

(RESEARCH ARTICLE)



Hydrocarbon solubilisation by oil and cellulose-degrading *Chitinophaga terrae* isolated from the rumen.

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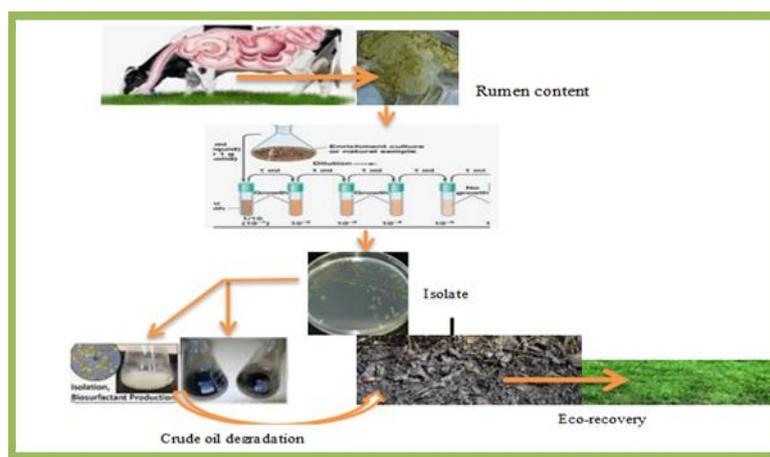
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Abstract

This study investigated the capacity of cellulose and hydrocarbon degrading bacterium isolated from the rumen of a cow to solubilise hydrocarbon. The bacterium was isolated from the rumen fluid of cow and its capacity to degrade cellulose was screened on carboxyl methyl cellulose (CMC) agar plate and the ability to degrade crude oil was carried out using Bonny Light crude. Solubilisation of hydrocarbon was determined by carrying out emulsification index (E_{24}) using kerosene. Other bio-surfactant characteristics such as blood haemolysis, tilted slide capacity and oil displacement were tested also. The bacterium was identified based on phenotypic, biochemical and molecular characteristics. The isolate achieved 48.17% degradation of total petroleum hydrocarbon (TPH) within 14 days with emulsification index of 54.5%. The isolate also produced clear zone on agar plate containing CMC as the sole carbon source. Phylogenetic tree analyses classified the bacterial isolate as *Chitinophaga terrae*. The sequences have been deposited to GenBank under the accession number KJ076216.1. This study has demonstrated that the novel strain of *Chitinophaga terrae* used in this study not only has the capacity for multiple substrate utilization, but also has the capacity to produce bio-surfactant. Considering that the isolate was obtained from the rumen of cow it shows that rumen content may harbour bacteria with diverse economical and ecologically-friendly product, which may be utilized for bioremediation of crude oil contaminated systems.

Keywords: Rumen; *Chitinophaga terrae*; Cellulose degradation, Hydrocarbon degradation, Bio-surfactants



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1. Introduction

In most developing countries, Nigeria inclusive, enormous health and environmental risk has been linked to hydrocarbon pollution of lands and water resulting from oil exploration activities [1]. Sadly, baseline levels of these oil pollution has surpassed World Health Organisation (WHO) bench mark and levels set by the Nigerian Department of Petroleum Resources (DPR) as stipulated in the Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN) [2].

In the bid to proffer lasting solution to this challenge, green bio-remediation options such as the use of microbes mined from different unique ecological niches are prospected for the recovery of these ecosystems [3, 4]. As microbes with genes capable of performing specialized functions can be quarried from different environment, these functions include cellulose-degrading, bio-surfactant production, and hydrocarbon degradation. The rumen chamber of herbivorous animals such as cattle, goats, sheep, buffalo, deer, and Carmel etcetera is one ecological niche that has always attracted researchers as potential sources of bacteria with unique properties [5].

In past years, researchers have isolated and characterised microbes with potential of producing from different sources such as insect guts, plant materials which are decaying, dropping of animals, different composts, soil [6,7]. Plant biomass is chiefly made of cellulose. This cellulose is considered as a renewable, inexpensive biopolymer which is also abundant on earth [8] Annual production of natural cellulose is about 100 billion metric tons and approximately 280 billion metric tons for the whole biomass [9] Polysaccharides, which are the major component of cellulose appear like fibrous crystals connected together by a β -1, 4- glycosidic bond of repeated D-glucose, units [10]. Microbes produce enzymes such as cellulase, endoglucanase, β 1-4 glycosidase and ligninase which are capable of breaking the β 1-4 glycoside bonds [10]. Microorganisms, mostly cellulolytic fungi and bacteria are those responsible for the breakdown of cellulosic materials [11] and converting it into economically viable product such as biofertilizer and biofuels and biogas through microbial and enzymatic actions [12, 13]. Cellulose-degrading bacteria also play an important role in the enzymatic degradation of organic compounds for a resultant release of nutrient. These nutrients sometimes are used as bio-stimulants for bioremediation purposes [14].

