

# Bioinformatics approach in speciation of oil degrading uncultured bacterium and its frequency recording

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## ABSTRACT

**Aim:** As per NCBI database search, number of oil degrading uncultured bacterial species reported to be targeted for their 16S rRNA region worldwide, but no systematic approach was made till date to relate all these isolates with sequence-based homology for a particular genus and species to which probably they belongs to as the data is so discrete. **Materials and Methods:** The present study put forward the frequency-based classification of the several oil-degrading bacteria into particular genus and species as per 16S rRNA homology and phylogenetic analysis by involving, BLASTN, CLUSTALW, and MAGA6 software in together and finally reported the major species dominated by the set of uncultured bacteria related with oil degradation studied. **Result:** In a result when 1123 uncultured bacteria related with oil degradation were sequence homolog for 16S rRNA with public database; isolates were majorly been identified as 320 bacterial species which were further grouped into 15 clades as per phylogenetic analysis. **Conclusion:** Adopted methodology certainly been useful to classify the set of oil-degrading uncultured bacteria with their close homolog as they grouped only into 320 species out of which few shares majorly percentage as recorded.

**KEY WORDS:** Bioinformatics, Homology, Oil degradation, Phylogenetics, Uncultured bacteria

## INTRODUCTION

The ability of each bacterium to grow in the laboratory conditions is not guaranteed and sometimes referred to them “unculturable/uncultured.” This term as it indicates that few bacteria will never grow in the medium could be explained as that we lack information about the medium, in which we can help them.<sup>[1]</sup> In an attempt, it has been showcased that uncultured bacteria are actually metabolically active even though they cannot multiply in the laboratory media.<sup>[2]</sup> The diversity of these uncultured bacteria is now easily understood by polymerase chain reaction targeting of 16S rRNA gene with given environmental samples directly, regardless of the viability of the organisms that harbored the DNA, and it share the added advantage for screening of real population present.<sup>[3]</sup> In a success since 1987 when only 11 bacteria phyla was known, now the number has reached to 85, the majority of them still remain uncultivable.<sup>[4-7]</sup> Surely, a diverse

group of bacteria are growing in an environment as evidenced by presence of their DNA in the samples and still not very well understood about them.<sup>[8]</sup>

The present study attempted to possibly answer the phyla-based classification of oil-degrading uncultured bacteria based on the 16S rRNA gene sequence available in NCBI received from all over the world by implementing few bioinformatics tools which could be in combination can strengthen the possibility of the species-specific classification of several uncultured species.

## MATERIALS AND METHODS

### Collection of 16S rRNA Gene Sequences

To collect the 16S rRNA gene sequences for the uncultured bacteria capable of oil degradation, NCBI Nucleotide database was used. Database possess the information about the cultures capable of oil degradation recorded worldwide. The query given to the database as “uncultured bacterium 16S rRNA oil degradation” and 16S sequences were saved in the FASTA format.

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## BLASTN Analysis

In total, 1123 sequences were retrieved for the uncultured bacteria capable of oil degradation were checked for the sequence homology using the BLASTN program available at website: [http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). During routine investigation BLASTN program selects default nonredundant database which includes all the sequences obtained from the various databases. Resultant the BLAST showed the similar sequences homology for the uncultured bacterium. Hence, in decision, default database was changed to 16S rRNA database which helped in aligning the uncultured sequences with the known bacterial sequences only and that assisted us to classify them into the given classified family. With this new protocol used, 1123 sequences of uncultured bacteria were recorded for the homology obtained according to the specialized BLAST performed.<sup>[9]</sup>

## Multiple Sequence Alignment (MSA)

Obtained homology for the previously uncultured bacteria were used to rename the cultures according to homology and further, these 1123 sequences were MSA using CLUSTALW to understand the phylogenetics relationship among the sequences capable of oil degradation. The DND file obtained from the CLUSTALW program was given as an input to understand the phylogenetics relationship in a MEGA6 phylogenetic analysis program.<sup>[9]</sup>

