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Crude oil hydrocarbon degradation efficiency of indigenous bacterial strains isolated from contaminated sites in Nigeria

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Abstract

The crude oil degradation potential of bacterial isolates from three contaminated sites in Nigeria were investigated. Seven bacterial isolates namely *Pseudomonas aeruginosa* strain W15*, Pseudomonas aeruginosa* strain N3R*, Serratia marcescens* strain N4, *Providencia vermicola* strain W8, *Serratia marcescens* strain W13, *Pseudomonas aeruginosa* strain W11 and *Pseudomonas protegens* strain P7 were isolated and identified using molecular methods. Isolates N4, N3 and W13 showed higher % total petroleum hydrocarbon (TPH) degradation of 79.26%, 78.96% and 78.69% respectively than W15, P7, W8 and W11 with % TPH degradation of 68.96%, 62.14%, 59.75% and 59.00% respectively. W13 showed the fastest degradation rate with 78.72% within the first 14 days of incubation; however, after the 14th day, there was no progressive change in % TPH. W11 showed degradation of wider range of hydrocarbon components originally in the crude oil as well as the complete degradation of most intermediates formed. The isolates showed good degradation potentials for bioremediation applications.

Keywords: Bacterial strains, Crude oil, Hydrocarbons, Bioremediation, Total petroleum hydrocarbon degradation.

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INTRODUCTION

Crude oil contamination as a result of leakages and spills has been an environmental issue for several decades (Hanafy *et al.,* 2015; Moss, 2010). In Nigeria, various oil spills, leakages and oil pipeline vandalism are common and the economy depends highly on the revenue derived from crude oil (Ite *et al.*, 2018; Nwilo and Badejo, 2006; UNEP, 2017). With the increase in

development of several small independently owned and government refineries, the problems with oil spills will definitely increase. Crude oils are toxic, carcinogenic and result in bioaccumulation and biomagnification, especially through aquatic foods (Afshar-Mohajer *et al.*, 2019; Shukla and Cameotra, 2012). According to Naik and Duraphe (2012), remediation of a contaminated site reduces the risk of adverse effect of the pollutant to the environment and health of individuals as well as redevelopment of the site for possible use. Sometimes, especially in water bodies, major volume of the spilled crude oil is removed through physical approach like the use of booms, skimmers and /or adsorbent materials (Michel and Fingas, 2015; Motta *et al.*, 2018). However, significant amounts still persist in the contaminated sites and are subject to degradation by indigenous living organisms in the site. A major limiting factor during degradation is the availability of the crude oil to the degrading microbes which is a characteristic of the hydrophobicity of the crude oil (Dasgupta *et al.*, 2013; Dejvakumari *et al.*, 2020).

In crude oil contaminated area, with limited or no amount of readily available carbon source, microorganisms are forced to degrade crude oil hydrocarbons to obtain carbon, energy and also reduce physiological stress presented by the crude oil (Ra *et al.*, 2019; Xu *et al.*, 2018). This is possible because microorganisms tend to have good adaptation mechanisms to changes in their environment which varies across species and/or strains. They achieve this either by employing specific enzymes or developing new metabolic pathways through genetic changes (Malaotva, 2005; Pawar, 2015). Furthermore, bacteria especially, can easily use alternate electron acceptors besides oxygen during crude oil degradation, and as a result, dominate other microorganisms in crude oil contaminated habitat (Boopathy, 2000; Paliwal *et al.*, 2011; Wartell *et al.*, 2021). Nigeria is a tropical country capable of encouraging the existence of significant biodiversity of microorganisms (Alsaffar *et al.*, 2020; Chaillan *et al.*, 2006; Obi *et al.*, 2016). As Nigeria continues to depend on oil as the major source of revenue, the problems of oil pollution will definitely increase and there is an urgent need to isolate and maintain microbial cultures of efficient crude oil degraders. The aim of this study was therefore, to isolate crude oil degraders from crude oil contaminated sites in Nigeria and to evaluate their potentials for crude oil degradation.

MATERIALS AND METHODS

Samples, crude oil, and media

Water samples (three each) for bacterial isolation were obtained from crude oil contaminated fields in Obagi community, Rivers State and Kolo creek, Bayelsa State Nigeria. These two sites are highly exposed to re-occurring hydrocarbon contamination from crude oil spills because of oil wells and pipelines situated in the area. Three wet soil samples were also obtained from crude oil contaminated site in Ikpandiagu, Nsukka, Enugu State and mixed to form a single sample. For routine cultures, nutrient agar (NA, from Oxoid Limited, Hampshire, United Kingdom) was used unless otherwise stated, while for biodegradation experiment, Bushnell Haas media (Bushnell and Haas, 1941) at pH 7.2 and supplemented with 1% (for isolation) and 2% (for degradation evaluation) filter-sterilized crude oil obtained from Obagi OMLC, Rivers State, Nigeria was used. Nutrient broth was used for revitalization of pure isolates prior to inocula preparations. Experiments were performed in triplicates.