Additionally, different ecological niches are been mined for bacteria with special potential such as ability to produce degrade crude oil as well as produce bio-surfactants and stable enzymes which can be used in the remediation of oil polluted sites and for industry use [15,16]. Some microbes have the capacity to degrade hydrophobic substances such as petroleum as well as produce natural emulsifiers [17]. Biosurfactants are surface-active substances which by their structure and functions are considered amphiphilic. They reduce tension between molecules at surfaces and interfaces [18]. These characteristic properties, therefore, make it important in biosystems and may be considered as the life-wire of the biological membranes which ensures the transport and exchange of the various important materials [19; 20]. Biosurfactants are economical and ecologically friendly products used for the remediation process. For remediation processes to be considered as effective, poly aromatic hydrocarbon which is recalcitrant should be made bioavailable to microorganisms. This limited bioavailability of polycyclic aromatic hydrocarbons (PAHs) is a critical factor of concern that limits biodegradation in contaminated sites. Studies conducted by Zang *et al* [21] show that bio-surfactant produced by soil microbes enhances the PAH bioavailability. Oil spills clean-ups that are aided by the use of bio-surfactant have in recent times brought to the forefront as a promising technology. This technology was adopted in recently contaminated soils and groundwater remediation projects done by Zhang *et al* [22] and Mao *et al* [23]. The area of prospecting organisms or multiple function such as ability to degrade cellulose, crude oil and sometimes bio-surfactant have not fully been explored, hence, the primary objective of this research which is isolation of a bacterium strain capable of degrading crude oil and cellulose.

This study was therefore aimed at investigating the capacity of cellulose- and hydrocarbon-degrading bacterium isolated from the rumen of a cow to solubilize hydrocarbon.

2. Material and methods

2.1. Reagents

Chemicals, reagents and standards used in the study were of analytical grade. They were purchased from a commercial distributor Joechem Ventures Limited, Choba, Rivers State.

2.2. Collection of cow rumen content

Rumen contents were obtained from a cow slaughtered at an abattoir situated at Choba Market, in Obio-Akpor local government area of Rivers state, Nigeria. The samples were collected using a properly labeled sterile container and thereafter transported to the laboratory in an ice pack for bacteria enrichment and isolation.

2.3. Enrichment and isolation of hydrocarbon degrading strains

The selective enrichments were carried out using Bushnell Haas medium containing Bonny light crude oil (1% (v v-1) as the lone source of energy & carbon. One ml of rumen juice (taken from 10⁻¹ dilution) was aseptically transferred into 100ml Erlenmeyer flasks containing 98 ml enrichment medium (KCl 0.5 g L⁻¹; KH₂PO₄ 1 g L⁻¹; FeSO₄.7H₂O 10 mg L⁻¹, MgSO₄.7H₂O 0.5 g L⁻¹, NaNO₃ 3 g L⁻¹) adjusted to pH 6.3 ± 0.5), the medium was sterilized at 121°C for 15 minutes with the Bonny Light crude oil added aseptically. The seeded broth was agitated on a bench top shaker incubator at 120 rpm, 37°C for 20 days. At intervals of 5 days, 0.1 ml was drawn from the enrichment broth, serially diluted, and plated out on Carboxyl methyl cellulose (CMC) agar and Bushnell Haas agar respectively. This was to monitor the progress of enrichment by enumeration and picking off the dominant viable isolate. These isolates were purified and screened for cellulose-degrading ability. The desired organism was sub-cultured and preserved in slant for further analysis

2.3.1. Isolation and culturing of bacterial isolates

Ten-fold serial dilutions were performed for enriched sample suspension and 1ml of each dilution was poured into mineral salt agar plates to isolate crude oil utilizing bacteria and incubated at 30 °C for three to seven days. The same dilution was also poured into nutrient agar plates to enumerate the total heterotrophic bacterial count. Pure colonies were sub-cultured on CMC agar and then sent to Nutrient agar slants for morphological, biochemical, and molecular identification.

2.3.2. Screening for cellulose degraders

Selected colonies were screened further for cellulose utilization by using the halo from Congo red and iodine reaction described by Kasana *et al* [24]. This flooding with iodine was done after incubation period of 48-72 hours. The cellulose-degrading potential of the positive isolates was also qualitatively estimated by calculating hydrolysis capacity (HC), that is, the ratio of the diameter of clearing zone and colony [25, 26]. The strain that exhibited maximum zone of clearance was selected for further culture dependent and molecular characterization. The isolate of interest was sub-cultured and preserved in nutrient agar slant at 4 °C.

2.3.3. Screening for hydrocarbon degraders

In order to investigate the crude oil biodegradability efficacy of the isolate about 98 ml of mineral salt medium was dispensed in a 250 ml conical flask to create room for adequate headspace, 1% Bonny Light crude oil was introduced into the media and sterilization was performed at 121°C for 15 minutes and 15 psi. Upon cooling, 1 ml of broth culture was inoculated into the setup which was agitated with the aid of an orbital shaker incubator (Stuart, Germany S150) set to be shaken at 170 r.p.m at 37°C at intervals of 7 days. pH reading was taken using a pH meter while the optical density (absorbance) was taken at 560nm with the aid of SP-OPTIMA Spectrometer (Optima Japan0 [27]. Percentage degradation of total petroleum hydrocarbon was calculated after 14th day monitoring period elapsed.