## Phylogenetic Analysis

Previously selected 1123 uncultured bacteria were classified according to BLASTN into the given best-scored genus and species to which they showed the best homology. In its further study, phylogenetics relationship was drawn based on the sequence homology and alignment using CLUSTALW2 and phylogram was developed using MEGA6 software. MEGA6 accepts the DND file developed by CLUSTAL program and creates the phylogram with relative distance. The developed phylogram further assisted us in classifying the 1123 cultures in the given CLADE, and resultant final cultures were broadly classified as the bacteria capable of oil-degrading which were previously nominated as uncultured bacteria capable of oil degradation.<sup>[9]</sup>

## RESULT

### Collection of 16S rRNA Gene Sequences

Till 1<sup>st</sup> January 2018, NCBI nucleotide database available at [www.ncbi.nlm.nih.gov/nucleotide](http://www.ncbi.nlm.nih.gov/nucleotide) recorded to possess 1123 hits for the term oil degradation and 16S RNA gene sequences as shown in Figure 1.

<input type="checkbox"/>	<a href="#">Uncultured bacterium, partial 16S rRNA gene</a>
1.	1,356 bp linear DNA Accession: CU915245.1 GI: 239913764 <a href="#">PubMed</a> <a href="#">Taxonomy</a> <a href="#">GenBank</a> <a href="#">FASTA</a> <a href="#">Graphics</a>
<input type="checkbox"/>	<a href="#">Uncultured bacterium, partial 16S rRNA gene</a>
2.	1,356 bp linear DNA Accession: CU915244.1 GI: 239913763 <a href="#">PubMed</a> <a href="#">Taxonomy</a> <a href="#">GenBank</a> <a href="#">FASTA</a> <a href="#">Graphics</a>
<input type="checkbox"/>	<a href="#">Uncultured bacterium, partial 16S rRNA gene</a>
3.	1,364 bp linear DNA Accession: CU915243.1 GI: 239913762 <a href="#">PubMed</a> <a href="#">Taxonomy</a> <a href="#">GenBank</a> <a href="#">FASTA</a> <a href="#">Graphics</a>
<input type="checkbox"/>	<a href="#">Uncultured bacterium, partial 16S rRNA gene</a>
4.	1,390 bp linear DNA Accession: CU915242.1 GI: 239913761 <a href="#">PubMed</a> <a href="#">Taxonomy</a> <a href="#">GenBank</a> <a href="#">FASTA</a> <a href="#">Graphics</a>
<input type="checkbox"/>	<a href="#">Uncultured bacterium, partial 16S rRNA gene</a>
5.	1,393 bp linear DNA Accession: CU915241.1 GI: 239913760 <a href="#">PubMed</a> <a href="#">Taxonomy</a> <a href="#">GenBank</a> <a href="#">FASTA</a> <a href="#">Graphics</a>

**Figure 1:** Reported hits for uncultured bacteria capable of oil degradation as per Genbank information linked with

## BLAST Analysis and Frequency match

Collected 1123 sequences individually screened by pair-wise alignment program BLASTN by searching in 16S rRNA gene sequence database. The results highlighted the defined homology with the bacterial sequences which have been previously identified and assigned with accession numbers in the database. The defined homology of each uncultured sequence with the subject sequence (bacterial species known for genus and species) were given (data not shown), and further, their frequency was denoted indicating the number of times sequences of uncultured bacteria aligned with particular bacterial species. Among 1132 bacterial isolates previously have been recorded as uncultured bacteria now they have been found to be getting classified into 320 different bacterial species [Table 1]. In all these bacterial species, frequency of repetition of many bacteria recorded on higher side for example: *Nitratireductor aquimarinus* strain CL-SC21 appeared to be matching with 108 uncultured bacterial 16S rRNA sequences reaching 9.62% of total population studied; *Actinomadura gamaensis* strain NEAU-Gz5 matching with 60 uncultured isolates

(5.34%); *Kocuria dechangensis* strain NEAU-ST5-33 shown frequency of 47 (4.19%); *Kocuria polaris* with frequency of 46 (4.10%), and others recorded on lower side as low as one time with given bacterial species [Table 1].

### MSA

In an MSA, all species-level identified uncultured sequences renamed as per BLASTN homology details by replacing the accession number of uncultured part with genus and species of close homolog and then allowed to undergo MSA to generate DND file. Given file was successfully created and used in phylogenetic analysis with few modifications (data not shown).

