Supporting Information for

High permeation rates in liposome systems explain rapid glyphosate biodegradation associated with strong isotope fractionation

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SUMMARY

5 pages, 3 figures and one table

CONTENT

Supporting experimental section

Chemicals and media composition

The following chemicals were used in the liposome permeation study: glyphosate (98%, Sigma Aldrich), D_2O (99.9%, Sigma Aldrich), sodium hydroxide (97%, Fisher scientific), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (99%, Avanti Polar Lipids), praseodymium(III)-chloride (99.99%, Fisher scientific), and 3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionic acid (98 atom % D, Sigma Aldrich).

The following chemicals were used for the isolation of *Ochrobactrum sp*. FrEM and for glyphosate degradation: glyphosate (99%, Sigma Aldrich), ammonium acetate (99%, Sigma Aldrich), sodium glutamate (99%, Sigma-Aldrich), sodium peroxodisulfate (99%, Fluka), potassium hydroxide (99%, Fluka), phosphoric acid (99%, Fluka), , potassium dihydrogenphosphate (99%, AppliChem), sodium hydrogenphosphate (98%, Sigma Aldrich), sodium hydroxide (99%, Sigma Aldrich), potassium sulfate (99%, Merck), ammonium chloride (99.5%, Sigma Aldrich), magnesium sulfate heptahydrate (99%, Fluka), calcium chloride hexahydrate (94%, Roth), boric acid (99.8%, Merck), manganese sulfate monohydrate (99%, Sigma Aldrich), zinc sulfate heptahydrate (99%, Fisher Scientific), nickel chloride hexahydrate (99%, Merck) sodium molybdate dihydrate (99.5%, Merck), and iron sulfate heptahydrate (98%, Sigma Aldrich).

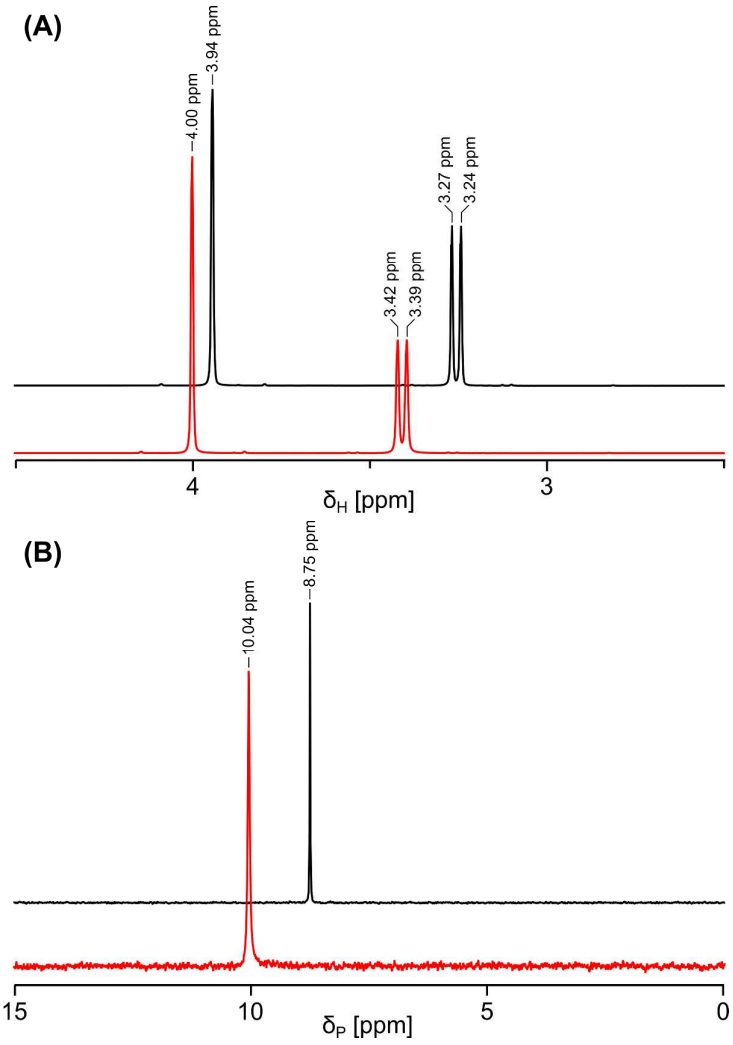
The medium contained (in gL^{-1}): NH_4Cl, 2.0; $MgSO_4 \times 7\ H_2O$, 0.2; K_2SO_4, 0.5; as well as trace elements (in mgL^{-1}): $FeSO_4 \times 7H_2O$, 2.5; $CaCl_2 \times 6H_2O$,10.0; $CuSO_4 \times 5H_2O$, 2.0; H_3BO_3, 0.06; $ZnSO_4 \times 7\ H_2O$,20.0; $MnSO_4 \times H_2O$, 1.0; $NiCl_2 \times 6H_2O$, 0.05; $Na_2MoO_4 \times 2H_2O$, 0.3.

