# Assessment of bilge water degradation by isolated *Citrobacter sp.* and two indigenous strains and identification of organic content by GC-MS

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A. **Supplementary Tables**

B. **Supplementary Figures**

C. **DNA analysis data**

D. **References**

## A. Supplementary Tables

**Table S1.** Physicochemical properties of real bilge water before treatment.

| Physicochemical Parameters | Concentration |
|---|---|
| TS | 908±15 (mg/L) |
| BOD | 4.147 ± 1.05 (g/L) |
| COD | 10.684 ± 1.8 (g/L) |
| $PO_4$ | 1.09 ± 0.24 (mg/L) |
| $NH_4$-N | 0.62± 0.1 (mg/L) |
| $NO_3$-N | 0.04± 0.01 (mg/L) |
| **pH:** 7.5-8.5 | - |

*Data are obtained from Ecofuel Ltd database.

**Table S2.** Hydrocarbon index of bilge water before treatment.

| Hydrocarbon content | Concentration ($mgL^{-1}$) |
|---|---|
| C6 – C10 (gasoline components) | 0.2 |
| C11 – C23 (Diesel) | 0.5 |
| C24 – C35 (Lubrication oil) | 0.3 |

*Data are obtained from Ecofuel Ltd database.
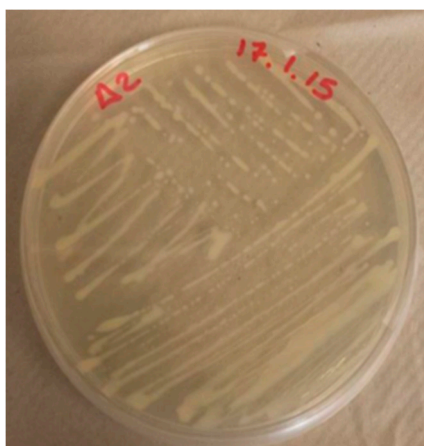
## B. Supplementary Figures



**Figure S1.** Colony formation by *Citrobacter sp. D2* strain in the presence of phenanthrene (150 mg L$^{-1}$).
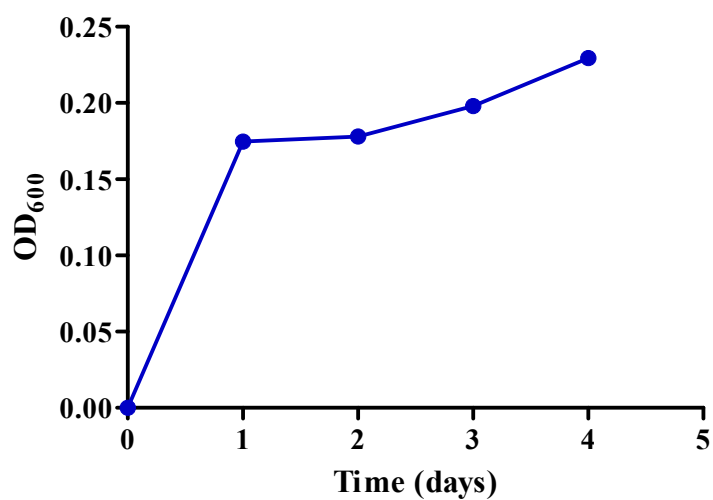


