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Isolation and Selection of Crude Oil-Degrading Bacteria from Coastal Soil of Ko Samet, Rayong Province, Thailand

Phichamon Noisuwan[1,2], Tavich Intaraphan[1], Petch Suthiporn[1,2], Wasana Phantewee[1, 2, *]

[1] Department of Biology, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, 2 Nang linchi Road, Sathorn, Bangkok 10120, Thailand.
[2] Biodiversity and Sustainable Utilization Research Unit, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, 2 Nang linchi Road, Sathorn, Bangkok 10120, Thailand.

* Corresponding author: *wassana.p@mail.rmutk.ac.th*

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Abstract. Oil spill incidents in Thailand's coastal areas, particularly in Rayong Province, pose significant ecological and economic threats. Bioremediation, utilizing indigenous hydrocarbon-degrading bacteria, is a sustainable and environmentally friendly alternative to conventional cleanup methods. This study aimed to isolate and evaluate such bacteria from coastal soils and sediments of Ko Samet, specifically from Ao Phrao, Ao Tawan, and Ao Kluai Na Nok. From a total of 144 initial isolates, twenty-eight demonstrated significant crude oil degradation ability when screened in Bushnell-Haas Mineral Salt (BHMS) medium supplemented with 1% crude oil. Isolate 1ATIII 4 showed the best performance, with the lowest residual oil mass (0.0866 g), indicating high degradation efficiency. Preliminary identification based on morphological and biochemical characteristics (including comparison with Bergey's Manual of Determinative Bacteriology) strongly suggests that the most efficient isolate belongs to the genus Pseudomonas. These findings provide promising indigenous bacterial candidates for developing a localized and effective bioremediation strategy for oil contamination in this ecologically sensitive region.

Keywords: Crude oil-degrading, biosurfactants microorganisms, bioremediation, Ko Samet, Rayong Province

1. Introduction

Coastal ecosystems, such as those surrounding Ko Samet in Rayong Province, Thailand, are vital for biodiversity and local economies, particularly tourism. Oil spills remain a significant environmental concern worldwide, with Thailand experiencing notable incidents that have impacted its coastal ecosystems. A prominent example is the oil spill on July 27, 2013, when a pipeline operated by PTT Global Chemical Public Company Limited ruptured, releasing crude oil into the Gulf of Thailand. The spill reached Ao Phrao Beach on Ko Samet, Rayong Province, causing extensive environmental damage, disrupting marine ecosystems, and adversely affecting the local tourism economy (Sathornwong, 2013). The Gulf's rich biodiversity is increasingly threatened by frequent oil spills, with 146 incidents documented between 2015 and 2021, and 22 leaks reported in 2022 alone (Fernandes et al., 2025), underscoring the urgent need for sustainable and effective remediation strategies.

Traditional remediation methods, including physical removal and chemical dispersants, often entail high costs and potential environmental risks, such as residual toxicity affecting marine life (Navarro & Caipang, 2024). Consequently, there is a growing interest in biological approaches. Bioremediation, which leverages the natural capabilities of indigenous microorganisms to break down pollutants, presents a cost-effective and sustainable solution for mitigating these impacts. Indigenous microorganisms, adapted to local environmental conditions, often possess superior hydrocarbon degradation capabilities compared to non-native strains. Recent studies have highlighted the efficacy of marine actinobacteria in biodegrading oil-derived hydrocarbons, suggesting their potential role in natural attenuation processes (Fernandes et al., 2025). Moreover, biosurfactants produced by certain microbes have emerged as environmentally friendly alternatives to chemical dispersants.

These biologically derived agents can effectively emulsify oil, enhancing its bioavailability for microbial degradation, while exhibiting lower toxicity and higher biodegradability (Mohammed et al., 2024).

This research focuses specifically on isolating and characterizing indigenous bacteria from the affected coastal soils and sediments of Ko Samet. This study aims to investigate the changes in microbial diversity across various coastal areas of Ko Samet Island by comparing sites heavily impacted by crude oil contamination with those less affected. The primary objectives are: 1) to isolate and identify indigenous hydrocarbon-degrading bacterial strains from distinct coastal sites; 2) to quantitatively evaluate their efficiency in degrading crude oil under laboratory conditions; and 3) to compare the differences in microbial species composition and diversity across sampling locations to inform location-specific bioremediation strategies.