Bacterial isolation and characterization

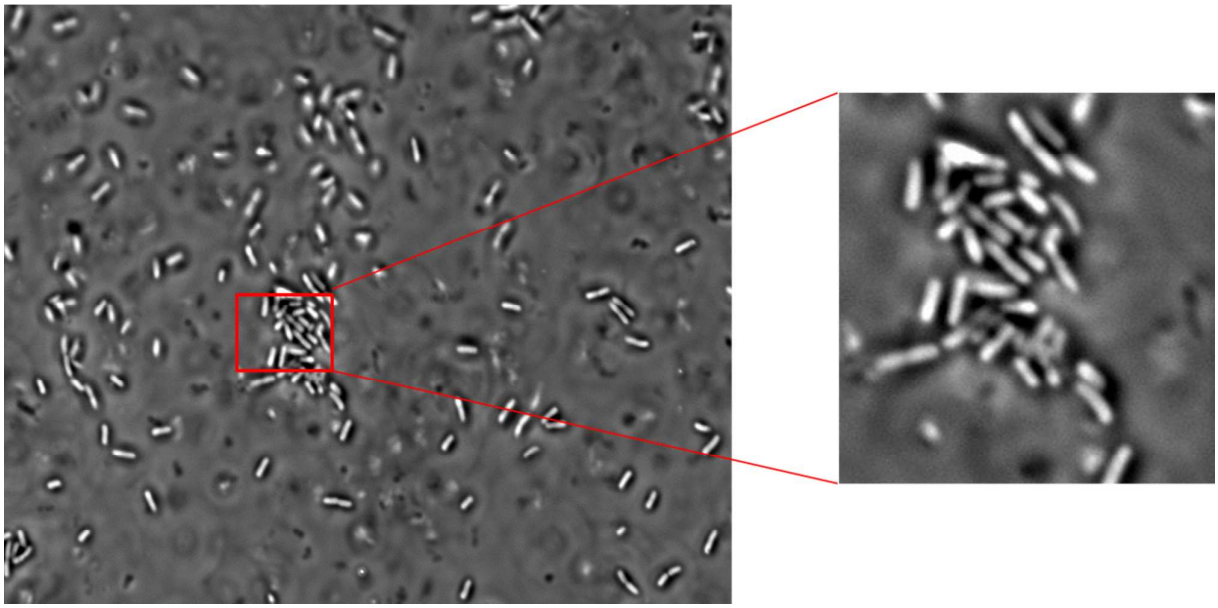
The soil samples were collected from different plots (and later combined) on a vineyard site in northern France (Agricultural and Viticultural College of Rouffach - Rouffach soil) where glyphosate was the most frequently used herbicide with a yearly application of between 18 and 61 $kg \cdot ha^{-1}$.[1] Soil samples from each plot location were thoroughly mixed in sterile bottles, sealed, transported to the laboratory and stored at 4 °C until use.

For bacterial isolation from soil, medium (see above) containing 60 mM sodium glutamate as carbon source was used. Ammonium chloride was used as nitrogen source, and glyphosate was the sole phosphorous source. Soil samples (10 g) were first sieved (> 2 mm). Then, 5 g of soil were suspended in 10 mL sterile water and centrifuged. A 1 mL aliquot of the supernatant was used to inoculate 50 mL medium containing 3 mM glyphosate and incubated at 30 °C at 160 rpm for 24 h. Several transfers were made and later streaked on agar plates containing 3 mM glyphosate. The single colonies formed were inoculated on agar plate to represent the pure isolated strain. The isolated bacteria were identified using 16S rRNA gene sequencing. For 16S rRNA gene amplification, the chromosomal DNA was isolated using a bacterial DNA extraction kit (Roche Applied Science, Germany) following the protocol of the manufacturer. PCR amplification was performed using universal primers (forward 27f and reverse 1492r). Standard PCR conditions was carried out in a 50 µL volume containing 1× PCR buffer, 1.5 mM $MgCl_2$, 2 mM dNTP mixture, 1 µM primers, 1 µM of P*fu* DNA polymerase (Fermentas, St. Leon-Rot, Germany), and 2 ng of template DNA. DNA was purified from a gel using an Agarose Gel Extraction kit (Roche Applied Sciences, Germany) and sequenced. Sequence homologies were evaluated using BLAST software (version 2.2.12). ClustalQ software was used to align the sequences. A neighbour-joining tress was constructed using Molecular Evolution Genetic Analysis (MEGA) software (version 6.0).