**Figure S2.** Growth curve of *Citrobacter* sp. D2 in MSM medium containing phenanthrene (150 mg L$^{-1}$).
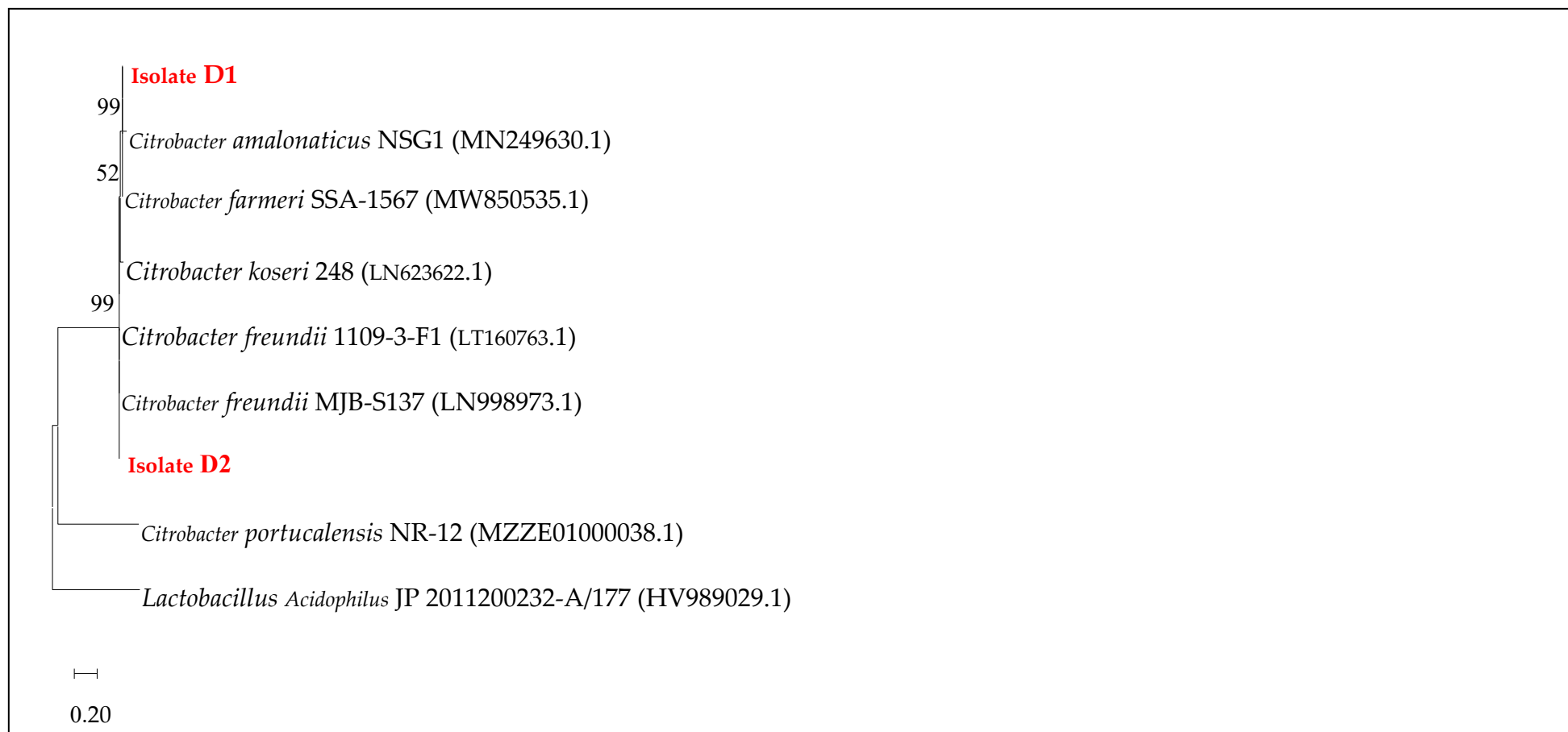
**Figure S3.** Phylogenetic tree diagram for Isolate D1 and D2. The evolutionary history was inferred using the Neighbor-Joining method [42]. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) are shown next to the branches [43]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [44] and are in the units of the number of base substitutions per site. This analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed

for each sequence pair (pairwise deletion option). There were a total of 791 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [45].
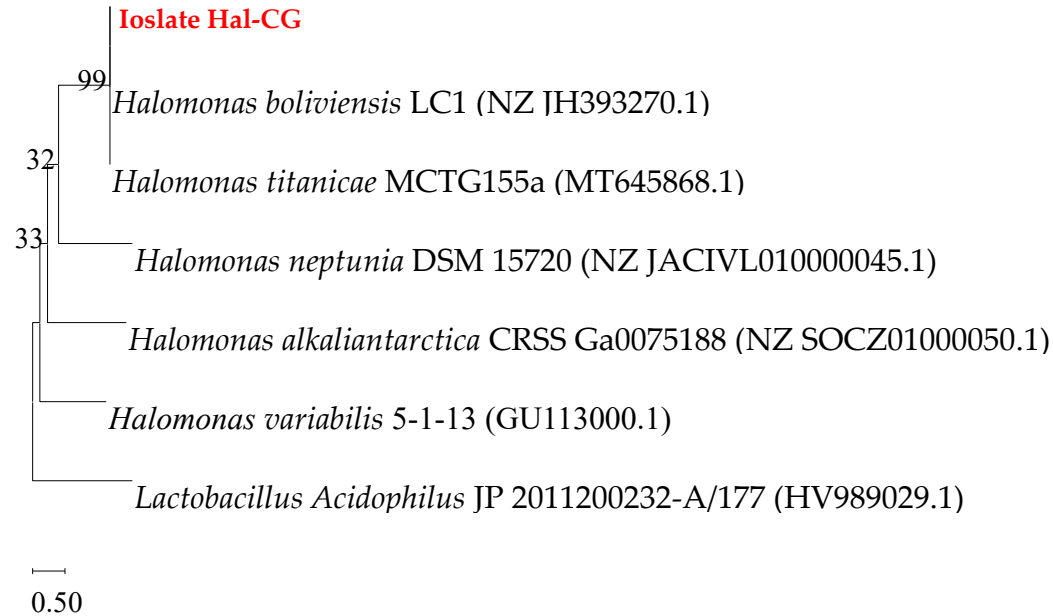


**Figure S4.** Phylogenetic tree diagram for islolate Hal-CG. The evolutionary history was inferred using the Neighbor-Joining method [42]. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) are shown next to the branches [43]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [44] and are in the units of the number of base substitutions per site. This analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed

for each sequence pair (pairwise deletion option). There were a total of 330 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [45].
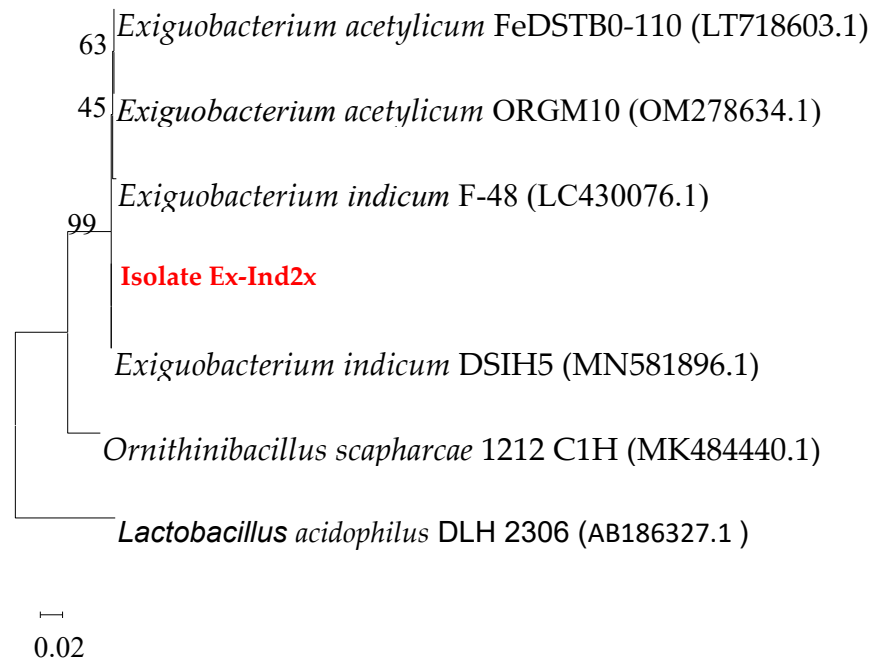


