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DEGRADATION PROFILE AND DIOXYGENASE ACTIVITY OF *CARNOBACTERIUM GALLINARUM* (MT350233) AND *ENTEROCOCCUS FAECALIS* (MT345788) ISOLATED FROM LANDFILL SOIL IN BENIN CITY

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ABSTRACT

Landfill is a potential microbial hub of hydrocarbon (HC) degrading bacterial population owing to nutrient availability and continuous enrichment by organic materials. The degradation and dioxygenase activity of Carnobacterium gallinarum and Enterococcus faecalis isolated from landfill soil in Benin City was investigated. Soil samples were collected from a Government approved landfill in Benin City at a depth of 0 to 10 cm. Standard microbiological and molecular methods were followed for the isolation and characterization of bacterial population. Bacterial isolates were standardized for degradation using spectrophotometer optical density (OD) 0.08 at 600 nm (equivalent to 1×10^8 cfu/ml). The gas chromatography with flame ionization detector (GC-FID) method was used to determine the total petroleum hydrocarbon (TPH) of waste engine oil (WEO) containing samples. The spectrophotometric methods were used for the assay of catechol 1,2-dioxygenase (C12O) and catechol 2,3-dioxygenase (C23O) activity. The result of the 16S rRNA analyses of the bacterial isolates confirmed the identity of Carnobacterium gallinarum as 98.71 % and Enterococcus faecalis as 100 %. The degradation profile of Carnobacterium gallinarum and Enterococcus faecalis at varied concentrations (20, 40, 60, 80 and 100 mg/L) of WEO showed significant decline in optical density (OD) values from day 1 to day 7. The decline in OD is an indication of utilization of Polycyclic Aromatic Hydrocarbons (PAHs) present in the WEO. The result of the gas Chromatography with Flame Ionization Detector (GC-FID analyses) of degraded WEO containing samples revealed the absence of low molecular weight and high molecular weight PAHs after the 7-days degradation study. The presence of HC degrading enzyme in the bacterial isolates was confirmed by the production of C12O and C23O as a result of the formation of cis, cis muconic acid and 2-hydroxymuconic semi-aldehyde respectively. This study revealed that the landfill soil is a potential natural microcosm for WEO degrading bacteria and possesses biologically active HC degrading bacteria population such as Carnobacterium gallinarum and Enterococcus faecalis.

Key words: Hydrocarbon, waste engine oil, optical density, catechol 1,2-dioxygenase, catechol 2,3-dioxygenase

INTRODUCTION

Hydrocarbon (HC) pollutants in the environment can be classified as organic or inorganic (Chikere, 2013). The organic pollutants are mainly HCs in different forms. The most common are petroleum HCs which include n-alkanes and other aliphatics, aromatic compounds and other components in trace amounts (Atlas and Philp, 2005; Sarkar *et al*., 2005).

Spillage from crude oil and refined fuel has damaged the natural ecosystems in the Niger Delta region, South-South Nigeria and many other places worldwide. The quantity of oil spilled during accidents has ranged from a few hundred tons to several hundred thousand tons (Deepwater Horizon Oil Spill, Atlantic Empress, Amoco Cadiz), but it is a limited measurement of damage or impact (Godleads *et al*., 2015). Adams *et al*. (2014) reported that oil spills in soil reduce the ability of soil to support the growing of plants and leaches into the ground to pollute ground-water. Waste engine oil increases the presence of heavy metals which bioaccumulate and biomagnify thereby causing adverse health effects to the receiving environment. Major constrain facing scientists and industrialists today is how to tackle the problems arising from pollution of soil and water by HCs; using environmental friendly, safe approach and cost implications of degrading these contaminants. Bioremediation technology is a veritable tool for HCs degradation that is intensively studied today (Godleads *et al*., 2015).

The major characteristics that define hydrocarbon-oxidizing microorganisms in the soil are; membrane-bound, group-specific oxygenases; and mechanisms for optimizing contact between microorganisms and the water-insoluble HCs (Chikere *et al*., 2011). HCs are broken down into less innocuous form through microbial oxidation by bioavailability and bioaccessibility (Semple *et al*., 2003). A compound is said to be bioavailable if it is freely available to cross an organism's membrane from the medium the organism inhabits at a given time. A bioaccessible compound is a compound available to cross an organism's membrane from the environment it inhabits, if the organism can have access to it. Bioremediation remains a highly potential approach since many studies have reported its effectiveness in removing numerous pollutants from contaminated sites (Nie *et al*., 2009; Rimmer *et al*., 2006; Li *et al*., 2013). Studies on petroleum HC biodegradation adopted various methodologies (Bidoia *et al*., 2010; Cerqueira *et al*. 2014; Zhang *et al.*2016), although they all indicated that degradation can occur in specific fractions of the substances to be degraded. There is no rule of thumb in petroleum HC biodegradation as most compound showed preferential remediation of lighter HC compounds, while others directed towards heavier HCs (Huang *et al*., 2004).

The microbial component of landfill soils is an area of interest for the remediation of hydrocarbon pollutants. Bacteria proliferate abundantly in landfills due to the abundance of organic nutrients; therefore, landfills are considered microbial pools (Song *et al*., 2015). Bacteria such as Gamma Proteobacteria, which include *Firmicutes*, *Bacteriodes* and *Fusobacteria*., have been implicated in landfill soils (Blake *et al*., 2016); the hydrocarbon degrading potentials of these organisms in landfill soils is yet to be fully studied. The utilization of bacteria diversity in landfill soils for the remediation of waste engine oil polluted soil is practicable due to the richness of the soil and its microbial community dynamics. This study was carried out to investigate the degradation and dioxygenase activities of *Carnobacterium gallinarum* (MT350233) and *Enterococcus faecalis* (MT345788) isolated from government approved landfill soil in Benin City.

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MATERIALS AND METHODS

Collection of Soil samples

Landfill soils were collected from three Government approved landfills in Benin City (Ovia North-East and Uhunmwode LGAs). The Global Position System (GPS) coordinates of sample location for the landfill soils included Ovia N/E (Oluku) LGA position A: N60 27' 44", E50 36' 0", B: N60 27' 48", E50 36'0.8" and Uhunmwode LGA position A: N60 19' 3.383", E50 44' 48.54", B: N6019'34.397", E50 44' 48.5"

Characterization and Identification of BacterialIsolates

Cultural, microscopic and biochemical characterization of bacterial isolates were carried out following standard microbiological methods (Cheesbrough, 2000)**.**

Molecular Characterization of bacterial isolates

The DNA extraction was carried out using QuickDNA™ Fungal/Bacterial MiniPrep Kit from Zymo Research, according to the manufacturer's protocol. Universal primer 27F: AGAGTTTGATCMTGGCTCAG, 1525R: AAGGAGGTGWTCCARCCGCA was used for PCR amplification of the extracted DNA. The PCR amplification was carried out using 5x Firepol® Master Mix from Solis BioDyne. The 16S rRNA gene was amplified in 20 µl reactions containing; Master mix (5x): 4 µl, Forward primer: 0.6 µl, Reverse primer: 0.6 µl, Template: 2 µl and nuclease free water: 12.8 µl. The PCR cycling conditions followed denaturation at 95 ^{o}C for 3 min, 25 cycles of denaturation at 95 ^{o}C for 30 s, annealing at 54 ^{o}C for 30 s and extension at 72 ^{o}C for 30 s, with a final extension at 72 ^{o}C for 10 min. Quality check of Plasmid/PCR products by gel electrophoresis was carried out and was followed by DNA sequencing with specific universal primers using Sanger Sequencing chemistry. After sequencing, Q20 Read Length up to 800 bases with ABI or SCF, FASTA File with Quality Control reports were obtained.