### Phylogenetic Analysis

To record the phylogenetic relationship of all uncultured bacteria showcasing homology with few species of bacterial kingdom as recorded in BLASTN study; phylogenetic approach further allowed us to cluster by putting DND file received from the MSA into an analysis. As per MEGA6 – a phylogenetic analysis software, all 1123 isolates were grouped to create 15 clades and it was further recorded that as confirmed in the BLAST N, number of uncultured bacteria remained clustered in a one clade as per homology recorded in BLASTN results for the given genus and species. Hence, phylogeny further strengthen the evidence of close homology for number of uncultured bacteria even though they were isolated from varied environment, but always kept related with

**Table 1: Frequency of match recorded for the given bacterial species with studied uncultured bacterial 16S rRNA sequences related with oil degradation**

Close homolog species	Frequency
<i>Hydrocarboniphaga effusa</i> strain AP103	1
<i>[Clostridium] bifermentans</i> strain JCM 1386	1
<i>[Clostridium] paradoxum</i> strain DSM 7308	4
<i>[Clostridium] straminisolvens</i> strain CSK1	1
<i>[Desulfobacterium] catecholicum</i> strain NZva20	3
<i>Acholeplasma palmae</i> strain J233	2
<i>Achromobacter anxifer</i> strain LMG 26857	1
<i>Achromobacter denitrificans</i> strain DSM 30026	1
<i>Acidaminobacter hydrogeniformans</i> strain glu 65	2
<i>Aciditerrimonas ferrireducens</i> strain IC-180	3
<i>Acinetobacter movanagherensis</i> strain Movanagher 4	1
<i>Actinomadura gamaensis</i> strain NEAU-Gz5	60
<i>Actinomadura rubrobrunea</i> strain DSM 43750	1
<i>Aequorivita viscosa</i> strain 8-1b	1
<i>Aeromicrobium fastidiosum</i> strain KCTC 9576	1
<i>Albidovulum inexpectatum</i> strain FRR-10	1
<i>Albirhodobacter marinus</i> strain N9	1
<i>Alcaligenes faecalis</i> strain NBRC 13111	46
<i>Alcanivorax dieselolei</i> strain B5	1
<i>Alcanivorax venustensis</i> strain ISO4	1
<i>Alcanivorax xenomutans</i> strain JCI09	1
<i>Algoriphagus confluentis</i> strain HJM-2	1
<i>Alkaliphilus metalliredigens</i> strain QYMF	1
<i>Altererythrobacter rigui</i> strain WW3	2
<i>Aminivibrio pyruvatiphilus</i> strain 4F6E	3
<i>Anaerobacterium chartisolvens</i> strain T-1-35	1
<i>Anaerobaculum hydrogeniformans</i> strain OS1	1
<i>Anaerobaculum mobile</i> strain DSM 13181	1
<i>Anaeromyxobacter dehalogenans</i> strain 2CP-1	1
<i>Anaerospira hongkongensis</i> strain HKU15	1
<i>Arcobacter nitrofigilis</i> strain DSM 7299	1
<i>Ardenticatena maritima</i> strain 110S	2
<i>Armatimonas rosea</i> strain YO-36	3
<i>Atopobium parvulum</i> strain DSM 20469	3
<i>Azonexus caeni</i> strain Slu-05	1
<i>Bacillus circulans</i> strain ATCC 4513	4
<i>Bacillus cytotoxicus</i> strain NVH 391-98	1
<i>Bacillus isronensis</i> strain B3W22	3
<i>Bacillus massilianorexius</i> strain AP8	2
<i>Bacillus mesophilus</i> strain SA4	1
<i>Bacillus oceanisediminis</i> strain H2	1
<i>Bacillus psychrosaccharolyticus</i> strain NBRC 101233	1
<i>Bacteroides graminisolvens</i> strain JCM 15093	1
<i>Bellilinea caldifistulae</i> strain GOMI-1	9
<i>Brevibacillus massiliensis</i> strain phR	1
<i>Burkholderia lata</i> strain 383	1

(Contd...)