63 *Exiguobacterium acetylicum* FeDSTB0-110 (LT718603.1)

45 *Exiguobacterium acetylicum* ORGM10 (OM278634.1)

*Exiguobacterium indicum* F-48 (LC430076.1)

99

**Isolate Ex-Ind2x**

*Exiguobacterium indicum* DSIH5 (MN581896.1)

*Ornithinibacillus scapharcae* 1212 C1H (MK484440.1)

*Lactobacillus* acidophilus DLH 2306 (AB186327.1 )

0.02

**Figure S5.** Phylogenetic tree diagram for islolate Ex-Ind2. The evolutionary history was inferred using the Neighbor-Joining method [42]. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) are shown next to the branches [43]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [44] and are in the units of the number of base substitutions per site. This analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 330 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [45].
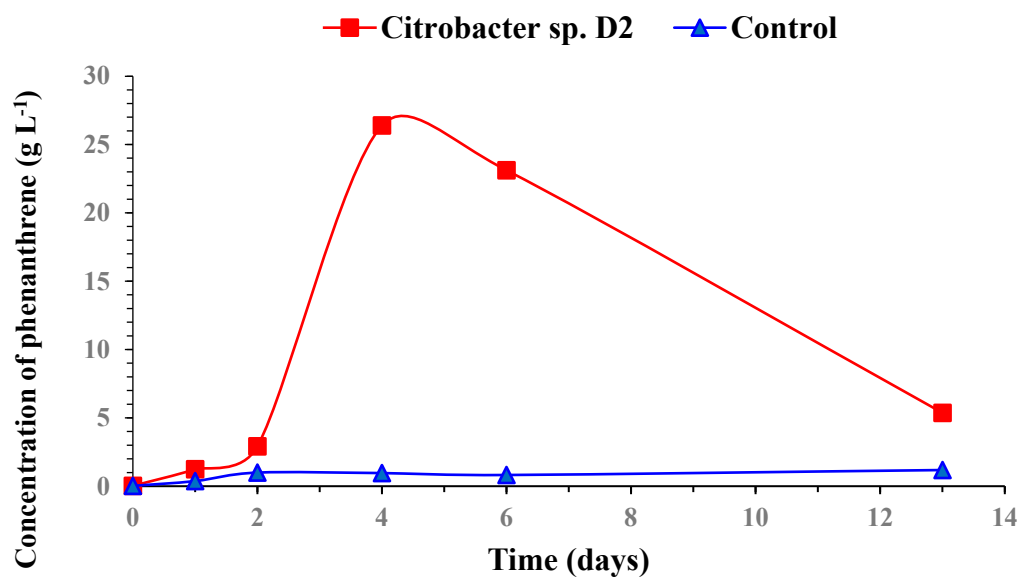
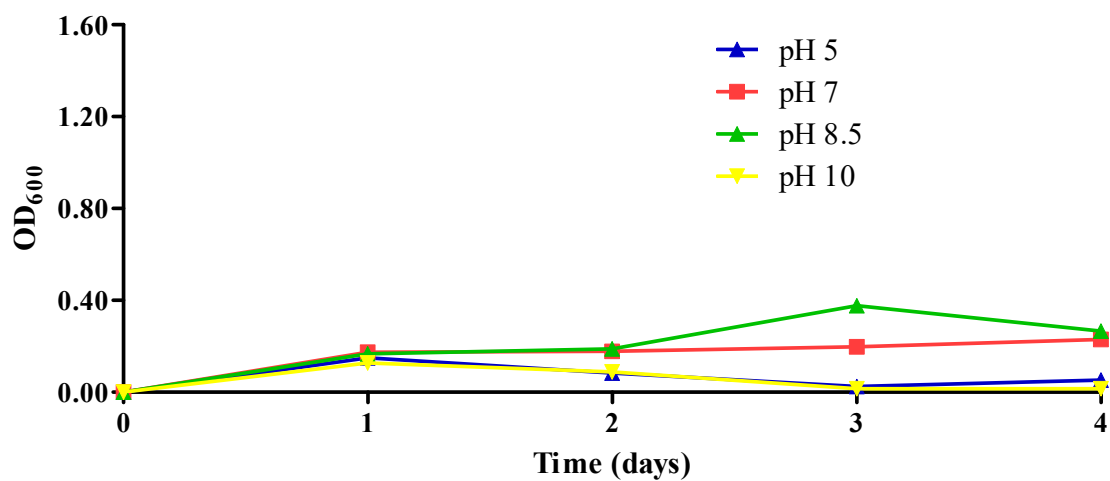**Figure S6.** Concentration of dissolved phenanthrene during its biodegradation by *Citrobacter* sp. D2.