Sequence Submission

Nucleotide sequence was screened for chimeras using DECIPHER 1.12.3. The sequence was assigned unique code to indicate the strain and deposited in the NCBI data base.

Phylogenetic Tree construction

The evolutionary history of *C. gallinarum* (MT350233) and *E. faecalis* (MT345788) was inferred using the Maximum Likelihood method. The tree was drawn to scale where branch length measured the number of substitution per site. Phylogenetic tree was drawn using computer algorithm "mega 7" according to the methods of Kumar *et al*. (2015).

Screening for waste engine oil degrading bacteria

Overnight grown bacterial cultures were standardized to 1.5×10^{8} cfu/ml. Two hundred (200) microliter (µL) of WEO was spread to the surface of solid Bushnell Haas (BH) agar where 10 mm well was bored and the base sealed with molten solid BH agar. Thereafter, the standardized cultures were used to fill the wells and allowed to stay for 2 hours on bench top to diffuse. The plates were incubated right-up for 3 days and the zones of clearance were measured on a daily basis using a meter rule.

Shake flask degradation of waste engine oil by *Carnobacterium gallinarum*

(MT350233) and *Enterococcus faecalis* (MT345788)

Bacterial isolates were standardized for degradation using spectrophotometer based on optical density of (OD) 0.08 at 600 nm (equivalent to 1×10^8 cfu/ml). The standardized culture was incorporated into 50 ml sterile BH broth containing varied concentrations (100, 80, 60, 40 and 20 mg/L) of waste engine oil as carbon source. The mixture was introduced into 250 ml Erlenmeyer flask capped with sterile cotton wool. The flask was shaken using water bath shaker (120 rpm) continuously for 7 days (168 h) at room temperature. Samples were aseptically collected from the set up using 5 ml micropipette and OD values were read on a daily basis for the period of the experiment.

Total Petroleum Hydrocarbons (TPH) determination

The Gas chromatography with Flame Ion Ionization detector (FID) model 6890 series and 6890 plus was used for this experiment according to the method of ASTM (2006). The GC-FID method was used to measure the collective concentrations of extractable aliphatic and aromatic petroleum hydrocarbons in the shake flask experiment. For this study, aliphatic hydrocarbons were quantitated within ranges C_9 through C_{18} and C_{19} through C_{36}. Aromatic hydrocarbons were quantitated within C_{11} through C_{22} range.

Dioxygenase activity Assay

The screened *Carnobacterium gallinarum* (MT350233) and *Enterococcus faecalis* (MT345788) were grown and populated in Tryptone Soya broth (TSB) and Bushnell Haas broth for 36 h. After growth, cells were harvested at the exponential phase of growth by centrifugation (model; D3024, DragonLab) (15,000 rpm, for 5 min at 25^{o}C). The cell pellets were washed twice with Tris-HCl buffer (50 mM, pH 8.0) and suspended with the same buffer. The cell suspension was sonicated (model; Bandelin Sonorex, RK 100H) in ice cooled condition at 15 min. After sonication, the cell debris was centrifuged at 15, 000 rpm for 10 min. The supernatant (cell-free extract) was used for the enzyme assay of catechol 1, 2-dioxygenase (C12O) and catechol 2, 3-dioxygenase (C23O) production by the bacterial isolates.

Catechol 1, 2-dioxygenase (C12O) Activity

The ortho-cleavage activity (C120) was measured following the formation of cis, cis-muconic acid (Mahiudddin *et al*., 2012; Olaniran *et al*., 2017). C12O activity was assayed by digesting 2 ml of 50 mM Trish-HCl buffer at pH 8.0, sterile distilled water (0.7 ml), 0.1 ml of 100 mM 2-Mercaptoethanol and 0.1 ml cell-free extract (free enzyme). Zeropoint one-milliliter (0.1 ml) 0.8 mM catechol was added as the substrate. The solution (3 ml volume) was mixed and incubated at 30^{o}C for 5 minutes and absorbance read at 260 nm using Spectronic Cemspec M501 Spectrophotometer. The control sample contained all reagents excluding cell free extract (free enzyme), which was replaced with distilled water.

Catechol 2, 3-dioxygenase (C23O) Activity

The meta cleavage product of catechol was measured by the formation of 2-

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hydroxymuconic semi-aldehyde (Mahiudddin *et al*., 2012; Olaniran *et al*., 2017). C23O activity was assayed by digesting 2 ml of 50 mM Tris-HCl buffer, 0.6 ml distilled water, 0.2 ml cell free extract (free enzyme) and 0.2 ml of 100 mM catechol as the substrate. The solution (3 ml volume) was mixed and incubated at 30ºC for 5 min and absorbance read at 375 nm using Spectronic Cemspec M501 Spectrophotometer. The control sample contained all reagents excluding cell free extract, which was replaced with distilled water.

Enzyme Activity Calculations

The activity of C12O and C23O was calculated using the following equations as reported by Sethare*et al*. (2019):

Enzyme Activity (µ moles of product formed/ minute) =

$$\frac{E \times L}{V} \times \frac{\Delta OD}{\text{time (min.)}} \quad (1)$$

Where E_{260}: Molar extinction coefficient for C12O = 16,800 L/mol/cm

E_{375}: Molar extinction coefficient for C23O = 14,700 L/mol/cm

L: path length of the cuvette (mm)

V: reaction volume of the mixture

ΔOD: change in optical density

$$\text{Total Protein } (mg/L) = \frac{\text{Sample}}{\text{Standard}} \times \text{ standard conc.} \quad (2)$$

where standard concentration (constant) = 59.83g/L

$$\text{Specific Activity } (\mu \text{ moles/min/mg}) = \frac{\text{Enzyme Activity}}{\text{Total Protein}} \quad (3)$$

$$\text{Relative Activity } (\%) = \frac{\text{Specific Activity of the sample}}{\text{Specific Activity of the control}} \times 100 \quad (4)$$

Effect of pH and Temperature on C12O and C23O activity

The optimum pH was obtained by measuring the enzyme activity at 30ºC over a pH range of 3.0 to 10.0 using 50 mM Tris-HCl buffer, 100 mM of 2-mercaptoethanol and 0.8 mM catechol for C12O and 50 mM Tris-HCl buffer, 100 mM catechol for C230. The optimum temperature was determined by assaying the activity of C12O and C23O at varied temperature (30ºC to 90ºC) using the same buffer formulations as applicable to pH.

