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Diesel Biodegradation Capacities of *Bacillus subtilis* OR632422, *Micrococcus luteus* OR632421 Isolated from Petroleum-Contaminated Soil

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BACTERIAL candidates were isolated from soil contaminated by petroleum and these isolates were evaluated for their ability to degrade diesel from which twelve bacterial isolates showed growth magnitude of ≤ 8.16 log CFU/ml. The two most promising microbial candidates (S1 and S3) showed the highest growth of 8.16 and 7.98 Log CFU/ml and were phylogenetically analyzed and uploaded in GenBank as *Bacillus subtilis* and *Micrococcus luteus* strains with GenBank accession no. OR632422 and OR632421, respectively. *B. subtilis* and *M. luteus* emulsification indices recorded were 72.22% and 65.56%, respectively. Worthy to mention that optimal growing conditions in presence of 4% diesel fuel (v/v) for both strains were achieved at pH 7, with optical densities maximum values of 1.007 and 0.85 for *B. subtilis* and *M. luteus*, respectively. After 14 days the maximum values for biodegradation percent of added diesel fuel achieved by *B. subtilis* and *M. luteus* were 70.74 and 54.63 %, respectively. Ultimately, the current study demonstrated that out of the twelve bacterial isolates only two identified strains of which *B. subtilis* was more effective in degrading diesel oil than *M. luteus*.

Keywords: Diesel fuel, biodegradation, *Bacillus subtilis* and *Micrococcus luteus*, Emulsification index (E_{24}).

1. Introduction

The most common organic environmental pollutants in the world are hydrocarbons, like diesel fuel. Major organic pollution in soils and groundwater are caused by this fuel. There are numerous reasons why hydrocarbon spills happen, including tank leaks and the disposal of leftover petroleum products. When motor vehicles, oil tanks, or ships transporting diesel oil are involved in accidents, diesel pollution of the environment arises. The soil and plant growth suffer damage by spills in agricultural regions. The aquatic living environment is impacted by spills in the marine environment (Oyewole et al., 2020) Plant development is often inhibited when diesel oil spills onto agricultural land. Reasons for the decreased plant growth in soil contaminated by diesel oil vary from direct toxic effects on plants and decreased germination to inadequate soil conditions triggered by diesel oil's displacement of air from the spaces between soil particles (Olukunle et al., 2022). The com ted fuel mixture that makes up diesel fuel includes minor amounts of sulfur, nitrogen, metals, and oxygen along with paraffin, olefin, and aromatic hydrocarbons. There are anywhere between 8 and 40 carbon atoms in each hydrocarbon molecule (Sarıkoç, 2020). Like oil, diesel differs depending on where it comes from and how it is produced. Diesel fuels can be colorless or brown in color and their many substances constitute diesel generated from petroleum, such as alkanes (42.7%), cycloalkanes (33.4%), and aromatic hydrocarbons (23.9%). Microbial action has been observed to conveniently degrade the comparatively low molecular weight n-alkanes and aromatic hydrocarbons found in diesel (Oyewole et al., 2020). Bioremediation has proven to be a suitable and efficient substitute for cleaning up hydrocarbon-contaminated sites (Lima et al., 2019). Microorganisms are a good choice for cleaning up petroleum contamination in the environment because of their potential for degradation. Microbial communities made up of bacteria, fungus, and algae are instances of biotic forces. Because Indigenous bacteria use petroleum hydrocarbons as sources of

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carbon and energy, they are able to degrade petroleum hydrocarbons (Ahmed et al., 2023). One of the greatest ways to degrade soil contaminated with diesel during the bioremediation process is to employ microorganisms (Nurza, 2023). Microbes that degrade diesel are commonly present in our surroundings. It is possible that there are not enough bacteria present for the degrading process to be successful. The capacity for degradation will be increased by inoculating contaminated areas with effective bacterial strains that degrade hydrocarbons (Kour et al., 2022). petroleum hydrocarbons degrading bacteria such as *Bacillus* , *Micrococcus*, *Mycobacterium* and *Pseudomonas* were used to eliminate hydrocarbon-polluted water and soils (Fadhil and Al Baldawi, 2020). Furthermore, it is commonly recognized that hydrocarbon-degrading bacteria also generate biosurfactants and can emulsify hydrocarbons in solution; Biosurfctants are surface-active agents that promote the surface area of hydrophobic water-insoluble substrates, so the hydrocarbon utilizing bacteria can use petroleum hydrocarbons more for its growth so the biodegradation is augmented. Bacteria that produce biosurfactants are highly efficient in degreding oil and are frequently isolated from contaminated areas (Zahed et al., 2022).

Nevertheless, hydrocarbon biodegradation in soil has been demonstrated to be controlled by a variety of parameters, including the type of microbe, nutrients, pH, temperature, moisture content, oxygen content, soil characteristics, and the number of contaminants (Nurza, 2023).

Employing microorganisms to manage environmental contamination is a promising technology that offers several benefits over other well-established traditional remediation procedures.

Therefore, it is imperative to develop practical bioremediation procedures and to ensure that petroleum materials are removed from soil ecosystems as effectively as possible. Consequently, the current study focuses on isolating and characterizing effective hydrocarbon-degrading bacteria that have sufficient capacity to degrade diesel oil.