2.3.4. Screening for emulsification potentials

To screen for potentials of biosurfactant production, the following activities and tests were carried out: (a) emulsification index (b) hemolytic activity and (c) oil spreading test. For Oil spreading test, a modified method of Zhang *et al* [22] and Padmapriya [28] were adopted which involved determination of diameter of the circle formed after the oil had expelled with a slide caliber after 30 sec; after 40 mL of distilled water + 20 ml crude oil + 10 ml free sell supernatant was gently dropped in the centre of the oil layer. For emulsification activity (E24) method of Nwaguma *et al* [18] and Satpute *et al* [20] where adopted where 2 ml of free cell obtained after 15 min centrifugation of the bacteria broth culture at 10,000rpm was added to 2 ml of kerosene then vigorously shaken or vortex at high speed for 2 min and allowed to stand for 24 hours before the height of the stable emulsion layer was measured. Positive control adopted in this study was tween 20 and sodium dodecyl sulphate (SDS) while water was used as negative controls. [18] and haemolytic activity by adding 5% (v/v) fresh sheep blood to a nutrient agar plates and observed for haemolysis after 37°C for 24 h incubation [18, 29,30].

2.4. Identification of the bacterium strain. (Morphology/Biochemical & Molecular).

Bacterial isolates were preliminarily identified according to their morphological features and biochemical characterization. The morphological features studied were colony characteristics, shape, size, spores, motility and elevation while the microscopic features were recorded for the isolate was according to Gram stain protocol. Biochemical properties investigated were MR-VP, catalase, oxidase, indole, H₂S production, Starch Hydrolysis, sucrose, maltose fructose and lactose. All the phenotypic characterization was carried out following the procedure described in Bergey's Manual of Determinative Bacteria [31, 32]. For the amplification of the 16S rRNA region of the rRNA genes of the bacterial isolates, PCR protocol was followed under the conditions of initial denaturation at 94°C for 5mins, 36 cycles of denaturation at 94°C for 30sec, annealed at 56°C for 30secs and elongated at 72°C for 45sec, final 72°C for 7 minutes elongation then 10 °C forever. Sequencing of the amplified DNA was done using the BigDye Terminator kit on a 3510 ABI sequencer (Inqaba Biotech., Pretoria, South Africa), edited by bioinformatics algorithm trace edit, similar sequences downloaded by BLAST (BLAST, www.ncbi.nlm.nih.gov/BLAST/Blast.cgi).and aligned using Clustal-X evolutionary relationships (construct phylogenetic trees) was deduced using Neighbor-Joining method in MEGA 6.0.

3. Results and discussion

3.1. Bacteria enumeration

The presence of microbial activity in the enrichment setup was determined by the enumeration of culturable cellulose degrading bacteria and total hydrocarbon utilizing bacteria .Sampling was done at intervals of 5 days over a 20-day period. The sample recorded highest cfu with a mean value of $2.92 \pm 0.098 \times 10^6$ cfu/g from organism grown in CMC agar plate and highest hydrocarbon utilising bacteria (HUB) count with a mean value of $1.68 \pm 0.1 \times 10^6$. These counts were recorded on the fifth day of the monitoring period. Continuous decline in the bacteria counts suggests that enrichment process helps to screen out organisms that are either unable to withstand the certain concentrations of the crude oil or are unable to utilise crude oil as carbon source.

3.1.1. Isolation of Cellulose -degrading Bacterium

Colony was isolated to have demonstrated cellulose degrading potential through the screening of size of halo zone around the colonies on carboxyl methyl cellulose agar plates after being stained with Congo. (**Figure1**). Isolate A7 identified as *Chitinophaga terrae* Strain KJ076216.1 had zones of clearance and colony diameter of 1.5cm and 0.1cm respectively.



Figure 1 Screening for cellulose degrading bacterium by CMC plate; and flooding with Congo red stain. Zone of clearance appeared around the cellulose degrading bacterium colony

3.1.2. Hydrocarbon degradation

In the biodegradability study, the presence of microbial activity was evident by the constant increase in turbidity of the setup as presented in **Figure 2**. Three isolates labelled as Wh1, A7 and A5 where studied. Sampling was done at intervals of 2 days within a forth night period. Within the first five days of the experiment, no significant change in turbidity was observed. However, a sharp rise in the OD value was seen between the 5th to the 14th day. On the contrary, pH values gradually increased until the 5th day before a decline was experienced. Thus, alkalinity increased as crude oil

degradation progressed as seen in **Figure 3**. Turbidity, consequently, increased with increase in bacteria count. This result agrees with the report of Latha and Kalaivani [33] which stated that the correlation between increased oil degradation and increase in microbial population is a pointer that the isolates were responsible for the crude oil degradation that occurred.