Table 1: (Continued)

Close homolog species	Frequency
<i>Burkholderia xenovorans</i> strain LB400	1
<i>Caedibacter caryophilus</i> strain 221	1
<i>Caldicoprobacter algeriensis</i> strain TH7C1	1
<i>Caldilinea tarbellica</i> strain D1-25-10-4	1
<i>Calditerricola satsumensis</i> strain YMO81	2
<i>Calditerrivibrio nitroreducens</i> strain DSM 19672	1
<i>Caldithrix abyssi</i> strain LF13	2
<i>Caldithrix palaeochoryensis</i> strain MC	1
<i>Caldivirga maquilingsensis</i> strain IC-167	1
<i>Carboxydocella manganica</i> strain SLM 61	1
<i>Cellulomonas bogoriensis</i> strain 69B4	1
<i>Chitinispirillum alkaliphilum</i> strain ACh6-1	1
<i>Chroococcidiopsis thermalis</i> strain PCC 7203	1
<i>Chryseobacterium halperniae</i> strain H1	1
<i>Citrobacter murlinae</i> strain CDC 2970-59	4
<i>Citrobacter youngae</i> strain GTC 1314	1
<i>Clostridium bowmanii</i> strain DSM 14206	1
<i>Clostridium merdae</i> strain Marseille-P2953	1
<i>Cocleimonas flava</i> strain KMM 3898	2
<i>Comamonas terrae</i> strain A3-3	1
<i>Congregibacter litoralis</i> strain KT71	1
<i>Corynebacterium marinum</i> strain D7015	1
<i>Cupriavidus basilensis</i> strain DSM 11853	1
<i>Cycloclasticus spirillensus</i> strain M4-6	3
<i>Cytophaga fermentans</i> strain NBRC 15936	8
<i>Dehalogenimonas alkenigignens</i> strain IP3-3	1
<i>Desulfatibacillum alkenivorans</i> strain PF2803	6
<i>Desulfatiglans parachlorophenolica</i> strain DS	1
<i>Desulfatitalea tepidiphila</i> strain S28bF	2
<i>Desulfitispora alkaliphila</i> strain AHT17	1
<i>Desulfitobacterium chlororespirans</i> strain Co23	3
<i>Desulfobacula toluolica</i> strain Tol2	2
<i>Desulfobotulus sapovorans</i> strain DSM 2055	1
<i>Desulfobulbus mediterraneus</i> strain 86FS1	1
<i>Desulfobulbus rhabdiformis</i> strain M16	3
<i>Desulfocapsa sulfexigens</i> strain DSM 10523	1
<i>Desulfocapsa thiozymogenes</i> strain Bra2	6
<i>Desulfococcus multivorans</i> strain DSM 2059	1
<i>Desulfoconvexum algidum</i> strain JHA1	1
<i>Desulfocurvus thunnarius</i> strain Olac 40	1
<i>Desulfofaba gelida</i> strain PSv29	1
<i>Desulfomicrobium hypogeiium</i> strain CN-A	1
<i>Desulfomonile limimaris</i> strain DCB-M	1
<i>Desulfomonile tiedjei</i> strain DSM 6799	2
<i>Desulfonatronobacter acetoxydans</i> strain APT3	2
<i>Desulfonema magnum</i> str. Montpellier strain 4be13	1
<i>Desulfopila inferna</i> strain JS_SRB250Lac	1
<i>Desulforhopalus singaporensis</i> strain S'pore T1	1
<i>Desulfosarcina ovata</i> strain oXyS1	1
<i>Desulfotignum toluenicum</i> strain H3	3
<i>Desulfotomaculum geothermicum</i> strain DSM 3669	1
<i>Desulfotomaculum thermosubterraneum</i> strain RL50JIII	2
<i>Desulfovibrio alkalitolerans</i> strain RT2	1
<i>Desulfuromonas michiganensis</i> strain BB1	3
<i>Desulfuromonas palmitatis</i> strain SDBY1	3
<i>Desulfuromonas svalbardensis</i> strain 112	1
<i>Desulfuromonas thiophila</i> strain NZ27	2
<i>Desulfuromusa ferrireducens</i> strain 102	1
<i>Dethiobacter alkaliphilus</i> strain AHT 1	1
<i>Dictyoglomus turgidum</i> strain DSM 6724	3
<i>Dinoroseobacter shibae</i> strain DFL 12	1
<i>Enterobacter asburiae</i> strain JCM6051	1
<i>Enterobacter cancerogenus</i> strain LMG 2693	2
<i>Enterobacter cloacae</i> strain ATCC 13047	2
<i>Enterobacter hormaechei</i> strain 0992-77	20
<i>Enterobacter ludwigii</i> strain EN-119	2
<i>Enterobacter tabaci</i> strain YIM Hb-3	1
<i>Enterobacter xiangfangensis</i> strain 10-17	14

(Contd...)