2. Materials and Methods

2.1. Soil Sample and Diesel Collection

Near petroleum well, a sample of soil polluted with crude oil was taken in, Badr El Din Petroleum Company, Well BED-3, Western Desert, Egypt (29°51"23.23'N, 27°56'21.78"E). Also, the local gas station provided the diesel used in the present study, Giza, Egypt. Soil sample was collected from the topsoil (5–10 cm). The sample was transferred into sterile polyethylene bags, kept in an icebox, and transported to the Laboratory and stored in a refrigerator at 4°C until use. The physical and chemical analyses of the soil are displayed in (Table 1), which were conducted in laboratories of Soils, Water and Environment Res. Inst. (SWERI); Agric. Res. Center (ARC), Giza, Egypt. Chemical parameters were determined using (Reeuwijk, 2002) methodology, whereas physical characteristics were determined using (Haluschak, 2006) approach.

2.2. Bacterial isolation from polluted soil

After serially diluting the soil sample, 0.1 ml of the diluted sample was spread out over the nutrient agar plate surface, and the plates were incubated for 24 h at 37°C. Using pouring dish techniques, the resultant microbial colonies were purified before being placed into nutritional agar plates. The obtained pure colonies were kept at 4°C in nutrient agar slants for preservation.

2.3. Preparation of Bacterial Suspension

Each bacterial isolate was inoculated into 50 ml of sterile nutritional broth medium with a loopful, and was shaken for 24 h at 30 °C at 100 rpm. Centrifugation at 4000 rpm for 10 min at 4°C was then used to harvest the cells. To achieve an O.D. of 0.5 at a wavelength of 600 nm, the produced bacterial pellets were once again suspended in 10 ml of saline solution. A final concentration of 10% (v/v) was obtained by adding bacterial inoculums (Mukred et al., 2008).

2.4. Screening and Selection of the most potent isolates for produced biosurfactant and diesel degradation

For their capacity to degrade the diesel fuel, the bacterial isolates were examined, when diesel fuel was used as the sole source of carbon in their growing liquid mineral medium. The degradation investigations were carried out in mineral salt medium (MSM) which contained (g/l) K_2HPO_4 1.8 NH_4Cl 4.0 $MgSO_4.7H_2O$ 0.2 NaCl 0.1 $FeSO_4.7H_2O$ 0.01, PH adjusted to 6.90) (Zajic and Supplisson, 1972). 5 ml of each individual bacterial solution was transferred in to a 100 ml conical flask containing 50 ml of sterile mineral salts medium (MSM), 0.1% yeast extract (Mnif et al., 2014) and 1 ml of diesel fuel (2%), which served as the only carbon source.

Table 1. Physico-chemical characteristics of the petroleum contaminated soil.

Soil analysis			
Particle size distribution		**Soluble cations (cmolc L^{-1})**	
Sand %	93.24	Ca^{2+}	3.12
Silt %	1.64	Mg^{2+}	5.56
Clay %	5.12	Na^{+}	17.1
Textural class	Sandy	K^{+}	0.94
Chemical analysis		**Soluble anions (cmolc L^{-1})**	
pH	7.77	CO_3^{2-}	0.00
CaCO3 %	1.35	HCO_3^{-}	1.12
Organic carbon	0.55%	Cl-	10.9
Total O.M	0.95%	SO_4^{2-}	14.7
ECe	0.67(dS m^{-1})		
CEC	4.32 $cmol_c$ kg^{-1} soil)		
Soil physical properties:			
Bulk density (g cm^{-3})	1.54	Total porosity%	41.77
Hydraulic conductivity (cm h^{-1})	13.98	Avail. Water %	6.74
wilting point %	5.18	Soil capacity%	11.92
Available Nutrients, ppm			
N	P	K	Cu
22.05	3.54	47.33	0.39
Fe	Mn	Zn	
3.75	0.92	0.77	

As a control, flasks without any bacterial isolate were maintained. For seven days, all flasks were incubated in a shaking incubator set at 30 °C and 150 rpm. By counting the number of bacteria in liquid cultures, the degradation of diesel fuel by selected isolates was evaluated. According to Khan et al., (2006), the plate count method was used to quantify the total bacterial counts in liquid culture; the bacterial counts were expressed as log CFU/ml.

The method of (Tabatabaee et al., 2005; Techaoei et al., 2011) was used to carry out the emulsification activity. Equal parts of cell-free supernatant were mixed with 4 ml of diesel fuel, and the mixture was vortexed for 10 minutes at 500 rpm. Using the meter rule, the height of the stable emulsion layer was determined after a 24-hour period. The height of the emulsion layer divided by the total height of liquid was used to compute the emulsification index (E_{24}), which was expressed as follows:

$$E_{24} = \frac{h\ emulsion}{h\ total}$$

Where:
E_{24} is the 24-hour emulsion index,
h emulsion is the emulsion layer height,
h total is the liquid's total height

2.5. Characterization and Molecular Identification of Selected Bacterial Isolates

Morphological and biochemical characterization of the selected bacterial isolates were studied, based on Morphological, biochemical characteristics and Gram reaction. Gram staining of each bacterial isolates was performed as described by (Abubakar YU et al., 2020) Biochemical tests were carried out using standard microbiological tests using Bergey's Manual of Systematic Bacteriology (Holt et al., 1994).

