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Bioremediation Potential of Cooking Oil-Degrading Microbes from Food Processing and Restaurant Wastewater

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Fat, oil, and grease (FOG) pollution from the food industry poses significant environmental and economic challenges, contributing to wastewater blockages and river contamination. Traditional physical treatments, such as precipitation and oil-water separation, are costly, labour-intensive, and generate secondary waste. Microbe-based biological treatment has emerged as a safer and more effective alternative. This study aims to isolate and characterise oil-degrading microbes from wastewater produced by food processing factories and restaurants in Malaysia. Four microbial strains—*Acinetobacter oryzae* MSMA-I1, *A. piscicola* MSMA-I2, *Candida palmioleophila* MSMA-A3, and *A. guillouiae* MSMA-A7—were identified through biochemical and molecular testing. The isolates demonstrated high growth rates and emulsification activity when incubated with 2% cooking oil as the sole carbon source for 72 hours. These strains also exhibited resilience at temperatures of 25–37 °C and pH 6–9, conditions typical of Malaysian wastewater. Gravimetric analysis showed that isolates I2, A3, and A7 achieved oil degradation rates exceeding 50%, while isolate I1 exhibited lower efficiency at 31.58%. This study provides valuable microbial candidates for the efficient bioremediation of FOG in food industry wastewater, offering a sustainable solution to environmental pollution.

Keywords: Oil-degrading microbes; wastewater; bioremediation; food industry

I. INTRODUCTION

Fat, oil, and grease (FOG) are significant pollutants in wastewater, posing substantial environmental and economic challenges globally. The formation of fatbergs, large masses of FOG, can lead to severe sewer blockages, as exemplified by the £100 million annual cost for fatberg removal in the UK (Hester, 2022). In Malaysia, FOG discharge remains a major source of river pollution, with small restaurants and eateries being the primary contributors (Zulaikha, Lau *et al.* 2014; Gurd, 2020). These establishments often dispose of FOG directly into sinks, sewer pipes, and drains, exacerbating the pollution problem.

The uncontrolled disposal of FOG can lead to water pollution, sewer overflows, stream oxygen depletion, and urban flooding, posing significant risks to aquatic ecosystems and the environment (Eljaiek-Urzola, Romero-Sierra *et al.*, 2019). In Malaysia, the cost of handling FOG in wastewater treatment is substantial, and effective management strategies are needed to reduce environmental hazards and operational costs.

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Traditional physical treatments for FOG, such as precipitation and oil-water separation, are often costly, labour-intensive, and generate secondary waste (Sena & Hicks, 2018; Xu, Wu *et al.*, 2022). Moreover, chemical treatments can have adverse health and environmental impacts. For example, aluminium sulphate, a chemical used in the coagulation and flocculation treatment of wastewater, can cause irritation to the eyes, respiratory tract, and gastrointestinal tract, and prolonged exposure has been associated with neurotoxic effects (Gao, Lu *et al.*, 2019; Zareimahmoudabady, Talebi *et al.*, 2019; Amin, Al Bazedi *et al.*, 2021). Additionally, chemical treatment costs range from $100 to $500 per cubic meter, making them significantly more expensive than biological treatment alternatives (Atemoagbo, 2024).

In contrast, microbial bioremediation offers a more efficient, cost-effective, and environmentally sustainable alternative to conventional FOG treatment methods. Studies indicate that bioremediation achieves contaminant removal rates between 85-95%, compared to 60-80% for chemical methods and only 40-70% for physical methods (Atemoagbo, 2024). Furthermore, bioremediation is the most economical option, with treatment costs ranging from $50 to $100 per cubic meter, significantly lower than chemical methods (Atemoagbo, 2024). Unlike physical and chemical techniques, which primarily remove FOG without breaking it down, bioremediation utilises microbial metabolism to degrade FOG into non-toxic byproducts, minimising secondary waste and reducing environmental impact.

Bioremediation, which utilises living organisms to degrade pollutants, offers a promising, cost-effective, and environmentally friendly alternative (He, Ni *et al.*, 2020). When combined with physical methods, such as the use of grease traps, bioremediation can effectively reduce the amount of FOG discharged into rivers or drainage systems. This approach involves the use of microbes to break down FOG into less harmful substances, making it a safe and effective solution for FOG issues in food wastewater (Kaur, Mavi *et al.*, 2019).

Despite the potential of bioremediation, there is a lack of research specifically addressing the unique composition of FOG in Malaysian wastewater and the identification of locally sourced microbes capable of efficient degradation under local conditions. This study aims to isolate and characterise oil-degrading microbes from wastewater produced by food processing factories and restaurants in Malaysia. By evaluating the growth rates, emulsification activities, and oil degradation capabilities of these isolates, this research seeks to identify potential bioremediation agents for FOG in Malaysian wastewater.

The identification of effective, locally sourced microbes for FOG bioremediation could significantly contribute to more sustainable and cost-effective wastewater management practices in Malaysia, potentially reducing environmental pollution and associated economic costs. This research addresses a critical gap in the current understanding of FOG bioremediation in the Malaysian context and has the potential to inform future wastewater treatment strategies in the region.

