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Acenaphthene biodegradation and structural and functional metagenomics of the microbial community of an acenaphthene-enriched animal charcoal polluted soil

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ABSTRACT

Animal charcoal from skin and hides cottage industries indiscriminately disposed in run offs and drainage channels harbors hazardous constituents that are mutagenic and toxic, and thus require bio-based eco-friendly depuration strategies. A microbial consortium (FN7) from an animal charcoal polluted site enriched with acenaphthene was structurally and functionally characterized via illumina next generation sequencing and annotation of their putative ORFs, and also studied for ability to degrade acenaphthene. Structurally, FN7 metagenome consists of 7 phyla, 13 classes, 38 orders, 49 families, 67 genera, 68 species, and 45 strains, respectively. The dominant phylum, class, order, family, genus, species, and strain in the metagenome are *Proteobacteria* (48.9%), *Actinobacteria* (31.8%), *Actinomycetales* (28.0%), *Enterobacteriaceae* (18.9%), *Paracoccus* (12.9%), *Bacillus cereus* group (13.5%), and *Methylobacterium radiotolerans* JCM 2831 (22.4%). The microbial consortium in the metagenome degraded 59.68% (29.84 mg l^{-1}) and 89.16% (44.58 mg l^{-1}) of the initial concentration of acenaphthene (50 mg l^{-1}) in 14 and 21 days. Functional annotation of the putative ORFs of the metagenome using KEGG KofamKOALA, NCBI's conserved domain database, BacMet, and Antibiotic Resistance Gene-ANNOTation (ARG-ANNOT) revealed the detection of hydrocarbon-degradation genes including salicylaldehyde dehydrogenase and catechol 1,2 dioxygenase involved in acenaphthene degradation, resistance genes for mercury, arsenic, cadmium, nickel, and several others, and antibiotic resistance genes for 15 antibiotic classes such as β-lactam, colistin, aminoglycoside, among others. This study revealed that members of FN7 metagenome are equipped with requisite gene batteries and could be veritable bioresources for *in vitro* biodegradation as well as on-site bioremediation of animal charcoal polluted sites.

1. Introduction

Acenaphthene is a three ring non-alternant polycyclic aromatic hydrocarbon with a five-membered alicyclic ring (Wise et al., 1988). It is naturally present in fossil fuel but also produced as a result of incomplete combustion of organic matter. It is abundant in creosotes and coal tar and is considered as a priority pollutant by EPA (Schocken and Gibson, 1984). It is used in the production of dyes, plastics, insecticides and fungicide, which are major sources of pollution in the environment (Marletta, 1985). It is one of the least studied PAH in terms of biodegradation both in soil and *in vitro* with only very few degraders reported in the literature (Schocken and Gibson, 1984; Selifonov et al., 1993; Kafilzadeh et al., 2012; Salam et al., 2016). The pathway of degradation of acenaphthene by bacteria was only recently clearly elucidated. Following an initial proposal that it usually proceeds by oxidation and dihydroxylation of the 5-membered alicyclic ring (Schoken and Gibson, 1984), Ghosal et al. (2013) showed that the lower pathway was essentially in consonance with the route of naphthalene degradation via salicylic acid and catechol. The key metabolites of the upper pathway are 1-acenaphthenol, 1-acenaphthenone, 1-hydroxy-2-ketoacenaphthene, 1,2-dihydroxyacenaphthylene, acenaphthene quinone, naphthalene-1,8-dicarboxylic acid. At the molecular level, the

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genes responsible for the initial dioxygenation step have also been cloned, identified and shown to bear much in common with genes with similar function in other PAH degrading bacteria (Pinyakong et al., 2004; Kouzuma et al., 2006).Whereas the desirability of isolation of bacteria capable of degrading PAH in pure culture cannot be overemphasized, it is usually difficult to have such isolate compete effectively in the natural environment and often in nature complete degradation is usually by group of organisms acting in concert as a consortium to effect complete mineralization. Within this milieu, different types of interaction are operational, some of them based on co-metabolism.

Animal charcoal polluted soil is not well reported in the literature. However, in recent time a few reports have shown that it is a potential source of novel degraders of hydrocarbons, including PAH (Obayori et al., 2017; Salam and Obayori, 2020). It has also been shown to be laden with heavy metals as a result of the diverse combustibles utilized in the process of roasting of animal skin and burning of livestock bones and hoofs which are the major raw materials used in the 'ponmo' cottage industry (Salam and Obayori, 2020). The length and breadth of Nigeria is dotted by thousands of such cottage industry which are usually located close to runoffs or waterways of drainage channels which debauched into waterways and equally impact surrounding soils. Till date not much is known about these polluted sites nor is there consideration for their reclamation (Obayori et al., 2017).

Metagenomics approach provides an effective means of discerning the community diversity and functional potential in an environment. Unlike the previous approaches of clone library, which is rather tedious, time consuming and expensive, metagenomic approach use next generation sequencing of the total genome in an environment and application of extensive bioinformatics resources to reveal structural and functional details. It is currently the most reliable approach for capturing both the culturable and non-culturable members of the microbiome (Sabale et al., 2019). This is crucial given that oftentimes the most functionally preponderant species are non-culturable. Metagenomics approach enables the discovery of novel metabolites and molecules with functionalities applicable in development of value added products, just as it may give insight into dead-end metabolites which may impact negatively on the overall process of biodegradation in the environment (Bashir et al., 2014; Salam et al., 2017; Salam, 2018). Previously it was shown that metabolites from incomplete degradation and dead-end products can accumulate in the environment and have potential to inhibit the degradation of other PAHs in the matrix (Kazunga and Aitken, 2000). Here we report the degradation of acenaphthene by a consortium enriched from an animal charcoal polluted soil, exploring metagenomic approach to unravel the composition and functional attributes of the culture.

2. Materials and methods

2.1. Sampling site description and acenaphthene enrichment

Composite soil samples were collected from an animal charcoal polluted site located at an abattoir in Ilorin, Kwara State, Nigeria. At the sampling site, slaughtered dairy animals hides and skin were burnt to remove the furs to produce 'ponmo', a local Nigerian delicacy. The coordinates of the sampling site were latitude 8.471498 N and longitude 4.531245 E. Soil samples were collected at a depth of 10–12 cm with sterile hand trowel after clearing debris from the soil surface, sieved using a 2-mm mesh size sieve and thoroughly mixed in a large plastic bag to ensure homogeneity. The physicochemical properties and heavy metal content of the polluted soil determined as described previously (Bray and Kurtz, 1945; Black, 1965; Chopra and Kanwar, 1998; Obayori et al., 2017; Salam et al., 2020) indicated a pH of 5.51 ± 0.02, moisture content of 8.59% (±0.16) and total organic content of 18.99 ± 0.18%. The nitrogen, phosphorus and potassium contents determined by macro-Kjeldahl digestion method, flame photometry and spectrophotometry are 5.99 ± 0.06, 5.55 ± 0.12, and 12.99 ± 0.04 mg/kg soil, respectively. Similarly, background heavy metal content of the polluted soil determined using atomic absorption spectrophotometer following mixed acid digestion and extraction of the soil sample revealed in mg kg^{-1} soil, the presence of iron (72.41 ± 0.72), lead (3.22 ± 0.11), zinc (12.50 ± 0.42, copper (8.99 ± 0.32), manganese (3.05 ± 0.01), cadmium (0.72 ± 0 .01), nickel (4.01 ± 0.01), and chromium (2.89 ± 0.02), respectively. Hydrocarbon content of the sampling site determined as described previously (Salam et al., 2018; Salam and Obayori, 2020) revealed a total hydrocarbon content (THC) of 1026 mg/kg of soil comprising 54% (554.98 mg/kg) aliphatics and 46% (471.56 mg/kg) aromatics, respectively.

Enrichment for acenaphthene-degrading microorganisms was prepared using carbon-free mineral medium (CFMM; g L^{-1}: NH_4NO_3, 3.0 g; Na_2HPO_4, 2.2 g; KH_2PO_4,0.8 g; $MgSO_4{\cdot}7H_2O$, 0.1 g; $FeCl_3{\cdot}6H_2O$, 0.05 g; and $CaCl_2{\cdot}2H_2O$, 0.05 g; pH 7.0) supplemented with yeast extract (0.005 g). Sieved and thoroughly mixed animal charcoal polluted soil (5 g) was added to 45 ml CFMM amended with 50 mg l^{-1} acenaphthene. Enrichment was carried out with shaking (180 rpm) at room temperature (25 ± 3 °C) in the dark for 3–4 weeks until there was turbidity. After four consecutive transfers, aliquots (0.5 ml) from final flasks were inoculated into 250-ml conical flasks containing CFMM (50 ml) and 50 mg l^{-1} acenaphthene. The set ups (in triplicate) designated FN7 was incubated under the same conditions as the enrichment for 3 weeks.

2.2. Extraction of residual acenaphthene and its analytical determination

Residual acenaphthene was extracted by liquid–liquid extraction. Briefly, 50 mL of CFMM broth culture was extracted twice with an equal volume of hexane. After removing the aqueous phase with separating funnel, the organic fraction was concentrated to 1 ml. Control flasks containing heat-killed broth culture sterilized at 121 °C for 15 min, supplemented with acenaphthene (50 mg l^{-1}) and incubated for 3 weeks as described above were also extracted similarly. Residual acenaphthene concentration was determined by injecting 1 μl of the resultant solution for gas chromatographic analysis.