2. Methodology

2.1 Study area

Ko Samet is a small island located at approximately 12°35'27" N, 101°25'01" E, about 6 kilometers off the coast of Rayong Province in the Gulf of Thailand. The island covers an area of approximately 13.1 square kilometers, stretching around 7 kilometers from north to south and 4 kilometers at its widest point. It is part of the Khao Laem Ya–Mu Ko Samet National Park, situated in Phe Subdistrict, Mueang District, Rayong Province (Phusantisampan et al., 2023). Topographically, Ko Samet is characterized by low hills and is predominantly forested. More than 80% of the island is covered by a mixture of tropical evergreen and deciduous forests, including the native cajeput tree (Melaleuca cajuputi), from which the island derives its name (Sutthiwong et al., 2024). The island is known for its relatively arid conditions compared to the Thai mainland, with a short rainy season from May to October and low annual rainfall, making it one of the driest islands in Thailand (Jitdeesuwan & Niyomdham, 2022). Ko Samet is fringed by more than 12 beaches along its coast. Most of these beaches, such as Hat Sai Kaew, Ao Phai, Ao Wong Duean, Ao Cho, and Ao Prao, are situated along the eastern shoreline, where the calm sea and white sandy beaches attract numerous tourists. The western beaches are less developed and known for their sunset views and ecological preservation zones (Thepsiriamnuay & Pumijumnong, 2024; (Rattanasathien et al., 2023). In recent years, the island has faced environmental pressures from tourism and coastal erosion. However, conservation efforts, such as plastic reduction campaigns and coastal rehabilitation programs, have been implemented to preserve its natural (Chandrapanya et al., 2025; Thonglim et al., 2023).

2.2 Field Data Collection and Soil sampling

Field data collection was carried out on Ko Samet, Rayong Province, Thailand, across three selected beach sites: Ao Phrao (AP), Ao Tawan (AT), and Ao Kluai Na Nok (AK). Soil and sediment samples were collected from three coastal sites on Ko Samet, Rayong Province, Thailand. These sites were selected based on their history of exposure to oil contamination from the 2013 spill and coastal environmental gradients. Site 1 (S1: Ao Phrao) was classified as the highly impacted site because it was the primary beach affected by the 2013 oil spill. In contrast, Site 2 (S2: Ao Tawan) and Site 3 (S3: Ao Kluai Na Nok) were classified as less impacted/ comparison sites. Sampling was performed in triplicate at each site during the intertidal zone, with sediment samples collected at four depth intervals: 0, 5, 10, and 15 centimeters. Each depth was sampled in triplicate to ensure statistical robustness and to account for local heterogeneity in microbial and physicochemical properties. Geographical coordinates of each sampling point were recorded using a GPS handheld device to ensure accurate spatial referencing (Table 1). Environmental parameters, including pH, salinity, temperature of sand, electrical conductivity (EC), and dissolved oxygen (DO), were measured in situ using a Portable Multi-parameter Water Quality Meter (Model: Bante 900P/901P). The statistical correlation analysis of water quality parameters across two sampling rounds revealed notable

relationships among environmental variables, particularly involving temperature, conductivity, and dissolved oxygen (DO). These findings offer insight into both natural coastal dynamics and anthropogenic influences at Ko Samet's shoreline. These variables are critical for assessing the physicochemical environment that influences microbial community structure and the biodegradation of hydrocarbons in coastal sediments. (Khan & Rajshekhar, 2020; Wang et al., 2023). All samples were collected under aseptic conditions, transferred to sterile containers, and immediately transported on ice to the laboratory for further microbial and molecular analyses. The methodology employed followed established protocols for marine sediment sampling and coastal microbial ecology to ensure reproducibility and data integrity (Zhang et al., 2022; Lee et al., 2023).

Figure 1. Study site: (a) Rayoung Provine of Tailand, (b) Ko Samet, (c) Ao Phrao (AP), (d) Ao Tan Tawan (AT), and (e) Ao Kiew Na NoK (AK) on Ko Samet.

Table 1. GPS coordinates of sampling sites around Ko Samet, located in Phe Subdistrict, Mueang District, Rayong Province, Thailand.