Isolation and enumeration of microbes from crude oil contaminated samples

For bacterial isolation, 1 ml of sample was inoculated into 50 ml of Bushnell Haas broth (BHB) supplemented with 1% filter-sterilized crude oil and incubated at room temperature with intermittent shaking for 14 days. For the wet soil sample, it was shaken and allowed to settle for an hour before 1 ml aliquot was used for inoculation. After the 14 days, serial dilution-spread plate method was employed in the inoculation of NA and Bushnell Haas Agar (BHA) using 1 ml of culture to determine the total heterotrophic count (THC) and total hydrocarbon utilizer count (THUC) respectively. The NA and BHA plates were incubated at 30˚C for 48 h and 7 days respectively. To subculture from BHA plates, colonies were randomly selected based on colony morphology, serial dilution-spread plate method using 0.1 ml was used to inoculate NA plates and incubated at room temperature for 24 h. Colonies were subsequently selected for subculture on NA plates by streaking method and incubated at room temperature for 24 h. The pure isolates were preserved in BHA slants with crude oil and kept in the refrigerator at 4˚C for future use.

Inocula preparations

Wire loopful of isolate cultures from BHA slants were inoculated into 10 ml nutrient broth each and incubated for 24 h at room temperature. Aliquots and bacterial cell count of the pure cultures were obtained as described in Nnabuife *et al.* (2021).

Determination of degradation potential and confirmation of carbon source

To determine the degradation potential and ascertain that the source of carbon for exponential cell growth was from the degradation of the crude oil hydrocarbon, 1.0 ml of each bacterial aliquot was used to inoculate 30 ml of sterilized Bushnell Haas Broth (BHB) at pH 7.2 and supplemented with 0.3 ml crude oil (1% v/v). Whereas, to confirm the carbon source in the media, 3.0 ml of bacterial aliquot was inoculated into 30 ml BHB of pH 7.2 fortified with 1% ammonium sulfate without crude oil. All setups were incubated at room temperature for 14 days with intermittent shaking. Enumeration of viable cells was performed using plate count method as earlier stated.

Identification and characterisation of the crude oil degrading bacterial isolates

Morphological and Biochemical identification

Pure cultures of the isolates were characterised based on their morphological and physiological properties when compared to Bergey's Manual for Determinative Bacteriology (Holt *et al.*, 1994; Caequeria *et al.*, 2011).

Molecular identification of the seven selected isolates by DNA sequencing

DNA extraction was performed using ZR fungal/bacterial DNA miniprep (ZYMO research, Hunan Province, China, cat number: d6005). PCR was carried out in a Gene Amp 9700 PCR System Thermal cycler (Applied Biosystem Inc., Massachusetts, USA). Primers (16S F: GTGCCAGCAGCCGCGCTAA and 16S R: AGACCCGGGAACGTATTCAC) were applied for bacterial identification. The amplification conditions used were as follows: 94˚C for 5 mins followed by 35 cycles of denaturation at 94˚C for 30 s, annealing at 60˚C for 30 s, extension at 72˚C for 45 s and final extension at 72˚C for 7 min. The integrity of the DNA and PCR amplicons were checked on 1% and 1.5% agarose gel respectively. Then, the 16S rRNA amplified fragments were ethanol purified in order to remove the PCR reagents and the purified fragment was checked on a 1.5% agarose gel ran on a voltage of 110V for about 1h, to confirm the presence of the purified product. It was quantified using a nanodrop (Model 2000 from Thermo Fisher Scientific Corporation, Massachusetts, USA). The purified samples were sequenced using a Genetic Analyzer 3130xl sequencer (Applied Biosystems Biotechnology Company, Massachusetts, USA). The sequencing reaction was prepared using the Big Dye terminator v3.1 cycle sequencing kit according to manufacturers' instructions. Bio- Edit software version 7.2 and MEGA 6 (2013) were used for all genetic analysis.

The blast tool of the NCBI was used to identify the species using the 16S ribosomal RNA gene sequences of the isolates. The sequences were then deposited to GenBank database.

Screening for crude oil biodegradation potentials of the selected isolates

Experiments were set up according to Nnabuife *et al.* (2021). Samples were analysed every seven days. Viable cell counts were determined by inoculating into NA plates, 0.1 ml of 1 ml serially diluted broth cultures using spread plate method. The plates were incubated at room temperature for 24 h.

Biodegradation screening by gravimetric analysis.

The residual crude oil extraction was performed using solvent extraction method with *n*-hexane as described in Nnabuife *et al.* (2021). The percentage crude oil removed was calculated according to Kaczorek and Olszanowski (2011) as:

% crude oil removed = $[(W_{CONTROL} - W_{TEST\ SAMPLE})/W_{CONTROL}] \times 100$

Where W = weight of crude oil

Biodegradation screening by gas chromatography-mass spectrometry (GC-MS) analysis.

The *n*-hexane extracted samples at 14th and 28th day were analyzed with a triple quadruple Gas

Chromatograph-Mass Spectrometer (GCMS – QP2010SE Shimadzu, Japan) according to Nnabuife *et al*. (2021).

The results of the GC-MS were analyzed and represented in percentages according to Michaud *et al.* (2004).

Percentage degradation = 100 – (total peak area of sample x 100 **/** total peak area of control)

Statistical analysis

One-way analysis of variance (ANOVA) in Microsoft excel (2017) was used to carry out the statistical analyses on the variations in viable cell growth, % crude oil removed and % TPH degradation. A probability value of < .05 was used as the criterion for statistical significance. The error bars represent standard error of mean (SEM).