Hexane extract (1 μl) of residual acenaphthene was analysed using gas chromatography equipped with flame ionization detector (GC/FID). A standard acenaphthene (1 μl; 50 mg l^{-1}) was first injected into the GC/FID to obtain a standard chromatogram and identify the run time and retention time for acenaphthene prior to injection of the test sample. acenaphthene concentrations in the hexane were determined using a Hewlett Packard 5890 Series II gas chromatograph equipped with flame ionization detector. The column HP-5 is 30 m long (internal diameter, 0.25 mm; film thickness, 0.25 μm). The carrier gas was nitrogen. The injector and detector temperature were maintained at 300 and 320 °C, respectively. The column was programmed at an initial oven temperature of 60 °C for 2 min, then ramped at 12 °C/min to 205 °C and held for 16 min.

2.3. Total DNA extraction, shotgun metagenomics, pre-processing of raw reads and taxonomic classification

Total DNA used for metagenomic analysis was extracted directly from the CFMM broth culture 3 weeks post amendment with 50 mg l^{-1} acenaphthene. Total DNA were extracted from the acenaphthene-enriched broth culture (250 μl) using ZymoBIOMICS™ DNA Miniprep kit (Zymo Research, USA) following manufacturer's instructions. The quality and concentration of the extracted total DNA was ascertained using NanoDrop spectrophotometer and electrophoresed on a 0.9% (w/v) agarose gel, respectively. Shotgun metagenomics of the extracted DNA was prepared using the Illumina Nextera XT sample processing kit

and sequenced on a MiSeq. The protocols for total DNA preparation for Illumina shotgun sequencing were as described previously (Salam, 2018; Salam and Ishaq, 2019).

Pre-processing and quality control of fastq raw reads, assembly, assembly validation by read mapping and taxonomic classification were carried out using the analysis tools in EDGE Bioinformatics web server (Li et al., 2017). Read-based classification in the EDGE Bioinformatics web-server were deployed for taxonomic classification of the FN7 metagenome. In the EDGE, Kraken taxonomic sequence classifier (Wood and Salzberg, 2014) was selected for read-based taxonomic classification of the metagenome due to the depth and accurateness of its database. Metagenomic data of FN7 have been deposited and made public in EDGE Bioinformatics web server. In addition, various alpha diversity indices were determined for FN7 metagenome using MOTHUR v. 1.30.2 (Schloss et al., 2009).

2.4. Functional annotation of FN7 metagenome

Sequence reads generated from FN7 metagenome were assembled individually using the make.contigs command in the MOTHUR metagenomic analysis suite (Schloss et al., 2009). Gene calling was performed on the assembled FN7 contigs using MetaGene ((Noguchi et al., 2006)) to predict open reading frames (ORFs). The predicted genes (ORFs) were functionally annotated using the KEGG KofamKOALA (Aramaki et al., 2020), which assigns K numbers to the predicted genes by HMMER/HMMSEARCH against KOfam (a customized HMM database of KEGG Orthologs), and the NCBI's conserved domain database CDSEARCH/cdd v 3.15 (CDD; Marchler-Bauer et al., 2015). Heavy metal resistance genes in the metagenome was annotated using BacMet (Pal et al., 2014), a function-specific bioinformatics resource for the detection of antibacterial biocide and metal-resistance genes. Antibiotic resistance genes in the metagenome was annotated using the Antibiotic Resistance Gene-ANNOTation (ARGANNOT V6; Gupta et al., 2014), a functional annotation resource, which uses NCBI blastp program to annotate the ORFs and provide information on the existing and putative new antibiotic resistance genes (ARG) detected in the protein sequences based on coverage and similarity.

3. Results

3.1. Acenaphthene degradation by FN7 microbiome

Gas chromatography technique was used to monitor acenaphthene depletion by FN7 microbiome. After 14 days incubation, the residual acenaphthene content (50 mg l^{-1}; 100%) decreased to 40.32% (20.16 mg l^{-1}) corresponding to removal of 59.68% (29.84 mg l^{-1}) acenaphthene. At the end of 21 days incubation period, the residual acenaphthene content decrease further to 10.84% (5.42 mg l^{-1}) corresponding to removal of 89.16% (44.58 mg l^{-1}) acenaphthene. No apparent decrease in acenaphthene concentration was observed in the heat-killed control flasks.

3.2. General features of FN7 metagenome

Illumina shotgun next-generation sequencing of the total DNA from FN7 metagenome revealed 4672 sequence reads consisting of 1,393,915 bp, mean read length of 298.36 ± 17.06 bp, and mean GC content of 57.45% ± 4.30, respectively. After trimming and quality control, sequence reads in FN7 reduced to 4655 (99.64%) with 1,389,274 bp (99.33%), mean read length of 298.45 ± 16.75 bp, and mean GC content of 57.46% ± 4.26, respectively. Of the total reads, 99.85% are paired, 79.72% are mapped, and the average fold coverage is 27.97X. Additional features of FN7 metagenome are indicated in Table 1. Statistical analysis of species richness and abundance in FN7 metagenome revealed abundance-based coverage estimator (ACE) of 201.5, Chao1 estimator of 126.2, and Jackknife estimator of 138.6. In addition, it revealed Simpson diversity index of 0.032, Shannon diversity index of 3.646, phylogenetic diversity index of 193, and Good's library coverage of 97.8%, respectively.

Table 1
General features of FN7 metagenome.

	Stats
1. Pre-processing	
a. Raw Reads	
Reads	4672
Total Bases (bp)	1,392,915
Mean Read Length (bp)	298.36 ± 17.06
Mean GC Content (%)	57.45 ± 4.30
b. Quality Trimming	
Trimmed Reads	
Reads	4655 (99.64%)
Total Bases (bp)	1,389,274 (99.67%)
Mean Read Length (bp)	298.45 ± 16.75
Mean GC Content (%)	57.46 ± 4.26
Paired Reads	4648 (99.85%)
Paired Total Bases	1,387,309 (99.86%)
Unpaired Reads	7 (0.15%)
Unpaired Total Bases	1965 (0.14%)
2. Assembly and Annotation	
a. De Novo Assembly by idba_ud	
Number of contigs	29
N50 (bp)	446
Max contig size (bp)	527
Min contig size (bp)	200
Total Assembly size (bp)	12,176
b. Assembly Validation by Read Mapping	
Number of Mapped Reads	3711
% of Total Reads	79.72%
Number of Unmapped Reads	944
% of Total Reads	20.28%
Average Fold Coverage	27.97 X

3.3. Structural characteristics of FN7 metagenome

Structural characterization of acenaphthene-enriched FN7 metagenome revealed 7 phyla, 13 classes, 38 orders, 49 families, 67 genera, 68 species, and 45 strains, respectively. Phylum classification revealed the dominance of the phyla *Proteobacteria* (49%), *Actinobacteria* (31%), and *Firmicutes* (12%) (Fig. 1A). In class delineation, *Actinobacteria* (32%), *Gammaproteobacteria* (24%) and *Alphaproteobacteria* (15%) classes were preponderant (Fig. 1B). Order classification of FN7 metagenome revealed *Actinomycetales* (28%), *Enterobacteriales* (12%) and *Burkholderiales* (9%) as orders with the highest representation (Fig. 2), while in family classification, the families *Enterobacteriaceae* (19%), *Rhodobacteraceae* (9%), and *Bacillaceae* (9%) were preponderant (Fig. 3). In genus delineation, the genera *Paracoccus* (13%), *Methylobacterium* (11%) and *Bacillus* (10%) were dominant (Fig. 4), while in species classification, *Bacillus cereus* group (13%), *Propionibacterium acnes* (10%) and *Methylobacterium radiotolerans* (10%) have the highest representation (Fig. 5). In strain classification, the strains with the highest representation in FN7 metagenome are *Methylobacterium radiotolerans* JCM 2831 (22%), *Paracoccus denitrificans* PD122 (20%) and *Blastococcus saxobsidens* DD2 (9%), respectively (Fig. 6).

3.4. Functional characteristics of FN7 metagenome

Functional characterization of putative ORFs of FN7 metagenome revealed the detection of diverse genes responsible for heavy metal resistance, transport and regulation of inorganic nutrients, resistance to antibiotics and hydrocarbon degradation (Table 2, Table 3, Fig. 6).

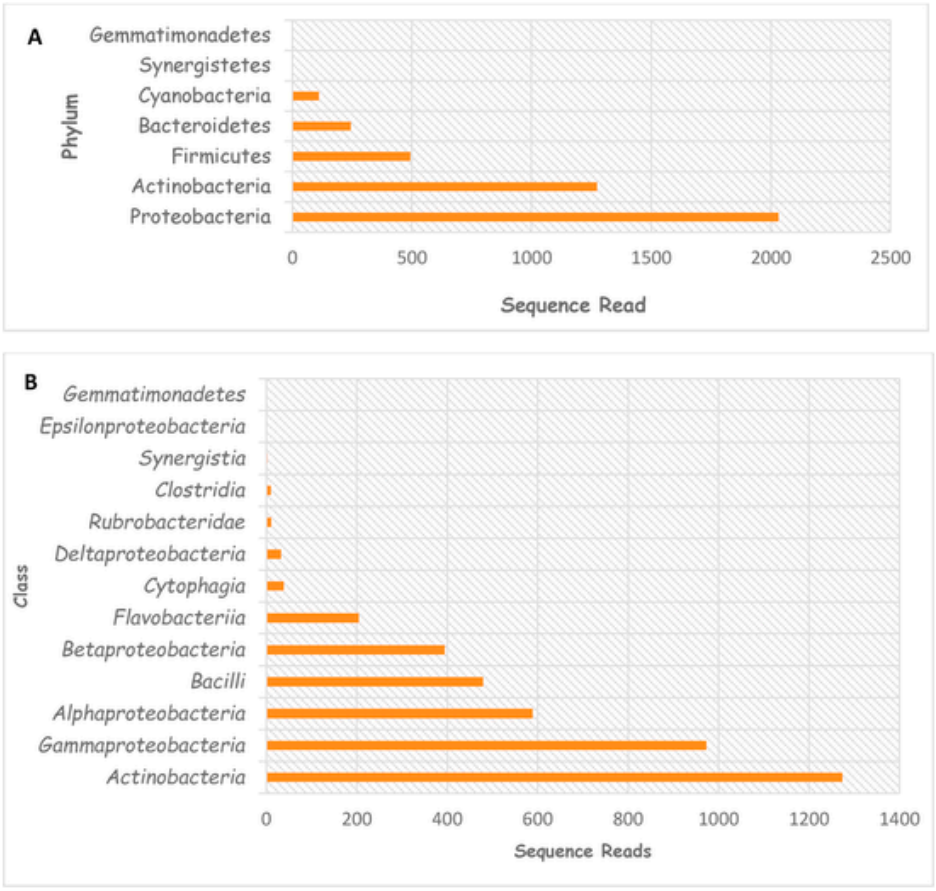

Fig. 1. Phylum (**A**) and Class (**B**) delineations of sequence read of acenaphthene-enriched FN7 metagenome. All the phyla and classes detected in FN7 metagenome were used. The phyla *Proteobacteria* (49%), *Actinobacteria* (31%), *and Firmicutes* (12%) and the classes *Actinobacteria* (32%), *Gammaproteobacteria* (24%) and *Alphaproteobacteria* (15%) have the highest representation.