Sampling Site	Sub-site	Latitude (N)	Longitude (E)
Ao Phrao	AP1	12°34' 17.71"N	101° 26' 54.40"E
	AP2	12°34' 11.72"N	101° 26' 58.79"E
	AP3	12°34' 04.89"N	101° 26' 55.95"E
Ao Tawan	AT1	12°33' 20.85"N	101° 27' 11.14"E
	AT2	12°33' 23.38"N	101° 27' 11.52"E
	AT3	12°33' 26.93"N	101° 27' 14.63"E
Ao Kluai Na Nok	AK1	12°31' 47.51"N	101° 26' 44.11"E
	AK2	12°31' 51.16"N	101° 26' 45.68"E
	AK3	12°31' 53.22"N	101° 26' 47.74"E

2.3 Isolation and Identification of Crude Oil-Degrading Microorganisms

The isolation and identification of crude oil-degrading microorganisms were conducted using soil and sand samples collected from Ko Samet. To stimulate the recovery of potentially stressed microorganisms due to environmental conditions, 10 grams of each sample were inoculated into Bushnell-Haas Mineral Salt (BHMS) medium supplemented with 1% crude oil as the sole carbon source. The cultures were incubated at 30°C for seven days. Subsequently, the resulting microbial suspensions were spread onto BHMS agar plates containing 1% crude oil and incubated at 30°C for 1–7 days. Colonies exhibiting growth were selected and preserved as stock cultures on slant agar. These isolates were further streaked onto nutrient agar (NA) plates and also cultured in BHMS liquid medium with 1% crude oil, followed by incubation at 30°C for 7–15 days. Isolates that formed clear zones around their colonies on BHMS agar plates indicated potential crude oil degradation capabilities. These promising strains underwent morphological characterization, Gram staining, biochemical tests, and identification using commercial test kits. The biochemical characterization of the bacterial isolates was carried out using a commercial biochemical identification kit (HiMedia, India) in accordance with the manufacturer's instructions (HiMedia Laboratories, Biochemical Identification Kit). The process began with the preparation of bacterial cultures, in which each isolate was inoculated into nutrient broth and incubated at 37 °C for 18 hours to promote active growth and ensure that the cells were in the exponential phase, which is optimal for metabolic testing. Following incubation, 50 µL of each bacterial suspension was aseptically transferred into the designated wells of the biochemical test strip using a sterile micropipette, ensuring accurate delivery to the specific test media contained within each compartment. Once inoculated, the biochemical test strips were placed in an incubator set at 32 °C and maintained for 18–24 hours under aerobic conditions to allow sufficient time for metabolic reactions to occur. After the incubation period, specific chemical reagents were added to certain wells, as outlined in the kit's protocol, to facilitate the visualization of enzymatic or metabolic activities. These reagents often induce characteristic color changes or other observable reactions, which were carefully recorded for each bacterial isolate. This methodology aligns with previous studies that have successfully isolated and characterized crude oil-degrading bacteria using similar approaches (Taher & Saeed, 2021; Mohammed et al., 2023).

2.4 Assessment of Crude Oil Biodegradation Potential by Isolated Microorganisms

To evaluate the crude oil biodegradation potential of isolated microorganisms, a standardized protocol was employed. Each bacterial isolate was inoculated into 100 mL of Bushnell-Haas Mineral Salt (BHMS) broth supplemented with 1% (v/v) crude oil as the sole carbon source. The cultures were incubated at 37°C with agitation at 120 rpm for 7 days to facilitate microbial growth and hydrocarbon degradation. This method aligns with protocols used in previous studies assessing hydrocarbon-degrading bacteria (Wu et al., 2023; Azuchukwuene, 2019). Post-incubation, microbial growth was quantified by measuring the optical density at 600 nm (OD600) using a UV-Vis spectrophotometer. An increase in OD600 compared to the uninoculated control indicated the utilization of crude oil as a carbon source by the microorganisms. This approach is consistent with methodologies reported in studies evaluating bacterial growth in hydrocarbon-enriched media (Wu et al., 2023; Azuchukwuene, 2019). Additionally, the total protein content produced by the microbial cells was determined using the Folin–Lowry method, employing commercially available assay kits. This colorimetric assay involved the reaction of protein samples with the Folin–Ciocalteu reagent, and absorbance was measured at 750 nm. Protein concentrations were calculated based on a standard curve generated using bovine serum albumin (BSA) standards. This method provides insights into the metabolic activity of the isolates during crude oil degradation (Lowry et al., 1951). Furthermore, to assess biosurfactant production, which facilitates hydrocarbon emulsification and