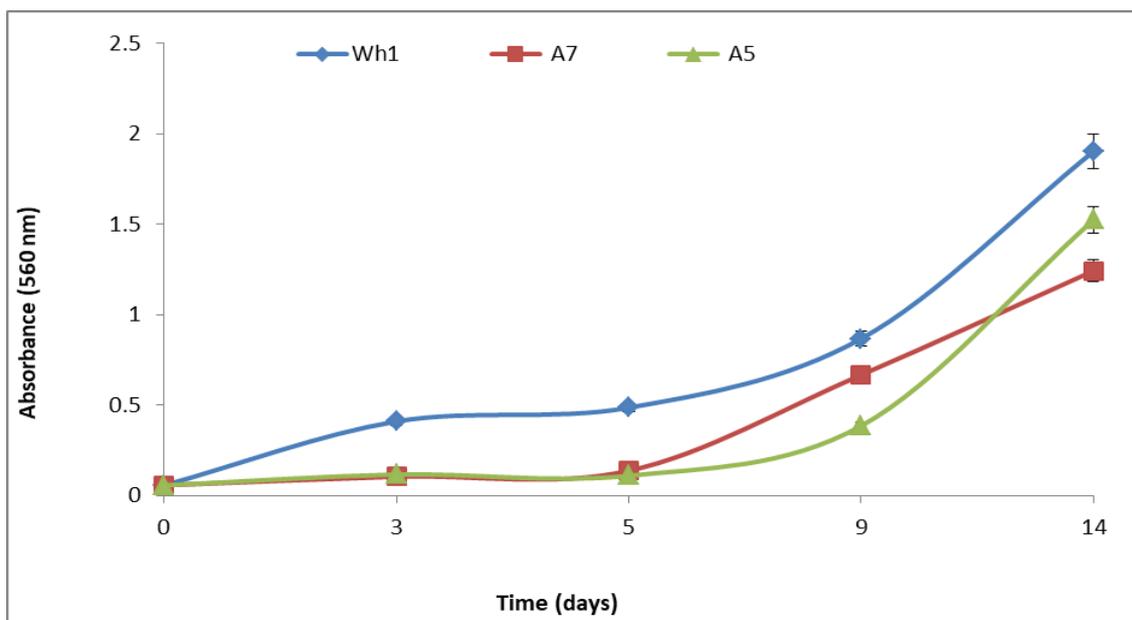


Figure 2 Biodegradability characteristics of the isolate based on changes in turbidity when grown on mineral salt broth over 14 days. A7= *Chitinophaga terrae*

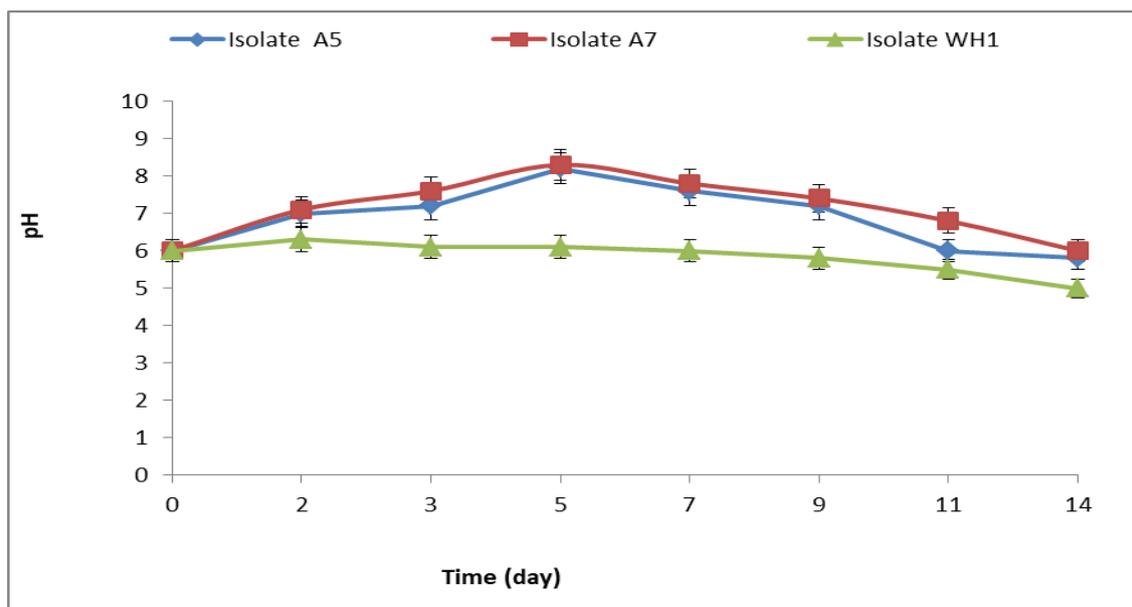


Figure 3 pH changes during crude oil degradation by the isolate

3.1.3. Total Petroleum Hydrocarbon degradation

To determine the biodegradability efficacy of isolate, changes in total petroleum hydrocarbon in the control setup was compared to that which was seeded with the isolate. This was ascertained using gas chromatograph. The carbon atoms present were C-8 to C-31, excluding C29 and C-32. Initially, TPH was 28,129.05 mg/kg but at the 14th day, setup A7 had TPH of 13,846.60mg/kg and Control 27,397.02 mg/kg with percentage degradation of 50.77mg/kg and 2.60mg/kg

respectively as shown in **Figure 4**, No significant degradation was observed for C-14 seen while; there was a total degradation of C-13, C24, C25 and C28 and the percentage residual carbon shown in **Table 1**.

Total petroleum hydrocarbon degradation as observed in this study provides evidence that the consortium of bacteria mined from the rumen chamber could possess a plethora of intrinsic ability including crude oil degradation as observed by means of GC-FID. This finding supports report of Okoye *et al* [34] and Agbaji *et al* [35] on the isolation of hydrocarbon degraders from unique environments.