**Figure S7.** Growth of *Citrobacter* sp. D2 under different pH conditions in the presence of phenanthrene (150 mg $L^{-1}$) and NaCl (15 g $L^{-1}$) at 30 °C.

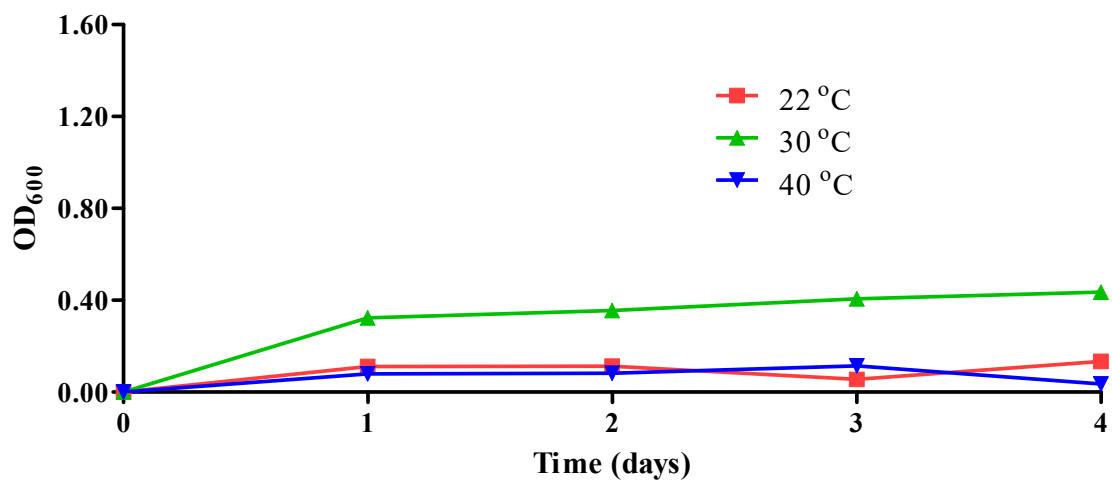**Figure S8.** Growth of *Citrobacter* sp. D2 under different temperatures in the presence of phenanthrene (150 mg L$^{-1}$) and NaCl (15 g L$^{-1}$).
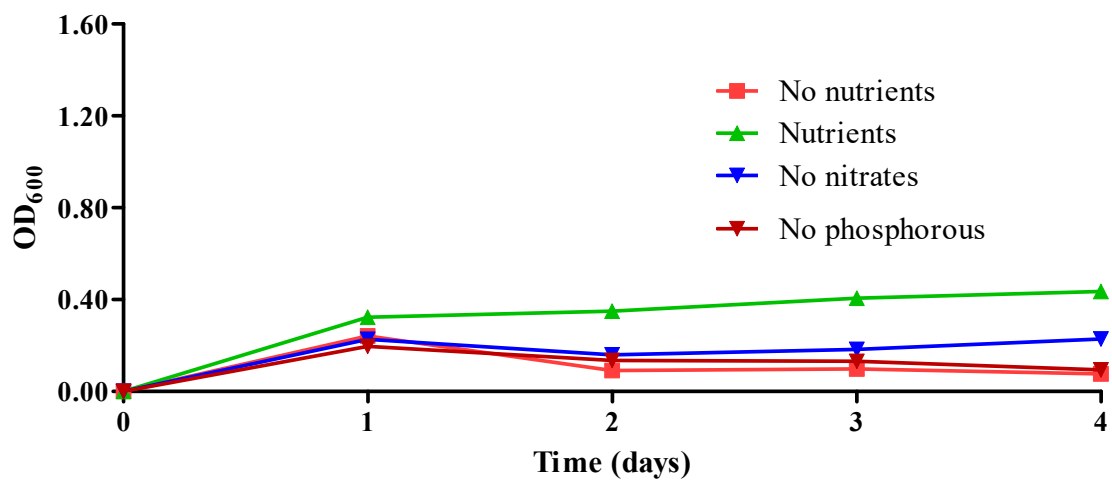