RESULTS

Isolation, enumeration and selection of crude oil degrading bacterial isolates

The samples from Obagi community, Kolo creek and Ikpandiagu, yielded THUC of 1.57 X 10^9 $CFUml^{-1}$, 1.83 X 10^9 CFU/ml and 2.43 X 10^{10} $CFUg^{-1,}$ whereas the THC obtained were 2.3 X 10^8 $CFUml^{-1}$, 4.1 X 10^8 $CFUml^{-1}$ and 9.6 X 10^8 $CFUg^{-1}$ respectively. A total of 40 bacterial isolates were obtained from three crude oil contaminated sites after randomly selected colonies with varying colony descriptions were sub-cultured on nutrient agar (NA) plates. From the 40 bacterial isolates, seven isolates designated as W15, W8, W13, W11 (isolates from Kolo creek); N3, N4 (isolates from Ikpandiagu) and P7 (isolate from Obagi) were selected as potentially efficient degraders based principally on their growth on 1 % (v/v) crude oil enriched Bushnell Haas Broth (BHB) and death rate in the absence of crude oil after 14 days of incubation (Figure 1). The selected isolates produced higher viable cell count in the presence of crude oil and lower cell count in the absence of crude oil.

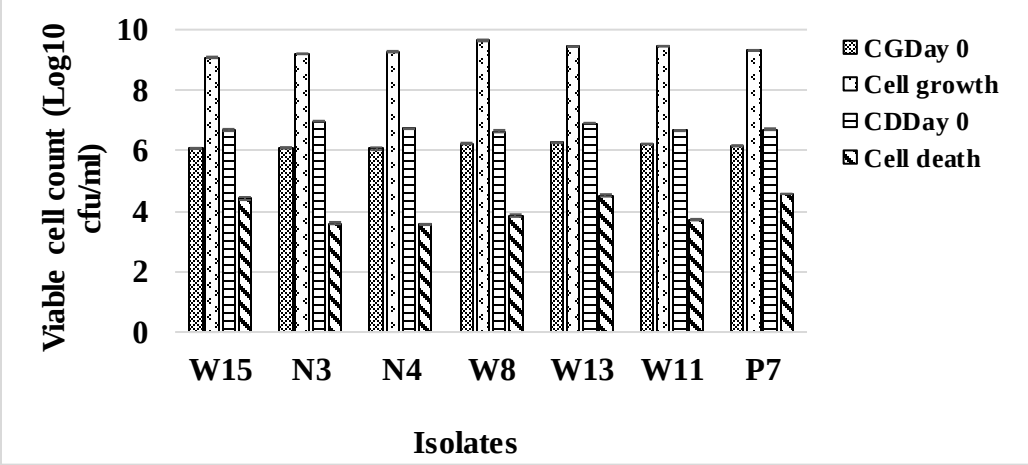

Figure 1: Growth response of the isolates in the presence and absence of crude oil as carbon and energy source

Identification and characterisation of the crude oil degrading bacterial isolates

The morphological and physiological characterisation showed the seven isolates to be Gram negative rods (Table 1). Except for N4 and W13 which appeared as red colonies, other selected isolates appeared as milk colonies on NA media. Figure 2 shows the constructed phylogenetic tree based on the 16S rRNA sequences of the isolates and other bacterial strains with minimum of 95% similarities from the

NCBI database using Mega X version 10.2.5 (Hansa log/MEGA Software, Dortmund, Germany, 2021). The calculations were performed according to the neighbor-joining algorithm (Bootstrap number = 1000). The 16S rDNA gene partial sequence data represent the named organisms below with the accession numbers in the NCBI GenBank database: *Pseudomonas aeruginosa* strain W15 (MW320658), *Pseudomonas aeruginosa* strain N3R (MW320659.1), *Serratia marcescens* strain N4 (MW320660.1), *Providencia vermicola* strain W8 (MW320661.1), *Serratia marcescens* strain W13 (MW320662.1), *Pseudomonas aeruginosa* strain W11 (MW332545.1) and *Pseudomonas protegens* strain P7 (MW320664.1).

Screening for crude oil biodegradation potentials of the selected isolates

Bacterial cell growth

Figure 3 shows the cell growth of the individual isolates during the incubation period with the zero-hour count indicating the inoculum size. The viable bacterial cell count of the various set-ups showed an exponential increase through the incubation period with the highest count produced at the end of the incubation period in the order W13 > P7 > N3 > W11 > N4 W8 > W15. The rate of cell growth was the lowest at the first seven days and the highest between Day 7 and Day 21 of incubation period for all the isolates; isolates W15 and W8 had two order (10^2) increase while N3, N4, W13, W11 and P7 had three order (10^3) increase. At the last seven days of the incubation period, there was drastic decline in growth rate that led to relative stability in viable cell number (Figure 3). At the end of the 28 days of incubation period, the differences in the increased cell number were statistically significant, but insignificant among the isolates (p = 0.55).

Table 1: Physiological and biochemical characteristics of the seven bacterial isolates recovered from crude oil contaminated sites.

Characteristics	W15	N3	N4	W8	W13	W11	P7
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Gram Reaction	-	-	-	-	-	-	-
Citrate test	+	+	+	+	+	+	+
Urease	+	+	-	+	-	+	+
Sulphur	-	-	-	-	-	-	-
Indole test	-	-	-	+	-	-	-
Motility	+	+	+	+	+	+	+
Pigmentation	-	-	Red	-	Red	-	-
Mannitol test	-	-	+	+	+	-	-
Catalase Activity	+	+	+	+	+	+	+
Oxidase test	+	-	-	+	-	+	+
Lactose Utilization	-	+	+	-	-	-	-

+, positive reaction; -, negative reaction. W15, N3, N4, W8, W13, W11 and P7 represent *Pseudomonas aeruginosa strain* W15, *Pseudomonas aeruginosa strain* N3R, *Serratia marcescens strain* N4, *Providencia vermicola strain W8, Serratia marcescens* strain *W13, Pseudomonas aeruginosa* strain W11 and *Pseudomonas protegens* strain P7 respectively.