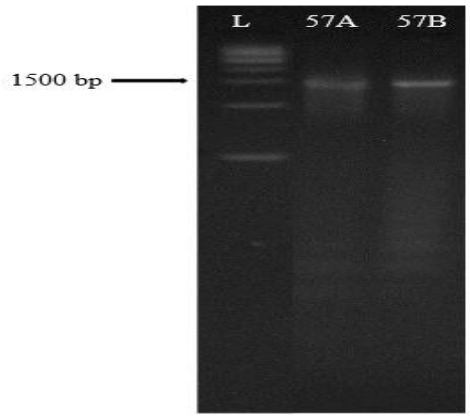

Figure 1: Purified amplicons of bacterial DNA before sequencing

Lane L: marker at 1500bp, lane 2 (**57A)**: *Enterococcus faecalis*
Lane 3 (**57B)**: *Carnobacterium gallinarum*

Table 1: Gene Bank confirmatory profile of 16S rRNA sequence of HC degrading bacterial isolates isolated from engine oil polluted and landfill soils

Isolate Codes	**Assigned Accession no.**	**Query Cover (%)**	**% Identity**	**Closets Match on Gene Bank**
57A	MT345788	100	100.00	*Enterococcus faecalis*
57B	MT350233	98	98.71	*Carnobacterium gallinarum*

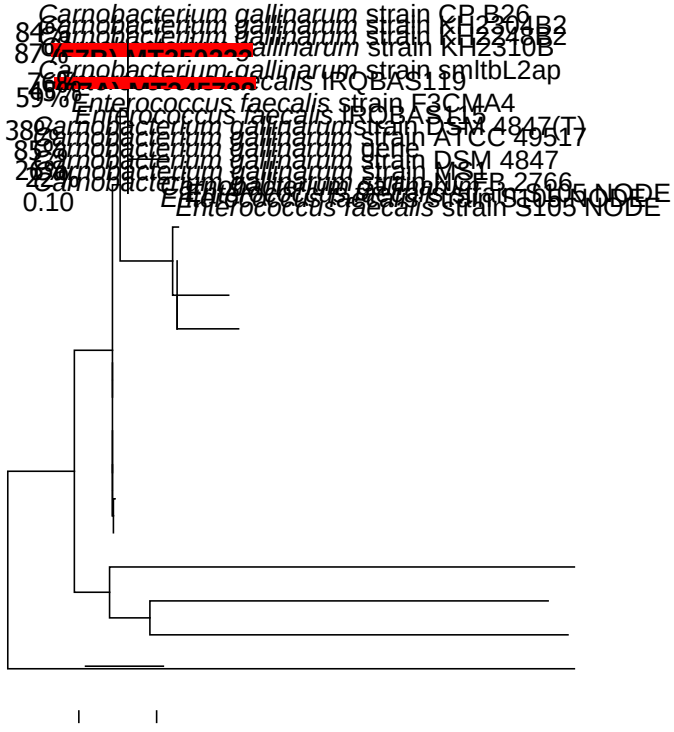

Figure 2: Maximum Composite Likelihood (MCL) consensus phylogenetic tree of *C. gallinarum* (MT350233) and *E. faecalis* (MT345788) showing evolutionary relationship with NCBI data base bacteria sequence.

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Table 2: Screened waste engine oil degrading bacteria

Isolate Code	Bacterial isolates	Initial OD	24 h	48 h	72 h
			Zone of Clearance (mm)		
57A	*Enterococcus faecalis*	0.173	25.00±0.50	TC	-
57B	*Carnobacterium gallinarum*	0.314	NC	18.20±0.45	TC

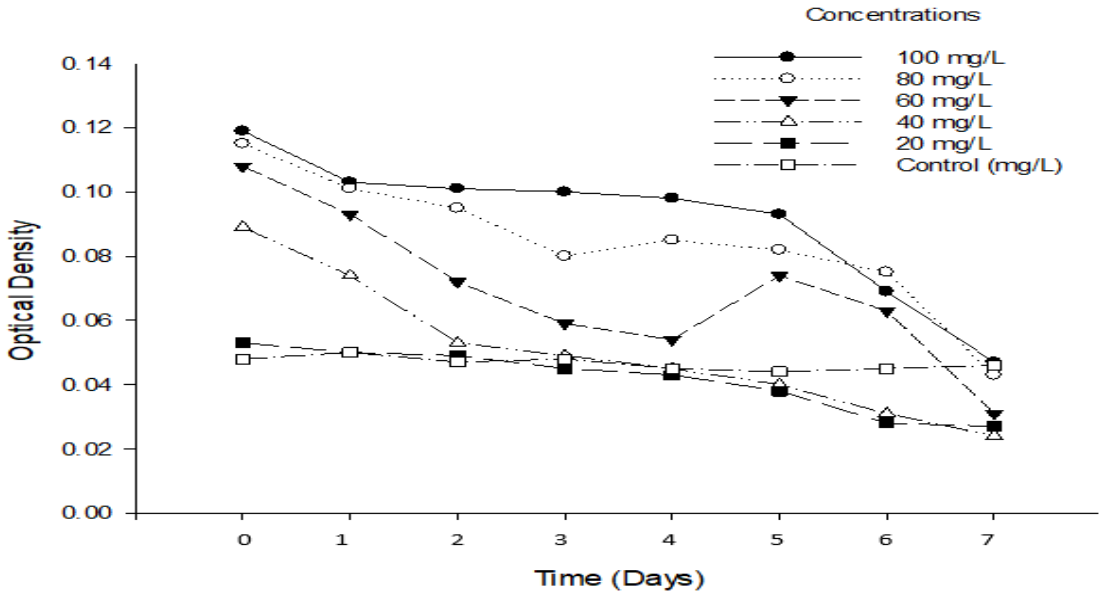


Figure 3: Growth curve of Enterococcus faecalis (MT345788) exposed to varied concentration of WEO at OD 540 nm.

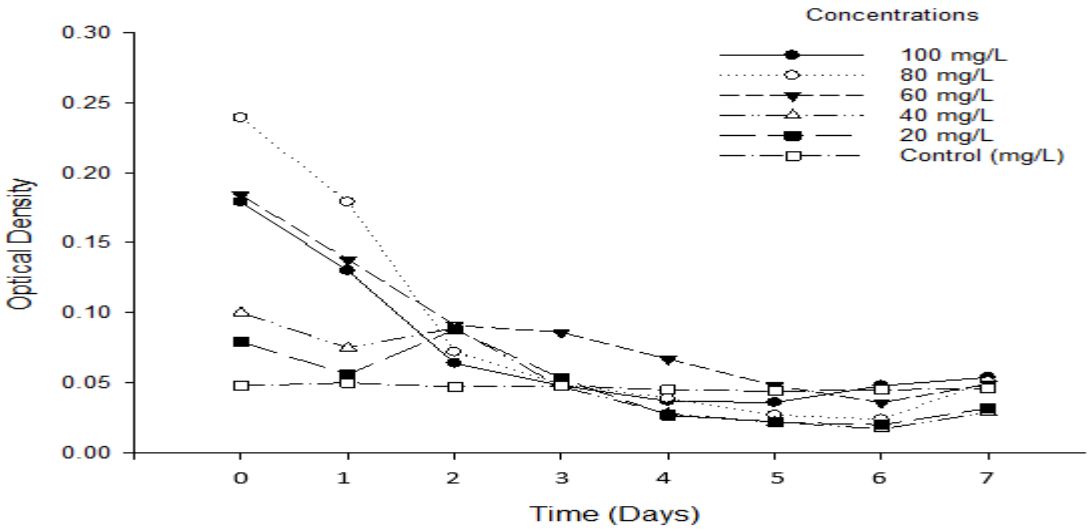
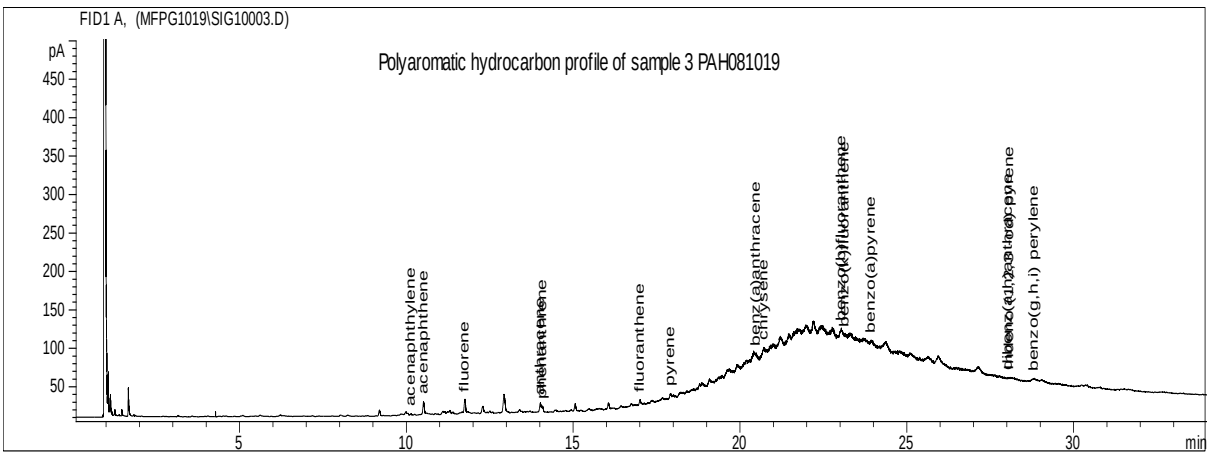


Figure 4: Growth curve of Carnobacterium gallinarum (MT350233) exposed to varied concentration of WEO at 540 nm.