**Figure S9.** Growth of *Citrobacter* sp. D2 under different nutrient conditions in the presence of phenanthrene (150 mg L$^{-1}$) and NaCl (15 g L$^{-1}$).

**C. DNA analysis data**

*1. Citrobacter* **sp. D2**

<u>**Sequence:**</u>

CTTGCTCCTTGGGTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTG
CCCGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGT
CGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCA
GATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCT
AGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGA
CTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGA
TGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCA
GCGAGGAGGAAGGCGTTGTGGTTAATAACCGCAGCGATTGACGTTACTCGC
AGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGT
GCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTC
AAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCGAAACTG
GCAGGCTAGAGTCTTGTAGAGGGGGGGTAGAATTCCAGGTGTAGCGGTGAA
ATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAA
AGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCC
TGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCG
TGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCA
AGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATG
TGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAG
AACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCA
TGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTATCCTTTGTTGCCAGCGGTTCGGCCGGGAACTCAAAGGAAACT
GCCATTGATAA

**Table S3.** BLASTn sequence alignment results for *Citrobacter* sp. D2.

| Scientific name | Max Score | Total score | Percent Identity | Gaps (0%) | GeneBank accession |
|---|---|---|---|---|---|
| *Citrobacter freundii* | 1993 | 15832 | 99.72% | | NZ_CP033744.1 |
| *Citrobacter portucalensis* | 1977 | 15727 | 99.45% | | NZ_CP044098.1 |

## 2. *Citrobacter* sp. S1

## Sequence (969 bp):

GCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCCGATGGAG
GGGGATAACTACTGGAAACGGTAGCTAATACCGCATAATGTCGCAAGACCA
AAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAG
CTTGTTGGTGAGGTAACGGCTCACCAAGGCGACGATCCCTAGCTGGTCTGAG
AGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAA
GGGGTTAAGGTTAATAACCTTAGCCATTGACGTTACCCGCAGAAGAAGCAC
CGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAA
TCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTG
AAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTTGAGTC
TCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCT
GGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAG
GTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC
GTAAACGATGTCTATTTGGAGGTTGTGCCCTTGAGGTGTGGCTTCCGGAGCTA
ACGCGTTAAATAGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAA
TGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGC
AACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTGGCAGAAATG
CCTTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTC

**Table S4.** BLASTn sequence alignment results for *Citrobacter* sp. S1.

| Scientific name | Max Score | Total score | Percent Identity | Gaps | GeneBank accession |
|---|---|---|---|---|---|
| *Citrobacter amalonaticus* | 1773 | 12417 | 99.69% | 1/970 (0%) | NZ_LT556085.1 |
| *Citrobacter farmeri* | 1768 | 1768 | 99.59% | 1/970 (0%) | NZ_BBMX01000029.1 |