The bacterial isolates (S1-S3) were cultured in a nutrient broth medium (Zimbro, 2009) and incubated for 48 hours at 28ºC before being sent to the Assiut University molecular biology research unit for DNA extraction. The Intron Biotechnology Company, Korea, supplied the patho-gene-spin DNA/RNA extraction kit, which was employed. Two universal primers were used for PCR, namely 27F (5'-AGAGTTTGATCC TGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTA CGACTT-3'). Using a size nucleotide marker (100 base pairs), the purified PCR products (amplicons) were reaffirmed using electrophoreses on 1% agarose gel. Dodeoxynucleotides (dd NTPs) were added to the reaction mixture and 27F and 1492R primers were used to sequence the purified amplicons in both the sense

and antisense directions (White et al., 1990). The National Center for Biotechnology Information (NCBI) website's Basic Local Alignment Search Tool (BLAST) was used to analyze the sequences. MegAlign (DNA Star) software version 5.05 was used to perform phylogenetic analysis on the sequences.

2.6. Effect of Different pH Values on the Growth of Selected Bacterial Isolates

Using MSM medium with pH values of 5, 6, 7, and 9, supplemented with 2% diesel, 0.1% yeast extract, and 5 ml of each bacterial suspension, the impact of various pH values on the growth of certain bacterial isolates and their biodegradation ability was established and were then shaken in an orbital incubator at 37 °C for eight days. At 600 nm, the growth patterns of selected bacterial isolates were determined at 2 days interval for 8 days.

2.7. Effect of Hydrocarbon (diesel) Concentration on B. subtilis and M. luteus Growth

The isolate's potential to degrade diesel was assessed based on an increase in microbial biomass, which was demonstrated by a rise in turbidity. The impact of diesel fuel concentrations on the growth of certain bacterial isolates and their capacity for biodegradation was assessed by the use of MSM medium supplemented with varying amounts of diesel fuel (*i.e*. 2%, 4%, 6%, 8% and 10%), 0.1% yeast extract and 5 ml of each bacterial suspension in 50 ml of MSM broth at pH 7 and 37ºC. Un-inoculated flasks with any bacterial isolate were kept as control. For eight days, all of the flasks were incubated in a shaking incubator at 30°C and 150 rpm. The growth patterns of selected bacterial isolates were determined at 600nm at 2-day intervals for 8 days.

2.8. Determination of Diesel Biodegradation Efficiency by Gravimetric Analysis

Every two days, the gravimetric analysis method was used to determine the level of diesel degradation. Every chosen bacterial strain (5 mL of 0.5 OD bacterial suspension) was cultured for 14 days at 30 °C on a rotator shaker (150 rpm) in 50 mL of MSM medium supplemented with 4% (v/v) diesel and 0.1% yeast extract for a period of 14 days, the gravimetric analysis method was used to determine the degree of diesel deterioration every two days. 50 millilitres of chloroform were used to extract the diesel from the medium (Lima et al., 2019). The residual diesel was then measured gravimetrically following the volatilization of the organic solvent (Varjani and Upasani, 2016). The residual diesel was calculated as the percentage of degradation (Jayanthi and Hemashenpagam, 2015) using the following formula as in (Ashikodi and Abu, 2019)**:**

Percentage degradation (%) =

(Initial concentration of the diesel − final concentration of diesel) / initial concentration of diesel

2.9. Statistical Analysis

Duncan's multiple range test was used to compare treatment means, and the analyses of variance approach described by (Snedecor and Cochran, 1982) was used to tabulate and analyze the data and (Duncan, 1955) level of 5% for one factor at a time (one-way analysis) using CoStat 6.45 program (CoHort software 6.311).

3. Results

3.1. Screening and Selection of the most Potent isolates for diesel degradation and Biosurfactant Production

Based on its capacity to use diesel oil, the isolated bacterial strain was screened. Twelve bacterial isolates were isolated from petroleum-polluted soil, and these isolates were evaluated for their ability to degrade diesel by measuring their growth on a mineral salt medium supplemented with 2% diesel oil. After seven days, the growth was expressed as a log of the bacterial count number (CFU/ml). Results in Fig. (1-A) showed variation in growth of 12 bacterial isolates, which recorded from 0 to 8.16 Log CFU/ml. As shown in Fig. (1-B), the isolates S1 and S3 had the greatest growth rates, at 8.16 and 7.98 Log CFU/ml, respectively. As shown in Fig. (2) variable E.I (24%) results that the bacterial isolates were screened for the potential for producing biosurfactants using the emulsification index (24%). Emulsification index results obtained in this study ranged from 0 to 72.22% S1 and S3 showed the highest values, which recorded 72.22% and 65.56%, respectively.

The bacteria produced biosurfactants, as demonstrated by the emulsification of diesel oil on a mineral growth media Fig. (2) displays the variable E.I. (24%) results for the 12 isolates, where only S1 and S3 exceeded 72.22% and 65.56%, respectively. Then the identification of the best bacterial isolates (S1 and S3) as a result of highest growth in liquid culture and the biosurfactants screening test was carried out.