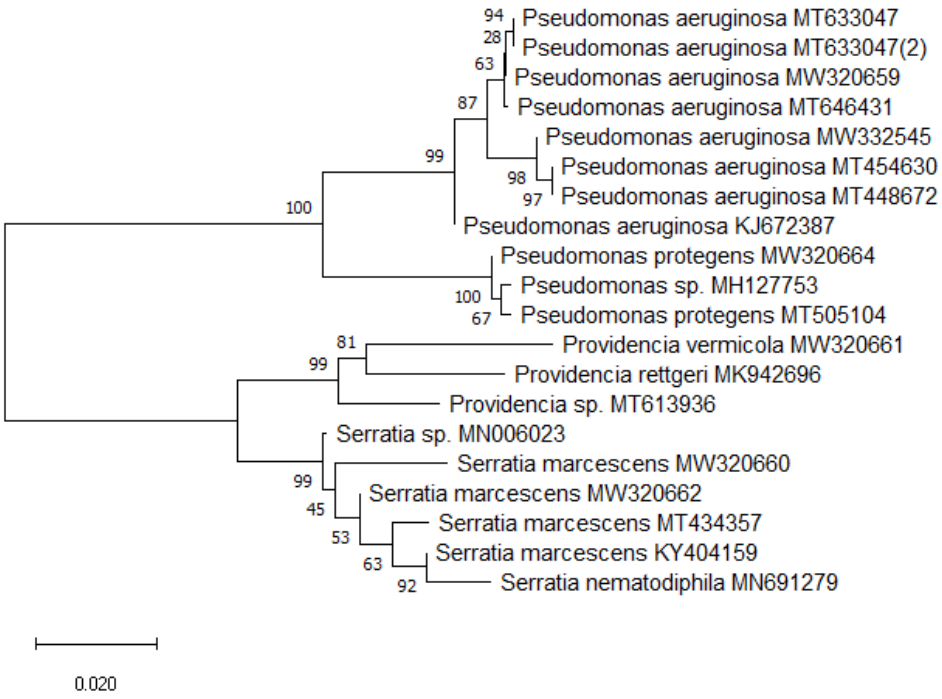

Figure 2: Phylogenetic tree construct of six of the bacterial isolates earlier mentioned using Mega X and based on 16S rRNA gene sequences showing the relationship of the strains and other bacterial strains from NCBI database

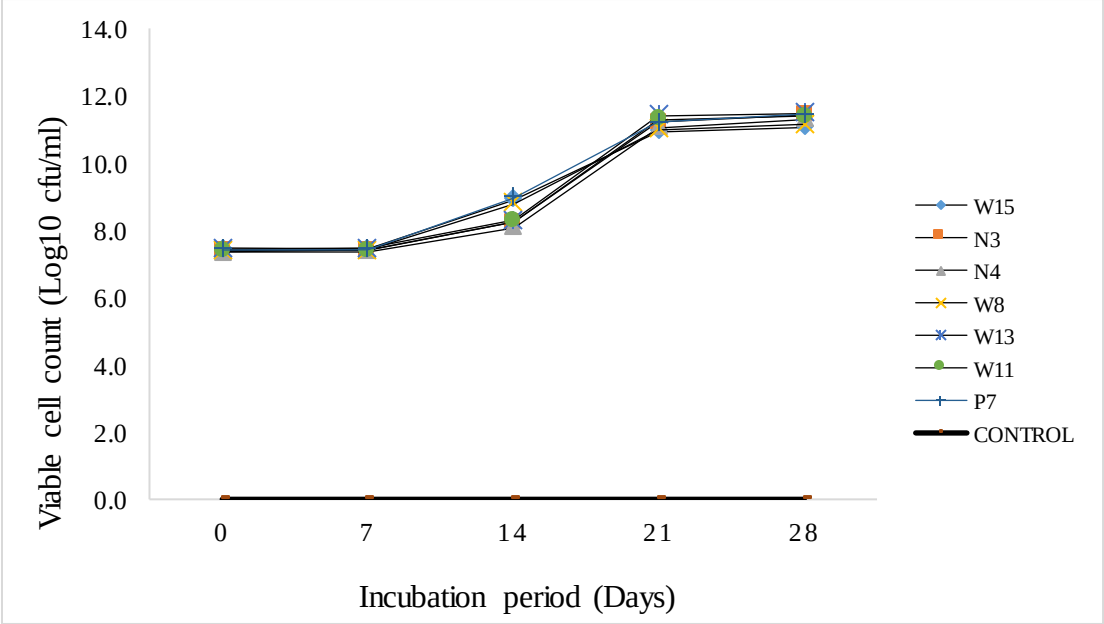

Figure 3: The growth of selected isolates on 2 % (vv^{-1}) crude oil supplemented BHB media. W15, N3, N4, W8, W13, W11 and P7 represent *Pseudomonas aeruginosa* strain W15*, Pseudomonas a eruginosa* strain N3R*, Serratia marcescens* strain N4, *Providencia vermicola* strain W8, *Serratia marcescens* strain W13, *Pseudomonas aeruginosa* strain W11 and *Pseudomonas protegens* strain P7 respectively

Biodegradation screening by gravimetric analysis

The gravimetric analysis was carried out to quantitatively determine the amount of crude oil degraded; calculated as percentage weight reduction. Based on the analysis, the crude oil degradation of the isolates showed increase in percentage crude oil removal through the incubation period with decreasing order of percentage crude oil removal by W11 > N4 > P7 > N3 > W15, W13 > W8 at the end of the incubation (Figure 4). Although, the percentage crude oil removal for organism W8, W13 and P7 were similar at the 7th day, the removal by organisms W13 and P7 exceeded that of W8 after 28 days of incubation. At the end of the 28 days, the differences in the percentage crude oil removal among the isolates was statistically significant ($p < .05$). Comparing the viable cell counts with result of the gravimetric analyses, there was no correlation among the selected isolates ($p = .41$).