Table 3: Polycyclic Aromatic and Aliphatic Hydrocarbon Content of samples after shake flask degradation by new strains of *Carnobacterium gallinarum* and *Enterococcus faecalis*

Components	***Carnobacterium gallinarum* (MT350233)**		**control (mg/L)**	***Enterococcus faecalis* (MT345788)**	
	(80mg/L)	**(40mg/L)**		**(80mg/L)**	**(40mg/L)**
Naphthalene	0.000	0.000	0.000	0.000	0.000
Acenaphthalene	0.000	0.000	0.001	0.000	0.000
Acenaphthene	0.000	0.000	0.009	0.000	0.000
Florene	0.000	0.000	0.010	0.000	0.000
Phenathrene	0.000	0.000	0.005	0.000	0.000
Anthracene	0.000	0.000	0.008	0.000	0.000
Fluoranthene	0.004	0.000	0.004	0.000	0.000
Pyrene	0.000	0.000	0.004	0.000	0.000
Benzo(a)anthracene	0.010	0.003	0.015	0.000	0.000
Crysene	0.004	0.002	0.032	0.000	0.000
Benzo(b)fluoranthrene	0.016	0.008	0.014	0.000	0.000
Benzo(a)pyrene	0.013	0.006	0.005	0.000	0.000
Benzo(k)fluoranthrene	0.009	0.003	0.016	0.000	0.000
Indeno(1,2,3) perylene	0.000	0.000	0.000	0.000	0.000
Dibenzo(a,h)anthracene	0.000	0.000	0.000	0.000	0.000
Benzo(g,h,i) perylene	0.000	0.000	0.000	0.000	0.000
Total PAH (mg/L)	0.055	0.022	0.123	0.000	0.000
Total Aliphatic (mg/L)	9.342	8.708	32.365	0.078	0.090
Total TPH (mg/L)	**9.397**	**8.729**	**32.489**	**0.078**	**0.090**

Accession number in parenthesis, control: 60 mg/L WEO + BH broth without isolate, THC: Total Hydrocarbon Content, PAH: Polycyclic aromatic Hydrocarbon



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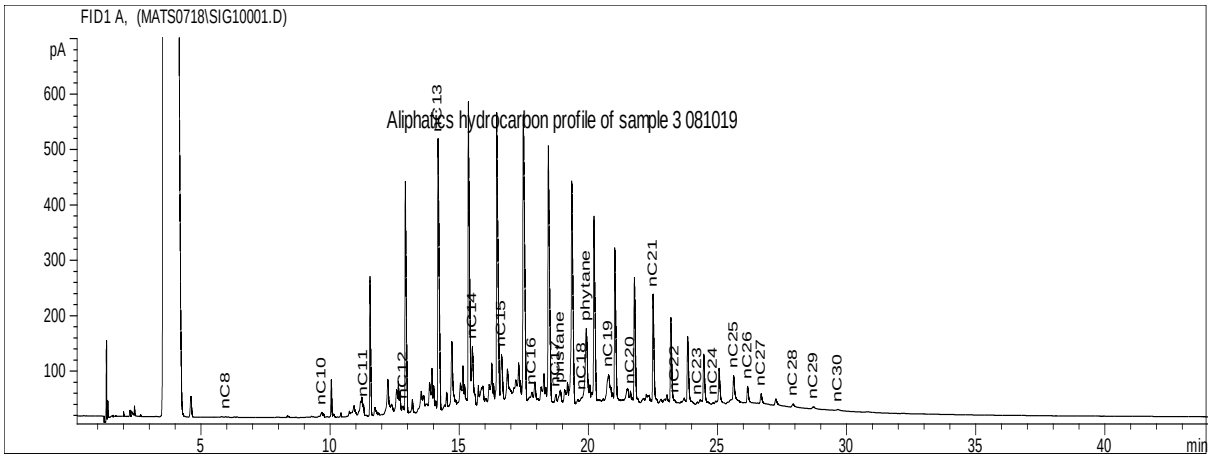
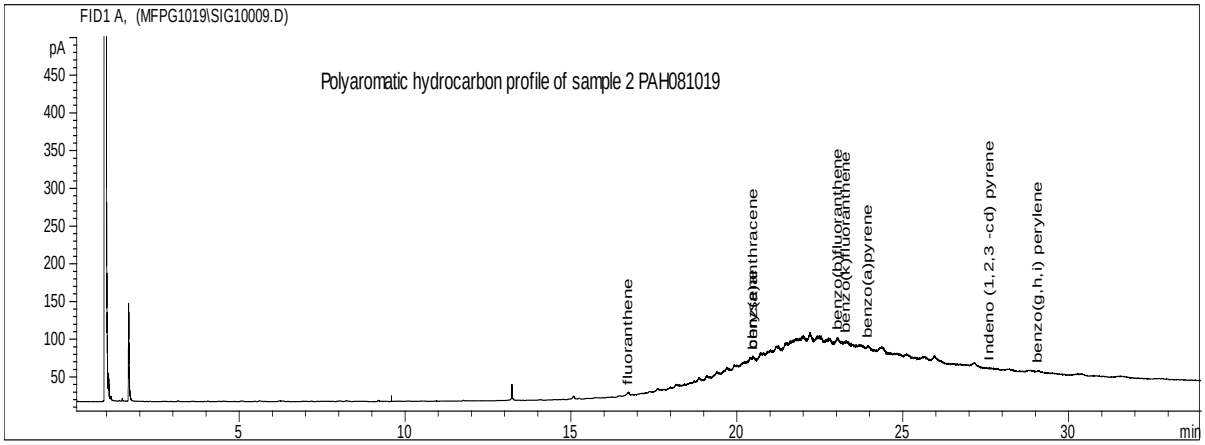


b

Figure 5: GC-FID chromatogram of used engine oil (control), a: polycyclic aromatic fraction, b: aliphatic hydrocarbon fraction