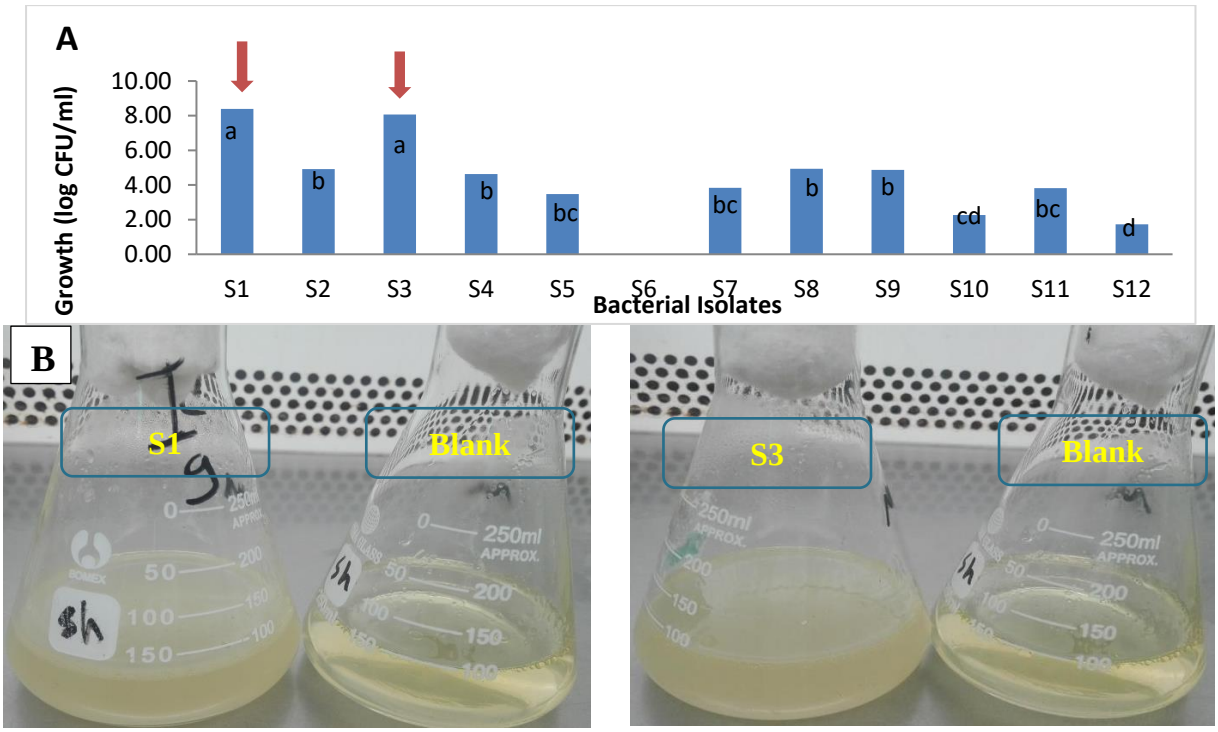

Fig. 1. (A) Bacterial count (Log CFU/ml) and (B) Growth of S1 and S3 isolates grown on MSM supplemented with 2% of diesel fuel after 7 days.

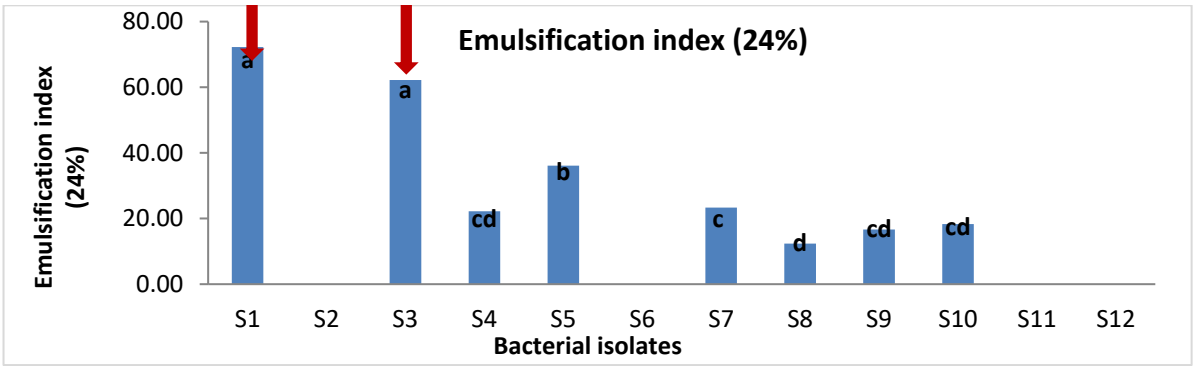

Fig. 2. Emulsification index (24%) of bacterial isolates (S1-12).

3.2. Characterization and Molecular Identification of Selected Bacterial Isolates by rRNA gene Sequencing Analysis: Morphological and biochemical characteristics of the potent bacterial isolates (S1 and S3) were presented in Table 2. Isolates S1 and S3, phylogenetic trees based on 16S rDNA sequences of the bacterial isolates as shown in Fig. (3 and 4), were identified in the present study as *Bacillus subtilis* AUMC B-530 with GenBank accession OR632422 and *Micrococcus luteus* AUMC B-529with GenBank accession OR632421 (red arrow) aligned closely related strains that were obtained from the GenBank.

Table 2. Morphological and biochemical characteristics of the potent isolates.

Test	S1	S3
Elevation	umbonate	convex
pigmentation	cream	yellow
Cell type	Rod	Cocci
Grams stain	+	+
Motility	+	-
Oxidase	+	+
Catalase	+	+
Gelatin	-	-
Urease test	-	-
Citrate test	-	+
Nitrate test	+	-
Indole	+	+

Key: (+) Positive, (-) Negative

M. luteus OR632421 showed 99.21% - 99.86% identity and 95% - 100% coverage with several strains of the same species, including the type material ATCC4698 (NR_114673), while, *B. subtilis* OR632422 showed 99.66% - 99.89% identity and 100% coverage with several strains of the same species, including the type material *B. subtilis* IAM 12118T with GenBank accession no. MG645260.