uptake, the oil-spreading technique was employed. In this assay, 10 µL of crude oil was added to the surface of 40 mL of distilled water in a Petri dish to form a thin oil layer. Subsequently, 10 µL of culture supernatant was gently placed at the center of the oil layer. The presence of biosurfactants was indicated by the displacement of oil and formation of a clear zone, whose diameter was measured after 30 seconds. This method correlates with surfactant activity and has been used in previous studies (Sivakumar et al., 2014). The crude oil-degrading microbial isolates were compared for their efficiency in crude oil degradation, biosurfactant production, and the determination of the residual oil dry weight after cultivation. These were compared with a reference strain obtained from the Thailand Institute of Scientific and Technological Research (TISTR). The identified bacterial isolates were cultured on slant nutrient agar and incubated at 37°C for 12–18 hours. After incubation, the slants were overlaid with sterile liquid paraffin and stored at 4°C for preservation.

3. Results and Discussion

3.1 Physicochemical parameters across different sampling sites around Koh Samet

The water quality assessment conducted across three bays—Ao Phrao (AP), Ao Tawan (AT), and Ao Kluai Na Nok (AK)—on Ko Samet was carried out over two sampling periods: the first sampling round (March) and the second sampling round (July). The measured parameters included temperature, pH, ion concentration, salinity, conductivity, dissolved oxygen (DO), and total dissolved solids (TDS) (see Figure 2). In the first sampling round, temperatures ranged from 25.8°C to 29.8°C, while the second round showed an increase to 28.2°C–31.2°C, likely due to seasonal or climatic variations, which can reduce DO levels and impact aquatic life (Ahmed et al., 2025). pH remained relatively stable at 7.7–8.5, indicating a balanced marine ecosystem. Ion concentrations varied across sites and sampling rounds, potentially influenced by freshwater influx or human activities. Salinity levels were generally consistent, though AK(3) decreased sharply from 12.79 ppt to 0.99 ppt during the second round, suggesting freshwater dilution, a factor affecting marine organism osmoregulation (Fondriest Environmental, 2024). Conductivity trends mirrored salinity and ion concentrations, with higher salinity corresponding to higher conductivity values. DO levels showed declines in some sites during the second round, such as AK(1) dropping from 7.02 mg/L to 5.01 mg/L, possibly due to elevated temperatures and organic matter leading to hypoxia (Ahmed et al., 2025). TDS values also fluctuated, affecting water clarity and potentially influencing photosynthetic organisms (Ahmed et al., 2025). These findings highlight temporal and spatial variations in water quality, driven by seasonal change, hydrological inputs, and anthropogenic impacts, underscoring the importance of continuous monitoring to safeguard the marine environment of Ko Samet.

3.2 Microbial Isolation

Microbial isolation from three coastal sites around Ko Samet—Ao Kluai Na Nok (AK), Ao Phrao (AP), and Ao Tan Tawan (AT)—yielded a total of 144 isolates (Table 2). Ao Kluai Na Nok and Ao Phrao each contributed 48 isolates, while Ao Tan Tawan contributed 45. These findings highlight the microbial diversity in coastal sediment environments, which may be influenced by site-specific environmental factors such as organic matter content, salinity, and anthropogenic activities. The higher isolate numbers at Ao Kluai Na Nok and Ao Phrao suggest potentially more favorable conditions for microbial proliferation. These results align with Khan et al., who reported that microbial diversity in marine sediments is significantly influenced by organic nutrient levels and physico-chemical properties (Khan et al., 2023). Moreover, the isolates from these coastal sites could serve as promising candidates for biotechnological applications, including oil biodegradation and bioactive compound production, reflecting the increasing focus on microbial-based environmental remediation strategies (Li et al., 2024; Nguyen et al., 2023). An analysis of bacterial growth rates across sampling sites revealed that the highest mean growth was observed in group 1AP from Ao Phrao, with

an average score of 2.71 (SD = 0.61), while the lowest growth occurred in group 2AK from Ao Kluai Na Nok, with a mean of 1.42 (SD = 0.70). The clear difference in growth between isolates from Ao Phrao and Ao Kluai Na Nok suggests that environmental factors such as nutrient availability, moisture, and the presence of hydrocarbons may significantly influence microbial proliferation. These findings are consistent with Zhang et al., who reported that hydrocarbon-degrading bacteria tend to thrive in environments enriched with petroleum derivatives, which serve as alternative carbon sources (Zhang et al., 2025). Moreover, Meng et al. The importance of oxygen levels and temperature in regulating anaerobic or facultative bacterial growth during oil biodegradation processes (Meng et al., 2024). The consistent advantage of Ao Phrao isolates implies that this site may offer optimal physicochemical conditions conducive to microbial expansion, indicating its potential as a natural reservoir for selecting high-performance strains for oil spill bioremediation.