Several genes responsible for transport, detoxification and efflux of heavy metals such as zinc, manganese, and cadmium (*zraP, zntA, mntA, znuA, ctpC*), Nickel/cobalt (*nrsD/nreB, nikC, nikB, dmeF, ctpD, rcnA*), chromium/molybdenum/copper (*chrA, chrB, modA, modB, modC, copA, copB, copD, cueO, cutO*), iron, arsenic, mercury (*afuB, fpvA, arsA, arsB, arsC, merA, merE*), among others were detected. Interestingly, several species and strains detected in FN7 metagenome are also taxonomically affiliated to the annotated heavy metal resistance genes (Table 2). Putative genes responsible for inorganic nutrient transport and regulation were also annotated in FN7 metagenome. These include genes responsible for the transport and regulation of sulfur (*cysW, tauC, ssuC*), phosphorus (*phnE, phnU, pstB, phoR, senX3*, *creC*), potassium (*kdpD*), and nitrogen (*glnL, ntrB, nac*).

Putative ORFs of FN7 metagenome were annotated for ARGs using ARGANNOT V6. ARGs for fifteen antibiotic classes were detected. These include β-lactam, aminoglycoside, glycopeptide, tetracycline, colistin, macrolide-lincosamide-streptogramin (MLS), fluoroquinolones, trimethoprim, phenicol, nitroimidazole, rifampicin, fosfomycin, fusidic acid, monocarboxylic acid, and tetracenomycin C, respectively. In β-lactam ARGs, fifty-four β-lactamase genes were detected in FN7 with the preponderance of *blaGES*, *blaSHV*, and *blaOXA*, respectively (Fig. 7A). Fifty-four aminoglycoside ARGs were also detected in FN7 with the predominance of aminoglycoside acetyltansferase *aac2-Ib*, and aminoglycoside nucleotidyltransferase *aadB* (Fig. 7B). Thirty-five MLS ARGs were detected in FN7 metagenome with the predominance of *msr(D)*, *srm(B)*, and *msr(A)*, respectively (Fig. 7C). Of the thirty tetracycline ARGs detected in FN7 metagenome, *tet(33)*, *tetL*, and *tetAB* were preponderant (Fig. 7D). The dfrA (and its variants) genes dominated the trimethoprim ARGs in FN7 metagenome (Fig. 7E) while the predominant glycopeptide ARGs detected in FN7 metagenome belong to *vanS-Re* and *vanR-C* (Fig. 7F). Also detected are ARGs for colistin (*mcr-1*, 52%; *mcr-3*, 33.3%; *mcr-4*, 8%; *mcr-5*, 2.67%; *mcr-6, mcr-7, mcr-8*, 1.33%), phenicol (*catA, catB, floR, pexA, cmr, cmxA, bcr1, cmlV, cmlB_variant 1, cpt_strepv*), fluoroquinolone (*oqxBgb, qnrB, qnrS*), nitroimidazole (*nimE* (39; 88.6%), *nimA, nimJ*), and fosfomycin (*fosB, fosD, fosE, fosH, fosI, fomA, fomB*). Solitary ARG for rifampicin (*rphD*), fusidic acid (*fusD*), monocarboxylic acid (*mupB*), and tetracenomycin C (*tcmA*) were also detected.

Sixteen putative genes encoding enzymes that mediate hydrocarbon (mostly aromatic) degradation were annotated in FN7 metagenome. As shown in Table 3, majority of the hydrocarbon degradation genes are involved in benzoate degradation (*acd, aliB*, Cyclohexane-1-carbonyl-CoA dehydrogenase, Cyclohex-1-ene-1-carbonyl-CoA dehydrogenase) and degradation of aromatic compounds. Also annotated in FN7 metagenome ORFs are the genes encoding salicylaldehyde dehydrogenase and catechol 1,2 dioxygenase, two major enzymes involved in acenaphthene degradation pathway as well as their transcriptional regulators (Table 3).

4. Discussion

Enrichment technique is usually geared towards selecting the strains most effective in degrading a sole source of carbon and energy. However, this may lead to exclusion of potential degraders since some of the otherwise very active strains may not be viable on medium usually employed in isolating pure strains from the enrichment or may be slow growers readily overshadowed by fast growing strains (Stach and Burns, 2002). Furthermore, cultural methods such as spread plate tech-

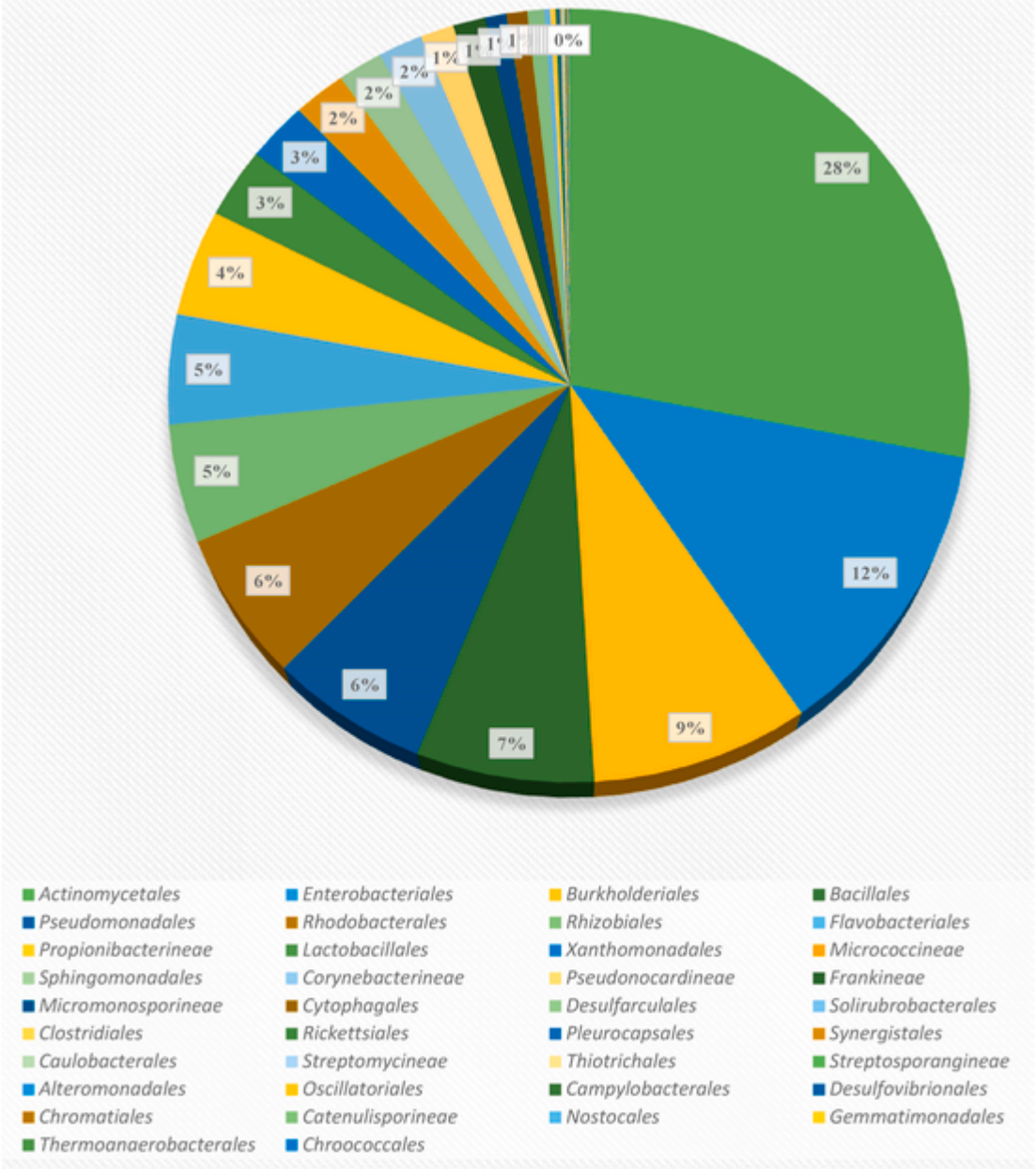

Fig. 2. Order delineation of sequence reads of acenaphthene-enriched FN7 metagenome. All the orders detected in FN7 metagenome was used. The orders *Actinomycetales* (28%), *Enterobacteriales* (12%), *Burkholderiales* (9%) were preponderant.

nique may be inadequate in revealing the overall impact of perturbation with PAHs on the community structure and functionality of the microbiome. A combination of the use of aliquot of enrichment as inoculum and use of next generation sequencing to discern community structure offers a double-barrel approach to addressing these shortcomings.