Table 1: (Continued)

Close homolog species	Frequency
<i>Erwinia billingiae</i> strain Billing E63	1
<i>Escherichia fergusonii</i> strain ATCC 35469	2
<i>Eudoraea adriatica</i> strain AS06/20a	1
<i>Euzebya tangerina</i> strain F10	1
<i>Fervidicella metallireducens</i> strain AeB	1
<i>Filomicrobium fusiforme</i> strain DSM 5304	2
<i>Flaviflexus huanghaiensis</i> strain H5	4
<i>Flaviflexus salsibiostraticola</i> strain EBR4-1-2	1
<i>Flavitalea gansuensis</i> strain JCN-23	2
<i>Fusibacter fontis</i> strain KhalAKB1	3
<i>Gaetbulibacter aquiaggeris</i> strain KEM-8	8
<i>Gaiella occulta</i> strain F2-233	1
<i>Geobacillus subterraneus</i> subsp. <i>aromaticivorans</i> strain Ge1	1
<i>Geobacter anodireducens</i> strain SD-1	1
<i>Geobacter daltonii</i> strain FRC-32	1
<i>Geobacter pelophilus</i> strain Dfr2	1
<i>Geodermatophilus terrae</i> strain PB261	2
<i>Geojedonia litorea</i> strain YCS-16	1
<i>Geosporobacter ferrireducens</i> strain IRF9	1
<i>Granulicella sapmiensis</i> strain S6CTX5A	1
<i>Haliea salexigens</i> strain 3X/A02/235	1
<i>Halochromatium roseum</i> strain JA134	1
<i>Halomonas alkaliphila</i> strain 18bAG	4
<i>Halomonas venusta</i> strain DSM 4743	47
<i>Halomonas zhaodongensis</i> strain NEAU-ST10-25	1
<i>Herbaspirillum seropedicae</i> strain NBRC 102524	1
<i>Hippea alviniae</i> strain EP5-r	1
<i>Hydrocarboniphaga effusa</i> strain AP103	7
<i>Hydrogenophaga taeniospiralis</i> strain 2K1	1
<i>Hydrogenophaga taeniospiralis</i> strain NBRC 102512	2
<i>Hyphomicrobium facile</i> subsp. <i>ureaphilum</i> strain IFAM CO-582	1
<i>Inmirania thermoithiophila</i> strain S2479	1
<i>Intestinibacter bartlettii</i> strain WAL 16138	5
<i>Kineosporia rhizophila</i> strain DSM 44389	2
<i>Kocuria dechangensis</i> strain NEAU-ST5-33	47
<i>Kocuria polaris</i> strain CMS 76or	46
<i>Leclercia adecarboxylata</i> strain CIP 82.92	4
<i>Legionella quateirensis</i> strain 1335	1
<i>Lentimicrobium saccharophilum</i> strain TBC1	6
<i>Leptolinea tardivitalis</i> strain YMTK-2	20
<i>Leptospira alexanderi</i> serovar <i>Manhao 3</i> strain L 60	1
<i>Leucobacter celer</i> subsp. <i>astrifaciens</i> strain CBX151	1
<i>Levilinea saccharolytica</i> strain KIB1-1	15
<i>Listeria innocua</i> strain NCTC 11288	1
<i>Litorilinea aerophila</i> strain PRI-4131	1
<i>Longilinea arvoryzae</i> strain KOME-1	1
<i>Loriellopsis cavernicola</i> strain LF-B5	1
<i>Luteimonas terricola</i> strain BZ92r	1
<i>Lutibacter flavus</i> strain IMCC1507	1
<i>Lutibacter maritimus</i> strain S7-2	1
<i>Lutimonas vermicola</i> strain NBRC 102041	1
<i>Lysobacter novalis</i> strain THG-PC7	1
<i>Maliponia aquimaris</i> strain MM-10	1
<i>Marinilabilia nitratreducens</i> strain AK2	1
<i>Mariniphaga anaerophila</i> strain Fu11-5	4
<i>Mariniphaga sediminis</i> strain SY21	2
<i>Marinobacter aquaeolei</i> VT8	6
<i>Marinobacter hydrocarbonoclasticus</i> strain ATCC 49840	3
<i>Marinobacterium georgiense</i> strain NBRC 102606	1
<i>Marinomonas pontica</i> strain 46-16	1
<i>Marinomonas profundimaris</i> strain 25BN12M-4	1
<i>Melioribacter roseus</i> strain P3M-2	1
<i>Mesotoga infera</i> strain VNs100	1
<i>Mesotoga prima</i> strain MesG1.Ag. 4.2	2
<i>Methanogenium cariaci</i> strain JR1	1
<i>Methyloligella solikamskensis</i> strain SK12	1
<i>Methylomicrobium alcaliphilum</i> strain 20Z	1
<i>Methylovorus mentalis</i> strain MM	1