3.3. Factors Affecting Bioremediation

The growth and development of microbial cells are influenced by various of environmental conditions, both biotic and abiotic, which impact the biological processes that occurred inside a microbial community.

3.3.1. Effect of Different pH Values on the Growth of Selected Bacterial Isolates

Results in Fig. 5 (A and B) showed that both the strains of *B. subtilis* OR632422 (*B. subtilis*) and *M. luteus* OR632421 (*M. luteus*) were capable of growing in a pH range of 5 to 8.

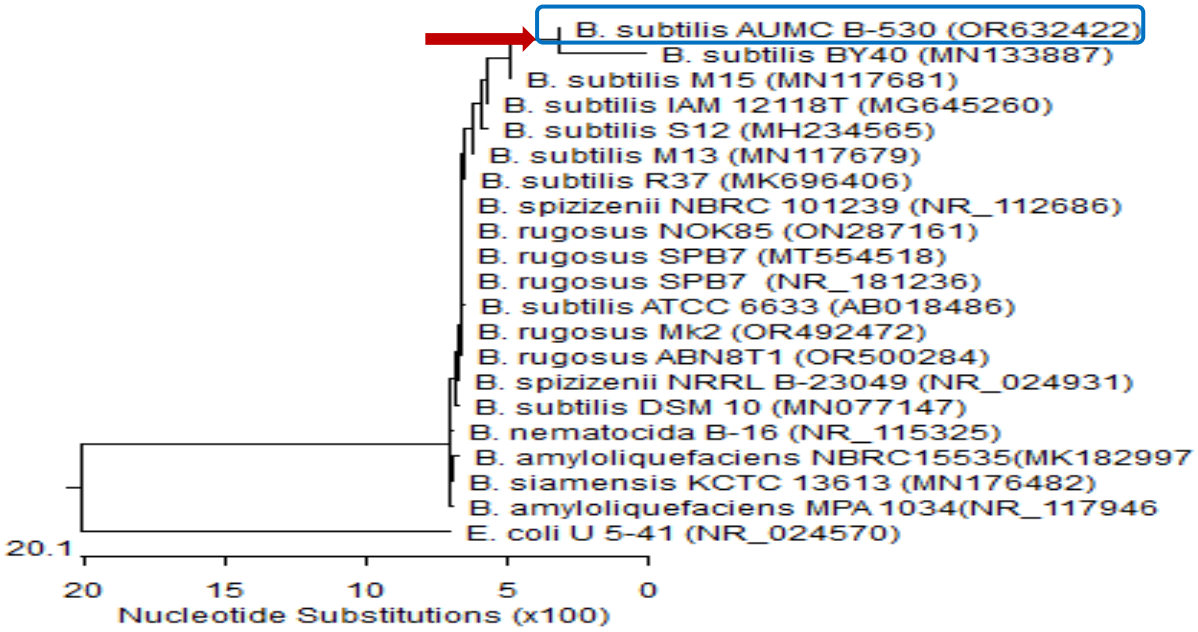

Fig. 3. Phylogenetic tree based on 16S rDNA sequences of the bacterial strain identified in the present study as *Bacillus subtilis* strain AUMC B-530, GenBank accession OR632422 (red arrow) aligned with closely related strains accessed from the GenBank. This strain showed 99.66% - 99.89% identity and 100 % coverage with several strains of the same species including the type material *B. subtilis* IAM 12118 with GenBank accession no. MG645260. *E. coli* is included in the tree as outgroup strain, *B.= Bacillus, E= Eschericia*

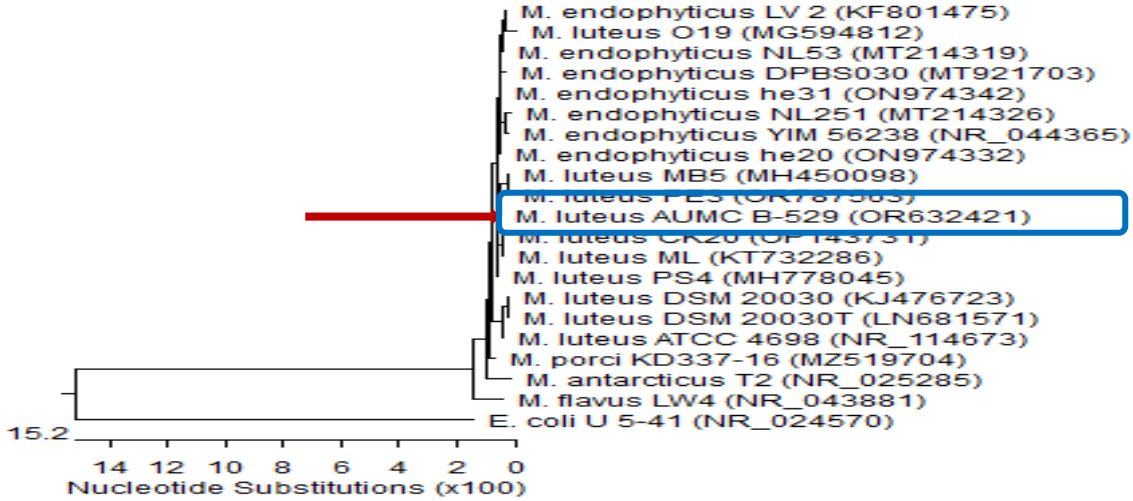

Fig. 4. Phylogenetic tree based on 16S rDNA sequences of the bacterial strain identified in the present study as *Micrococcus luteus* isolate SH-5, strain AUMC B-529 with GenBank accession OR632421 (arrowed) aligned with closely related strains accessed from the GenBank. This strain showed 99.21% - 99.86% identity and 95% - 100 % coverage with several strains of the same species including the type material ATCC4698 (NR_114673). *E. coli* is included in the tree as outgroup strain, *E= Escherichia*, M.= *Micrococcus.*

