

1 **Supporting information**

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4 **An uncultivated nitrate-reducing member of the genus *Herminiimonas* degrades toluene**

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12  
13 This file includes:

14 Tables S1 to S3

15 Supplementary Figure Legends

16 Figures S1 to S7

17

18 **Table S1.** Release of  $^{13}\text{CO}_2$  after the degradation of  $^{13}\text{C}$ -toluene in stable isotope-probing  
19 microcosms. The  $^{13}\text{CO}_2/^{12}\text{CO}_2$  isotopic composition of  $\text{CO}_2$  is indicated as atoms percent  
20 (AT%)

| Culture condition                     | $^{13}\text{CO}_2/^{12}\text{CO}_2$ (%) |
|---------------------------------------|---|
| $^{13}\text{C}$ -toluene              | 24.9                                    |
| $^{12}\text{C}$ -toluene              | 1.8                                     |
| $^{13}\text{C}$ -toluene (autoclaved) | 2.1                                     |
| Atmosphere                            | 1.2                                     |

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25 **Table S2.** Composition of bacterial 16S rRNA gene clone libraries from heavy fraction DNA  
 26 of <sup>13</sup>C-toluene-degrading microcosms

| Phylogenetic affiliation   | Number of clones (%) | T-RF (bp) |          |
|----------------------------|----------------------|-----------|----------|
|                            |                      | Predicted | Measured |
| <i>Alphaproteobacteria</i> |                      |           |          |
| <i>Rhodobacteraceae</i>    | 1 (4.3)              | 112       | 108      |
| <i>Betaproteobacteria</i>  |                      |           |          |
| <i>Comamonadaceae</i>      | 1 (4.3)              | NA        | 399      |
| <i>Gallionellaceae</i>     | -                    | -         | -        |
| <i>Oxalobacteraceae</i>    |                      |           |          |
| <i>Herminiimonas</i>       | 19 (82.6)            | 117       | 120      |
| <i>Herbaspirillum</i>      | -                    | -         | -        |
| Unclassified               | 1 (4.3)              | 428       | 429      |
| <i>Burkholderiales</i>     |                      |           |          |
| <i>Gammaproteobacteria</i> |                      |           |          |
| Unclassified               | 1 (4.3)              | NA        | 882      |
| <i>Gammaproteobacteria</i> |                      |           |          |

27 NA, not applicable; T-RF, terminal restriction fragment.

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31 **Table S3.** General features of CN and reference genomes

|                               | CN genome | <i>Herminiimonas<br/>arsenicoxydans</i><br>ULPAs1 | <i>Herbaspirillum<br/>seropedicae</i><br>SmR1 | <i>Aromatoleum<br/>aromaticum</i><br>EbN1 |
|-------------------------------|-----------|---|---|---|
| Size (Mb)                     | 3.38      | 3.42  | 5.51  | 4.30                                      |
| GC content (%)                | 58.8      | 54.3  | 63.4  | 65.1                                      |
| No. of ORFs                   | 3,196     | 3,333   | 4,735   | 4,133                                     |
| Protein coding<br>regions (%) | 87.1      | 88.5  | 88.3  | 90.9                                      |
| No. of tRNAs                  | 38        | 45  | 55  | 58  |
| No. of rRNA<br>operons        | 1         | 2   | 3   | 4   |

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34 **Supplementary tables are provided as separate excel files**

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36 **Table S4.** Genes involved in the degradation of aromatic hydrocarbons in the CN genome.

37 **Table S5.** Genes involved in respiration in the CN genome.

38 **Table S6.** Genes involved in detoxification in the CN genome.