(Contd...)

Table 1: (Continued)

Close homolog species	Frequency
<i>Microbacter margulisiae</i> strain ADRI	1
<i>Microbacterium oleivorans</i> strain BAS69	1
<i>Moorella glycerini</i> strain JW/AS-Y6	1
<i>Moorella humiferrea</i> strain 64-FGQ	39
<i>Moorella perchloratireducens</i> strain An10	2
<i>Moorella thermoacetica</i> strain JCM 9319	1
<i>Mucilaginibacter myungsuensis</i> strain HMD1056	1
<i>Mycoplasma glycophilum</i> strain 486	1
<i>Namhaecicola litoreus</i> strain DPG-25	2
<i>Natranaerobaculum magadiense</i> strain Z-1001	1
<i>Nereida ignava</i> strain 2SM4	1
<i>Nevskia terrae</i> strain KIS13-15	1
<i>Nitratireductor aquibiodomus</i> strain NL21	30
<i>Nitratireductor aquimarinus</i> strain CL-SC21	108
<i>Nitratireductor indicus</i> strain C115	1
<i>Novosphingobium endophyticum</i> strain EGI 60015	1
<i>Nubsella zeaxanthinifaciens</i> strain NBRC 102579	1
<i>Oceanicola flagellatus</i> strain DY470	1
<i>Ochrobactrum anthropi</i> strain ATCC 49188	1
<i>Olegusella massiliensis</i> strain KHD7	2
<i>Oleispira antarctica</i> strain RB-8	1
<i>Oleispira lenta</i> strain DFH11	1
<i>Ornatilinea apprima</i> strain P3M-1	15
<i>Ornithinibacter aureus</i> strain HB09001	1
<i>Paracoccus aminophilus</i> strain DM-15	1
<i>Paracoccus thiocyanatus</i> strain NBRC 14569	1
<i>Paraperlucidibaca baekdonensis</i> strain RL-2	1
<i>Paucimonas lemoignei</i> strain LMG 2207	1
<i>Pelobacter acetylenicus</i> strain WoAcy1	1
<i>Pelobacter carbinolicus</i> strain DSM 2380	3
<i>Pelobacter propionicus</i> strain DSM 2379	1
<i>Pelolinea submarina</i> strain MO-CFX1	4
<i>Pelosinus fermentans</i> strain R7	1
<i>Pelotomaculum isophthalicum</i> strain JI	1
<i>Petrimonas sulfuriphila</i> strain BN3	1
<i>Phaeobacter inhibens</i> strain T5	1
<i>Pontibacter actiniarum</i> strain KMM 6156	1
<i>Prolixibacter bellariivorans</i> strain JCM 13498	2
<i>Prolixibacter denitrificans</i> strain MIC1-1	8
<i>Prosthecochloris aestuarii</i> strain DSM 271	1
<i>Prosthecochloris vibrioformis</i> strain 6030	19
<i>Proteiniphilum acetatigenes</i> strain TB107	2
<i>Pseudoalteromonas nigrifaciens</i> strain NBRC 103036	2
<i>Pseudolabrys taiwanensis</i> strain CC-BB4	1
<i>Pseudomonas aeruginosa</i> strain DSM 50071	1
<i>Pseudomonas balearica</i> strain SP1402	1
<i>Pseudomonas chloritidismutans</i> strain AW-1	8
<i>Pseudomonas guangdongensis</i> strain SgZ-6	3
<i>Pseudomonas knackmussii</i> strain B13	2
<i>Pseudomonas kunmingensis</i> strain HL22-2	1
<i>Pseudomonas linyingensis</i> strain LYBRD3-7	4
<i>Pseudomonas nitroreducens</i> strain NBRC 12694	1
<i>Pseudomonas oryzae</i> strain WM-3	1
<i>Pseudomonas sagittaria</i> strain CC-OPY-1	3
<i>Pseudonocardia hydrocarbonoxydans</i> strain NRRL B-16171	1
<i>Pseudorhodobacter ferrugineus</i> strain NBRC 15767	4
<i>Pseudorhodobacter wandonensis</i> strain WT-MW11	1
<i>Pseudoruegeria haliotis</i> strain WM67	1
<i>Pseudoxanthomonas mexicana</i> strain AMX 26B	1
<i>Psychrobacillus insolitus</i> strain DSM 5	1
<i>Psychromonas arctica</i> strain strain Pull 5.3	2
<i>Racemicystis crocea</i> strain MSr9521	2
<i>Raoultibacter timonensis</i> strain Marseille-P3277	1
<i>Rhodanobacter denitrificans</i> strain 2APBS1	1
<i>Rhodanobacter soli</i> strain DCY45	1
<i>Rhodococcus aetherivorans</i> strain DSM 44752	4
<i>Romboutsia timonensis</i> strain DR1	6
<i>Roseivivax roseus</i> strain BH87090	1