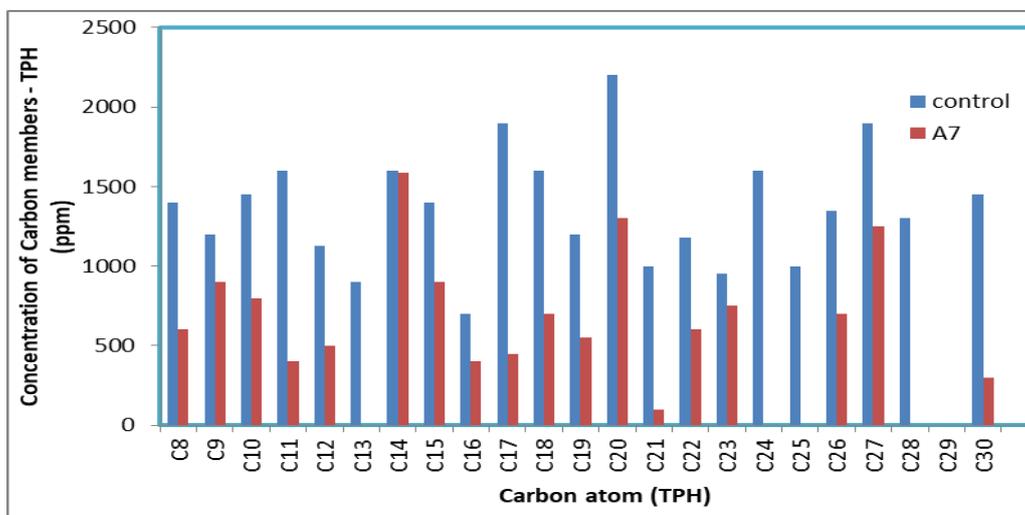


Figure 4 Total petroleum hydrocarbon (TPH) after 14 days of degradation.

Table 1 Percentage TPH degradation of individual carbon atom carbon.

| Carbon atom | control | Isolate A7 | % degradation | residual |
|-------------|---------|------------|---------------|----------|
| C8 | 1400 | 600 | 57% | |
| C9 | 1200 | 900 | 25% | |
| C10 | 1452 | 800 | 45% | |
| C11 | 1600 | 400 | 75% | |
| C12 | 1125 | 500 | 56% | |
| C13 | 900 | - | 100% | |
| C14 | 1600 | 1590 | 6% | |
| C15 | 1400 | 900 | 36% | |
| C16 | 700 | 400 | 43% | |
| C17 | 1900 | 450 | 76% | |
| C18 | 1600 | 700 | 56% | |
| C19 | 1200 | 550 | 54% | |
| C20 | 2200 | 1300 | 41% | |
| C21 | 1000 | 100 | 90% | |
| C22 | 1180 | 600 | 49% | |
| C23 | 950 | 750 | 21% | |
| C24 | 1600 | - | | 100% |
| C25 | 1000 | - | | 100% |
| C26 | 1350 | 700 | 48% | |
| C27 | 1900 | 1250 | 34% | |
| C28 | 1300 | - | 100% | |
| C29 | - | - | | - |
| C30 | 1450 | 300 | 79% | |
| C31 | 1500 | 900 | 40% | |

3.2. Isolate Identification

The bacteria isolated from the rumen content of cow obtained from an abattoir situated at Choba Market, at Choba in Obio/akpor Local Government Area of Rivers state Nigeria was initially labelled as A7. **Table 2** illustrated morphological characteristics for isolates. Isolate A7 was distinguished with cocci, golden yellow, smooth, round and opaque. Biochemical assays shows positive result for catalase, Voges- Proskauer and starch test and negative outcome for indole, oxidase and hydrogen sulphide production. 16S rDNA fingerprinting method which bacteria is based on highly conserved regions, which help in the analysis. The amplicons which were amplified, eluted and sequenced were almost 1.2 Kb. From the results, the morphological, biochemical and molecular characteristic features of the isolate A7 is shown in Table 1 and figure 4 respectively. The organism was identified as *chitinophaga terrae* with a 96% homology percentage as shown by **Figure 5**.

Table 2 Morphological and Biochemical test performed on the isolate.A7

| Test/Characteristics | Result | Test/Characteristics | Result |
|----------------------|---------------|--------------------------|--------|
| Size | 0.3-0.35 | MR | - |
| Shape | cocci | Voges- Proskauer | + |
| colour | Golden yellow | catalase | + |
| elevation | smooth | oxidase | - |
| margin | round | Indole | - |
| Opacity | opaque | H2S production | - |
| motility | none | Starch Hydrolysis | + |
| Gram nature | Negative | Sucrose | - |
| Growth at 370C | Positive | Maltose/fructose lactose | - |