Biodegradation screening by Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis was performed to evaluate the degradation efficiency and type analysis based on the percentage degradation of the various hydrocarbon components of the crude oil. When compared with the control, the % TPH of 79.26%, 78.96%, 78.69%, 68.69%, 62.14%, 59.75% and 59.00% were obtained for N4, N3, W13, W15, P7, W8 and W11 respectively after the incubation period. Generally, the rate of degradation was high during the first 14 days for all the isolates (Figure 5) and corresponds with the results of the bacterial growth rate. At the end of the 28 days of incubation, the differences in the percentage total petroleum hydrocarbon degradation among the isolates were statistically significant ($p < .05$).

Samples of all the isolates showed a degree of degradation of most of the hydrocarbons originally present in the crude oil sample especially the linear alkanes with W11 degrading all hydrocarbons except 1,4-Bis(trimethylsilyl) benzene at the end of the incubation period as shown in Table 2.

All isolates efficiently degraded Di-n-decylsulfone which constitutes 18% of the crude oil hydrocarbon, with the highest and the lowest degradation of 100 % and 21.28 % obtained for W15 and W11 respectively. Varying number of PAHs especially naphthalene and their derivatives were detected in the control and test samples. Some PAHs were also reduced significantly but not completely by the individual isolates (Table 2). From the PAHs detected, W11 was able to completely degrade the entire naphthalene and their derivatives after 28 days of incubation; however, their alkane degradation was less compared to some of the other isolates (Figure 6 and Table 2).

The number of intermediates produced varied among the treated samples with isolates W15, W13, N4, W8, W11, N3 and P7 producing 15, 14, 12, 12, 12, 10 and 8 intermediates respectively after 28 days of incubation; however, W13 had the highest number of non-degraded new compounds and/or intermediates (Table 3). W11 and P7 were able to completely remove 50% of the intermediates produced whereas, N3 removed 20% of the intermediate produced. However, the number of intermediates produced by P7 was the minimum compared to other isolates. This result also proves W11 an efficient hydrocarbon degrader.

DISCUSSION

In this present study, bacterial species capable of utilizing crude oil hydrocarbon as the sole source of carbon on Bushnell Haas media supplemented with 1 – 2% Bonny light crude oil were isolated from three crude oil contaminated sites in Nigeria**.** Growth of an organism on mineral salt media enriched with crude oil indicates that an organism is a hydrocarbon utilizer/degrader (Azamjon *et al.*, 2011; Dhaegheem *et al.*, 2021). The THUC was lower than the THC obtained; in addition, the THC obtained for Ikpandiagu was highest compared to Obagi and Kolo creek. This may be because Obagi and Kolo creek have had re-occurring contamination over the years which resulted in more population dynamics that reduces the population and existence of microbial strains.

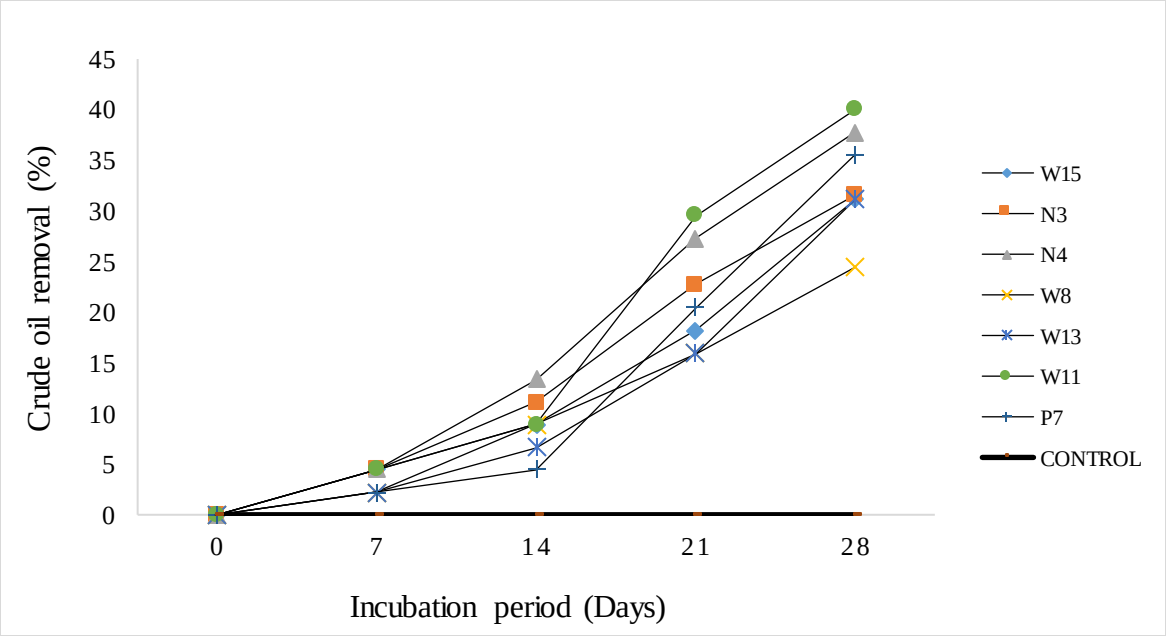

Figure 4: Percentage crude oil removal from the 2% crude oil supplemented BHB by the selected bacterial isolates