The 28 microbial isolates revealed that isolates 1ATIII 4, 1APII 6, and 2ATI 15 exhibited the highest oil degradation potential, with the lowest residual oil amounts of 0.0866 g, 0.0924 g, and 0.0965 g, respectively. These values were markedly lower than that of the control group, which showed no degradation (1.0695 g). This indicates the ability of certain microbial strains to utilize hydrocarbons as an energy source. Isolates classified with a “4+” degradation rating generally demonstrated superior oil-degrading capacity compared to those rated “3+” or “2+”. This observation supports the hypothesis that these bacteria possess the ability to produce biosurfactants and oil-degrading enzymes. The crude oil degradation capability of the isolated strains was evaluated in comparison with four standard reference microorganisms—Pseudomonas sp. TISTR 554, Acetobacter sp. TISTR 975, Micrococcus sp. TISTR 1404, and Aeromonas hydrophila TISTR 1321—by measuring the residual dry weight of crude oil after the biodegradation assay. The results indicated that among the isolates tested, strain 1ATIII4 exhibited the highest crude oil degradation efficiency, outperforming all reference strains. These findings are consistent with previous studies, such as Zhang et al., who reported that a microbial consortium could degrade up to 75% of soil oil contamination within 60 days (Zhang et al., 2025). Similarly, Yakimov et al. isolated novel bacterial strains capable of efficiently degrading hydrocarbons in extreme environments, including deep-sea and industrial zones (Yakimov et al., 2023). Furthermore, Meng et al. demonstrated that efficient oil degradation could still occur under anaerobic conditions when specialized microbial consortia were employed (Meng et al., 2024). The results of the present study align with these reports, suggesting that the selected isolates hold significant potential for the development of bioremediation technologies, both at the laboratory and field application levels.

The biochemical profiling of the 10 bacterial isolates revealed varied carbon source utilization capacities, categorized based on response rates: positive over 90% (+), moderately positive (11–89%) as (v), and negative over 90% (-). Isolate 1APIII 1 exhibited the broadest carbon utilization spectrum, showing strong positive results for malonate, esculin, arabinose, xylose, adonitol, rhamnose, cellobiose, trehalose, raffinose, lactose, and glucose. This indicates a highly versatile metabolic system that can support survival in diverse and fluctuating environments (Zhang et al., 2025). Conversely, isolates such as 1ATI13 and 1API 6 showed negative responses to several complex sugars, suggesting a limited capability to metabolize structurally complex carbon sources. Isolates like 1APII 6, 2API 6, and 2ATI 15 showed intermediate positive (v) reactions in multiple tests, implying that their metabolic activity might be conditional upon environmental parameters like pH, oxygen levels, or carbon concentration (Meng et al., 2024). Several isolates including 2API 6 and 1API 6 were highly positive for citrate and glucose utilization, supporting their potential as efficient degraders in environments enriched with simple carbohydrates and inorganic substrates—common features in oil-contaminated ecosystems (Yakimov et al., 2023). Overall, this detailed carbon source profiling aids in the strategic selection of bacterial strains for bioremediation applications targeting hydrocarbon pollutant.