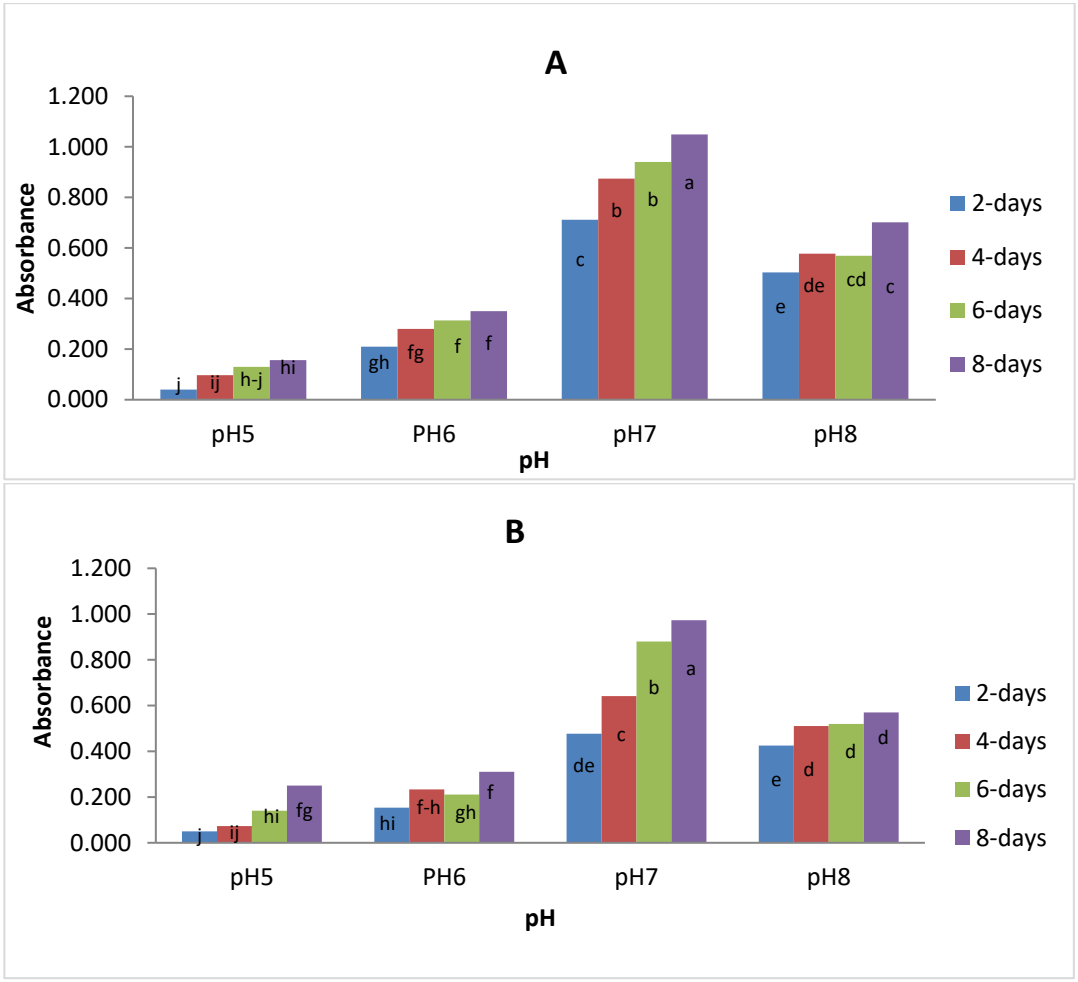

Fig. 5. Effect of pH values on *Bacillus subtilis* OR632422 (A) and *Micrococcus luteus* OR632421 (B) growth.

In addition, the maximum optical density was 1.049 and 0.973 in respective to *B. subtilis* and *M. luteus* was achieved at pH 7 after 8- days. Results also confirmed that increasing the pH range from 5 to 7 increased the optical density for the both strains, thereafter, and then this optical density decreased with the pH value 8. The minimum values of optical density were with pH 5. Finally, the data indicated that pH 7 was shown to be the optimal growth condition for both cultures and the lowest growth was observed at pH values of 5 and 6.

3.3.2 Effect of Hydrocarbon (diesel) Concentration on B. subtilis and M. luteus Growth

Data illustrated in Fig. 6 (A and B) the impact of hydrocarbon concentration on the growth of *B. subtilis* and *M. luteus* at concentrations ranging from 2 to 10%. The strain growth was measured at equal intervals over eight days. It was found that *B. subtilis* and *M. luteus* tolerated high concentrations of diesel oil and continued to grow even in 10% (v/v) of the oil present. Also, it was observed that the maximum values of optical density were 1.007 and 0.850 for *B. subtilis* and *M. luteus*, respectively in the presence of 4% (v/v) at 8 days. Diesel fuel toxicity was suggested because both cultures' growth was suppressed at concentrations higher than 8%. Meanwhile, *M. luteus* showed slight decrease in growth than *B. subtilis*. Since 4% of the hydrocarbon concentration was shown to promote optimal growth, this concentration was selected for additional study. Also, an increase in the concentration of diesel led to an increase in the growth of the bacterial species. At concentrations of diesel oil above 4%, the bacterial species did not exhibit increased growth.