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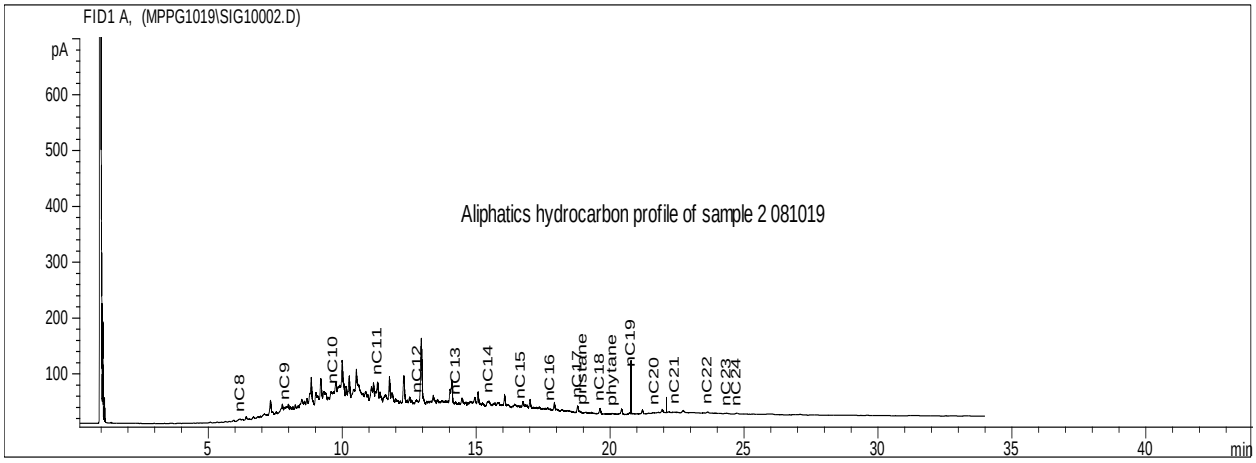
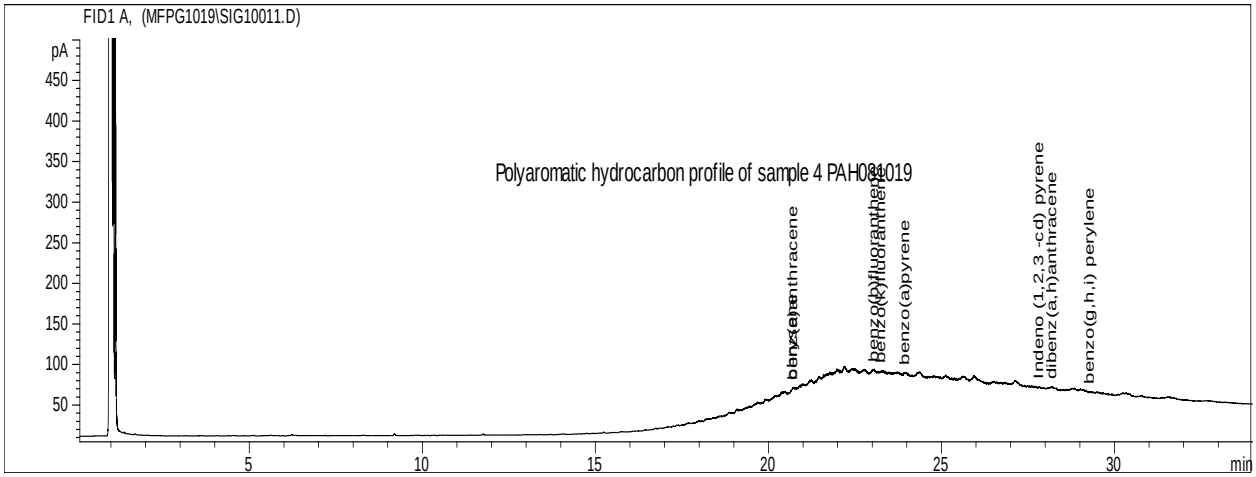


b

Figure 6:GC-FID chromatogram of WEO (**80 mg/L**) after degradation by Carnobacterium gallinarum (MT350233) for 7 days, a: polyaromatic hydrocarbon fraction, b: aliphatic hydrocarbon fraction



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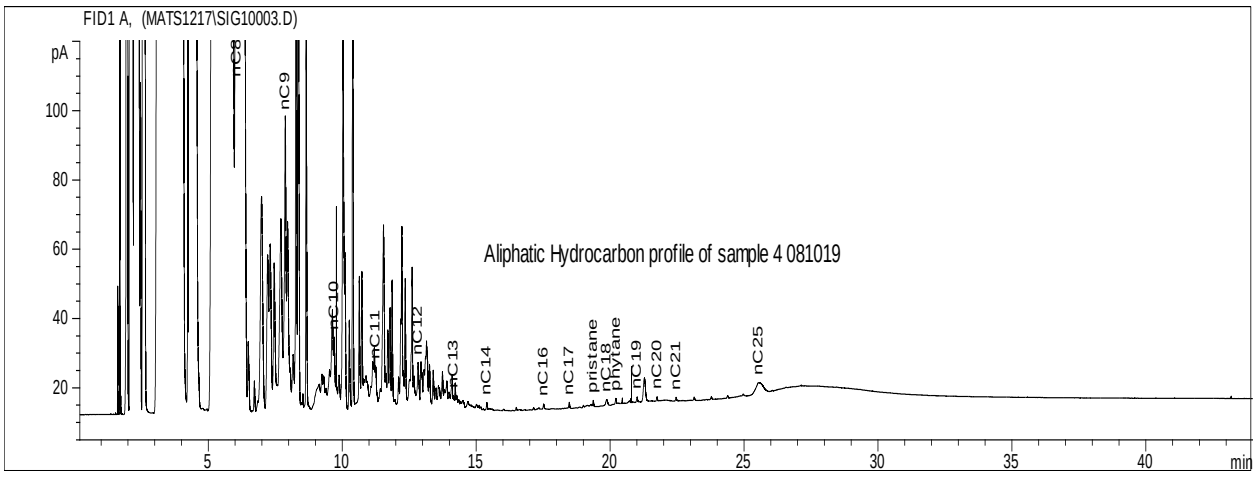
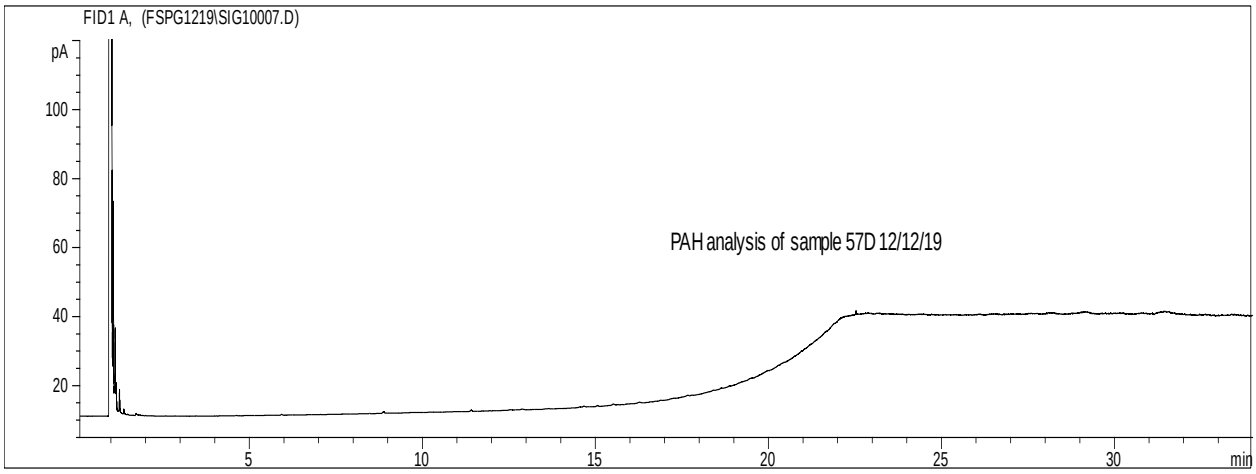


b

Figure 7: GC-FID chromatogram of WEO (40 mg/L) after degradation by Carnobacterium gallinarum (MT350233) for 7 days, a: polyaromatic hydrocarbon fraction, b: aliphatic hydrocarbon fraction