II. MATERIALS AND METHOD

A. Sample Collection

Wastewater samples containing fat, oil, and grease (FOG) were collected from two locations to isolate potential FOG-degrading microbes. The first sample was collected on August 7th, 2022, from a restaurant drain in Sri Serdang, Selangor, Malaysia, and the second from the food processing wastewater of Ramly Food Industries Sdn. Bhd, in Port Klang, Selangor. Samples were collected at 12:00 noon to coincide with peak wastewater flow and maximise FOG concentration. Each 25 mL sample was stored at 4 °C immediately after collection to preserve integrity and minimise changes in FOG composition.

B. Media

Mineral Salt Media (MSM), supplemented with 20 g/L bacteriological agar and 2% (v/v) cooking oil as the sole carbon source, was used for microbial cultivation (Table 1). The preparation of MSM and the Trace Element Mixture (TEM) followed the protocol described by Tian, Wang *et al.* (2018), with MSM solidified using agar for plate cultivation (Table 1 and 2).

Table 1. Ingredients for Trace Element Mixture (TEM). The composition includes essential elements for microbial growth in a controlled environment, with adjustments made to pH 7.0 before topping up with distilled water to a final volume of 1 litre.

Ingredient	Amount
$CaCl_2$	2.00 mg
$FeCl_3 \cdot 6H_2O$	50.00 mg
$MnCl_2 \cdot 4H_2O$	0.50 mg
$ZnSO_4 \cdot 7H_2O$	10.00 mg
$CuSO_4$	0.50 mg
Adjust to pH 7.0	
Top up distilled water to 1 L	

Table 2. Ingredients for Mineral Salt Medium (MSM). This medium was prepared by combining TEM and additional nutrients for microbial cultivation. The pH was adjusted to 7.0 before topping up with distilled water to a final volume of 1 litre.

Ingredient	Amount
TEM	5.00 mL
NH_4NO_3	1.00 g
$CaCl_2$	0.02 g
Mg_2SO_4	0.05 g
K_2HPO_4	1.00 g
Adjust to pH 7.0.	
Top up distilled water to 1 L	

C. Isolation and Screening of FOG-degrading Microbes

For direct isolation, a serial dilution plating method was employed to reduce microbial density and obtain well-separated colonies. Wastewater samples were diluted in sterile saline solution (0.85% NaCl) up to 10^{-6}, and 100 µL from the 10^{-3} to 10^{-6} dilutions was spread onto Mineral Salt Medium (MSM) agar plates supplemented with 2% (v/v) cooking oil as the sole carbon source. The plates were incubated at 30 °C for 72 h to allow the growth of FOG-degrading microbes. Distinct colonies were selected and subjected to three successive rounds of streak plate purification to ensure the isolation of pure cultures.

For enrichment culture, 3 mL of each wastewater sample was inoculated into 100 mL MSM broth containing 2% (v/v) cooking oil and incubated under identical conditions (30°C, 72 h, 150 rpm). After the incubation period, 1 mL of the enriched culture was transferred to fresh MSM broth for subculturing, enhancing the growth of dominant oil-degrading microbes. The enriched cultures were then streaked onto MSM agar to isolate distinct colonies.

Negative controls included MSM without inoculum and blank plates incubated under the same conditions to monitor contamination and ensure microbial specificity.

D. Screening of Effective Isolates based on Growth Rate and Emulsification Activity

The screening procedure utilised in this study was previously described by Gao, Lu *et al.* (2019). The evaluation was based on two key parameters: growth rate and emulsification activity. Growth rate was evaluated by assessing the ability of isolates to utilise palm oil as the sole carbon source, identifying isolates with enhanced growth. Additionally, the isolates' emulsification activity was measured, which reflects their ability to degrade and disperse FOG by reducing the surface tension between oil and water. Two isolates from each location (restaurant drain and food processing wastewater) exhibiting the highest growth rates and emulsification activities were selected for further study. Overnight cultures were prepared in LB broth, and 1 mL of the log-phase culture (OD 600 nm = 0.5-0.8) was transferred into 250 mL sterile conical flasks containing 100 mL of MSM broth. Control flasks included one with cooking oil (CO) and another without CO. Cultures were incubated at 30 °C and 150 rpm for 72 hours, during which the absorbance at OD 595 nm was recorded to monitor growth. For emulsification activity assessment, log-phase cultures (OD 600 nm = 0.5-0.8) were similarly transferred into sterile conical flasks containing 100 mL of MSM broth. After 72 hours of incubation, 1 mL of each isolate was centrifuged at 8000 × *g* for 10 minutes, and 100 mL of supernatant was transferred to a bottle containing 5.2 mL of potassium phosphate buffer (pH 7.0) and 0.2 mL of cooking oil. The mixture was vigorously shaken for 2 minutes and left to rest for 5 minutes at room temperature.

Emulsification activity was measured by recording absorbance at OD 540 nm.

E. Identification of Isolates

1. Morphological and biochemical tests

Four selected isolates underwent morphological observation and various biochemical tests, including Gram staining, hydrogen peroxide test, oxidase test, methyl red test, and lipid hydrolysis test. For the hydrogen peroxide test, overnight cultures were grown on LB agar, and hydrogen peroxide was added to observe bubble formation. *E. coli* served as a positive control. The oxidase test involved applying an oxidase strip to LB agar-grown isolates. A purple colour change indicated a positive result for cytochrome c oxidase. The methyl red test was performed using MR-VP broth, with a colour change after the addition of methyl red indicating a positive result for mixed acid fermentation. Lipase production was tested using 1% tributyrin agar, with clear zones around isolates indicating positive lipase activity. *Staphylococcus aureus* was the positive control, and *E. coli* was the negative control. All tests were performed in triplicate to ensure accuracy.