3.4. Determination of Diesel Biodegradation Efficiency by Gravimetric Analysis

As shown in Fig. (7) revealed that the two organisms can degrade diesel fuel and *B. subtilis* degraded diesel fuel efficiently than *M. luteus*. The degradation rate of diesel fuel increased gradually with increasing the time of

incubation with the both strains, hence the maximum values of biodegradation percent were 70.74 and 54.63 % of added diesel fuel, respectively for *B. subtilis* and *M. luteus* after 14 days. Finally, it's noticed that the treatment with *B. subtilis* was much more efficient efficient statistically than with *M. luteu.*

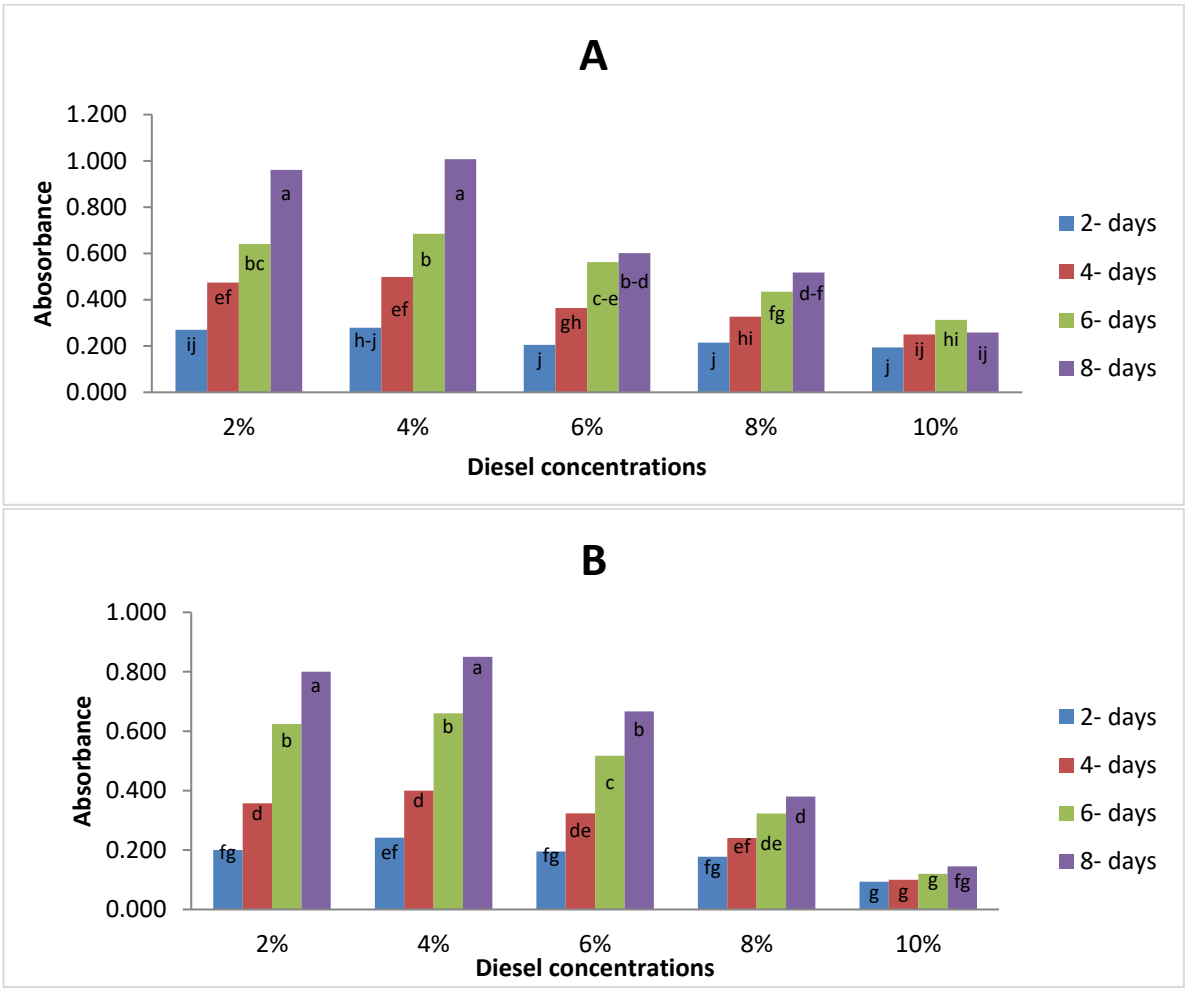

Fig. 6. Effect of different diesel Concentrations on *Bacillus subtilis* OR632422 (A) and *Micrococcus luteus* OR632421 (B) growth.