(Contd...)

Table 1: (Continued)

Close homolog species	Frequency
<i>Rubrobacter naiadicus</i> strain RG-3	1
<i>Rubrobacter xylanophilus</i> strain DSM 9941	1
<i>Saccharicrinis marinus</i> strain Y11	1
<i>Saccharofermentans acetigenes</i> strain P6	1
<i>Salmonella enterica</i> subsp. <i>diarizonae</i> strain DSM 14847	1
<i>Seohaecicola saemankumensis</i> strain SD-15	2
<i>Shewanella xiamenensis</i> strain S4	2
<i>Smithella propionica</i> strain LYP	35
<i>Soehngenia saccharolytica</i> strain DSM 12858	1
<i>Sphaerochaeta associata</i> strain GLS2	1
<i>Sphingobium phenoxybenzoativorans</i>	6
<i>Sphingobium phenoxybenzoativorans</i>	1
<i>Spirochaeta africana</i> strain DSM 8902	1
<i>Spirochaeta bajacaliforniensis</i> strain BA-2	1
<i>Spirochaeta halophila</i> strain RS1	1
<i>Sporichthya polymorpha</i> strain DSM 43042	1
<i>Sporosarcina luteola</i> strain NBRC 105378	1
<i>Stappia indica</i> strain B106	3
<i>Stenotrophomonas humi</i> strain R-32729	2
<i>Steroidobacter denitrificans</i> strain FS	2
<i>Streptomyces fukangensis</i> strain EGI 80050	1
<i>Sulfurimonas paralvinellae</i> strain GO25	2
<i>Sulfurovum aggregans</i> strain Monchim33	8
<i>Sulfurovum lithotrophicum</i> strain 42BKT	5
<i>Sunxiuqinia rutila</i> strain HG677	1
<i>Syntrophomonas bryantii</i> strain CuCal	4
<i>Syntrophomonas curvata</i> strain GB8-1	1
<i>Syntrophomonas wolfei</i> subsp. <i>saponavida</i> strain DSM 4212	2
<i>Syntrophomonas zehnderi</i> strain OL-4	7
<i>Syntrophorhabdus aromaticivorans</i> UI	1
<i>Syntrophothermus lipocalidus</i> strain DSM 12680	1
<i>Syntrophus aciditrophicus</i> strain SB	3
<i>Syntrophus gentianae</i> strain HQgoe1	4
<i>Taonella mepensis</i> strain H1	1
<i>Tepidanaerobacter syntrophicus</i> strain JL	1
<i>Tepidibacter mesophilus</i> strain B1	15
<i>Tepidisphaera mucosa</i> strain 2842	2
<i>Terrimonas pekingensis</i> strain QH	1
<i>Tetrasphaera vanveenii</i> strain Ben 70	1
<i>Thauera linaloolentis</i> strain 47Lol	2
<i>Thauera phenylacetica</i> strain B4P	6
<i>Thermaerobacter composti</i> strain Ni80	1
<i>Thermaerobacter subterraneus</i> strain C21	2
<i>Thermanaeromonas toyohensis</i> strain ToBE	1
<i>Thermanaerovibrio acidaminovorans</i> strain DSM 6589	1
<i>Thermanaerovibrio velox</i> strain Z-9701	1
<i>Thermoanaerobaculum aquaticum</i> strain MP-01	1
<i>Thermodesulfobacterium commune</i> strain strain DSM 2178	1
<i>Thermogutta terrifontis</i> strain R1	1
<i>Thermohalobacter berrensensis</i> strain CTT3	1
<i>Thermolithobacter ferrireducens</i> strain JW/KA-2	2
<i>Thermomarinilinea lacunifontana</i> strain SW7	6
<i>Thermosediminibacter oceani</i> strain DSM 16646	1
<i>Thermostilla marina</i> strain SVX8	1
<i>Thermosulfidibacter takaii</i> strain ABI70S6	1
<i>Thermotoga profunda</i> strain AZM34c06	1
<i>Thermovirga lienii</i> strain DSM 17291	6
<i>Thiolapillus brandeum</i> strain Hiromi 1	2
<i>Thiopfundum lithotrophicum</i> strain 106	1
<i>Tissierella creatinini</i> strain BN11	1
<i>Treponema caldarium</i> strain DSM 7334	2
<i>Turcibacter sanguinis</i> strain MOL361	2
<i>Ulvibacter litoralis</i> strain KMM 3912	1
<i>Undibacterium oligocarbonophilum</i> strain EM 1	1
<i>Vampirovibrio chlorellavorus</i> strain ICPB 3707	2
<i>Vitellibacter vladivostokensis</i> strain NBRC 16718	3
<i>Wandonia haliotis</i> strain NBRC 105642	1
<i>Woeseia oceani</i> strain XK5	7
<i>Youngiibacter fragilis</i> strain 232.1	1
<i>Kocuria dechangensis</i> strain NEAU-ST5-33	1