2. DNA extraction and sequencing of isolates

DNA extraction was performed using the colony PCR method as described by Pereira, Silva *et al.* (2023) . A 200 µL aliquot of the overnight culture was centrifuged at 8000 × *g* for 5 minutes, and the cell pellet was resuspended in 20 mM sodium hydroxide. The suspension was heated at 100 °C for 8 minutes before undergoing a final centrifugation. For the PCR process, the 16S rRNA gene was amplified using two primers: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The ITS gene was amplified using two primers: TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′). The total PCR volume was 50 µL, consisting of 25 µL of DreamTaq Green PCR Master Mix, 15 µL of ultrapure water, 1 µM of the 27F primer, 1 µM of the 1492R primer, and 6 µL of genomic DNA template. The amplification process involved 30 cycles under the following conditions: initial denaturation at 95 °C for 2 minutes, denaturation at 95 °C for 30 seconds, annealing at 53 °C for 30 seconds, extension at 72 °C for 1 minute, and a final extension at 72 °C for 7 minutes. The amplification of the 16S rRNA and ITS genes was confirmed by loading 1 µL of the PCR product onto a 1% agarose gel for electrophoresis. Successful amplification was indicated by the presence of a PCR product of approximately 1500 base pairs for the 16S rRNA gene and 550 base pairs for the ITS gene. Phylogenetic analysis was conducted to construct a phylogenetic tree based on reference sequences obtained from the National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). These sequences were then imported into MEGA11 software for further analysis and tree construction. The microbial sequences were subsequently submitted to the GenBank database, where they were assigned accession numbers.

F. Influence of pH and Temperature on the Degradation of FOG

To evaluate the influence of pH on FOG degradation, 100 µL of an overnight culture was inoculated into four separate tubes, each containing 10 mL of LB broth adjusted to distinct pH levels: 3, 6, 7, and 9. The tubes were incubated at 30 °C for 48 hours. For the temperature study, the overnight culture was streaked onto LB agar plates and incubated at four different temperatures: 4 °C, 27 °C, 37 °C, and 60 °C for 24 hours. Growth was visually evaluated and categorised using the following scale: (1+) for scant growth, (2+) for moderate growth, (3+) for abundant growth, and (-) for no detectable growth. All experiments were performed in triplicate to ensure reproducibility and statistical reliability.

G. Evaluation of the Oil-degrading Ability of Microbes through Gravimetric Analysis

The oil-degrading ability of the microbial isolates was evaluated using the gravimetric method, as described by Gao, Lu *et al.* (2019) and Ke, Hua *et al.* (2021). A 3 mL aliquot of the log-phase culture from each isolate was transferred into 100 mL of Mineral Salt Medium (MSM) in a 250 mL conical flask. The flasks were then incubated at 30 °C for 72 hours at 150 rpm. After the incubation period, the cultures were centrifuged at 8000 × g for 10 minutes. The supernatant was carefully discarded, and the cell pellet was washed twice with 0.85% sodium chloride solution to remove any residual media components. Subsequently, 1 mL of the washed cell suspension was transferred into 100 mL of fresh MSM broth. The cultures were incubated again at 30 °C for an additional 72 hours at 150 rpm to promote further oil degradation. After this second incubation, the cultures were centrifuged again at 8000 × g for 10 minutes, and the supernatant was collected. A 25 mL volume of *n*-hexane was added to a separating funnel, and the supernatant was vigorously shaken. The mixture was allowed to settle for 20 minutes, after which the organic layer containing the extracted oil was carefully collected. The extracted oil was transferred to a pre-weighed container and dried in a fume hood for **1–2 days** to remove any residual n-hexane. The dry weight of the remaining oil was measured using an electronic balance. The percentage of oil degradation was calculated using the following formula:

$$\text{Oil degradation percentage} = \frac{A-B}{A} \times 100\%$$

where A is the initial weight of the oil (g) used in the MSM broth as the carbon source, and B is the weight of the remaining oil after the biodegradation process.

H. Statistical Analysis

All statistical analyses were performed using GraphPad Prism 10 software (GraphPad Software, San Diego, CA, USA). One-way analysis of variance (ANOVA) was used to determine significant differences among treatments. Tukey's Honest Significant Difference (HSD) test was applied as a post-hoc analysis to identify pairwise differences between groups. Results are presented as mean ± standard deviation (SD) of three independent replicates. Different superscript letters indicate statistically significant differences at $p < 0.05$.

III. RESULT AND DISCUSSION

A. Isolation of Potential FOG-degrading Microbes from Food Processing Wastewater and Restaurant Drains

In this study, we successfully isolated three pure isolates (I1, I2, and I3) from food processing wastewater and seven isolates (A1, A2, A3, A4, A5, A6, and A7) from restaurant drainage. The morphological characteristics of these isolates on Mineral Salt Media (MSM) agar are presented in Table 3. MSM was selected as the isolation medium due to its minimalistic composition, providing only essential nutrients for microbial growth while lacking organic carbon sources. Palm oil, commonly used in Malaysian cooking, was introduced as the sole carbon source and selective agent, ensuring that only microbes capable of metabolising oil would proliferate.