*3.* *Halomonas* **sp. Hal-CG**

**Sequence:**

GCGGCGGACGGGTGAGTAATGCATAGGAATCTGCCCGGTAGTGGGGGATAA
CCTGGGGAAACCCAGGCTAATACCGCATACGTCCTACGGGAGAAAGGGGGC
TTCGGCTCCCGCTATTGGATGAGCCTATGTCGGATTAGCTAGTTGGTGAGGTA
ACGGCTCACCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCA
CATCGGGACTGAGACACGGCCCGAACTCCTACGGGAGGCAGCAGTGGGGAA
TATTGGACAATGGGGGCAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAA
GGCCCTCGGGTTGTAAAGCACTTTCAGCGAGGAAGAACGCCTAGTGGTTAAT
ACCCATTAGGAAAGACATCACTCGCAGAAGAAGCACCGGCTAACTCCGTGC
CAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGC
GTAAAGCGCGCGTAGGTGGCTTGATAAGCCGGTTGTGAAAGCCCCGGGCTC
AACCTGGGAACGGCATCCGGAACTGTCAGGCTAGAGTGCAGGAGAGGAAG
GTAGAATTCCCGGTGTAGCGGTGAAATGCGTAGAGATCGGGAGGAATACCA
GTGGCGAAGGCGGCCTTCTGGACTGACACTGACACTGAGGTGCGAAAGCGT
GGGTAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTC
GACCAGCCGTTGGGTGCCTAGAGCACTTTGTGGCGAAGTTAACGCGATAAGT
CGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGG
GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGA
ACCTTACCTACCCTTGACATCTACAGAAGCCGGAAGAGATTCTGGTGTGCCT
TCGGGAACTGTAAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAA
ATGTTG GGTTAAGTCCCGTAACGAGCGCAA

**Table S5.** BLASTn sequence alignments results *Halomonas* sp. Hal-CG.

| Scientific name | Max Score | Total score | Percent Identity | Gaps (%) | GeneBank accession |
|---|---|---|---|---|---|
| *Halomonas boliviensis* | 1709 | 1709 | 99.70% | 0/1004 (0%) | NZ_NPEY01000013.1 |
| *Halomonas titanicae* | 1816 | 10876 | 99.30% | 0/1004 (0%) | NZ_CP059082.1 |

### 4. *Exiguobacterium* sp. Ex-Ind2

**Sequence (785 bp):**

CGTCACCTTGAGATGGCCTTGCGGTGCATTAGCTAGTTGGTGGGGTAACGGC
CCACCAAGGCGACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTG
GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGATGAAGGTTT
TCGGATCGTAAAACTCTGTTGTAAGGGAAGAACACGTACGAGAGGAAATGC
TCGTACCTTGACGGTACCTTACGAGAAAGCCACGGCTAACTACGTGCCAGCA
GCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAG
CGCGCGCAGGCGGCCTTTTAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGG
AGGGCCATTGGAAACTGGAAGGCTTGAGTACAGAAGAGAAGAGTGGAATTC
CACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAG
GCGACTCTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACTCGATGAGTGCTAGGT
GTTGGGGGGTTTCCGCCCCTCAGTGCTGCAGCTAACGCATTAAGCACTCCGC
CTGGGGAGTACGCGCCGCAAGGCTGAAACTCAAAGCGCTTGAGAGATCAAG
TTTTCCCTTCGGGGACAATGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGT
GTCGTGAGATG

**Table S6.** BLASTn sequence alignment results for *Halomonas* sp. Hal-CG.

| Scientific name | Max Score | Total score | Percent Identity | Gaps (%) | GeneBank accession |
|---|---|---|---|---|---|
| *Exiguobacterium indicum* | 1271 | 1420 | 99.29% | 3/705 (0%) | NZ_MPSZ01000021.1 |
| *Exiguobacterium acetilicum* | 1271 | 12730 | 99.29% | 3/705 (0%) | NZ_JNIR01000001.1 |

### D. References:

42. Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

43. Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

44. Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.

45. Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution https://doi.org/10.1093/molbev/msab120.