقياس نشاط جسيمات ألفا (α) وبيتا (β) وأشعة جاما (γ). في هذا العمل ولأول مرة تمت دراسة المجتمع الميكروبي لحقل الفارغ، وذلك باستخدام تقنية التضخيم الجيني لجين 16 S rRNA. أظهرت النتائج أن المياه المصاحبة تحتوي على تركيزات عالية من الأملاح الذائبة الكلية (8443 ± 42.75 ملجم / لتر) ، الهيدروكربونات بترولية كلية (98.5 ± 2.18 ملجم / لتر) ، الهيدروكربونات المتطايرة بتكس (97.03 ± 0.2 جزء في المليون) والمعادن الثقيلة (باريوم2.42 ± 0.26، حديد 79.5±0.95 ، سيلينيوم 3.49±0.39، كادميوم 0.02±0.002، سترنشيوم 39±0.36 جزء في المليون). تم عزل سلالتين من البكتيريا المحللة للنفط ووجد أنهما مرتبطان إرتباطًا وثيقًا بسلالة *Microbacterium ureisolvens* CFH S00084 مع نسبة تشابه في التسلسل الجيني 98.96٪ وسلالة *Exiguobacterium aquaticum* IMTB-3094 مع نسبة تشابه في التسلسل الجيني 99.36٪. لمعرفة المزيد عن هذه المجموعات البكتيرية توصي هذه الدراسة باستخدام المزيد من التقنيات غير المعتمدة على الزراعة مثل إجراء تحليل تسلسل كامل الجينوم .

الكلمات الدالة: المياه المصاحبة، حقل غاز الفارغ ، السلالة البكتيرية *M. ureisolvens* CFH S00084 ، السلالة البكتيرية *E. aquaticum* IMTB-3094، ليبيا.

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