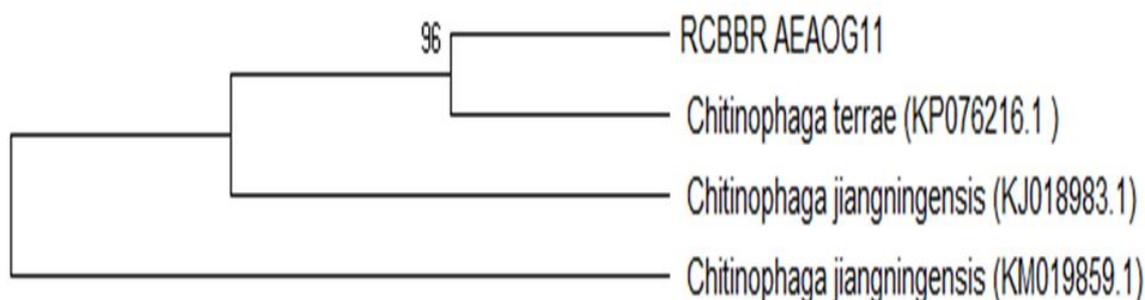


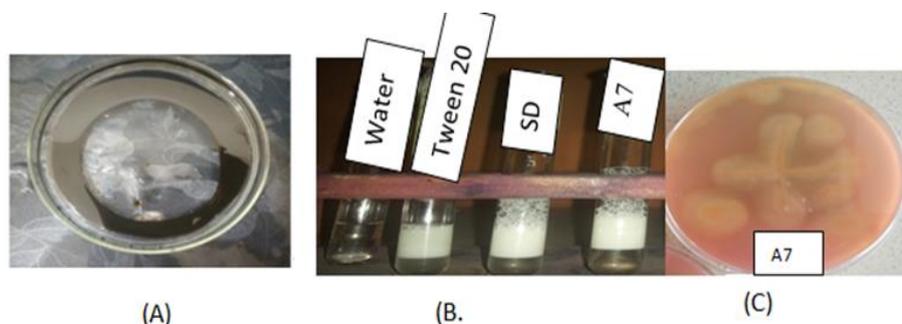
Figure 5 Phylogenetic tree constructed by the neighbour-joining method based on 16S rRNA gene sequences showing the relationship of the isolated stain as *Chitinophaga terrae* KP076216.1 retrieved from NCBI GenBank.

3.3. Bio-surfactant screening

Some tests had been recognized for the screening of potential bio-surfactant producing microbes. These tests have been broadly explored by different authors and consist of the following: haemolytic test, a measure of surface/interfacial tension, emulsification index/assay and oil displacement [19, 3, 37]. By utilizing some of these screening approaches, the potential for bio-surfactant production efficacy of isolate A7 was investigated. **Table 3** shows outcomes of the investigations for bio surfactant production potential test performed on the isolate.

Table 3 Bio-surfactant production characteristics of the isolate.

| Parameter | Result |
|---|--------------|
| Height of solution (cm) | 3.2± 0.34 |
| Height of emulsion layer(cm) | 1.75± 0.52 |
| Height of emulsion layer/Total height of the solution. | 0.547 |
| E ₂₄ % (Height of emulsion layer/Total height of solution)x 100 | 54.7 ± 0.5%, |
| Oil spreading(mm) | 50.50 ± 0.57 |
| Haemolysis | Beta |

**Figure 6** Evaluation of biosurfactant-producing ability and activity of *Chitinophaga terrae* Strain KJ076216.1 (a) Oil displacement test (b) Emulsification activity test and (c) Haemolysis test

The oil displacement test measures the surface activity of the culture supernatant. This is measured by the diameter of the circle formed (clear zone) after the oil has been expelled displacement of oil. The wider diameter of the oil displacement, indicate higher surface activity of the bio-surfactant. Oil dispersed by the supernatant of *Chitinophaga terrae* gave a diameter of 50.50 ± 0.57 mm, which indicates the presence of high concentrations of surface-active compounds. In a similar work done by Nwaguma *et al* [18] *Pseudomonas* sp. G4 isolated from hydrocarbon polluted soil in Ogoni land, Nigeria has an oil spreading value of 23.8mm (crude-oil). Nwaguma *et al* [18] also reported beta haemolysis on blood agar. The microbial production of surfactants by microorganisms isolated from hydrocarbon polluted soil and other hydrophobic substances has been often reported [38, 39] From the result biosurfactant screening test, Culture supernatant for the isolated strain gave an emulsification index (E₂₄) of 54.7 ± 0.5%, this result was not in large variance with positive control (SDS) (64.77 ± 1.6%). In a similar work done by Nwaguma *et al* [18], *Pseudomonas* sp. G4 isolated from hydrocarbon polluted soil in Ogoni land, Nigeria had an E₂₄ of 11.1%. This shows that the *Chitinophaga terrae* have a promising ability for hydrocarbon degradation. There are other reasons for disparities in the result of emulsification index. The nature and type of oil should be considered. In the work of Nayak *et al* [40] bio-surfactant production and engine oil degradation of a halotolerant marine bacterium *Bacillus licheniformis* LRK1 was studied using various screening methods. It showed an Emulsification Index (EI₂₄) of 70%, a positive haemolysis test. Bio-surfactant production was confirmed by visualizing oil the spreading. The oil displacement generated a clear zone with a diameter of 6.8 ± 0.2 cm. The variance in the Emulsification index and Oil spreading value here could be attributed to differences in media in which they were tested. Therefore, hydrocarbon emulsification in water is essential to pave way for the biodegradation. This helps to make the pollutant in the environment more bioavailable to microorganisms and hence, increase the rate of biodegradation [41, 42, 43]. Beta haemolysis was observed using the haemolytic activity test of the culture supernatant which is an indication of bio-surfactant production, haemolytic test, however, is not precise as there had been cases of either false negative or positive outcome [36].