oil degradation as per literature submitted by number of submitter (data not shown).

## DISCUSSION

In the present study as per bioinformatics approach, it has been reported that data on uncultured bacteria relating them with oil degradation surely been available at NCBI. We have attempted to relate the discrete 16S rRNA sequence data submitted by number of authors across the world by involving a defined protocol already suggested by the Satija and Gore,<sup>[9]</sup> and successfully grouped 1123 uncultured bacteria into 320 bacterial species as per respective homology they showcased with 16S rRNA database sequences which gives a unique opportunity to relate them with known species data available till date.

In the present study, the high frequency recorded *N. aquimarinus* strain CL SC21 bacteria although not found to be directly related with the oil degradation but in one of the study worker Pan *et al.*<sup>[10]</sup> reported close species *Nitratireductor shengliensis* isolated from the oil-contaminated saline soil which certainly indicated some role of the given genus in oil degradation, but probably due to lack in ability to grow in common nutrient medium by this organism it's exact role is less understood and hence recommended that further. Another high-frequency bacterium identified as *A. gamaensis* represented as novel species of the genus of *Actinomadura*, isolated from soil of gama, chad, and appears as novel single spore-producing actinomycetes.<sup>[11]</sup> With no earlier report with this oil degradation, bacterium clearly directed us to make a focus on this bacterium as its frequency with oil-contaminated samples recorded to be on higher side worldwide and probably been reported for the first time as a evidence as per bacterium classification system study.

## CONCLUSION

Protocol designed will assist every biologist to easily investigate the classification of the uncultured bacterium into given genus and species, and further evolutionary and neighborhood study could be possible if the altered protocol followed for the investigation. With respect to subject line, study protocol successfully recorded the ability of the many new bacteria species which were not related earlier with any such oil-degrading function, and hence, put forward the strength of the protocol for the directed study toward the uncultured bacteria in coming time.

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