# Tracking the fate of microbially sequestered carbon dioxide in soil organic matter

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**Section S1: Soil Incubations**

Dry soil (32.06 g; Grey Brown Podzolic retrieved from an open public area located within Hampstead Park (Albert College Park), Glasnevin, Dublin, Ireland (53° 22' 54.63" N 6° 15' 43.72" W) with a field pH 7.1.) was placed into a sterile amber jar and 300 ml of autoclaved minimal salts medium (MSM; 0.5 g $l^{-1}$ $K_2HPO_4$, 0.5 g $l^{-1}$ $KH_2PO_4$, 0.5 g $l^{-1}$ $NH_4Cl$, 0.5 g $l^{-1}$ $MgSO_4.7H_2O$, 0.12 g $l^{-1}$ NaCl, 0.05 g $l^{-1}$ $CaCl.2H_2O$ and 1 ml of a trace metal solution [0.1 g $l^{-1}$ $ZnSO_4.7H_2O$, 0.3 g $l^{-1}$ $MnCl_2.4H_2O$, 0.3 g $l^{-1}$ $H_3BO_3$, 0.1 g $l^{-1}$ $CuCl_2.2H_2O$, 0.2 g $l^{-1}$ $NiCl_2.6H_2O$, 0.3 g $l^{-1}$ $NaMoO_4.2H_2O$, 1.0 g $l^{-1}$ $FeSO_4.7H_2O$])[1,2] was added aseptically. 6.0 ml (0.2 μm filtered) of a 1000 mM $Na_2S_2O_3$ stock was prepared and added to each jar (20 mM $Na_2S_2O_3$). A central hole was inserted into the lid of the 900 ml amber jar, with four surrounding exit vents. The amber jar was then autoclaved (121$^oC$ / 15 minutes). Air was pumped through a 0.2 μm Millex-FG filter unit (Millipore, Molsheim, France) and then into soil solutions for 48 h using a battery air pump (Hagen Elite, Castleford, UK). Unamended soil (0 mM $Na_2S_2O_3$) was used as a control and to determine basal respiration (n = 2). This procedure was repeated for $^{12}CO_2$ (Air Products 99% $CO_2$ Industrial grade) incubations (n = 3) and $^{13}CO_2$ (Sigma Aldrich 99% atom $^{13}C$) incubations (n = 3). All incubations took place over 48 h under an atmosphere of 1000 ppm $CO_2$ (0.1 vol%). Separate unamended controls were also exposed to $^{12}C$ and $^{13}CO_2$ atmospheres to provide background data and demonstrate the requirement of $Na_2S_2O_3$.

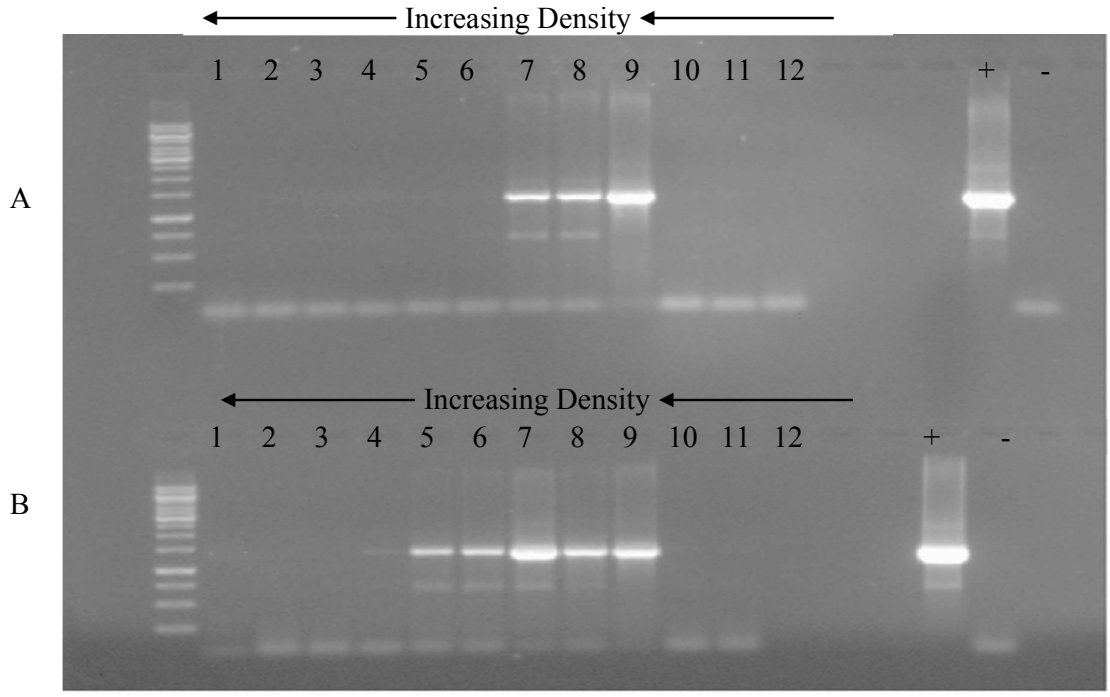
**References**


1. Madigan, M. M.; Martinko, J. M.; Dunlap, P. V.; Clark, D. P. Brock Biology of Microorganisms. Pearson Prentice Hall, New Jersey, 2000.
2. Shiers, D.; Blight, K. R.; Ralph, D. E. Sodium sulphate and sodium chloride effects on batch culture of iron oxidising bacteria. *Hydrometallurgy* **2005**, *80*, 75-82.