4. Conclusion

This study was the first research about introducing - *Chitinophaga terrae* KJ076216.1 as a crude oil-degrading bacteria strain which can degrade cellulosic material and also as a potential of producing bio-surfactant. It can therefore be concluded by the outcome of this study that the rumen content/fluid is an ecological niche that could be mined for economical and ecologically friendly products and for bio-prospecting aimed at industrial as well as bioremediation purpose.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest is hereby declared by the authors.

References

- [1] Akcha F, Burgeot J, Narbonne JFP. Garrigues, PAHs: An Ecotoxicological Perspective, John Wiley & Sons, Ltd, Chichester, UK. 2003.
- [2] Department of Petroleum Resources, Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN). 2002.
- [3] Naeem U, Qazi MA. Leading edges in bioremediation technologies for removal of petroleum hydrocarbons, Environ. Sci. Pollut. Res. 2020; 27: 27370–27382.
- [4] Azubuike C, Chikere CB Okpokasili GO. in: Bioremediation: An Eco-Friendly Sustainable Technology for Environmental Management, Bioremediation of Industrial Waste for Environmental Safety, Springer Nature Singapore Pte Ltd, Singapore. 2020; 19–32.
- [5] Saranya Kuppusamy, Palanisami Thavamani, Surender Singh, Ravi Naidu and Mallavarapu Meghara. j Polycyclic aromatic hydrocarbons (PAHs) degradation potential, surfactant production, metal resistance and enzymatic activity of two novel cellulose-degrading bacteria isolated from koala faeces. Environmental Earth Science. 2017; 76: 14.
- [6] Doi RH. Cellulase of mesophilic microbes: cellulosome and non-cellulosome producers. Ann. N.Y.Acad. Sci. 2008; 1125: 267–279.
- [7] Stanley HO, Ogbonna CB, Abu GO. Exploration of one- Factor RSM to optimize concentration of organic fraction of municipal soil waste (OFMSW) for biogas production. International Journal of waste resources. 2017; 7: 293.
- [8] Khaleel HL, Abd AN, Ali KM. Preparation of Nano-Cellulose from Industrial Waste by Ultrasonic Device. Journal of Biochemical Technology. 2018; 9: 35.
- [9] Cheng Q, Wang J, McNeel JF, Jacobson PM. Water Retention Value Measurements of Cellulosic Materials Using a Centrifuge Technique. BioResources. 2010; 5: 1945-1954.
- [10] Zaghoud L, Gouamid M, Benmenine A, Khanblouche A. (.Kinetic and Thermodynamic of Gentian Violet Removal by 2, 3-Dialdehyde Nanocellulose. Journal of Biochemical Technology. 2019; 10: 38-42.
- [11] Ojumu T, Solomon V, Bamidele O, Betiku E, Layokun SK. Cellulase Production by *Aspergillus flavus* Linn Isolate NSPR 101 fermented in sawdust, bagasse and corncob. African J. Biotechnol. 2003; 2: 150–152.
- [12] Dubey Sumit Kumar, Meena Rakesh Kumar, Sao Shweta, Patel Jitendra, Sanket Thakur, Prashant Shukla. Isolation and Characterization of Cellulose Degrading Bacteria from Biogas Slurry and Their RAPD Profiling. Current Research in Microbiology and Biotechnology. 2014; 4: 416-421.
- [13] Yucai, Wang Xiaofen, Li Ning, Wang Xiaojuan, Ishii Masaharu, et al. Characterization of the effective cellulose degrading strain CTL-6, Journal of Environmental Sciences. 2011; 23: 649–655.
- [14] Romanus AA, Ikechukwu EF, Patrick AS, Goddey U, Helen O. Efficiency of plantain peels and guinea corn shaft for bioremediation of crude oil polluted soil, J. Microbiol. Res. 2015; 5: 31–40.
- [15] Haakana H, Mittinen-Oinonen A, Joutsjoki V, Mantyla A, Souminen P, Vahmaanpera J. Cloning of cellulase from *Melanocarpus albomyces* and their efficient expression in *Trichoderma reesei*. Enzyme Microbial Technology. 2004; 34: 159-167.
- [16] Ashjaran A, Sheybani S. Drug Release of Bacterial Cellulose as Antibacterial Nano Wound Dressing. International Journal of Pharmaceutical Research & Allied Sciences. 2019; 8: 137-143.