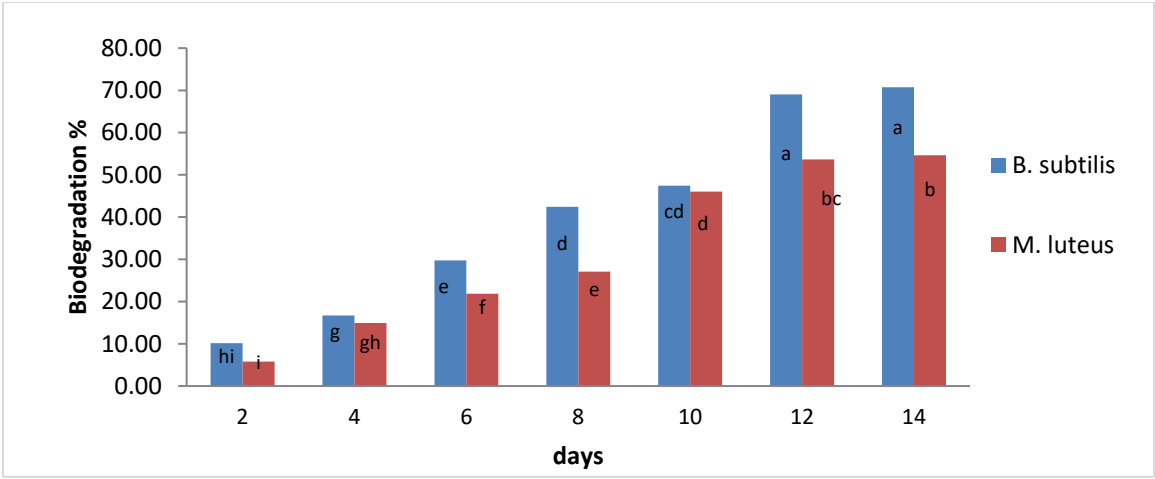

Fig. 7. Diesel biodegradation (%) of *Bacillus subtilis* OR632422 and *Micrococcus luteus* OR632421.

4. Discussion

The isolated bacterial strain was screened according to the capability to use diesel oil. It uses hydrocarbons as the sole source of carbon and degrades it to a maximum extent (Bekele et al., 2022) prior to conducting field trials, it is crucial to isolate and evaluate microorganisms for their effectiveness in utilizing hydrocarbons in the bioremediation process (Zhang et al., 2024). Ranjani et al., (2024) have documented the development of several methods for the screening of bacteria that degrade hydrocarbons, including the use of liquid media containing hydrocarbons. Imron et al., (2020) showed that many bacteria are able to use hydrocarbons as their only carbon source, which allows them to convert harmful materials into non-dangerous, biodegradable, and eco-friendly molecules. Bacteria isolated from contaminated places have a different metabolism which can make them more effective in diesel degradation.

In present studies, emulsification had crucial role in degradation of nonpolar wastes by bacteria (Hazaimeh et al., 2024; Sar et al., 2023). The bacteria produced biosurfactants, as demonstrated by the emulsification of diesel oil in the growth media (Machado et al., 2022). The majority of oil-degrading bacteria, according to Mustafa and Oktaviani, (2021), release biosurfactants to lower the water's interfacial tension and increase the oil's solubility in the water phase. Role of surfactant in bioremediation Since hydrophobic organic molecules are generally difficult to desorb and make accessible, biologically produced surfactants increase the accessibility and desorption efficiency of hydrophobic organic compounds, making organic contaminants more readily bioavailable (Ling et al., 2023). Kridi et al., (2021) found that *Micrococcus* species were considers as petroleum hydrocarbons degraders. In addition, Gaikwad et al., (2023) discovered that *M. luteus* considers as biosurfactants producers and it produces the lipopeptide biosurfactants. The current study's result was consistent with that of Maiyadi et al., (2022), who found that certain *Bacillus* species were more effective in degrading diesel oil than other soil-found bacterial isolates , so bacillus species are powerful hydrocarbon degraders (gasoline and diesel), as evidenced by their ability to utilize diesel as a source of carbon and energy for metabolism. According to Diallo et al., (2021), discovered that diesel biodegradation and microbial growth occurred at a pH of 7. Likewise, Oyewole et al., (2020) who also founded that 7 was the optimum pH for biodegradation of diesel by *B. subtilis* and *B. cereus*.

Additionally, Ahmed et al., (2023b) said that neutral pH is optimum for the growth of bacteria on diesel-contaminated soil. While, Hossain et al., (2022) revealed that the reduction of isolates occurred with increasing the diesel concentration and also, all the isolates showed their degradation efficiency in presence of 4% v/v diesel. Also, Oyewole, et al., (2020) showed that the optimum hydrocarbon concentration was 5% for *B. subtilis.*

Maiyadi et al., (2022) found that the biodegradation potential of diesel was approximately 80% when *B. subtilis* used the only source of carbon and energy. Oil hydrocarbons are biodegraded by *Bacillus* and *Micrococcus* species (Abdul-Ameer Ali, 2019; Al-Dhabaan, 2021). After 25 days, *B. subtilis* was able to degrade up to 80% of diesel. According to research, the two most efficient bacterial isolates for degrading diesel are *B. subtilis* and *Bacillus megaterium* (Maiyadi et al., 2022)

5. Conclusion

This study provided a general overview of the current situation and highlighted the prospects for bioremediation technologies that can be used to restore a polluted area more effectively in the future. In this work, the superiority of the degradation of diesel by the both efficient isolates (*B. subtilis* OR632422 and *M. luteus* OR632421) was proved. Diesel could be degraded by both strains at a pH of 7, with 4% diesel concentration resulting in the maximum degradation for the both strains. And the biodegradation potencies were about 70.74 and 54.63 % for *B. subtilis* OR632422 and *M. luteus* OR632421, respectively. The findings demonstrated that these isolates can degrade fuel and *B. subtilis* is the more effective diesel fuel degraders, than *M. luteus*. And can be applied to larger-scale bioremediation studies in soil affected by diesel.

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