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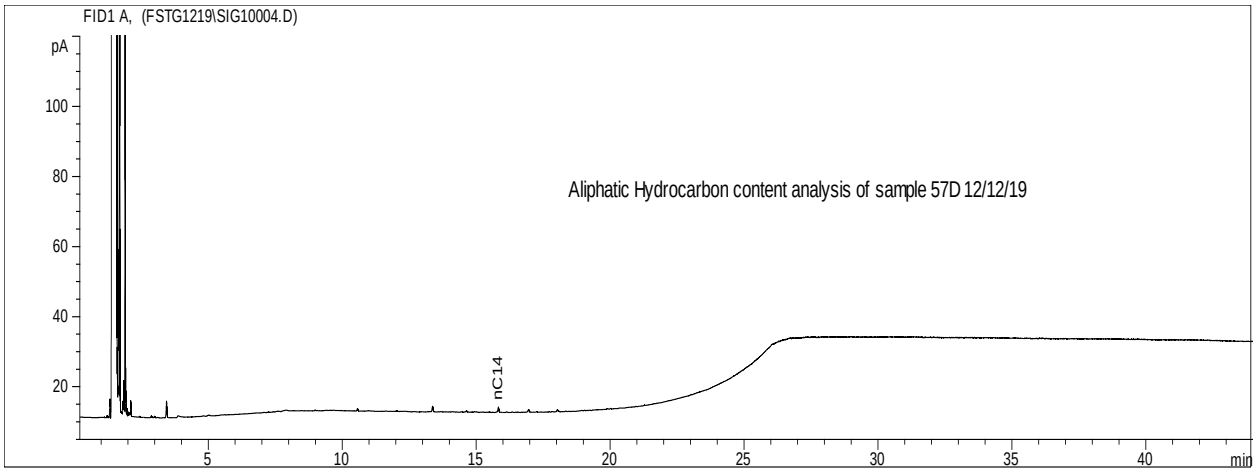

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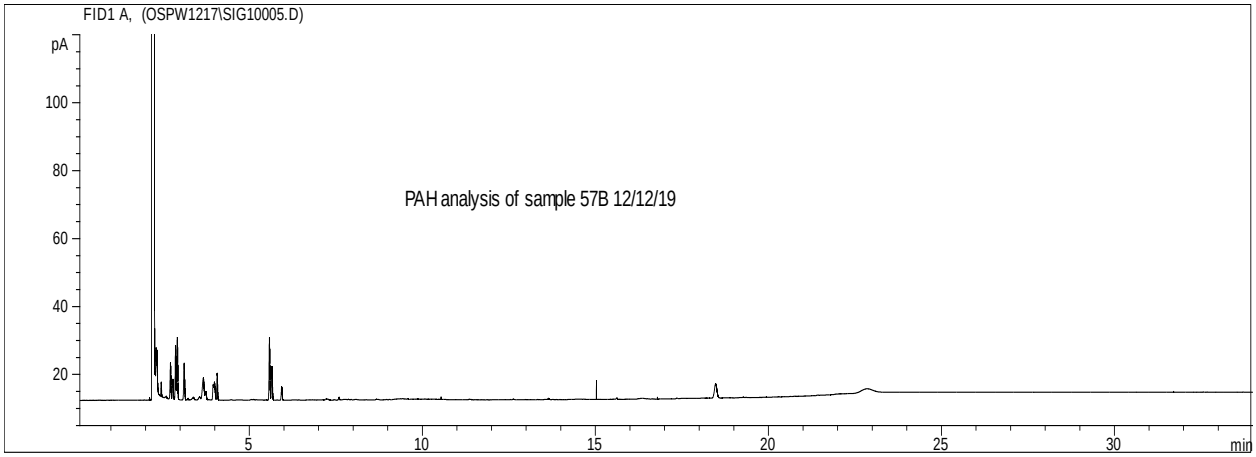
Figure 8: GC-FID chromatogram of WEO (80 mg/L) after degradation by Enterococcus faecalis (MT345788) for 7 days, a: polyaromatic hydrocarbon fraction, b: aliphatic hydrocarbon fraction



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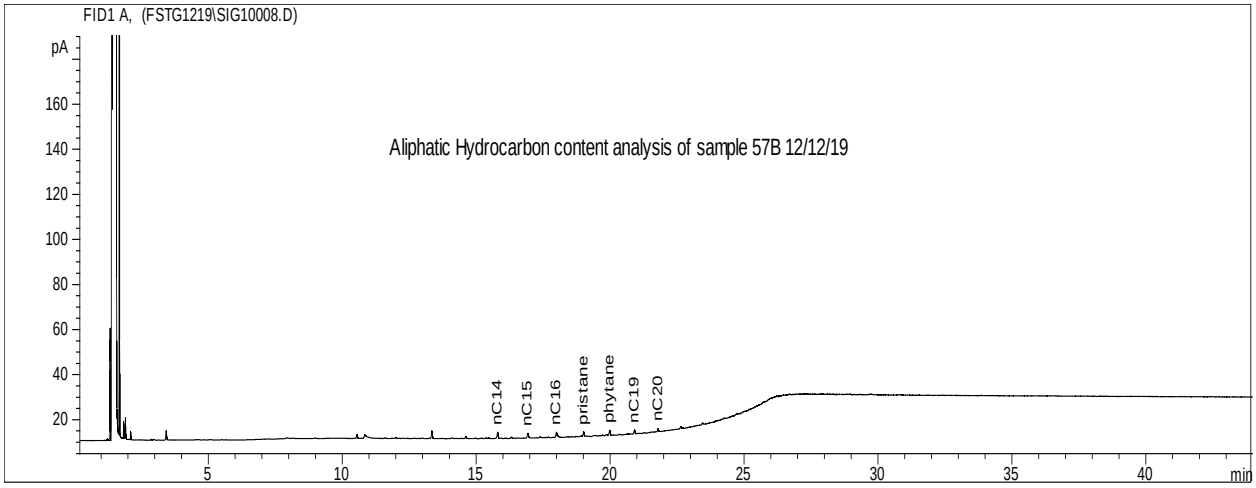

b

Figure 9: GC-FID chromatogram of WEO (40 mg/L) after degradation by Enterococcus faecalis (MT345788) for 7 days, a: polyaromatic hydrocarbon fraction, b: aliphatic hydrocarbon fraction