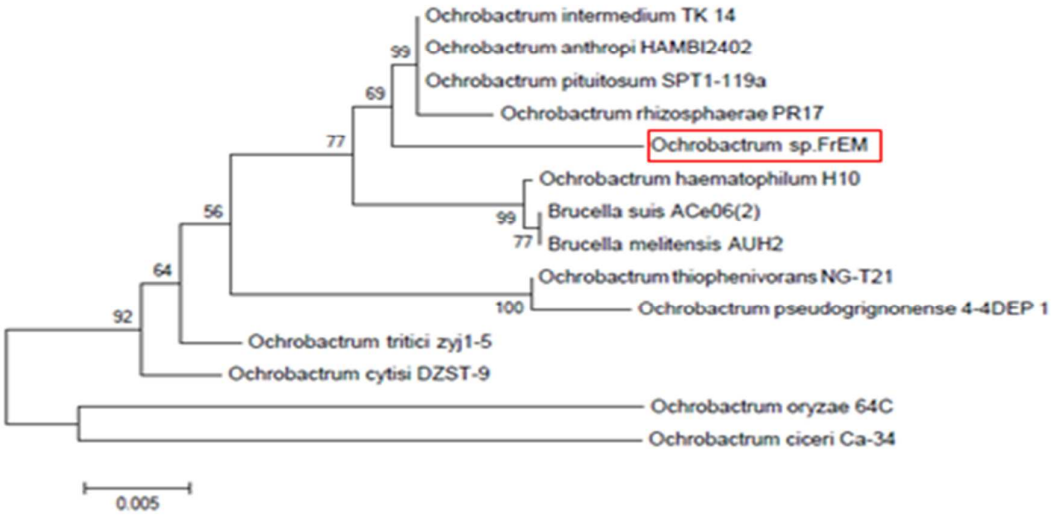
Supporting Figures and Tables



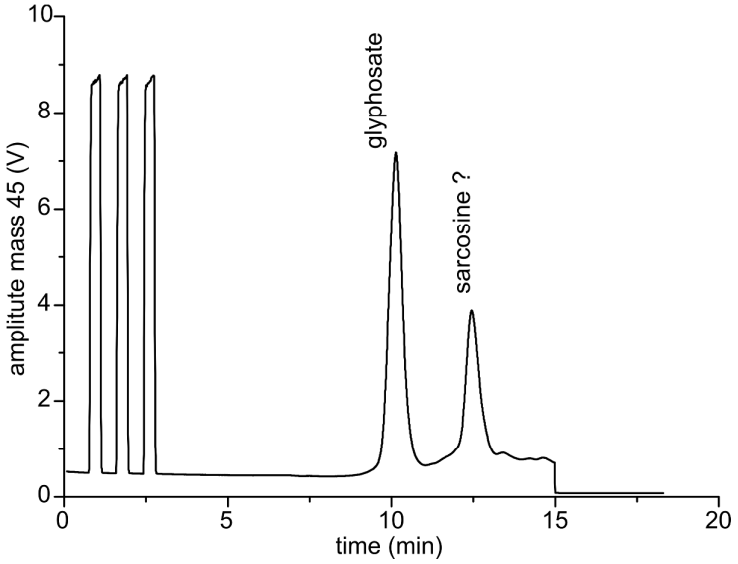
SI Figure S1. (A) ^{1}H with solvent suppression and **(B)** ^{31}P{^{1}H} spectra of glyphosate without (black) and with addition of 1 mM $PrCl_3$ (red). The NMR spectra were obtained glyphosate in D_2O without pH adjustment where glyphosate dissociates into the monoanionic and the zwitterionic form.[2] The chemical shifts of glyphosate depend on pH. Therefore, the chemical shifts in these spectra differ from those presented in **Figure 1** and **Figure 2**.



SI Figure S2. Micrograph of *Ochrobactrum sp* FrEM cells by light microscope (Axioskop Plus2, ZEISS, Germany (×100 resolution oil emulsion), AxioVision 4.1



SI Figure S3. Phylogenetic position of the strain FrEM within *Ochrobactrum* species. Neighbour-joining tree based on partial 16S rRNA sequence. The bar indicates 0.005 substitutions per nucleotide.



SI Figure S4. LC-IRMS chromatogram of ongoing glyphosate biodegradation. The three peaks between 0 s and 200 s are the CO_2 peaks of the reference gas. Glyphosate elutes around 10 min and is separated (resolution 2.3) from the peak around 12.5 min which could be the metabolite sarcosine. The CO_2 background increases after 11 minutes, probably due to overloading the column with the carbon source glutamate which elutes after 900 s when the split to the IRMS is already closed.

SI Table S1.Summary of NMR spectra collection parameters

experiment	transmitter frequency offset	relaxation delay (s)	spectral width (Hz)	acquisition time (s)	number of scans
^{1}H Standard	3165.1 Hz	1.0	9973.4	2.855	8
^{31}P{^{1}H} Standard	10028.7 Hz	2.0	81521.7	0.99	16
^{1}H with solvent suppression (watergate W5)	HOD freq.	1.5	7978.7	2.4009	128
^{1}H{^{31}P} with solvent suppression prior $PrCl_3$ addition	HOD freq.	1.5	10000.0	1.499	16
^{1}H{^{31}P} with solvent suppression after $PrCl_3$ addition	HOD freq.	1.5	10000.0	1.499	64

Supporting Information References

1. Gregoire, C.; Payraudeau, S.; Domange, N., Use and fate of 17 pesticides applied on a vineyard catchment. *International Journal of Environmental and Analytical Chemistry* **2010,** *90*, (3-6), 406-420.

97 2. Sprankle, P.; Meggitt, W.; Penner, D., Adsorption, mobility, and microbial degradation of
98 glyphosate in the soil. *Weed Science* **1975**, 229-234.