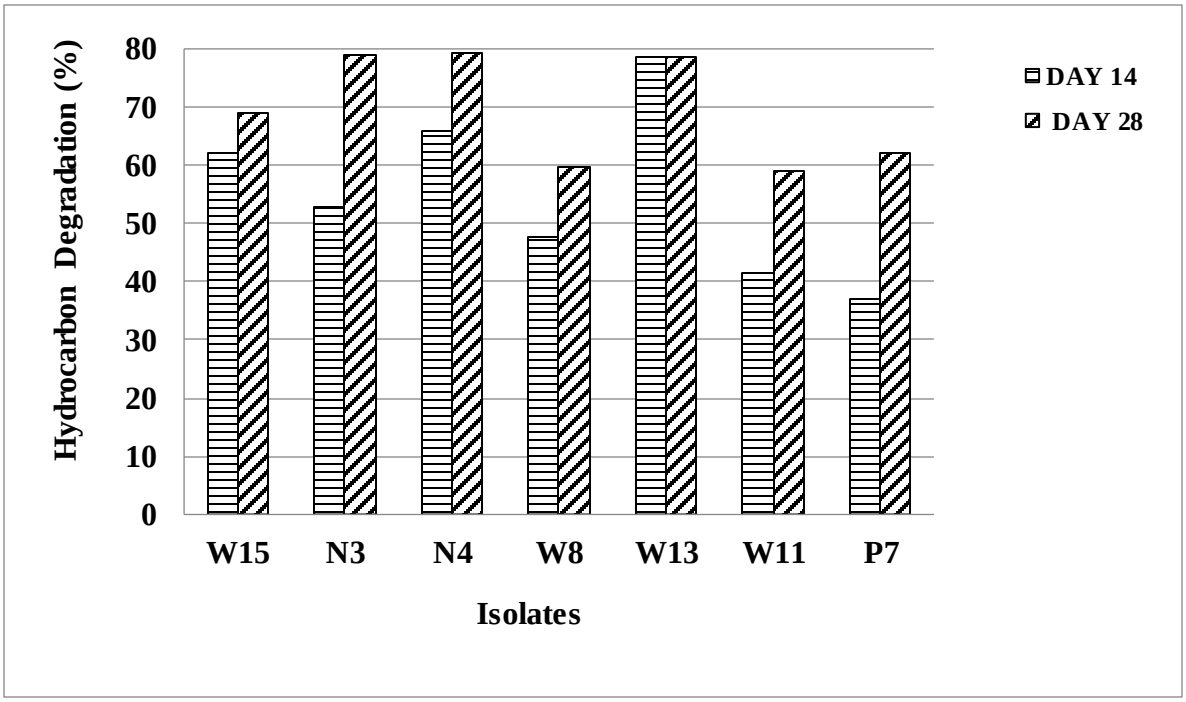

Figure 5: Percentage total petroleum hydrocarbon degraded by the isolates at Day 14 and Day 28 of incubation

Table 2: Percentage degradation of crude oil hydrocarbons after the 28 days of incubation by selected isolates

Hydrocarbon compounds	W15	N3	N4	W8	W13	W11	P7
Benzene, 1,2,4-trimethyl-	ND	ND	ND	100	ND	43.17	ND
Benzene, 1,4-diethyl-	ND	ND	ND	ND	ND	33.97	ND
2,4-Dimethylstyrene	ND	ND	ND	ND	ND	100	ND
Octane, 2,3,7-trimethyl-	100.00	100.00	100.00	61.27	100.00	100.00	100.00
Naphthalene, 2-methyl-	100.00	59.94	70.28	69.16	73.14	100.00	58.71
Naphthalene, 1-methyl-	ND	37.03	100.00	57.23	100.00	100.00	100.00
1-(2-Hydroxyethyl)-1,2,5,5-tetramethyl-cis-decalin	30.04	100.00	100.00	100.00	100.00	100.00	ND
(1R,2S,4as-							
Octacosane	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Naphthalene, 2,6-dimethyl-	100.00	75.17	100.00	100.00	100.00	100.00	100.00
Tetratetracontane	100.00	100.00	41.25	33.32	100.00	50.92	42.32
1H-Indene, octahydro-2,2,4,4,7,7-hexamethyl-, trans-	25.60	57.62	64.18	21.27	63.07	66.93	13.04
Heptadecane, 2,6,10,15-tetramethyl-	60.09	61.56	81.77	69.59	80.03	84.11	66.88
11,12-Dibromo-tetradecan-1-ol-	17.18	51.34	55.37	39.89	50.21	53.46	100.00
Dodecane	100.00	100.00	100.00	100.00	100.00	100.00	100.00
3-(2-Methyl-propenyl)-1H-indene	66.81	78.47	82.22	100.00	100.00	100.00	100.00
10-Nonadecanone	24.96	100.00	61.22	45.19	63.31	100.00	37.94
Pentadecane, 2,6,10,14-tetramethyl-	42.93	66.58	61.71	52.77	65.60	66.71	45.10
Hexadecane	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Naphthalene, 2,3,6-trimethyl-	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Pentadecane, 2,6,10-trimethyl-	31.26	56.43	56.88	43.10	58.98	61.26	35.63
2-methyltetracosane	86.22	94.80	94.85	86.43	94.66	86.08	64.69
Di-n-decylsulfone	100.00	98.29	98.46	92.48	99.91	21.28	91.54
1,2-Bis(trimethylsilyl)benzene	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1,4-Benzenediol,2,5-bis(1,1 dimethylethyl)-	100.00	100.00	96.91	68.18	92.09	61.69	74.19
1,4-Bis(trimethylsilyl)benzene	100.00	100.00	100.00	100.00	100.00	ND	100.00