**Section 2: Figure S1**.

Gel electrophoresis analysis of 16s rRNA PCR amplified soil DNA after 48 h incubation under chemoautotrophic conditions. DNA was separated according to its isopycnic correlation to CsCl gradient induced under ultracentrifugation. Positive (+)

and negative (-) controls were performed using *Pseudomonas putida* G7 DNA PCR product and ultra-pure autoclaved water respectively. A) Soil exposed to 99% $^{12}CO_2$ for 48 h, run against a 1 kb ladder. B) Soil exposed to 99% $^{13}CO_2$ for 48 h, run against a 1 kb ladder. The observable presence of DNA in a density range between 1.592-1.583 g $ml^{-1}$ for the $^{13}CO_2$ gradient fractions provides the first step evidence of higher density DNA penetration and indicates $^{13}C$ incorporation into genomic DNA.




**Section S3: Figure 1 – Expanded caption**

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model.[1] The bootstrap consensus tree inferred from 1000 replicates[2] is taken to represent the evolutionary history of the taxa analyzed.[2] Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.[2] Initial tree(s) for the heuristic search were obtained automatically as follows. When the

number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise BIONJ method with MCL distance matrix was used. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 68 nucleotide sequences. All positions with less than 95% site coverage were eliminated. There were a total of 808 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.[3]

**References**


1. Tamura, K.; Nei, M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **1993**, *10*, 512-526.
2. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783-791.
3. Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei, M.; Kumar, S. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **2011**, *28*, 3731-2739.

**Table S.1:** 16S rRNA Clones identified from the gene library from density fraction 1.592 g $ml^{-1}$. Purified nucleic acids retrieved from CsCl isopycnic SIP-$^{13}CO_2$ DNA experiment.

| OTU Group | Accession Number | No. of Clones | Closest sequence match with BLAST (Affiliated Group) (% match) (Accession No.) | Autotroph or Facultative Autotroph (+/-) | Reference |
|---|---|---|---|---|---|
| I | a) | 24 | *Ralstonia eutropha* JMP134 (β-proteobacteria) (97%) (NC 007348.1) | + | 34 |
| | b) | 15 | *Ralstonia solanacearum* GMI1000 (β-proteobacteria) (97%) (NC 003295.1) | **?** | 35* |
| II | | 4 | *Tsukamurella paurometabola* DSM20162 (Actinobacteria) (94%) (NC 014158.1) | - | 36 |
| III | | 4 | *Acidimicrobium ferrooxidans* DSM10331 (Actinobacteria) (89%) (NC 013124.1) | + | 37 |
| IV | | 1 | *Solibacter usitatus* Ellin 6076 (Acidobacteria) (91%) (NC 008536.1) | - | 38 |
| V | | 4 | *Pseudomonas mendocina* ymp (γ- proteobacteria**) (**98%) (NC 009439.1) | - | 39 |
| VI | | 12 | *Cupriavidus metallidurans* CH34 (β-proteobacteria**) (**99%) (NC 007973.1) | + | 40 |
| VIII | | 4 | *Sphingomonas wittichii* RW1 (α-proteobacteria) (94%) (NC 009511.1) | - | 41 |
| IX | | 10 | *Rhodoferax ferrireducens* T118 (β-proteobacteria) (95%) (NC 007908.1) | + | 42 |
| X | | 8 | *Thioalkalivibrio sp*. K90mix (γ-proteobacteria) (84-85%) (NC 013889.1) | + | 43 |

| | | | | |
|---|---|---|---|---|
| XI | 2 | *Agrobacterium tumefaciens* str. C58 (α-proteobacteria) (98%) (NC 003062) | - | 44 |
| XII | 3 | *Ralstonia eutropha* JMP134 (β-proteobacteria) (98%) (NC 007348.1) | + | 34 |
| XIII | 3 | *Achromobacter xylosoxidans* A8 (β-proteobacteria) (99%) (NC 014640.1) | - | 45 |
| XIV | 5 | *Oligotropha carboxidovorans* OM5 (α-proteobacteria) (95-97%) (NC 011386.1) | + | 46 |
| XV | 3 | *Methylobacterium radiotolerans* JCM2831 (α-proteobacteria) (99%) (NC 010505.1) | - | 47 |
| XVI | 2 | *Brucella microti* CCM4915 (α-proteobacteria) (90%) (NC 013119.1) | - | 48 |
| XVII | 1 | *Stenotrophomonas maltophilia* K279a (β-proteobacteria) (99%) (NC 010943.1) | - | 49 |
| XVIII | 1 | *Bradyrhizobium japonicum* USDA110 (α-proteobacteria) (99%) (NC 004463.1) | + | 50 |

*Footnotes:* *Description given by accompanying reference under the name of *Burkholderia vandii* sp. nov., since transferred to *Ralstonia Solanacearum* by Yabuuchi et al. 1995.

**Reference**

Yabuuchi, E.; Kosako, Y.; Yano, I.; Hotta, H.; Nishiuchi, Y. Transfer of two *Burlholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. *Microbiol. Immun.* **1995**, *39*, 897-904.