Table 3. Cultural characteristics of isolates on Mineral Salt Media (MSM) agar. The table summarises the colony morphology of microbial isolates sourced from food processing wastewater and restaurant drainage.

Isolate	Source	Morphology on agar
I1	Food processing wastewater	White, convex, circular
I2	Food processing wastewater	Slightly translucent, wavy margin
I3	Food processing wastewater	Raised elevation
A1	Restaurant drainage	Yellow, entire, circular form, convex
A2	Restaurant drainage	White, entire, circular form, convex
A3	Restaurant drainage	Circular, small, entire, convex, white
A4	Restaurant drainage	Big colony, white, entire, circular form, convex
A5	Restaurant drainage	Small colony, white, entire, circular form, flat
A6	Restaurant drainage	White, moderate, entire, circular, flat
A7	Restaurant drainage	Moderate, entire, flat, white, circular form

The isolation of FOG-degrading microbes from these sources aligns with previous research findings (Gao, Lu *et al.*, 2019; Md Badrul Hisham, Ibrahim *et al.*, 2019; Ren, Fan *et al.*, 2020). For instance, Gao, Lu *et al.* (2019) isolated eight bacteria from restaurant and food processing industry wastewater, all demonstrating high oil degradation capacity. Similarly, Ren, Fan *et al.* (2020) reported the isolation of a novel oil-degrading bacterium, *Klebsiella quasivariicola* IUMR-B53, from kitchen wastewater, which exhibited over 95% oil degradation capability. Furthermore, Md Badrul Hisham, Ibrahim *et al.* (2019) identified *Bacillus* sp. HIP3, isolated from used cooking oil (palm oil), as a potential bioremediation agent for heavy metal removal.

B. Screening of Effective Microbes based on Growth and Emulsification Activity

All 10 isolates were assessed to determine the most effective FOG-degrading microbes. Two isolates from each sampling location were selected based on superior growth rate and emulsification activity. Growth rate was considered a crucial factor as microbes capable of utilising FOG components as nutrient sources tend to exhibit faster growth rates. This ability allows them to outcompete other species and establish dominance in the environment, making them more effective in FOG degradation. Emulsification capability, the second test, targeted the production of biosurfactants by the microbes. Effective FOG-degrading microbes should possess the ability to produce biosurfactants, which can break down hydrophobic compounds in FOG, enhancing their degradation and increasing bioavailability (Adrion, Nakamura *et al.*, 2016).

Isolates I1 and I2, derived from food processing wastewater, exhibited the highest growth rate and emulsification activity after 72 h of incubation (Figure 1). I1 showed absorbance values of 0.70 and 0.75 for growth and emulsification, respectively, while I2 displayed values of 0.32 and 0.42. From the restaurant drainage samples, isolates A3 and A7 showed the most promising results, with A3 displaying a growth absorbance of 1.94 and an emulsification absorbance of 0.50, and A7 exhibiting values of 1.54 and 0.51, respectively.

These findings align with those of Gao, Lu *et al.* (2019), who found that three out of five isolates exhibited significant growth and emulsification activity, suggesting the potential of these bacteria in oil degradation. Similarly, Ahmed, Ahmad *et al.* (2022) observed increased growth of *Burkholderia vietnamiensis* AQ5-12 and *Burkholderia* sp. AQ5-13 in the presence of oil, indicating their potential to utilise oil as a carbon source.

C. Morphological, Biochemical and Molecular Identification of Isolates

The four isolates (I1, I2, A3, and A7) underwent morphological and biochemical characterisation as summarised in Table 4. Microscopically, I1, I2, and A7 were identified as Gram-negative coccobacilli, while A3 was Gram-positive. All isolates, except for I2, were catalase-positive, and all but A3 tested negative for oxidase activity. The methyl-red test yielded negative results for all isolates, and starch hydrolysis was observed only for A3.

Table 4. Morphological and biochemical tests of selected isolates. The table lists the Gram staining, cell shape, and biochemical test results, including catalase, oxidase, methyl-red, starch hydrolysis, and lipid hydrolysis tests, for the selected isolates.

Test	**Isolate**			
	I1	I2	A3	A7
Gram staining	Gram-negative	Gram-negative	Gram-positive	Gram-negative
Cell Shape	Coccobacilli	Coccobacilli	Coccobacilli	Coccobacilli
Catalase test	+	-	+	+
Oxidase test	-	-	+	-
Methyl-red test	-	-	-	-
Starch hydrolysis test	-	-	+	-
Lipid hydrolysis test	+	-	+	-