The degradation of 89.16% of the acenaphthene supplied by the consortium in the inoculum is similar to results previously obtained for single organisms and consortium. Salam et al. (2016) reported 91.78% degradation of 50 mg l^{-1} acenaphthene supplied to an isolate identified as *Pseudomonas* sp. strain KR3 as sole carbon and energy source within 21 days. Sikdar et al. (2016) reported 82% and 65.25% degradation of acenaphthene respectively for *Bacillus* sp. PD5 and *Halomonas* sp. PD4 respectively. In another study *Bacillus valensensis* and *Stenotrophomonas maltophilia* strains isolated from an abandoned coal gasification plant site were found to degrade singly and in combination up to 80% of acenaphthene supplied at even high concentration of 500 mg l^{-1} (Safitri et al., 2019). Since we did not isolate individual axenic culture, it can at least be surmised that the community of FN7 had capability to use acenaphthene as source of carbon and energy.

The domination of FN7 microcosm by the phyla *Proteobacteria* (48.9%), *Actinobacteria* (30.6%), and *Firmicutes* (11.9%) is not surprising because these phyla, particularly the *Proteobacteria* and *Actinobacteria,* are well known for their predominance in systems supplied with hydrocarbons as sole source of carbon and energy, as they are equipped with the necessary battery of genes and physiological capability to adapt to the sparingly available substrates, their detoxification and utilization of the uncommon peripheral pathway metabolites associated with them (Greer et al., 2010; Zhang et al., 2012; Yang et al., 2014; Salam et al., 2017). Furthermore, the *Proteobacteria* are remarkable for their pliability with respect to enzymes with broad specificity for diverse hydrocarbons including aromatic hydrocarbons (Pinyakong et al., 2003; Ezekoye et al., 2018; Galazka et al., 2018) just as the *Actinobacteria* are notable for their oligotrophic adaptations and diverse mechanisms for accessing substrates with low bioavailability (Yuan et

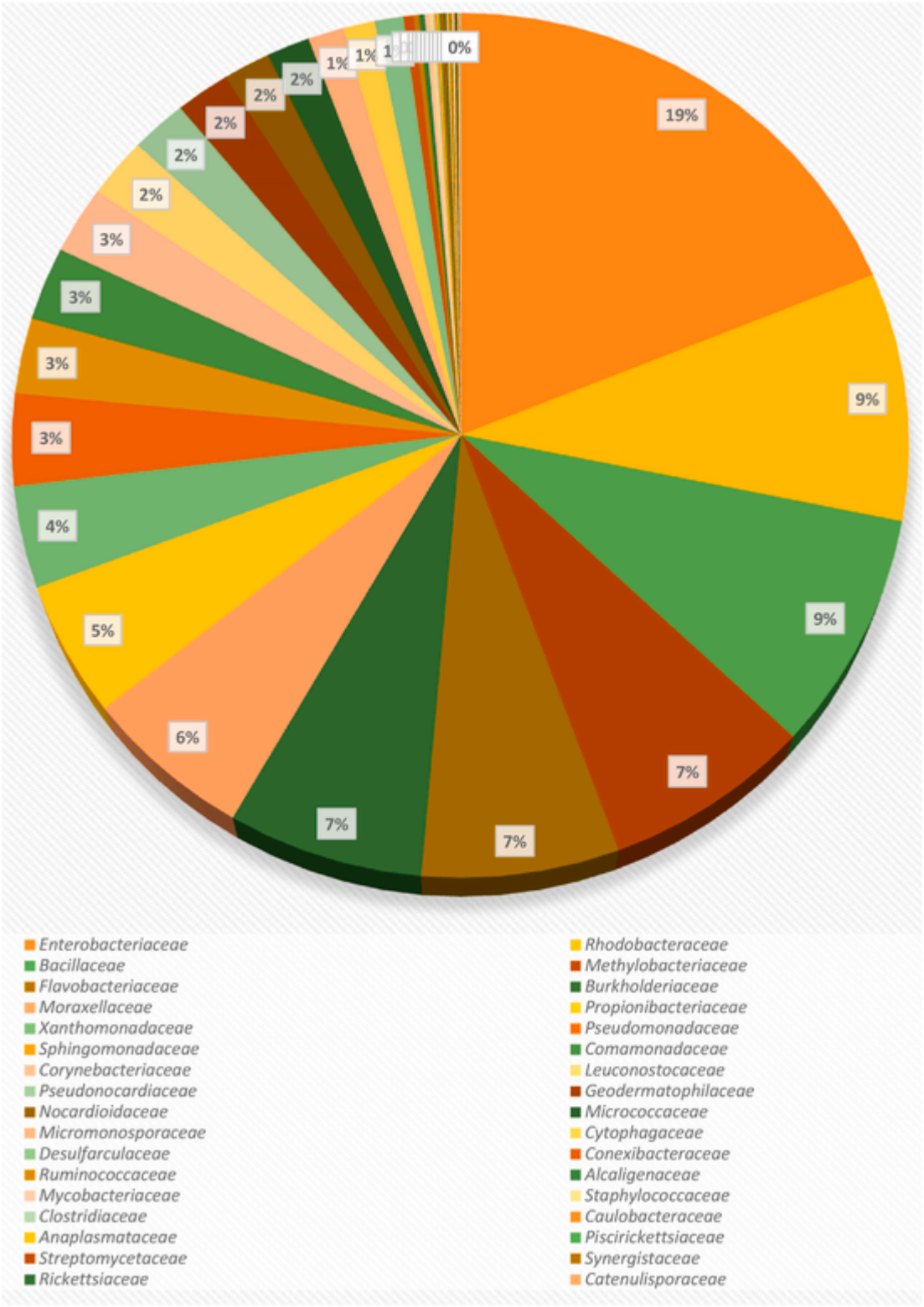

Fig. 3. Family delineation of sequence reads of acenaphthene-enriched FN7 metagenome. All the families detected in FN7 metagenome was used. The families *Enterobacteriaceae* (19%), *Rhodobacteraceae* (9%), and *Bacillaceae* (9%) have the highest representation.

al., 2015; Qin et al., 2016). The *Firmicutes*, unlike the *Actinobacteria*, are a group of low G + C Gram-positive bacteria most of which not only form spores but are also known to deploy a broad spectral of strategies to resist adverse conditions (Merchant and Helmann, 2012; Filippidou et al., 2016). This perhaps explains why they readily thrive in the acenaphthene-enriched animal charcoal polluted soil.

The dominant genera in the consortium are *Paracoccus* and *Methylobacterium*, both members of the class α-*Proteobacteria* which are endowed with a variety of metabolic strategies for adaptation in diverse environments, including extreme temperature, low water activity and poor nutrient availability amongst others (Williams et al., 2007, 2010) and also notable for their propensity to adapt to hydrocarbon polluted

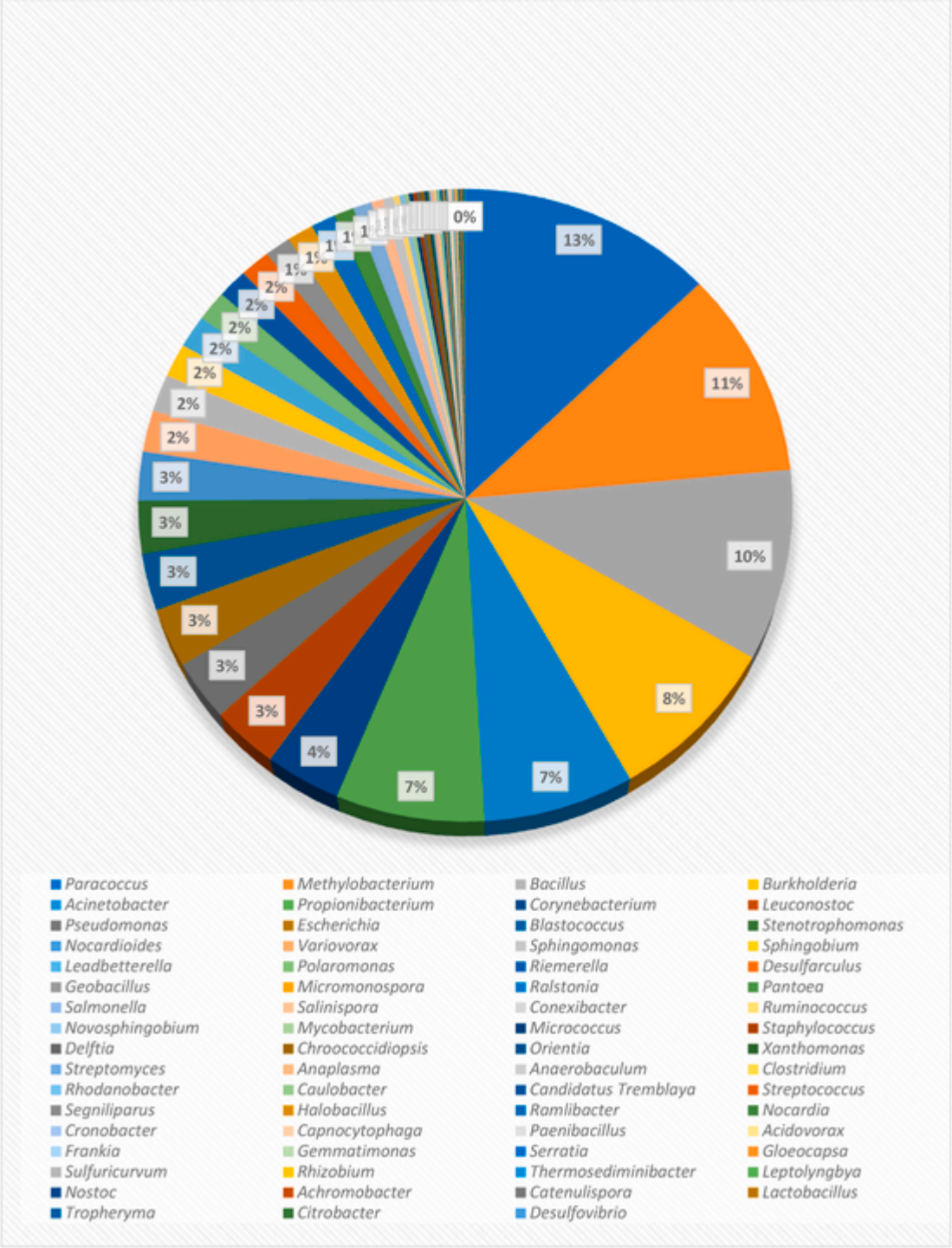

Fig. 4. Genus delineation of sequence reads of acenaphthene-enriched FN7 metagenome. All the genera detected in the metagenome was used. The genera *Paracoccus* (13%), *Methylobacterium* (11%) and *Bacillus* (10%) were dominant.