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42 **Fig. S1.** Bacterial community profile reflected by T-RFLP analysis of 16S rRNA genes from  
43 DNA of microcosms amended with labeled ( $^{13}\text{C}$ ) or unlabeled ( $^{12}\text{C}$ ) toluene. A) Light DNA  
44 and B) heavy DNA fractions of the metagenomic DNA separated by CsCl gradient-  
45 ultracentrifuged preparations from  $^{13}\text{C}$ -toluene amended nitrate reducing microcosm; C)  
46 Light DNA fraction of  $^{12}\text{C}$ -toluene amended nitrate reducing microcosm; D) Light DNA  
47 fraction of  $^{12}\text{C}$ -toluene amended but nitrate non-amended microcosm.

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49 **Fig. S2.** Phylogenetic tree showing the affiliation of representative bacterial 16S rRNA gene  
50 clones as detected in clone libraries of heavy fraction DNA from the CN microcosm.  
51 Obtained clones are highlighted in bold. The number in parentheses indicates the number of  
52 repeated clones in the clone libraries.

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54 **Fig. S3.** Cluster of genes involved in *p*-cresol degradation in the CN genome, *Candidatus*  
55 *Nitrospira defluvii* and *Aromatoleum aromaticum* EbN1.

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57 **Fig. S4.** Cluster of genes involved in 4-hydroxybenzoate degradation in the CN genome.

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59 **Fig. S5.** Cluster of genes involved in phenylacetate degradation in the CN genome,  
60 *Rhodopseudomonas palustris* BisB5 and *Aromatoleum aromaticum* EbN1.

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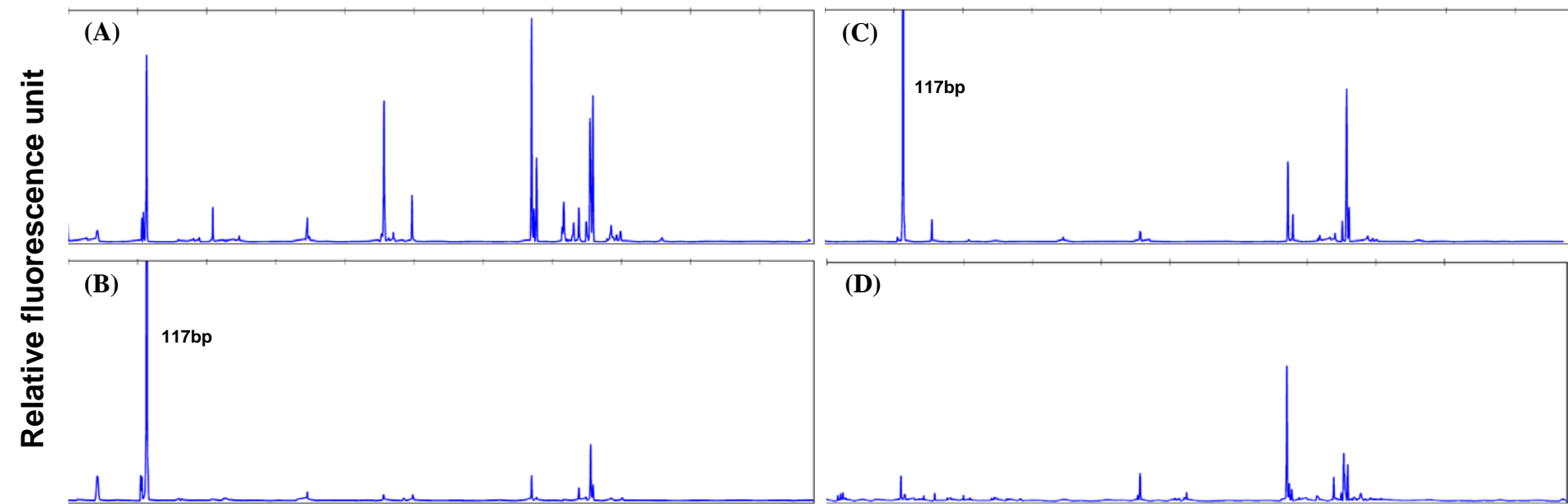
62 **Fig. S6.** Cluster of genes involved in cyclohexane carboxylate degradation in the CN genome