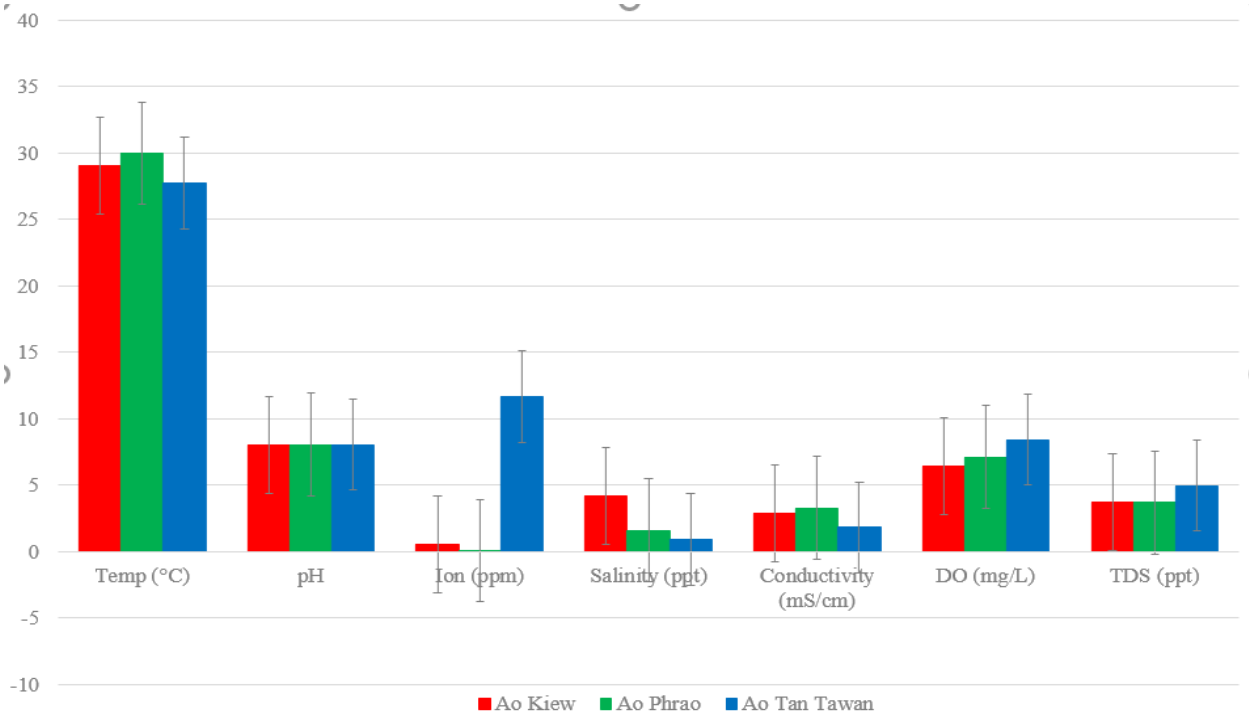

Figure 2. Comparison of key water quality parameters—temperature, salinity, dissolved oxygen (DO), pH, conductivity, ion concentration, and total dissolved solids (TDS)—measured during two sampling rounds in Ao Phrao (AP), Ao Tan Tawan (AT), and Ao Kiew Na NoK (AK) on Ko Samet.

Table 2. Distribution of Microbial Isolates and Their Codes from Coastal Sampling Sites around Koh Samet Ko Samet, located in Phe Subdistrict, Mueang District, Rayong Province, Thailand.