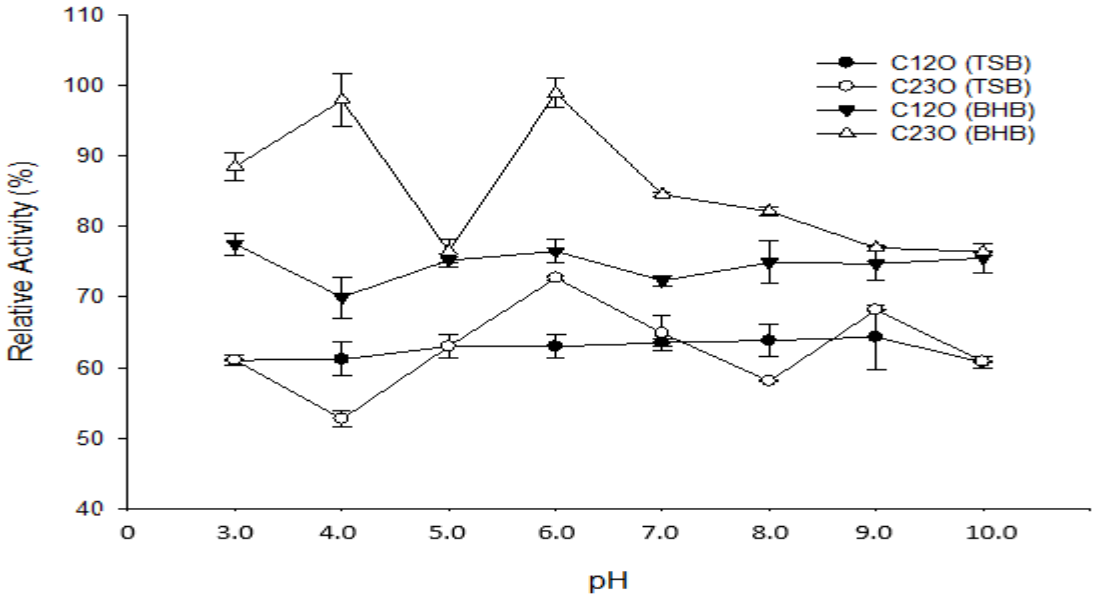

Figure 10: Effect of pH on the activity of catechol 1, 2-dioxygenase (C12O) and catechol 2, 3-dioxygenase (C23O) produced by Carnobacterium gallinarum (MT350233) cell free extract. Error bars are standard deviation of mean, TSB: tryptone soya broth, BHB: Bushnell Haas broth.

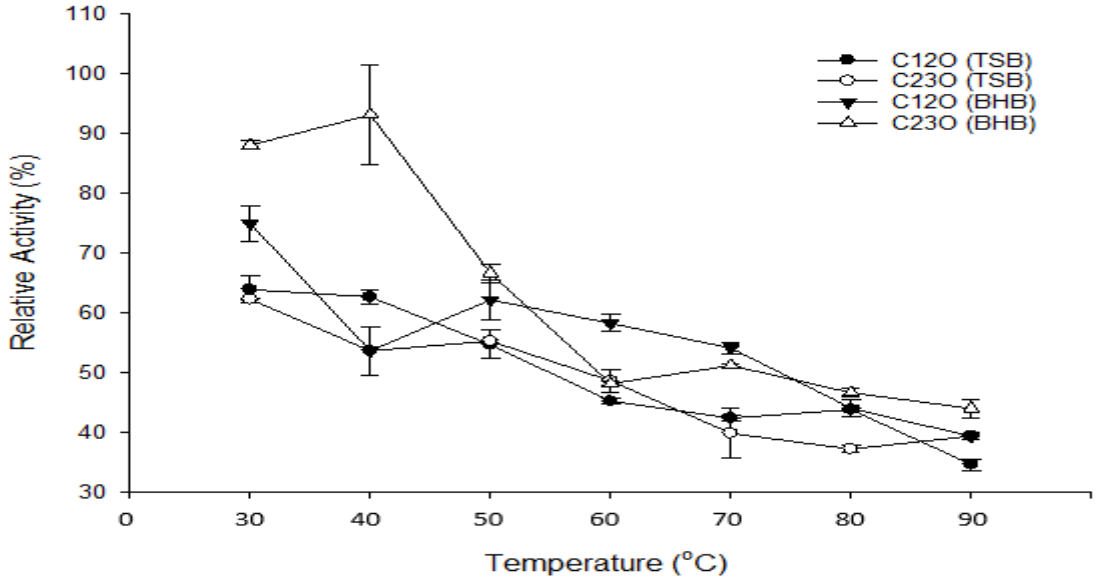

Figure 11: Effect of temperature on the activity of catechol 1, 2-dioxygenase (C12O) and catechol 2, 3-dioxygenase (C23O) produced by Carnobacterium gallinarum (MT350233) cell free extract. Error bars are standard deviation of mean, TSB: tryptone soya broth, BHB: Bushnell Haas broth.

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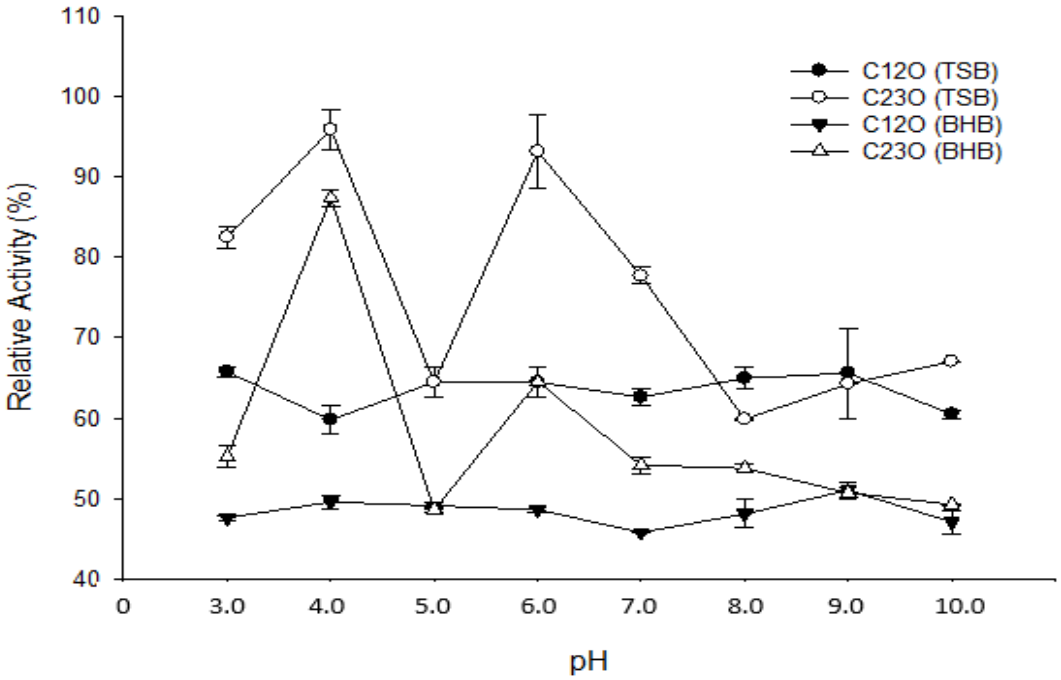

Figure 12: Effect of pH on the activity of catechol 1, 2-dioxygenase (C12O) and catechol 2, 3-dioxygenase (C23O) produced by Enterococcus faecalis (MT345788) cell free extract. Error bars are standard deviation of mean, TSB: tryptone soya broth, BHB: Bushnell Haas broth.