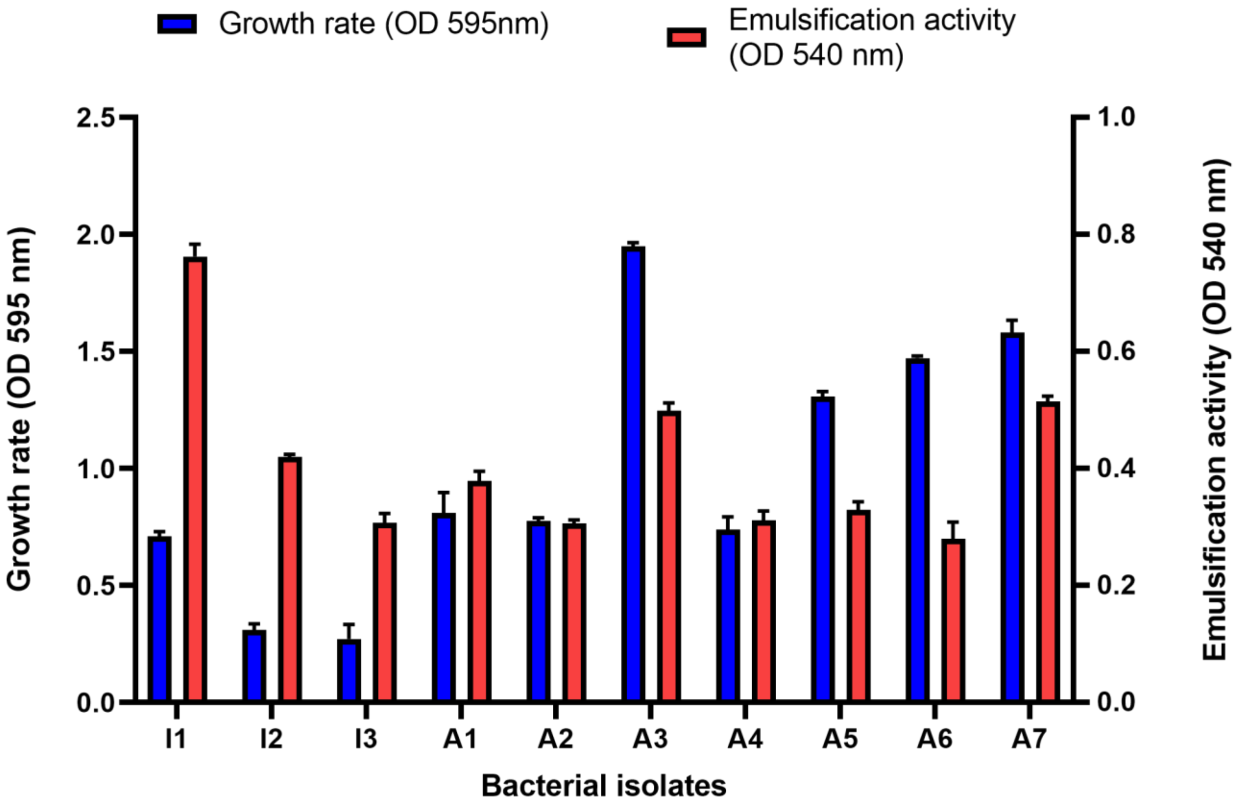

Figure 1. Growth rate and emulsification activity of microbial isolates. The experiment was conducted by incubating the isolates at 30 °C and 150 rpm for 72 hours. Growth rates were measured using a spectrophotometer at OD 595 nm, and emulsification activity was assessed at OD 540 nm. Error bars represent the mean of triplicate readings.

Lipid hydrolysis tests revealed positive results for I1 and A3, indicating their ability to secrete exo-lipase enzymes that break down triacylglycerols into glycerol and fatty acids, a crucial step in the degradation of oil. However, I2 and A7 did not demonstrate lipid hydrolysis, suggesting they may degrade oil through other metabolic pathways.

The lipid hydrolysis test is an extracellular enzyme assay used to assess the capability of organisms to hydrolyse lipids. In this test, lipase enzymes secreted by microorganisms break down triacylglycerol (TAG) into glycerol and free fatty acids (Cesario, Pires *et al.* 2021). A clear zone surrounding the organism on tributyrin agar indicates lipid hydrolysis ability. *Staphylococcus aureus* and *Escherichia coli* served as positive and negative controls, respectively.

While biochemical tests based on phenotypic characteristics provide initial identification, they may not be sufficient for species confirmation due to the influence of environmental factors on phenotypic trait expression (Adrion, Nakamura *et al.*, 2016). Therefore, molecular identification techniques were employed for more accurate species confirmation.

The isolates were further identified using 16S rRNA and ITS primers. BLAST analysis results are summarised in Table 5, providing information on the identity and similarity between the query and subject sequences. A phylogenetic tree was constructed to further investigate the relationships between the sequences (Figure 2).