environments as early colonizers (Salam and Ishaq, 2019). Members of the genus *Paracoccus* are highly metabolically pliable bacteria associated with both pristine and polluted environments (Dziewit et al., 2015). Although *Paracoccus denitrificans* is basically an autotrophic methylotrophs utilizing nitrate reduction as source of energy and metabolizing mono carbon compounds, some *Paracoccus* have been found to be useful in bioremediation of PAHs and xenobiotic compounds (Li et al., 2011). Also ubiquitous and capable of utilizing a variety of methyl and multi-carbon substrates are members of the genus *Methylobacterium* as they are adapted to a variety of environments, including leaf surfaces where they utilize methanol, rice grains, root nodules, soil, dust, water and air (Gallego et al., 2005; Podolich et al., 2009; Cordovana et al., 2019). They are also found in the hospital environments where they assume importance as agents on nosocomial infections, especially in immunocompromised patients (Truant et al., 1998). It is not surprising that a strain of *M. radiotolerans* is one of the preponderant in a consortium enriched from animal charcoal soil given the fact that the species is known to be resistant to gamma radiation. Although there is no report yet in the literature of degradation of acenaphthene by this organism, recently, a strain with ability to use naph-

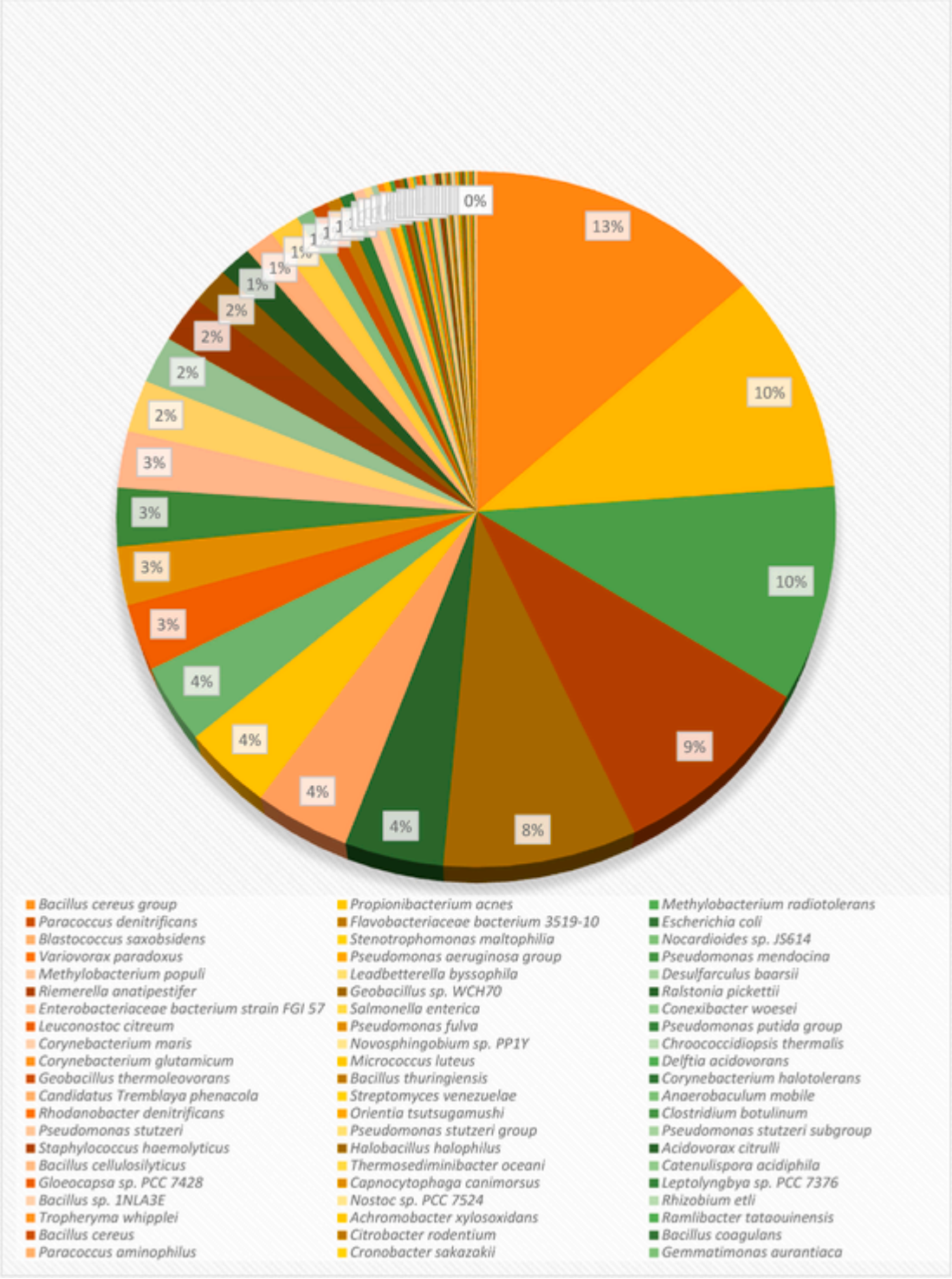

Fig. 5. Species delineation of sequence reads of acenaphthene-enriched FN7 metagenome. All the species detected in the metagenome was used. The species *Bacillus cereus* group (13%), *Propionibacterium acnes* (10%) and *Methylobacterium radiotolerans* (10%) have the highest representation.

thalene as sole source of carbon and energy was isolated from a petroleum hydrocarbon impacted soil in Saudi Arabia (Nzila et al., 2016). This should command attention not only because there had not been a prior report of degradation of PAH by *M. radiotolerans* in the literature, but also because naphthalene is known to share pathways with acenaphthene. *Propionibacterium acne* which is the second most represented species in the consortium is a facultatively anaerobic actinomycetes found in oral cavity, intestinal tracts, conjunctiva and most abundant member of the human skin flora, especially at the subcutaneous region where is sometimes associated with opportunistic infections (Mollerup et al., 2016). Leung et al. (2020) reported remarkable changes in the abundance of *Propionibacterium* on the skin of over 200 individuals following chronic exposure to PAHs. According to the authors, functional analysis identified associations between levels of PAHs and abundance of microbial genes of metabolic and other pathways with potential importance in host-microbe interactions as well as degradation of aromatic compounds. Genetic analysis of this organism