63

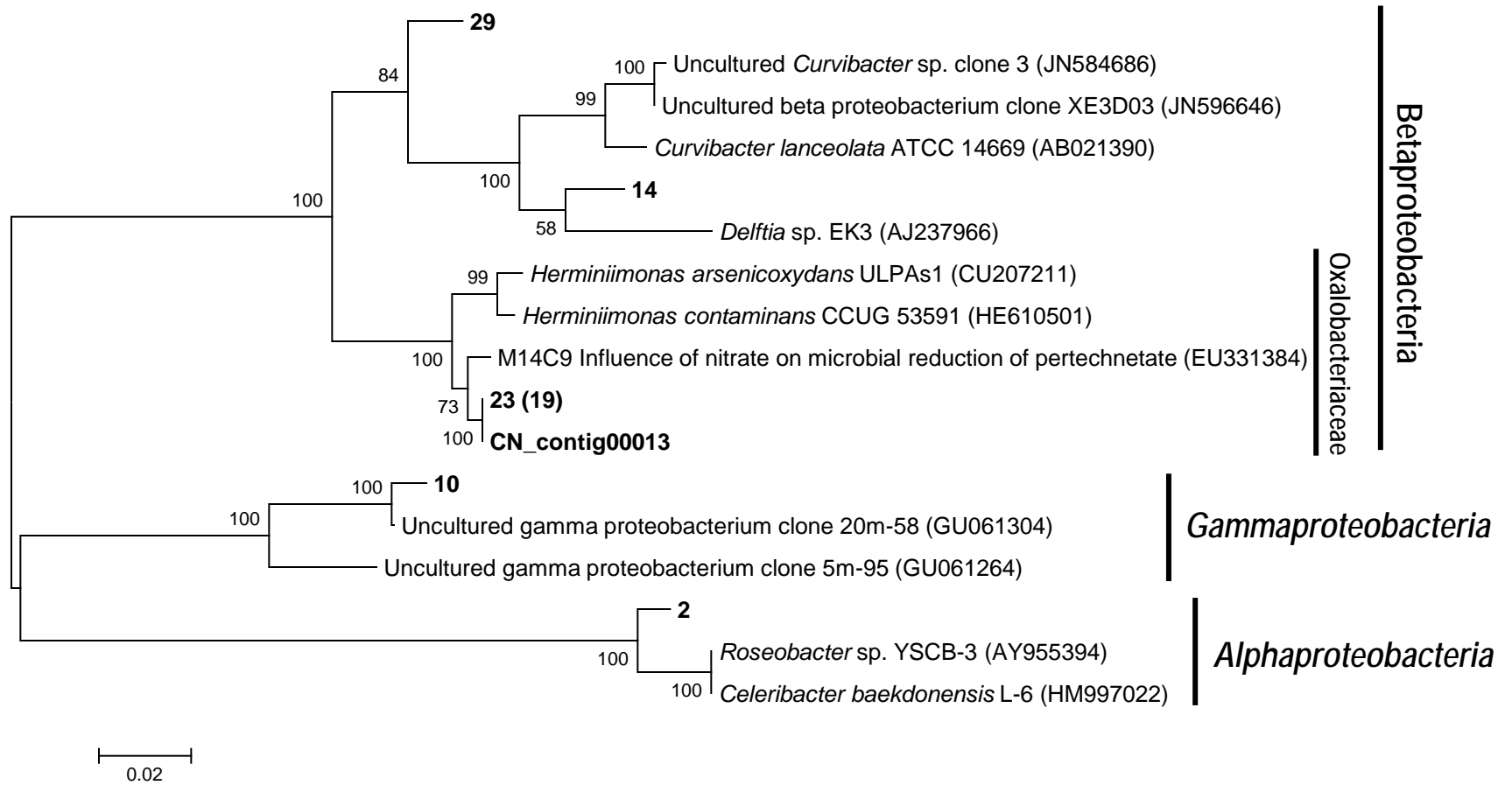
64 **Fig. S7.** Organization of gene clusters related to arsenate resistance in the CN and other  
65 bacterial genomes.