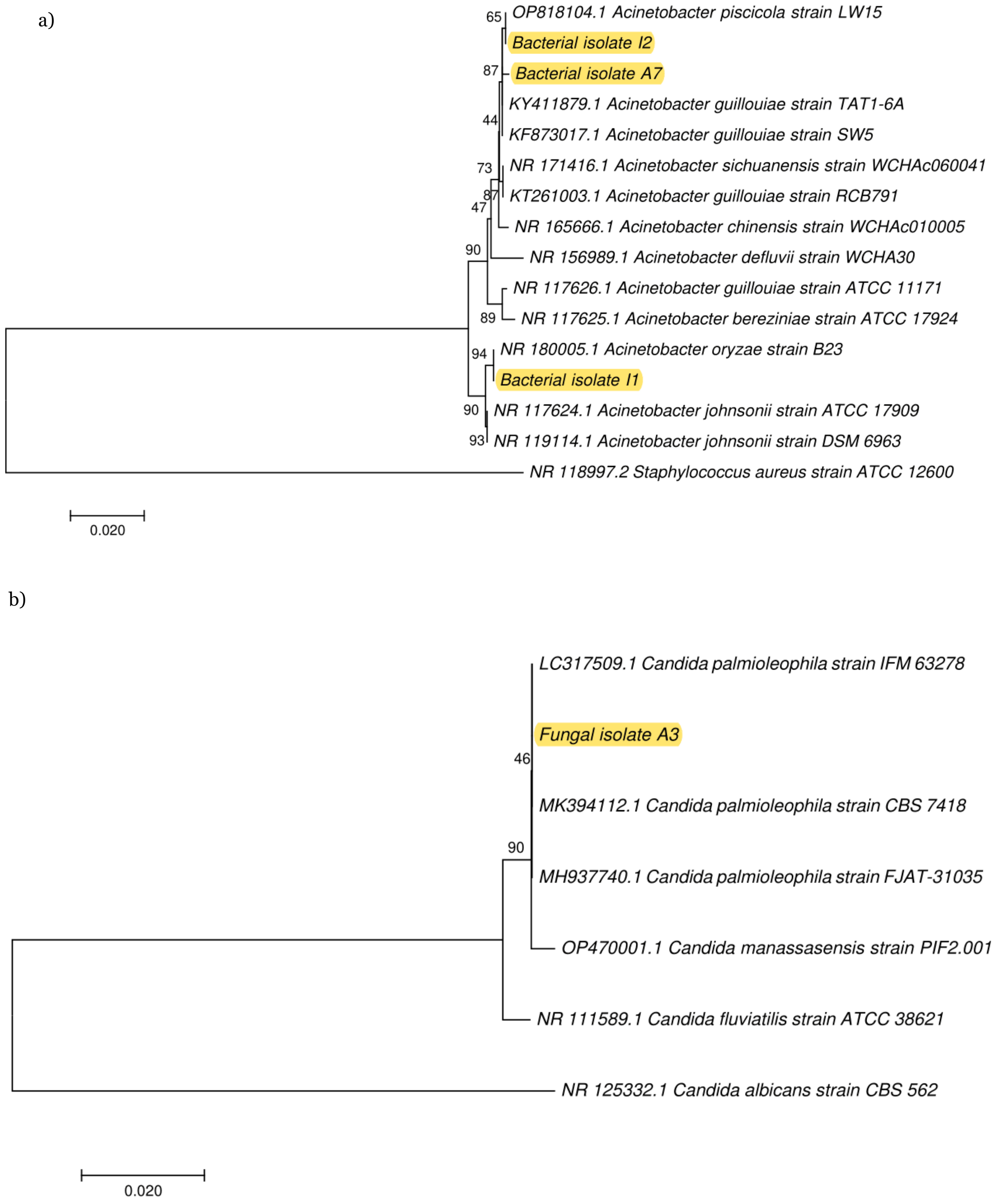

Figure 2. Phylogenetic tree of microbial isolates. The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap test (1000 replicates) shows the percentage of replicate trees in which the associated taxa clustered together. The phylogenetic tree was constructed using a) the 16S rRNA primer and b) the ITS primer. *Staphylococcus aureus* strain ATCC 12600 and *Candida albicans* strain CBS 562 were used as outgroups for evolutionary distance. Isolates from this study are highlighted in yellow.

Table 5. Molecular identification report of selected isolates. The table presents the species, strain, and accession numbers of isolates I1, I2, A3, and A7, along with their sequence similarity to reference strains from the NCBI database.

Report	**Microbial isolates**			
	I1	**I2**	**A3**	**A7**
Species	*Acinetobacter oryzae*	*Acinetobacter piscicola*	*Candida palmioleophila*	*Acinetobacter guillouiae*
Strain	MSMA-I1	MSMA-I2	MSMA-A3	MSMA-A7
Size	1404 bp	1382 bp	606 bp	1307 bp
Accession number	OR727192	OR727284	OR727302	OR727301
Reference bacteria	*Acinetobacter oryzae* ATCC 12600	*Acinetobacter piscicola* LW15	*Candida palmioleophila* IFM 63278	*Acinetobacter guillouiae* TAT1-6A
Accession number (Reference)	NR117624.1	OP818104.1	LC317509.1	KY411879.1
Similarity	99.29 %	100.00 %	100.00 %	99.77 %

Three bacterial isolates were identified from the *Acinetobacter* genus, and one fungal isolate from the *Candida* genus. Specifically, I1, I2, A3, and A7 were identified as *Acinetobacter oryzae*, *A. piscicola*, *Candida palmioleophila*, and *A. guillouiae*, respectively. These isolates showed high similarity (99.29% - 100.00%) to reference sequences in the NCBI database. The sequences were submitted to GenBank and assigned accession numbers OR727192, OR727284, OR727302, and OR727301, respectively.

Acinetobacter spp. are typically Gram-negative coccobacilli with variable oxidase and catalase test results. However, some inconsistencies were observed in our biochemical tests, such as I1's negative starch hydrolysis and I2's negative catalase reaction, which are atypical for *Acinetobacter* spp. (Cai, Zhang *et al.*, 2015). Similarly, A3's positive oxidase test is inconsistent with typical *Candida* characteristics (Kumar, Arora *et al.*, 2000).