Sampling Site	Number of isolates	Isolate code
Ao Phrao	48	1AKI 0.1, 1AKI 0.3, 1AKI 5.1, 1AKI 5.3, 1A I 10.1, 1AKI 10.3,1AKI 15.1, 1AKI 15.3, 1AKII 0.1, 1AKII 0.3, 1AKII 5.1, 1AKII 5.3, 1AKII 10.1, 1AKII 10.3, 1AKII 15.1, 1AKII 15.3, 1AKIII 0.1, 1AKIII 0.3, 1AKIII 5.1, 1AKIII 5.3, 1AKIII 10.1, 1AKIII 10.3, 1AKIII 15.1, 1AKIII 15.3, 2AKI 0.1, 2AKI 0.3, 2AKI 5.1, 2AKI 5.3, 2AKI 10.1, 2AKI 10.3, 2AKI 15.1, 2AKI 15.3, 2AKII 0.1, 2AKII 0.3, 2AKII 5.1, 2AKII 5.3, 2AKII 10.1, 2AKII 10.3, 2AKII 15.1, 2AKII 15.3, 2AKIII 0.1, 2AKIII 0.3, 2AKIII 5.1, 2AKIII 5.3, 2AKIII 10.1, 2 AKIII10.3, 2AKIII 15.1, 2AKIII 15.3
Ao Kluai Na Nok	48	1ATI 0.1, 1ATI 0.3, 1ATI 5.1, 1ATI 5.3, 1ATI 10.1, 1ATI 10.3, 1ATI 15.1, 1ATI 15.3, 1ATII 0.1, 1ATII 0.3, 1ATII 5.1, 1ATII 5.3, 1ATII 10.1, 1ATII 10.3, 1ATII 15.1, 1ATII 15.3, 1ATIII 0.1, 1ATIII 0.3, 1ATIII 5.1, 1ATIII 5.3, 1ATIII 10.1, 1ATIII 10.3, 1ATIII 15.1, 1AT III 15.3, 2API 0.1, 2API 0.3, 2API 5.1, 2API 5.3, 2API 0.1, 2API 0.3, 2API 5.1, 2API 5.3, 2API 0.1, 2API 0.3, 2APII 5.1, 2APII 5.3, 2APII 10.1, 2APII 10.3, 2APII 15.1, 2APII 15.3, 2APIII 0.1, 2APIII 0.3, 2APIII 5.1, 2APIII 5.3, 2APIII 10.1, 2APIII 10.3, 2APIII 15.1, 2APIII 15.3
Ao Phrao	48	1AKI 0.1, 1AKI 0.3, 1AKI 5.1, 1AKI 5.3, 1A I 10.1, 1AKI 10.3,1AKI 15.1, 1AKI 15.3, 1AKII 0.1, 1AKII 0.3, 1AKII 5.1, 1AKII 5.3, 1AKII 10.1, 1AKII 10.3, 1AKII 15.1, 1AKII 15.3, 1AKIII 0.1, 1AKIII 0.3, 1AKIII 5.1, 1AKIII 5.3, 1AKIII 10.1, 1AKIII 10.3, 1AKIII 15.1, 1AKIII 15.3, 2AKI 0.1, 2AKI 0.3, 2AKI 5.1, 2AKI 5.3, 2AKI 10.1, 2AKI 10.3, 2AKI 15.1, 2AKI 15.3, 2AKII 0.1, 2AKII 0.3, 2AKII 5.1, 2AKII 5.3, 2AKII 10.1, 2AKII 10.3, 2AKII 15.1, 2AKII 15.3, 2AKIII 0.1, 2AKIII 0.3, 2AKIII 5.1, 2AKIII 5.3, 2AKIII 10.1, 2 AKIII10.3, 2AKIII 15.1, 2AKIII 15.3

Table 3. Average Growth Rate and Standard Deviation of Bacterial Isolates from Different Sampling Sites

Area code	Study site	Growth Rate	Standard Deviation (SD)
1AK	Ao Kluai Na Nok	2.38	0.77
2AK	Ao Kluai Na Nok	1.42	0.70
1AP	Ao Phrao	2.71	0.61
2AP	Ao Phrao	2.08	0.74
1AT	Ao Tawan	2.08	0.83
2AT	Ao Tawan	2.42	0.65

Table 4. Oil degradation potential of 28 microbial isolates

Isolate	Oil Degradation	Residual Oil after Degradation (g)	Oil Degradation Rank
Control	0	1.0695	0
1ATIII 4	++++	0.0866	1
1APII 6	+++	0.0924	2
2ATI 15	++++	0.0965	3
1APIII 1	++++	0.1465	4
1ATI 13	++	0.1495	5
2API 6	+++	0.1577	6
1API 6	+++	0.1663	7
2ATII 5	++++	0.1710	8
2API 1	++++	0.1831	9
1ATII 9	++++	0.1835	10
1ATI 10	+++	0.1897	11
1ATI 13	+++	0.1495	12
2API 1	+++	0.2003	13
1AKIII 5	+++	0.2029	14
1APII 2	++++	0.2112	15
1API 1	++++	0.2125	16
1ATIII 5	+++	0.2136	17
1APIII 7	++++	0.2153	18
1ATIII 8	++++	0.2199	19

Table 4. Oil degradation potential of 28 microbial isolates (continue)

Isolate	Oil Degradation	Residual Oil after Degradation (g)	Oil Degradation Rank
1ATII 5	+ + +	0.2206	20
1ATI 3	+ + + +	0.2258	21
2APII 4	+ + +	0.2321	22
1AKI 7	+ + +	0.2473	23
1ATI 14	+ + +	0.2540	24
2APII 2	+ + +	0.2557	25
2ATIII 4	+ + +	0.2951	26
2ATII 9	+ + +	0.3187	27
1ATIII 5	+ + + +	0.3262	28

Table 5. The biochemical profiling of the 10 bacterial isolates revealed varied carbon source utilization capacities, categorized based on response rates: positive over 90% (+), moderately positive (11– 89%) as (v), and negative over 90% (-)