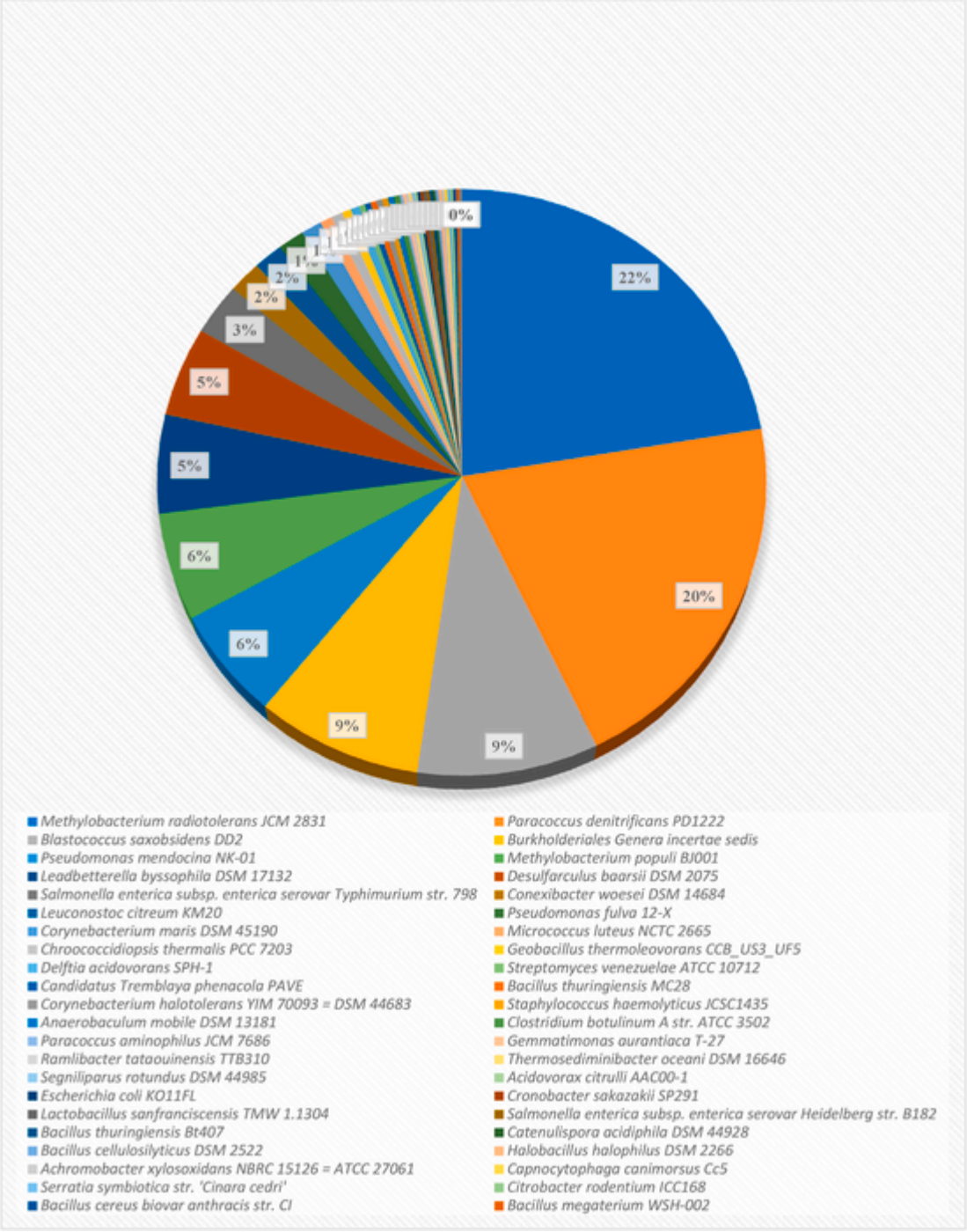

Fig. 6. Strain classification of sequence reads of acenaphthene-enriched FN7 metagenome. All the strains detected in the metagenome was used. The strains *Methylobacterium radiotolerans* JCM 2831 (22%), *Paracoccus denitrificans* PD122 (20%) and *Blastococcus saxobsidens* DD2 (9%) were dominant.

revealed genomic islands and genetic markers indicative of propensity for horizontal gene transfer (Brüggemann, 2010).

Although *Bacillus cereus* are predominantly soil microorganisms associated with spoilage of food and food poisoning, reports abound on their capability to produce biosurfactant and degrade petroleum hydrocarbons (Poornachander et al., 2016; Janaki et al., 2016; Christova et al., 2019).

The preponderance of *Blastococcus saxobsidens* in the consortium is of interest because of the unique morphology and ecology of the species which was first described by Urzì et al. (2004). The cells of the organism are coccoid and aggregated in tetrads buds that give the genus its name and the colonies are orange-pigmented and irregular (Chouaia et al., 2012). This organism is an actinobaterium in the family *Geodermatophilaceae*. It uniquely has the ability to withstand adverse environmental conditions, radiation, desiccation and heavy metals and are widely spread in arid or desert climate as well as calcified stones (Sghaier et al., 2016). It is doubtless that this combination of characteristics must have played a role in its surviving the extreme environmental pressure associated with the animal charcoal soil environment. Overall, the detection of species not commonly isolated is not unexpected, as animal charcoal polluted soil previously assayed by metagenomic approach yielded rare or difficult to culture species in the genera *Anoxybacillus* and *Solibacillus* (Salam and Obayori, 2020) and was also the source of a *Proteus mirabilis* capable of utilizing PAHs pyrene, an-

Table 2
Predicted heavy metals resistance genes and genes involved in inorganic nutrient transport and regulation detected in FN7 metagenome and their taxonomic affiliations.

Heavy metals	Enzyme/genes	Taxonomic affiliation
Zinc (Zn), Manganese (Mn), Cadmium (Cd), Nickel (Ni), Cobalt (Co)	Zinc resistance-associated protein zraP (Zn); ZntA gene product (Zn, Cd, Pb); periplasmic solute binding protein mntA (Mn, Cd); Zur-transcriptional regulator (Zn); zinc/manganese/iron ABC transporter, periplasmic Zn/Mn/Fe binding protein (Mn, Cd); zinc ABC transporter, periplasmic zinc-binding protein, znuA (Zn); heavy metal translocating P-type ATPase, ctpC (Mn, Zn); , nickel transporter permease, nikC (Ni); major facilitator superfamily transporter, nrsD/nreB (Ni/Co); cation diffusion facilitator, dmeF (Ni. Co); heavy metal translocating P-type ATPase, ctpD (Ni, Co); nickel/cobalt efflux protein, rcnA (Co, Ni, Fe); nickel transport system permease protein, nikB	*Citrobacter* sp. 30_2; *Xenorhabdus bovienii* SS-2004; *Thermobacillus composti* KWC4; *Methylococcus capsulatus* str. Bath; *Roseovarius* sp. 217; *Photobacterium profundum* 3TCK; *Kineococcus radiotolerans* SRS30216; *Azospirillum* sp. B510; *Pseudomonas putida* KT2440; *Methylobacterium radiotolerans* JCM 2831; *Desulfovibrio magneticus* RS-1; *Klebsiella oxytoca* 10–5246; *Arthrobacter* sp. FB24; *Conexibacter woesei* DSM 14684; *Arthrobacter phenanthrenivorans* Sphe3; *Rhodobacter sphaeroides* KD131; *Actinosynnema mirum* DSM 43827; *Escherichia* sp. TW09308; *Verminephrobacter eiseniae* EF01-2
Chromium (Cr), Magnesium (Mg), Tungsten (W), Molybdenum (Mo)	Chromate transporter, chrA (Cr); ATP-dependent DNA helicase, recG (Cr); NADPH-dependent FMN reductase, chrB (Cr); malic enzyme, ruvB (Cr); chromate resistance exported protein, chrB (Cr); magnesium-transporting ATPase, mgtA (Mg, Co); iron (metal) dependent repressor, DtxR family, mntR (Mg, Mn); molybdate/tungstate import ATP-binding protein WtpC/ModC (W, Mo); ABC-type molybdate transport system, periplasmic component, modA (W, Mo); ModE family transcriptional regulator, modE (W, Mo); molybdate ABC transporter membrane protein, modB (W, Mo); molybdate/tungstate transport system permease protein, wtpB (W, Mo); tungstate transport system permease protein, tupB	*Sphingomonas* sp. S17; *Micromonospora* sp. ATCC 39149; *Methylobacterium populi* BJ001; *Haemophilus parasuis* 29755; *Stenotrophomonas maltophilia* R551-3; *Caulobacter* sp. K31; *Burkholderia oklahomensis* E0; *Enterobacter cloacae* subsp. cloacae ATCC 13047; *Rahnella* sp. Y9602; *Paracoccus furiosus* DSM 3638; *Brevibacillus laterosporus* LMG 15441; *Acetonema longum* DSM 6540; *Shewanella baltica* OS195; *Cupriavidus metallidurans* CH34

Table 2 (*continued*)

Heavy metals	Enzyme/genes	Taxonomic affiliation
Copper (Cu)	Copper resistance protein D, copD; copper resistance protein, mmco; blue copper oxidase, cueO; apoliprotein N-acetyltrabsferase, actA (Cu, Zn, HCl); copper resistance protein A, copA; copper resistance protein B, copB; heavy metal translocating P-type ATPase, actP; multicopper oxidase type 2, type 3, cutO; molecular chaperone, DnaK; heavy metal translocating P-type ATPase, copP; P-ATPase superfamily, P-type ATPase copper transporter, ctpV; CopS sensor protein (plasmid); transcriptional regulator, ycnK; metal cation transporter P-type ATPase, ctpG; two-component system, OmpR family, heavy metal sensor histidine kinase cusS, copS, silS	*Klebsiella oxytoca* 10–5246; *Stenotrophomonas maltophilia* R551-3; *Sporolactobacillus inulinus* CASD; *Roseibium* sp. TrichSKD4; *Thalassiobium* sp. R2A62; *Nitrosomonas europaea* ATCC 19718; *Cupriavidus taiwanensis* LMG 19424; *Ralstonia eutropha* H16; *Sphingobium japonicum* UT26S; *Ralstonia pickettii* 12J; *Pseudomonas putida* F1; *Methanosaeta harundinacea* 6Ac; *Methylobacterium populi* BJ001; *Xanthobacter autotrophicus* Py2; *Haloadaptus paucihalophilus* DX253; *Verrucosispora maris* AB-18-032; *Thermus aquaticus* Y51MC23; *Mycobacterium parascrofulaceum* ATCC BAA-614; *Desmospora* sp. 8437; *Mycobacterium marinum* M
Iron (Fe), tellurium (Te)	Aconitase, acn; TonB-dependent siderophore receptor, fpvA (Mn, Fe, Co, Zn, Ni, Cu, Cd, Ga); NRAMP family Mn^{2+}/Fe^{2+} transporter, mntH (Mn, Fe, d, Co, Zn); Fe^{2+}-dependent transcriptional regulator, ideR (Fe, H_2O_2); ferritin, DPs family protein, dpsA (Fe, H_2O_2); peroxide-responsive repressor, Fur family protein, per (H_2O_2); fur family ferric uptake regulator, furA; pmrB/BasS sensor protein; tellurite resistance protein, tehA; tellurite resistance protein, tehB; tellurite resistance protein, trgB (Te, Cu); iron(III) transport system permease protein, afuB, fbpB	*Acidothermus cellulolyticus* 11B; *Patulibacter* sp. 111; *Streptomyces clavuligerus* ATCC 27064; *Frankia* sp. CcI3; *Streptomyces venezuelae* ATCC 10712; *Propionibacterium acnes*; *Nocardioides* sp. JS614; *Serratia proteomaculans* 568; *Meiothermus silvanus* DSM 9946; *Amycolicicoccus subflavus* DQS3-9A1; *Nostoc punctiforme* PCC 73102; *Bacillus* sp. 1NLA3E; *Geobacillus* sp. WCH70; *Bacillus cereus* biovar anthracis str. CI; *Bacillus cytotoxicus* NVH 391-98; *Bacillus cereus* Rock3-44; *Sporosarcina newyorkensis* 2681; *Anoxybacillus flavithermus* WK1; *Acidimicrobium ferrooxidans* DSM 10331; *Gordonia polyisoprenivorans* NBRC 16320; *Conexibacter woesei* DSM 14684; *Salmonella enterica* subsp. arizonae serovar 62:z4,z23:..str. RSK2987; *Pasteurella dagmatis* ATCC 43325; *Yersinia ruckeri* ATCC 29473; *Citreicella* sp. SE45
Mercury (Hg)	Mercuric reductase, merA (Hg, phenylmercury, methylmercury); disulfide bond formation protein B, dsbB (Cd, Hg); Hg (II)-responsive transcriptional regulator, merR1; alkylmercury lyase, merB1 (Hg, phenylmercury acetate); mercuric ion transport protein, merE	*Nitrosomonas eutropha* C91; *Enterobacter cloacae* subsp. cloacae ATCC 13047; *Vibrio tubiashi* ATCC 19109; *Alteromonas macleodi* str. 'Deep ecotype'; *Streptomyces zinciresistens* K42