The identification of I1, I2, and A7 as *Acinetobacter* spp. aligns with previous studies demonstrating their oil-degrading capabilities (Adetunji & Olaniran, 2019; Gao, Lu *et al.*, 2019; Fernandez, Callegari *et al.*, 2023). For instance, Adetunji and Olaniran (2019) reported high emulsifying activities (over 80%) for *Acinetobacter* sp. Ab9-ES and *Acinetobacter* sp. Ab33-ES in the presence of edible oil. Fernandez, Callegari *et al.* (2023) highlighted *A. guillouiae* SFC 500-1 potential as a bioremediation agent for tannery wastewater. Gao, Lu *et al.* (2019) identified *A. dijkshoorniae* LYC46-2 and *A. pittii* LYC73-4b as efficient oil degraders.

The identification of A3 as *Candida palmioleophila* is consistent with previous findings. Rodríguez Mateus, Agualimpia Valderrama *et al.* (2016) isolated five *C. palmioleophila* strains from palm oil refinery wastes, demonstrating their potential for oil and grease degradation. Theerachat, Tanapong *et al.* (2017) further corroborated this by showing that the co-cultivation of *Yarrowia lipolytica* and *Candida rugosa* effectively reduced the chemical oxygen demand and triglycerides in palm oil mill effluent. Tangsombatvichit, Pisapak *et al.* (2020) reported that a microbial mixture, including *Candida palmioleophila*, *Bacillus* sp., *Yarrowia* sp., and *Pseudomonas* sp., significantly reduced organic matter, oil, and grease in palm oil mill effluent by over 70%. These studies collectively suggest that *Candida palmioleophila* could serve as a potential bioremediation agent for reducing fat, oil, and grease in wastewater. This further supports the potential application of the isolate A3 in bioremediation.

D. Influence of Temperature and pH on the Degradation of FOG

Our study demonstrated that the growth and activity of isolates I1, I2, A3, and A7 were optimal at temperatures between 25 °C and 37 °C, with no growth observed at 4 °C or 60 °C (Table 6). This temperature range aligns with the typical ambient conditions in Malaysian wastewater systems, suggesting that these isolates are well-suited for practical bioremediation applications in local food processing and restaurant drainage environments. The results of this study align with those of Ahmed, Ahmad *et al.* (2022), who found that the growth and used cooking oil degradation of *Burkholderia* sp. AQ5-13 and *Burkholderia vietnamiensis* AQ5-12 were optimal at temperatures between 25 °C and 40 °C. Similarly, Tanaka, Takashima *et al.* (2010) reported that *Acinetobacter* sp. strain Ud-4 exhibited significant growth and degradation of various edible oils, including canola oil, olive oil, sesame oil, soybean oil, and lard oil, at an optimal temperature of 25 °C. Gao, Lu *et al.* (2019) also found that the optimal temperatures for the isolates *A. dijkshoorniae* LYC46-2, *Kluyvera cryocrescens* LYC50-1a, and *A. pittii* LYC73-4b ranged from 25 °C to 35 °C. These findings, along with those from other studies, suggest that most oil-degrading bacteria thrive at temperatures ranging from 25 °C to 40 °C, a range that is consistent with the findings of our study.

Table 6. Growth of isolates at various temperatures. The table shows the growth performance of isolates I1, I2, A3, and A7 at 4 °C, 25 °C, 37 °C, and 60 °C. Growth was assessed on a scale from (1+) for scant growth to (3+) for abundant growth, with (-) indicating no growth.

Isolate	**Temperature**			
	4 °C	25 °C	37 °C	60 °C
I1	-	+++	++	-
I2	-	+++	++	-
A3	-	++	+++	-
A7	-	++	+++	-
Negative control	-	-	-	-

Additionally, all isolates demonstrated adaptability to pH levels ranging from 6 to 9 (Table 7), with A3 and A7 showing remarkable acid tolerance, surviving at a pH as low as 3. This tolerance suggests that these strains may be especially effective in environments where wastewater exhibits slight acidity, such as in certain food processing industries. These pH findings are consistent with previous studies. Ibrahim, Abdul Khalil *et al.* (2020) found that *Rhodococcus erythropolis* AQ5-07 showed optimal emulsification activity in canola oil remediation at a pH of 7.5. Similarly, Ahmed, Ahmad *et al.* (2022) reported that the isolates *Burkholderia* sp. AQ5-13 and *B. vietnamiensis* AQ5-12 demonstrated optimal growth and used cooking oil degradation at a pH of 6.8. Gao, Lu *et al.* (2019) further indicated that the bacterial isolates *Acinetobacter dijkshoorniae* LYC46-2, *Kluyvera cryocrescens* LYC50-1a, and *A. pittii* LYC73-4b were effective for oil degradation at pH ranges of 7 to 10. Additionally, Ren, Fan *et al.* (2020) reported that *Klebsiella quasivariicola* IUMR-B53 achieved maximum degradation of soybean oil at a pH of 7. These collective findings suggest that the optimal pH for oil degradation by various bacterial isolates typically falls within the neutral range, further supporting the potential application of these isolates in bioremediation.

Table 7. Growth of isolates at different pH levels. The table illustrates the ability of the isolates to grow at pH 3, 6, 7 and 9. Growth is represented on a scale from (+) for growth to (-) for no growth.