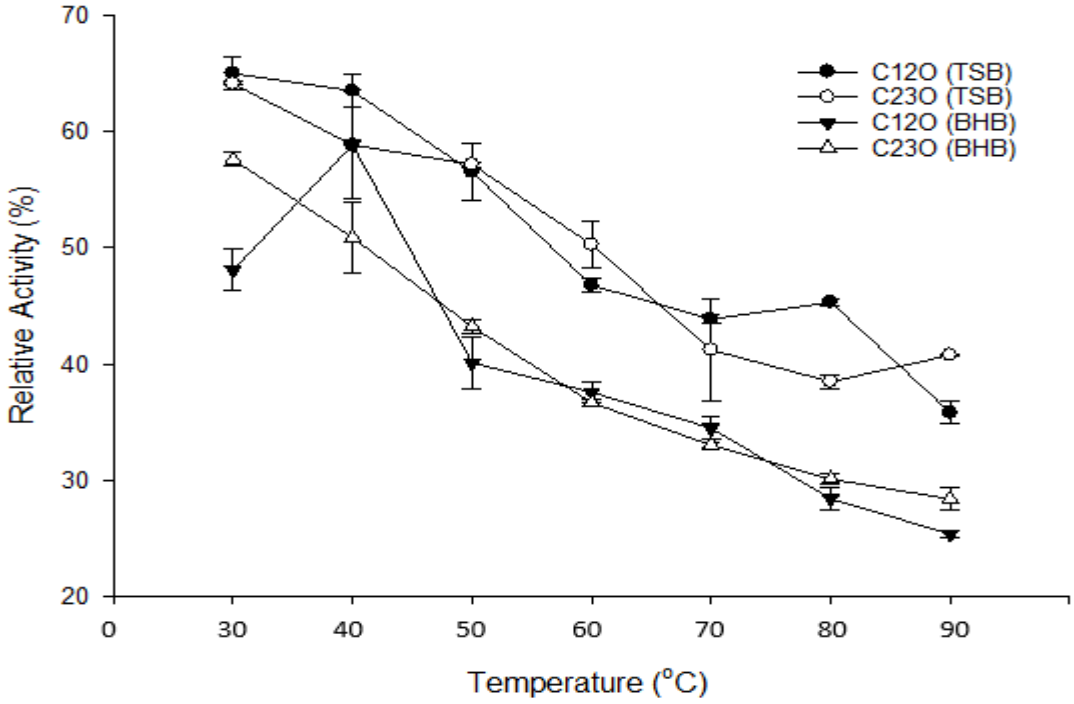

Figure 13: Effect of temperature on the activity of catechol 1, 2-dioxygenase (C12O) and catechol 2, 3-dioxygenase (C23O) produced by Enterococcus faecalis (MT345788) cell free extract. Error bars are standard deviation of mean, TSB: tryptone soya broth, BHB: Bushnell Haas broth.

RESULTS AND DISCUSSION

HC degrading bacterial isolates from landfill soils in Benin City was amplified using the 16S rRNA method on poly-acrylamide. The result of the amplification showed that the bacterial isolates match at 1500 bp on the DNA ladder (Figure 1) indicating that they belong to the genus bacteria and they are closely related. The Gene-Bank confirmatory identity of the 16S rRNA sequence output of HC bacteria isolated from landfill soil (Table 1) revealed the presence of new strains of *Enterococcus faecalis* (MT345788) and *Carnobacterium gallinarum* (MT350233). *Enterococcus faecalis* have been reported to be present in potential hydrocarbon contaminated environments (Ariole and George-West, 2020; Gangar *et al*., 2019). The activity of *Carnobacterium gallinarum* in HC utilization is lacking in literature.*Enterococcus faecalis* have been reported to degrade benzene, toluene, ethylbenzene, and xylene (BTEX) compounds (Yavas and Icgen, 2018); kerosene (Aydin and Icgen, 2020) and HC polluted soils (Dilmi *et al*., 2017).

The maximum composite likelihood consensus phylogenetic tree of bacterial isolates (Figure 2) showed evolutionary relationship comparing *Carnobacterium gallinarum* (MT350233) and *Enterococcus faecalis* (MT345788) with National Centre for Biotechnology Information (NCBI) gene bank database organisms. *C. gallinarum* (MT350233) revealed 87% relatedness to *C. gallinarum* strain CP B26, KH2304B2, KH2248B2 and KH2310B, while *E. faecalis* (MT345788) showed 76% relatedness to *E. faecalis* strain IRQBAS119. *Carnobacterium gallinarum* (MT350233) and *Enterococcus faecalis* (MT345788) isolated in this study are new strains of these bacteria which have been deposited in the NCBI data base.

Enterococcus faecalis (MT345788) and *Carnobacterium gallinarum* (MT350233) were screened for 72 h to determine their potential to utilize waste engine oil (WEO). After 24 h incubation, zone of clearance on Bushnell Haas (BH) agar was observed against *E. faecalis* (MT345788), whereas *C. gallinarum* (MT350233) showed no zone of clearance within 24 h. Total clearance of WEO on BH agar plate was observed within 48 h for *E. faecalis* (MT345788) and 72 h for *C. gallinarum.* The slow utilization of WEO by *C. gallinarum* (MT350233) is an indication that the bacterial isolate needed to acclimatize to the hydrocarbonoclastic environment. The ability of the bacteria to clear WEO on the surface of solid media may suggest their HC utilizing ability.

WEO contains potentially toxic high and low molecular weight aromatic hydrocarbons such as acenaphthalene, acenaphthene, florene, phenathrene, anthracene, fluoranthene, pyrene, chrysene, benzo(a)pyrene, benzo(k)fluoranthrene, indeno(1,2,3) perylene, dibenzo(a,h)anthracene and benzo(g,h,i) perylene as reported in our earlier study (Okolafor and Ekhaise, 2021). Shake flask experiment on the degradation of *C. gallinarum* (MT350233) and *E. faecalis* (MT345788) showed exponential reduction of optical density (OD) values from day 1 to day 7, which is an indication of utilization of WEO by the bacterial isolates investigated. As the bacterial isolates utilized WEO as source of carbon and energy, progressive reduction in turbidity was observed (Figures 3 and 4). The high and low molecular weight aromatic

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hydrocarbons were observed to have been degraded and utilized by *C. gallinarum* (MT350233) and *E. faecalis* (MT345788) after 7 days (Figures 6 to 9) compared to the control (Figure 5). This further proved the hydrocarbonoclastic potentials of these bacterial isolate.

The use of microbial enzyme in the clean-up and biodegradation of toxic organic compounds is promoting excellent results (Guo *et al*., 2010). C12O activity have been extensively characterized and studied onvariety of organisms such as Alcaligenes, Pseudomonas,Ralstonia, Acinetobacter, Rhodococcus, and Candida albicans (Saxena and Thakur, 2005; Sauret-Ignazi *et al*., 1996; Aoki *et al*., 1984; Strachan *et al*., 1998; Briganti *et al*., 1997; Tsai and Li, 2007). However, there is paucity of studies on dioxygenase activity of *C. gallinarum* and *E. faecalis.*The enzyme activities in this study revealed that catechol 2,3-dioxygenase (C23O) activity was higher than catechol 1,2-dioxygenase (C12O) activity. *C. gallinarum* (MT350233) and *E. faecalis* (MT345788) produced C12O by the formation of cis,cis-muconic acid (ortho-cleavage) and C23O by the formation of 2-hydroxymuconic semialdehyde (meta-cleavage). Progressive reduction in the activity of the enzyme was observed as the temperature increased from 40 ^{o}C to 90 ^{o}C. Enzyme activity is dependent on environmental factors such as temperature and pH (Whiteley and Lee, 2006). Fernandez-Lafeunte *et al.* (2000) reported that optimum temperature of degrading enzymes could be related to the temperature of the environment, which the microorganisms were isolated. Growth media used in the cultivation of the bacteria significantly influenced changes in pH of C12O and C23O produced by *C. gallinarum* (MT350233) and *E. faecalis* (MT345788) investigated, whereas temperature significantly influenced the activity of C12O and C23O in this study. Since degrading bacteria population required varied pH to initiate catabolic activity (Saxena *et al.*, 2005), pH of C12O and C23O is expected to fluctuate significantly depending on the bacteria investigated.

CONCLUSION

The bacterial species isolated and characterized from this study can serve as the promising remediation agent for future mineralization of waste engine oil when properly harnessed. The ortho-cleavage and meta-cleavage activities of C12O and C23O from this study is a confirmation of the hydrocarbonoclastic potentials of *Carnobacterium gallinarum* (MT350233) and *Enterococcus faecalis* (MT345788).

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