ND: Not degraded. W15, N3, N4, W8, W13, W11 and P7 represent *Pseudomonas aeruginosa* strain W15, *Pseudomonas aeruginosa* strain N3R, *Serratia marcescens* strain N4, *Providencia vermicola* strain W8, *Serratia marcescens* strain W13, *Pseudomonas aeruginosa* strain W11 and *Pseudomonas protegens* strain P7 respectively.

Table 3: Representation of the number of hydrocarbons degraded and produced under the category of hydrocarbons for the selected isolates

Isolates	A	B	C	D	E
W15	12	13	6	1	8
N3	11	14	2	5	3
N4	10	15	4	4	4
W8	10	15	4	2	6
W13	12	13	3	1	10
W11	13	12	6	2	4
P7	11	14	4	2	2

A - Compounds completely degraded after 28 days of incubation, **B** - Compounds incompletely degraded after 28 days of incubation, **C** - New compounds synthesised day 14 but not found after the day 28 of incubation, **D** - New compounds synthesised by day 14 and not completely degraded after the day 28 of incubation and **E** - New compounds synthesised and not degraded after the day 28 of incubation. C, D and E represent the new compounds/intermediates.

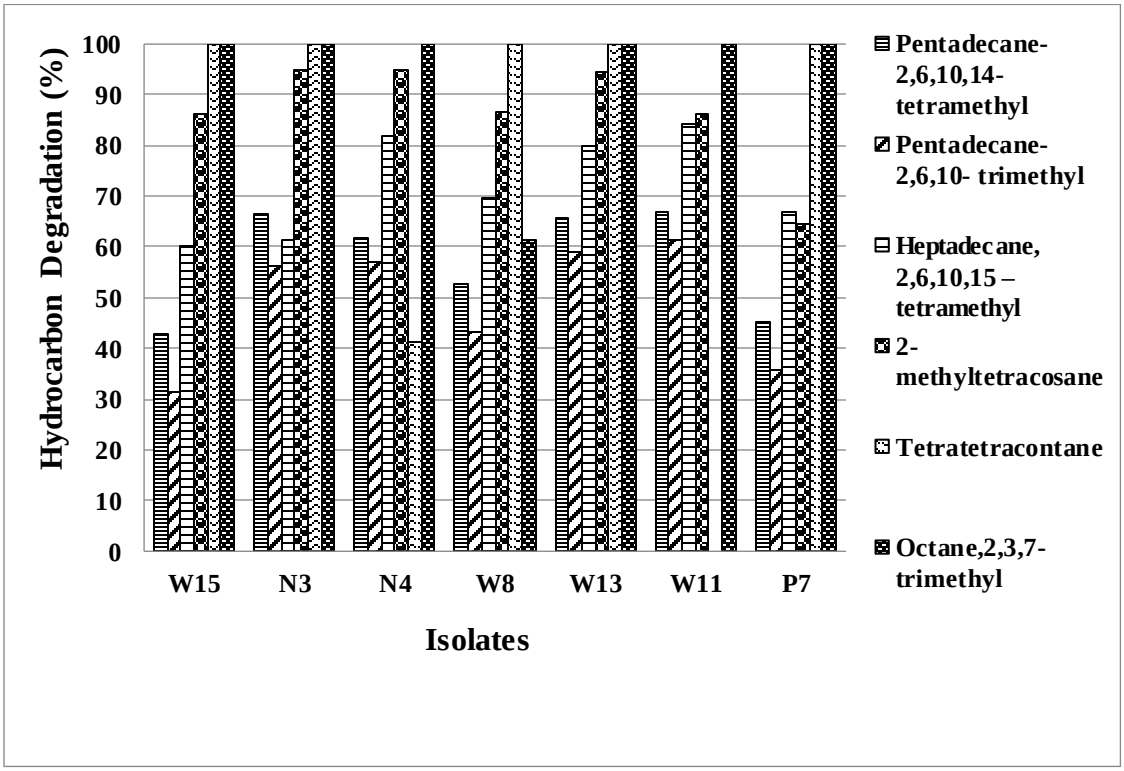

Figure 6: Percentage degradation of alkane fractions by single isolates after the 28 days of incubation

Of all the 40 isolates obtained, seven were selected based on their growth on supplemented BHB media and they were identified based on molecular properties. The predominant organisms were mainly strains of *Pseudomonas aeruginosa* and *Serratia marcescens*. Their growth ability shows the level of nutrient requirement and diversity or substrate specificity of their enzyme system (Wackett, 2003; Varjani *et al.*, 2017; Liu *et al.*, 2020).

The death of cells in the absence of crude oil and exponential growth in the presence of crude oil indicated that the organisms obtained carbon for growth by degrading the crude oil. However, while working with bacterial consortia, Horowitz *et al.* (1975) and Nnabuife *et al.* (2021) suggested that organisms may source for carbon from sugars and organic matter from dead cells in the culture broth.