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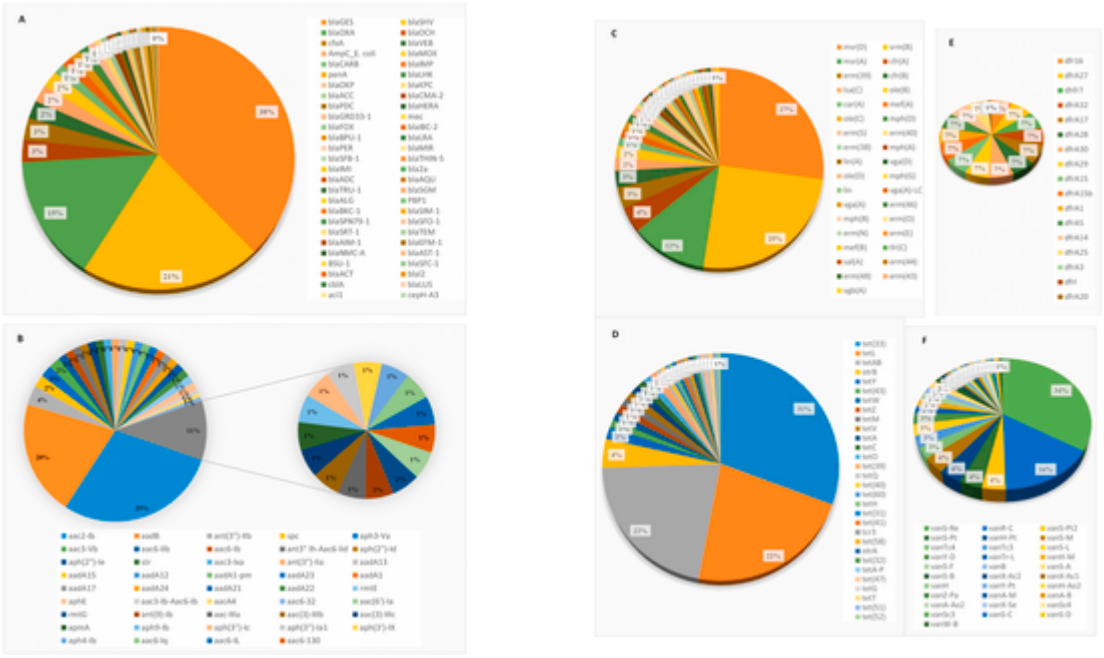

Fig. 7. Distribution of β-lactam (**A**), aminoglycoside (**B**), macrolide-lincosamide-streptogramin (MLS) (**C**), tetracycline (**D**), trimethoprim (**E**) and glycopeptide (**F**), resistance genes in FN7 metagenome as determined by ARG-ANNOT. In (**A**), *blaGES* (38%), *blaSHV* (21%), and *blaOXA* (15%) are the dominant resistance genes. In (**B**), aminoglycoside acetyltansferase *aac2-Ib* (29%), and aminoglycoside nucleotidyltransferase *aadB* (20%) were preponderant. In (**C**), *msr(D)* (27%), *srm(B)* (25%), and *msr(A)* (12%) resistance genes were dominant. In (**D**), *tet(33)* (31%), *tetAB* (22%), and *tetL* (22%) resistance genes have the highest representation. In (**E**), *dfrA* (and its variants) genes equally contributed (7%) to the trimethoprim ARGs in the metagenome while each of dfrA3, dfrl, and dfrA20 contributes only 1% to the trimethoprim ARGs. In (F), *vanS-Re* (34%) and *vanR-C* (16%) were the predominant glycopeptide ARGs in the metagenome.

Table 2 (*continued*)

Heavy metals	Enzyme/genes	Taxonomic affiliation
Arsenic (As), antimony (Sb)	Arsenic resistance protein, arsB (As, Sb); phosphate ABC transporter, ATP-binding protein, pstB (As); arsenate reductase, arsC (9As, Sb); arsenite oxidase, aioA/aoxB (As); arsenical pump-driving ATPase, arsA:plasmid (As, Sb); arsenic methyltransferase, arsM; putative ABC subfamily C, member 1, pgpA/ltpgpA (As, Sb)	*Methylomicrobium alcaliphilum*; *Blastopirellula marina* DSM 3645; *Planctomyces brasiliensis* DSM 5302; *Chlorobium phaeobacteroides* BS1; *Bacillus cellulosilyticus* DSM 2522; *Burkholderiales* bacterium JOSHI-001; *Cellvibrio gilvus* ATCC 13127; *Microbacterium* sp. A33; *Roseomonas cervicalis* ATCC 49957; *Klebsiella oxytoca* 10–5245; *Streptomyces viridochromogenes* DSM 40736; *Leishmania Mexicana* MHOM/GT/2001/U1103
Inorganic Nutrients		
Sulfur, Phosphorus, Nitrogen, Potassium	Sulfur (sulfate/thiosulfate transport system permease protein, cysW; taurine transport system permease protein, tauC; sulfonate transport system permease protein, ssuC); Phosphorus (phosphonate transport system permease protein, phnE; 2-aminoethylphosphonate transport system permease protein, phnU); two-component system, OmpR family, phosphate regulon sensor histidine kinase, phoR (phosphate starvation response); two-component system, OmpR family, sensor histidine kinase, senX3 (phosphate starvation response); two-component system, OmpR family, sensor histidine kinase creC (phosphate regulation); Potassium (two-component system, OmpR family, sensor histidine kinase kdpD); Nitrogen (two-component system, NtrC family, nitrogen regulation sensor histidine kinase GlnL, glnL, ntrB; LysR family transcriptional regulator, nitrogen assimilation regulatory protein, nac)	*Variovorax* sp. KBW07; *Nocardioides euryhalodurans*; *Brevundimonas naejangsanensis*; *Escherichia coli*

thracene, fluoranthene and dibenzothiophene individually as carbon and energy source (Obayori et al., 2017).

Essential heavy metals are required in varying amounts by microorganisms as co-factors in enzymatic reactions and other metabolic processes. While their absence hinders essential metabolic processes, their presence at very high concentration induce impairment of metabolic processes via DNA damage, inhibition of transcription, inhibition of translation, protein denaturation, inhibition of enzyme activity and inhibition of cell division. Such sub-lethal or lethal impairment often affect biodegradation of pollutants in the environment (Said and Lewis, 1991; Sandrin et al., 2000; Bamforth and Singleton, 2005). Diverse heavy metals resistance mechanisms have been devised by microorganisms to counteract the toxic effects effect of the metals (Oves, 2016) and changes in community structure and functions have often been found to correlate strongly with increased heavy metal contamination with consequent reduced biodegradation of hydrocarbon pollutants and xenobiotics (Tipayno et al., 2018). The resistance genes identified in this study are some of the best reported in the literature from polluted environments in relation to transport, detoxification and efflux of heavy metals.

Table 3
Hydrocarbon degradation genes detected in FN7 metagenome.

Hydrocarbon degradation genes	Enzyme function	E value
Glutaryl-CoA dehydrogenase (non-decarboxylating), acd [EC:1.3.99.32]	Benzoate degradation	4.2 e^{-11}
Naphthyl-2-methylsuccinyl-CoA dehydrogenase, bnsG [EC:1.3.99.-]	Naphthalene degradation, degradation of aromatic compounds	8.8e^{-07}
Cyclohexane-1-carbonyl-CoA dehydrogenase [EC:1.3.8.11]	Benzoate degradation	1.1e^{-08}
Cyclohexanecarboxyl-CoA dehydrogenase, aliB [EC:1.3.99.-]	Benzoate degradation	4.9e^{-06}
Pimeloyl-CoA dehydrogenase [EC:1.3.1.62]	Benzoate degradation	9.0e^{-04}
Cyclohex-1-ene-1-carbonyl-CoA dehydrogenase [EC:1.3.8.10]	Benzoate degradation	9.7e^{-06}
(R)-benzylsuccinyl-CoA dehydrogenase, bbsG [EC:1.3.8.3]	Toluene degradation, degradation of aromatic compounds	2.8e^{-14}
Butyryl-CoA dehydrogenase [EC:1.3.8.1]	Benzoyl-CoA degradation	2.2e^{-11}
Acyl-CoA dehydrogenase	β-oxidation of long chain fatty acid during alkane metabolism	3.3e^{-13}
Salicylaldehyde dehydrogenase [EC:1.2.1.65]	Upper naphthalene degradation pathway, acenaphthene degradation, acenaphthylene degradation, degradation of aromatic compounds	8.7e^{-18}
Catechol 1,2-dioxygenase [EC:1.13.11.1]	Acenaphthene degradation, chlorocyclohexane and chlorobenzene degradation, benzoate degradation, fluorobenzoate degradation, toluene degradation, degradation of aromatic compounds	9.3e^{-23}
LysR family transcriptional regulator, regulator for genes of the gallate degradation pathway	Gallate degradation	2.4e^{-03}
LysR family transcriptional regulator, salicylic acid-responsive activator of bsdBCD	Salicylate degradation	1.8e^{-03}
LysR family transcriptional regulator, benzoate and cis,cis-muconate-responsive activator of ben and cat genes, benM	Positive regulator of *ben* and *cat* genes for benzoate degradation. It is only required for expression of *ben* gene but not *cat* genes	4.7e^{-10}
LysR family transcriptional regulator, cis,cis-muconate-responsive activator of cat and ben genes, catM	Positive regulator of expression of *catA*, *catBCIJFD* and *benPK* genes in response to cis,cis-muconate.	8.1e^{-05}
LysR family transcriptional regulator, pca operon transcriptional activator, pcaQ	Activate transcription of the pcaDCHGB operon for the catabolism of protocatechuate	8.0e^{-04}

Among the resistance genes identified in relation to zinc, manganese, and cadmium (*zraP, zntA, mntA, znuA, ctpC*), *zraP, zntA, znuA,*

are genes that function normally in ABC transport system and efflux that keep the level of essential and ubiquitous metal below toxic threshold (Blencowe and Morby, 2003; Padilla-Benavides et al., 2013; Porcheron et al., 2013). However, *mtnA* and *ctpC* are also involved in the detoxification of cadmium. This is important considering that cadmium is a non-essential heavy metal with deleterious effect on microorganisms (Zhai et al., 2017).