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**Fig. S1**



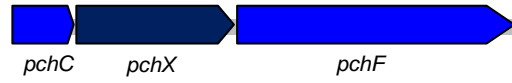
**Fig. S2**



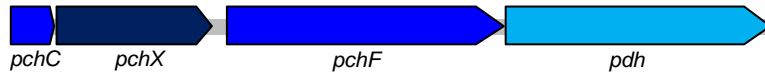


**Fig. S3**

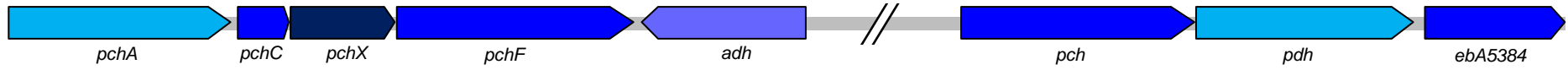
***Candidatus Nitrospira defluvii***



**CN (contig00022)**



***Aromatoleum aromaticum* EbN1**



1.5kb

**Fig. S4**

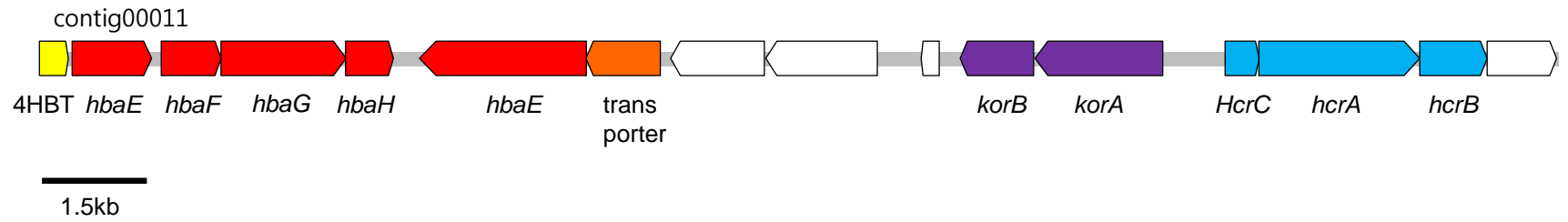
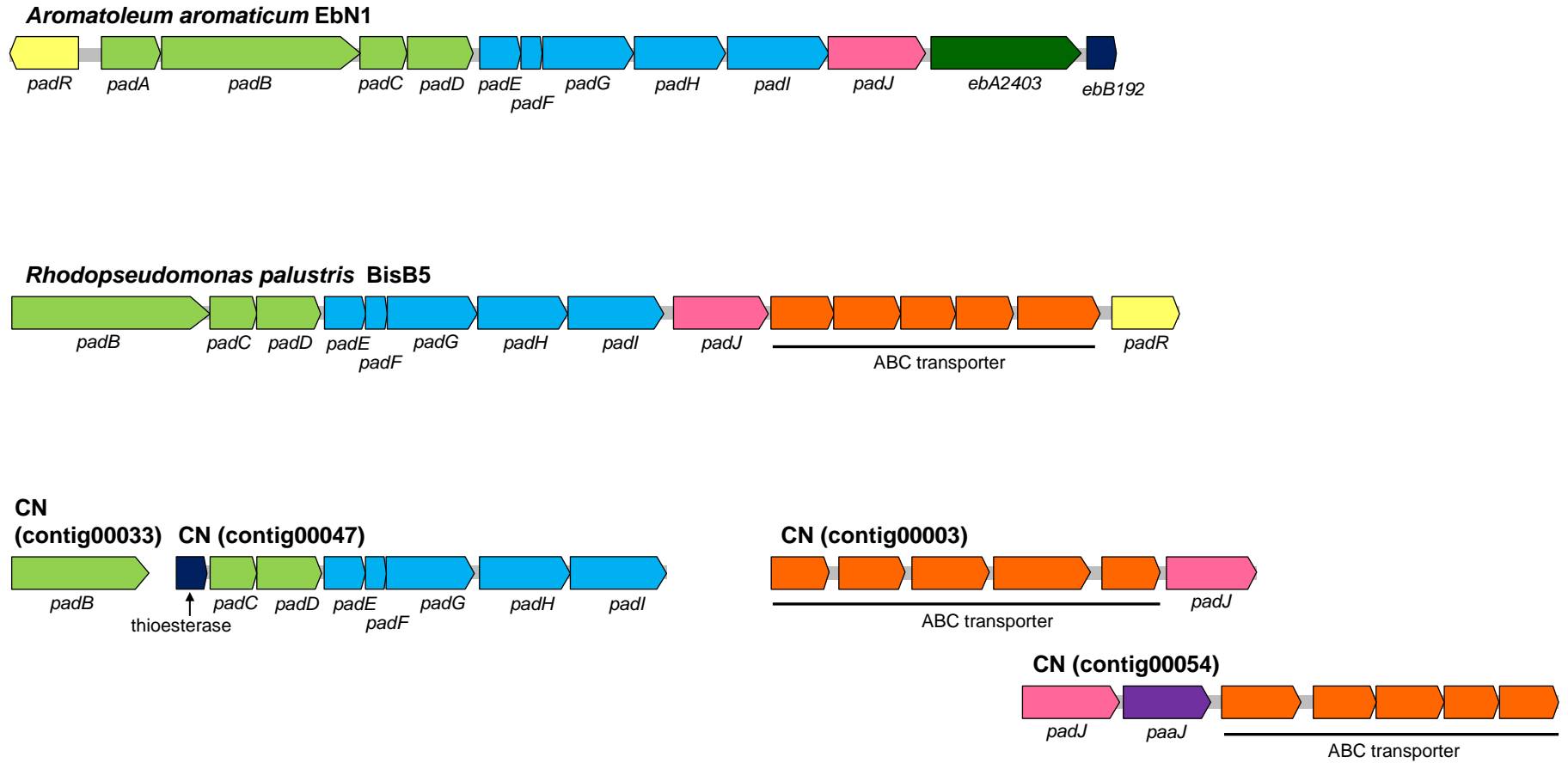
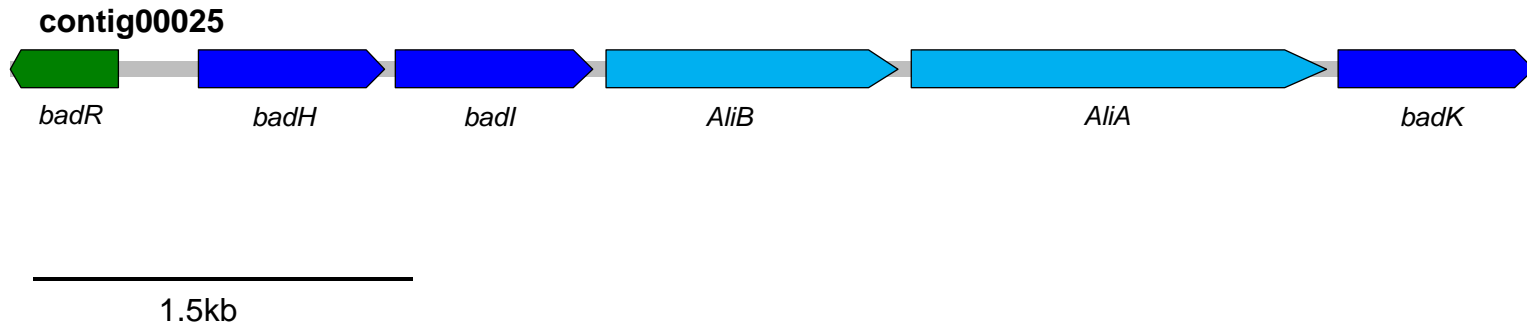


Fig. S5



**Fig. S6**



**Fig. S7**