No	Tests	Isolate									
		1ATIII 4	1APII 6	2ATI 15	1APIII 1	1ATI 13	2API 6	1API 6	2ATII 5	2API 1	1ATII 9
1	ONPG	-	-	-	-	-	-	-	-	-	-
2	Lysine utilization	v	v	+	+	v	+	+	v	+	+
3	Ornithine utilization	v	v		v	v	v	v	v		
4	Urease	+	+	-	-	-	-	-	-	-	-
5	Phenylalanine Deamination	v	v	v	v	v	v	v	v	v	v
6	Nitrate reduction	+	-	-	-	-	-	-	-	v	v
7	H_2S production	-	-	v	-	-	-	-	v	-	-
8	Citrate utilization	+	+	v	v	+	+	+	-	+	+
9	Voges Proskauer's	-	-	-		v	-	-	-	-	-
10	Methyl red	+	v	+	v	-	-	-	-	-	+
11	Indole	v	v	v	v	v	v	v	v	v	v
12	Malonate utilization	-	-	-	+	+	+	+	+	+	-

Table 5. The biochemical profiling of the 10 bacterial isolates revealed varied carbon source utilization capacities, categorized based on response rates: positive over 90% (+), moderately positive (11– 89%) as (v), and negative over 90% (-) (continue)

No	Tests	Isolate									
		1ATIII 4	1APII 6	2ATI 15	1APIII 1	1ATI 13	2API 6	1API 6	2ATII 5	2API 1	1ATII 9
13	Esculin hydrolysis	-	v	v	+	v	-	-	+	+	+
14	Arabinose	-	+	-	v	-	-	-	-	-	-
15	Xylose	v	v	-	+	v	v	-	v	-	-
16	Adonitol	v	-	v	+	-	v	-	-	-	v
17	Rhamnose	-	-	-	+	-	-	-	-	-	-
18	Cellobiose	-	-	-	+	-	-	-	+	-	v
19	Melibiose	-	-	-	-	-	-	-	-	-	-
20	Saccharose	v	-	-	v	-	v	-	v	v	+
21	Trehalose	-	-	v	+	-	v	-	+	+	+
22	Raffinose	-	-	-	+	-	-	-	-	-	+
23	Lactose	-	-	-	+	-	-	-	-	-	+
24	Glucose	+	v	v	v	+	v	+	v	v	+
25	Oxidase	-	+	-	-	+	+	+	+	+	-

4. Conclusion

Isolation and Selection of Crude Oil-Degrading Microorganisms from Coastal Soils of Ko Samet, Rayong Province, marine microorganisms isolated from coastal environments of Ao Phrao, Ao Tawan, and Ao Kluai Na Nok demonstrated significant potential for crude oil degradation. Soil samples were collected from intertidal zones at three different depths, and key environmental parameters—including temperature, salinity, pH, dissolved oxygen (DO), and electrical conductivity. (EC)—were measured to evaluate their influence on microbial diversity and growth. The analysis revealed site-specific variations in these parameters, particularly in areas with low salinity and reduced DO levels, which may affect both ecosystem balance and microbial efficiency. A total of 144 isolates were obtained, with 28 strains exhibiting crude oil degradation capability. Among them, isolates 1ATIII 4, 1APII 6, and 2ATI 15 showed the highest degradation efficiency, reducing residual oil levels. to 0.0866 g, 0.0924 g, and 0.0965 g, respectively. These strains also demonstrated the ability to produce biosurfactants and utilize a wide range of carbon sources—an important mechanism contributing to their hydrocarbon-degrading performance. Although the findings underscore the remarkable potential of indigenous microorganisms in crude oil bioremediation, further investigations are necessary to expand their applicability. Molecular techniques, such as 16S rRNA gene sequencing, should be employed to identify the isolates at the genetic level and better understand their functional roles. Developing microbial consortia may enhance degradation efficiency through synergistic interactions among strains. Field-scale trials under real environmental conditions are essential to assess the robustness of these microorganisms amidst fluctuating natural parameters. Moreover, the characterization of biosurfactants produced by selected strains, along with evaluations of their safety, stability, and environmental impact, is crucial for the formulation of effective bio-based remediation products. This research provides a

foundational step toward advancing biotechnological solutions for sustainable environmental management in oil-contaminated coastal regions of Thailand

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