Resistance genes for Nickel/cobalt (*nrsD/nreB, nikC, nikB, dmeF, ctpD, rcnA*), chromium/molybdenum/copper (*chrA, chrB, modA, modB, modC, copA, copB, copD, cueO, cutO*), iron, arsenic, mercury (*afuB, fpvA, arsA, arsB, arsC, merA, merE*), among others were detected. Nickel is sparingly needed by organisms that need it for the activity of their enzymes such as dehydrogenases, hydrogenases and urease and is often toxic at higher concentration, causing damages to proteins and nucleic acid and inhibiting several metabolic processes (Grass et al., 2001). Thus, together with cobalt, which is a regular contaminant of Nigerian crude (Oyetibo et al., 2017) nickel is a pollutant of interest. Molybdenum and copper are essential minerals involved in various cell function but which become toxic at high concentrations. The *modA, modB, and modC,* genes are part of the *modABCD* operon involved in uptake and homeostasis of molybdate (Rech et al., 1995), just as the copper tolerance genes *copA, copB* and *copD* are part of the *cop* operon for copper homeostasis (Ladomersky and Petris, 2015; Salam, 2020).

Although a few bacteria use arsenic as electron donor or final electron acceptor, arsenic is toxic to most organisms and metabolic mechanism for tolerance and resistance are common (Kruger et al., 2013). The arsenic resistance genes *arsA, arsB and arsC* detected in this study are part of the *ars* operon found in diverse bacteria including *Proteobacteria*, *Actinobacteria* and *Firmicutes* (Rosen, 2002). These gene mediate ATP-dependent arsenic extrusion. Mercury is also a non-essential metal, which in the oxidized and monomethyl form have strong affinity to sulfur atom of cysteine and interfere with protein structure and function (Møller et al., 2014; Yamamura and Amachi, 2014). The *merA* and *merB* genes are part of the *mer* operon which encode proteins involved in detection, scavenging, transport and reduction of mercury (Barkay et al., 2003).

The diversity of organisms in the consortium harboring heavy metal resistance genes and the multiplicity of genes harbored by these organisms suggest pronounced horizontal transfer of the genes involved in resistance in animal charcoal microbial community from which this consortium was enriched. Evidently, the organisms that constitute the consortium are well endowed with genes needed to survive and function effectively in environment laced with high concentration of diverse toxic heavy metals, an attribute possibly acquired during long period of acclimation to the polluted matrix. The implication of this is that although such heavy metals are not in the acenaphthene supplemented medium at toxic level, the consortium may well readily surmount the challenge of heavy metal toxicity when applied in bioaugmentation of animal charcoal sites which are usually heavy metals laden (Obayori et al., 2017; Salam and Obayori, 2020). Secondly, it is also evident that these organisms have evolved multiple resistance mechanisms, including energy dependent efflux, thus putting them in better position to compete in the polluted environment. The other metals and inorganic nutrients which genes encoding permeases and translocators are annotated and shown to be affiliated with several species is evidence that the organisms in the consortium can make effective use these nutrients even when such nutrients are in short supply.

Perhaps, more concerning is the detection of diverse acquired ARGs for 15 antibiotic classes in the acenaphthene-enriched culture. Aside from possible co-selection of ARGs by heavy metal resistance determinants (Salam, 2020), which are abundant in the enriched culture, the sampling site located at an abattoir consistently receive via indiscriminate disposal ensuing wastes (blood, faeces) emanating from the slaughtered animals. These wastes are rich in antibiotics and its residues and their disposal for several years possibly accentuate the spread and dissemination of acquired ARGs, which are mostly borne on mobile genetic elements and readily exchanged within the microbial community via horizontal gene transfer (Van Hoek et al., 2011; Patel and Bonomo, 2013; Toth et al., 2016; Haenni et al., 2018; Surette and Wright, 2017; Eiamphungporn et al., 2018; Xu et al., 2018; Salam, 2020; Wu et al., 2020). The detection of these ARGs is of serious environmental and public health concern as it revealed that animal charcoal polluted sites not only contributed to the environmental burden of toxic and mutagenic hydrocarbon constituents but is also a potential hotspot for the spread and dissemination of acquired ARGs.

The detection in the FN7 microcosm of genes encoding diverse oxygenases and dehydrogenases involved in the degradation of benzoate, naphthalene, toluene and other aromatic compounds is not surprising. Benzoate is a common intermediate in the biodegradation of many aromatic compounds. It is therefore a model compound for the study of biodegradation of aromatic compound, including PAHs, natural moieties found in lignin, xenobiotics and agrochemicals (Valderrama et al., 2012). The genes for benzoate degradation are very ancient and widely distributed in the biosphere due to preponderance of aromatic compounds in the living system.

Benzoate degradation can take place under aerobic as well as anaerobic conditions; hence there are two classical routes of its metabolism, namely the aerobic and the anaerobic. This distinction while undoubtedly highlighting the dependence on oxygen as a final electron acceptor or otherwise, speaks more to the role oxygen plays in ring activation preparatory to cleavage. In the aerobic process, oxygen is used in activation by way of hydroxylation followed by dearomatization, whereas in the anaerobic process Coenzyme A activation to Benzoyl-CoA is adopted (Elder and Kelly, 1994; Valderrama et al., 2012; Zhuang et al., 2019). Apart from these two major pathways, there is a hybrid pathway that incorporates elements of both aerobic and anaerobic (Rather et al., 2010). Thus, although enriched on acenaphthene, the FN7 metagenome is endowed with diverse genes affiliated with the various species in it, making it potentially suited as candidate inoculum for acenaphthene degradation but also for application in multi-contaminated matrix.

Salicylaldehyde dehydrogenase which gene was annotated in FN7 metagenome ORFs is an enzyme of the upper pathway of degradation of acenaphthene which it shares with acenaphthylene and naphthalene and one of three key enzymes earlier confirmed in the acenaphthene pathway, alongside 1-acenaphthenol dehydrogenase and catechol 1,2-dioxygenase (Ghosal et al., 2013). Salicylaldehyde dehydrogenase oxidizes salicylaldehyde to salicyclate which is further oxidized by salicyclate hydroxylase to catechol, a signature metabolite in the degradation of many aromatic compounds. It is worth mentioning that the catechol 1,2-dioxygenase which gene was annotated in this study has specificity for not only acenaphthene but, chlorocyclohexane, chlorobenzene, benzoate, degradation, toluene and other aromatic compounds degradation. This is consistent with earlier reports which demonstrated the broad specificity of catechol 1,2 dioxygenases for diverse aromatics (Jindrová et al., 2002; Shumkova et al., 2009; Silva et al., 2012). Catechol 1,2 –dioxygenase is a ring cleavage enzyme that compromises the integrity of the aromatic ring converting it to *cis,cis* muconic acid (Guzik et al., 2013) which is lactonised and processed through Succinyl-CoA into the tricarboxylic acid cycle.

Five different transcriptional regulators genes associated with salicyclate and other hydrocarbon metabolite catabolism were annotated and all in the LysR family. The LysR family of transcriptional regulators represents the most abundant type of transcriptional regulator in the prokaryotic kingdom. Highly conservative both structurally and functionally, LysR-type transcriptional regulators are known to regulate a diverse set of genes (Maddocks and Oyston, 2008). Needless to say, transcription regulators control the transcription of genetic information encoded in the DNA and ensures that the biological system is able to respond appropriately to metabolic needs.

5. Conclusion

We developed by enrichment of sample from an animal charcoal soil a consortium able to degrade *in vitro* within 21 days about ninety percent of supplied acenaphthene. The complex consortium which is also endowed with gene batteries for metabolism of diverse hydrocarbons and resistance to heavy metal and antibiotics was dominated by *Proteobacteria* and *Actinobacteria* and at the species level by *Methylobacterium radiotolerans* and *Paracoccus denitrificans*. We surmised from our findings that such consortium could be veritable bioresources for *in vitro* biodegradation as well as on-site bioremediation of animal charcoal polluted sites.

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Ethical approval

Neither animal nor human was used in part or whole during this study, as regards the procedures performed during the present investigation. Therefore, this article does not contain any studies with human participants or animals performed by any of the authors.

Authors' contribution

LBS participated in experimental design, collation of data, manuscript preparation, and overseeing execution of experimentation; OSO participated in data collection and manuscript preparation; MOI and OOA contributed to the Discussion section and overseeing experimentation and manuscript preparation. All authors read and approved the final manuscript.

Declaration of competing interest

Each of the Authors has no conflict of interest.

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