- [17] Yalaoui-Guellal, Drifa, Fella-Temzi Samira, Djafri-Dib Salima, Sahu Sunil Kumar, Irerere Victor U, Banat Ibrahim M, Madani Khodir .The petroleum-degrading bacteria *Alcaligenes aquatilis* strain YGD 2906 as a potential source of lipopeptide biosurfactant. *Fuel*. 2021; 285: 112–119.
- [18] Ijeoma Vivian Nwaguma, Chioma Blaise Chikere, Gideon Chijioke Okpokwasili .Isolation, Screening and Identification of Biosurfactant-producing Bacteria from Hydrocarbon-polluted and Pristine Soils within Ogoniland, Nigeria *British Microbiology Research Journal*. 2016; 15: 2231-0886.
- [19] Liang X, Guo C, Wei Y, Lin W, Yi X, Lu G, Dang Z. Cosolubilization synergism occurrence in codesorption of PAH mixtures during surfactant enhanced remediation of contaminated soil. *Chemosphere*. 2016; 144: 583-590.
- [20] Satpute SK, Arun GB, Prashant KD, Banat IM, Chopade AC. Methods for investigating biosurfactants and bioemulsifiers: A review.*Critical Reviews in Biotechnology*. 2010; 30: 127–144.
- [21] Zang T, Wu H, Yan B, Zhang Y, Wei C. Enhancement of PAHs biodegradation in biosurfactant/phenol system by increasing the bioavailability of PAHs. *Chemosphere*. 2020; 128941.
- [22] Zhang X, Xu D, Zhu C, Lundaa T, Scherr KE. Isolation and identification of biosurfactant producing and crude oil-degrading *Pseudomonas aeruginosa* strains. *Chemical Engineering Journal*. 2012; 209: 138-146.
- [23] Mao X, Jiang R, Xiao W, Yu J. Use of surfactants for the remediation of contaminated soils: a Review journal of hazard material. 2015; 285: 419- 435.
- [24] Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A. A rapid and easy method for the detection of microbial cellulases on agar plates using gram's iodine. *Current Microbiology*. 2008; 57: 503-507.
- [25] Pratima Gupta, Kalpana Samant, Avinash Sahu. Isolation of cellulose degrading bacteria and determination of their cellulolytic potential. *International Journal of Microbiology*. 2011; 2012: 5.
- [26] Hendricks WC, Doyle DJ, Hugley B. A new solid medium for enumerating cellulose-utilizing bacteria in soil," *Applied and Environmental Microbiology*. 1995; 5: 2016–2019.
- [27] Effiong E Agwa OK, Abu GO. Niche-proxies of Hydrocarbon-impacted Rhizosphere Soil of Weeds of Bodo in Gokana, Rivers State, Nigeria. *Archives of Current Research International*. 2019; 19: 1-14.
- [28] Padmapriya B, Isolation and screening of biosurfactants produced by *Pseudomonas aeruginosa* from oil spilled soils. *International Journal of Pharmacy and Biological sciences*. 2012; 3.
- [29] Seema Dhail. Isolation of potent biosurfactant producing bacteria from oil spilled marine water and marine sediments *African Journal of Biotechnology*. 2012; 11: 16751-16757.
- [30] Carrillo PG, Mardaraz C, Pitta – Alvarez SI, Giulietti AM. Isolation and selection biosurfactant – producing bacteria. *World Journal of Microbiology Biotechnology*. 1996; 12: 82-84.
- [31] Holt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST. *Bergey's Manual of Determinative Bacteriology*. 9th Ed. Williams and Wilkins Company, Baltimore, USA. 1994; 71-561.
- [32] De Ley J, J Swings, F. Gossele NR, Krieg JG. Holt (eds.) (9th ed.) *Bergey's Manual of Systematic Bacteriology*. 1984; 215: 268-74.
- [33] Latha R, Kalaivani R. Bacterial degradation of crude oil by gravimetric analysis. *Advances in Applied Science Research*. 2012; 3: 2789-2795.
- [34] Okoye AU, Chikere CB, Okpokwasili GC. Isolation and Characterisation of hexadecane Degrading Bacteria from Oil-Polluted soil in Gio Community, Niger Delta, Nigeria. *Scientific African*. 2020; 8: 2468-2276.
- [35] Agbaji JE, Abu GO, Nwaichi EO. Catechol-2,3-Dioxygenase and Crude Oil Biodegradation Screening of Rhizo-Bacterial Endophytes from Bodo-Gokana, Rivers State *Current Journal of Applied Science and Technology*. 2019; 38: 1-12.
- [36] Walter V, Syldatk C, Hausmann R. Screening concepts for the isolation of biosurfactant producing microorganisms. *Biosurfactants*. 2010; 1–13.
- [37] Varjani SJ, Upasani VN. Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant. *BioresourceTechnology*. 2017; 232: 389–97.
- [38] Iqbal S, Khalid Z, Malik K. Enhanced biodegradation and emulsification of crude oil and hyperproduction of biosurfactants by a gamma ray-induced mutant of *Pseudomonas aeruginosa*. *Letters in Applied Microbiology*. 1995; 21: 176–9.

- [39] Kumar M, Leon V, Materano ADS, Ilzins OA, Galindo-Castro I, Fuenmayor SL. Polycyclic aromatic hydrocarbon degradation by biosurfactant-producing *Pseudomonas* sp. IR1. *Zeitschrift für Naturforschung C*. 2006; 61: 203–12.
- [40] Nayak NS, Purohit MS, Tipre DR, Dave SR. Biosurfactant production and engine oil degradation by marine halotolerant *Bacillus licheniformis* LRK1. *Biocatalysis and Agricultural Biotechnology*. 2020; 101808.
- [41] Quek E, Ting YP, Tan HM. *Rhodococcus* sp. F92 immobilized on polyurethane foam shows ability to degrade various petroleum products. *Bioresource Technology*. 2006; 97: 32–8.
- [42] Bredholt H, Josefsen K, Vatland A, Bruheim P, Eimhjellen K. Emulsification of crude oil by an alkane-oxidizing *Rhodococcus* species isolated from seawater. *Canadian journal of Microbiology*. 1998; 44: 330–40.
- [43] Mnif S, Chamkha M, Labat M, Sayadi S. Simultaneous hydrocarbon biodegradation and biosurfactant production by oilfield selected bacteria. *Journal of Applied Microbiology*. 2011; 111: 525–36.