Organisms react to various changes in media conditions in different ways which subsequently affect their degradation efficiency. The differences in the degradation potentials of the isolates can be due to inherent factors (Dasgupta *et al.*, 2016; Xia *et al.*, 2017; Xu *et al.*, 2018; Wang *et al.*, 2020). *Serratia marcescens* strain W13 yielded percentage TPH of 78.72% by the 14th day; although, the increase in percentage degradation was stabilized between 14th day and 28th day of incubation, a higher significant increase was recorded for other isolates at the same period. The high activity may have resulted in a drastic change in the pH of the media which was unfavorable for the cells; leading to cell stress, increased cell death and withdrawal from obtaining carbon through breakdown of the crude oil hydrocarbon. Possibly, the surviving cells disengaged from crude oil hydrocarbon or their intermediates degradation and sourced for carbon from sugars and organic matter from dead cells. This is evident in Table 2 where the degraded components were high as well as the number of non-degraded intermediates and new compounds. Various scientific reports have suggested that the efficiency in degradation of hydrocarbons by different organisms is dependent on the type of culture method employed and duration of incubation. This is

because the bacterial cells react differently to environmental changes, resulting from new and toxic metabolites and/or accumulation of preferred alternative source of carbon resulting from the initial degradation of the hydrocarbons present in the crude oil or compounds from dead cells (Camacho-Montealegre *et al.*, 2021; Horowitz *et al.*, 1975; Ogbonna *et al.*, 2000; Xu *et al.*, 2017).

Serratia marcescens strains N4 and W13 showed faster and higher efficiency in degradation of total hydrocarbons than most of the isolates. This is in agreement with the report of Rajaesekar *et al.* (2007) that *Serratia marcescens* was able to degrade all the hydrocarbon components of diesel with a 69% degradation efficiency. Also, Xia *et al.* (2017) stated that among the 3 most efficient degraders out of 30 isolates studied, *Serratia proteamaculans* showed the highest degradation of 68.0 % with wide spectrum of hydrocarbon depletion within a shorter incubation period of 15 days. Contrary to the findings of this present study, Wongsa *et al.* (2004) also reported the efficiency of *Pseudomonas aeruginosa* and *Serratia marcescens* in the degradation of gasoline, kerosene, diesel oil and lubricating oil. In their findings, *Pseudomonas* sp performed better with degradation between 60-95% compared to 50 – 60% for *Serratia* sp. Liu *et al.* (2020) also reported the efficiency and quick degradation of n-alkanes of *P. aeruginosa* over *Bacillus cereus* and *Acinetobacter lwoffii*. It has been reported also, that *Pseudomonads* have the capacity to degrade diversities of hydrocarbon compounds owing to their possession of multiple dioxygenases. In addition, the degradation of hydrocarbons is not only dependent on their enzymes but also on their capability of metabolic regulation (Wackett, 2003). The variation in the result of this present study may be because of the initial pH which may have favoured *Serratia* sp better than *Pseudomonas* sp (Wang *et al.*, 2011). The poor degradation of the compound Di-n-decylsulfone may have contributed to the low % TPH obtained with *Pseudomonas aeruginosa* strain W11. This may suggest the susceptibility of the isoenzyme of W11 to changes in pH of the media (Kertesz *et al.*, 1999; Wang *et al.*, 2011). However, W11 also showed efficiency in degrading the crude oil hydrocarbon components and by having the highest number of completely removed compounds originally in the crude oil as well as the intermediates produced (Tables 1 and 2). According to the findings of Ridgway *et al.* (1990), *Pseudomonas* sp utilized more of the aromatic components of the crude oil than the cyclic and branched alkanes. However, Koma *et al.* (2003) reported a preference of alkanes and cyclic alkanes as sole carbon source than aromatics hydrocarbons.

Considering all the criteria employed in evaluating the efficiencies of the isolates, *Providencia vermicola* W8 isolated from water source performed the lowest. Few scientists have reported the crude oil degradation potential of this organism (Bhagobaty, 2020; Chikere *et al.*, 2017; Uba *et al.*, 2018). Contrary to the findings of this present study, Chikere *et al.* (2017) worked with isolates from crude oil contaminated soil in Gokana, Rivers State, Nigeria and reported higher degradation potential by *Providencia* sp than *Pseudomonas* sp. The variation in efficiency of isolate obtained in this study compared to the above may have been influenced by the isolation habitat.

CONCLUSION

The bacterial isolates from the crude oil contaminated sites showed dependence on crude oil hydrocarbons in mineral salt media supplemented with crude oil as the only source of carbon and energy. The strains of *Serratia marcescens* isolated were the fastest degraders with higher total petroleum hydrocarbon degradation compared to other isolates; however, many intermediates were not completely degraded. *Pseudomonas aeruginosa* strain W11 showed degradation of wider range of hydrocarbon components originally in the crude oil as well as the complete degradation of most intermediates formed. One limitation of this study was that it was set-up under unified media conditions rather than optimum media conditions of the various isolates studied. Future studies on the efficiencies of the isolates using various hydrocarbon compounds and various consortia formulations are necessary to ascertain their potentials for applications in bioremediation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Author contributions:

NOO performed the experiment and wrote the first draft of the manuscript. NOO and OJC

conceived the experiment, analyzed the data, and edited the manuscript. NOO and AC supervised the experimental process and curated data. All authors designed, validated the experiment, read, and approved the manuscript.

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