Isolates	**pH**			
	3	6	7	9
I1	-	+	+	+
I2	-	+	+	+
A3	+	+	+	+
A7	+	+	+	+
Control	-	-	-	-

The combination of temperature and pH adaptability indicates that these isolates could be effectively used in a wide range of wastewater treatment settings, particularly those involving food industry effluents. However, further testing is required under real-world conditions to validate these findings and optimise bioremediation processes for industrial applications.

E. Evaluation of Microbes' Oil-degrading Ability via Gravimetric Analysis

The gravimetric analysis was conducted to quantitatively assess the oil-degrading capabilities of the isolated microbes. This method measured the residual oil left after microbial treatment, using n-hexane to extract oil remnants. The oil degradation ratios for the tested isolates after 3 days of incubation at 30 °C and 150 rpm are presented in Figure 3.

Isolates I2, A3, and A7 exhibited significant oil degradation, with ratios of 57.90%, 52.63%, and 73.69%, respectively, positioning them as highly effective oil-degrading agents. In contrast, isolate I1 showed a lower degradation ratio of 31.58%, indicating a more limited bioremediation potential. The varying degradation capacities among the isolates suggest differences in lipid metabolism or adaptation to oil-rich environments.

Our findings are consistent with previous studies, such as Adetunji and Olaniran (2019), where *Acinetobacter* sp. Ab9-ES and *Acinetobacter* sp. Ab33-ES degraded oil by over 70%. Similarly, Gao, Lu *et al.* (2019), reported degradation rates of canola oil exceeding 90% in some bacterial strains. In addition, Ren, Fan *et al.* (2020) revealed that *Klebsiella quasivariicola* IUMR-B53 could degrade soybean oil at an impressive rate of over 95%. Despite these higher degradation rates in other studies, isolate A7's performance (73.69%) remains noteworthy and shows promise for practical application in FOG-laden wastewater treatment.

The limited performance of I1 may stem from its reliance on alternative degradation pathways or its lower enzymatic activity in breaking down oil. Future studies should explore the mechanisms behind these differences to optimise isolate selection for targeted bioremediation efforts.

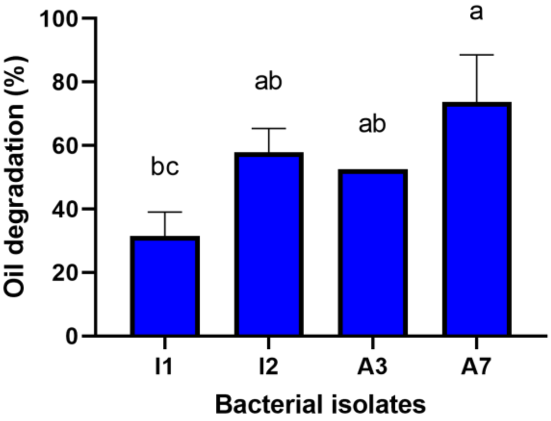

Figure 3. Oil degradation capability of bacterial isolates. Four bacterial isolates (I1, I2, A3, and A7) were tested for oil degradation after incubation for 72 hours at 30 °C and 150 rpm. The results are presented as the mean ± SD of three replicates. Different superscript letters indicate significant differences ($p < 0.05$) as determined by Tukey's HSD test.

The findings of this study provide a foundation for future research into the use of microbial consortia, which may enhance FOG degradation through the complementary action of multiple strains. Additionally, further testing across diverse wastewater conditions, including varying FOG compositions, would offer valuable insights into the practical adaptability of these isolates for industrial applications.

IV. CONCLUSION

This study successfully isolated and identified four microbial strains—*Acinetobacter oryzae* MSMA-I1, *A. piscicola* MSMA-I2, *Candida palmioleophila* MSMA-A3, and *A. guillouiae* MSMA-A7—from food processing and restaurant wastewater. Among these, isolates I2, A3, and A7 demonstrated superior oil-degrading capabilities, with degradation ratios exceeding 50%, making them strong candidates for FOG bioremediation. The ability of these isolates to thrive in typical Malaysian wastewater conditions (25–37 °C, pH 6–9) further supports their potential for real-world application in wastewater treatment. However, this study is not without limitations. The limited number of isolates reviewed reflects the exploratory nature of this research and the resource constraints associated with broader sampling efforts. Future studies should focus on expanding microbial diversity by sampling from diverse wastewater sources across different industries and geographic regions to enhance the applicability of findings. Additionally, while this study focused on evaluating the oil-degrading capabilities of the isolates, detailed investigations into their specific metabolic pathways and enzyme mechanisms were not included. Such analyses require advanced molecular techniques and were beyond the scope of this initial study. Future research should prioritise elucidating these pathways using approaches such as metagenomics and transcriptomics to optimise strain selection and enhance bioremediation efficiency. Furthermore, while this study was conducted under controlled laboratory conditions, real-world testing in pilot-scale or industrial wastewater treatment systems is necessary to validate the scalability and effectiveness of these microbes in large-scale applications. Environmental risks such as microbial containment and toxicity should also be evaluated to ensure safe implementation during industrial-scale operations. Overall, this research provides a valuable foundation for developing bioremediation strategies using locally sourced microbes, offering a promising solution to FOG pollution in industrial wastewater settings.

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