Reverse and multiple stable isotope probing to study bacterial metabolism and interactions at the single cell level

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ABSTRACT

The interactions between microorganisms driven by substrate metabolism and energy flow are important to shape diversity, abundance, and structure of a microbial community. Single cell technologies are useful tools for dissecting the functions of individual members and their interactions in microbial communities. Here, we developed a novel Raman stable isotope probing (Raman-SIP), which uses Raman micro-spectroscopy coupled with reverse and D_2O co-labelling to study metabolic interactions in a two-species community consisting of *Acinetobacter baylyi* ADP1 and *Escherichia coli*. This Raman-SIP approach is able to detect carbon assimilation and general metabolic activity simultaneously. Taking advantage of Raman shift of single cell Raman spectra (SCRS) mediated by incorporation of stable-isotopic substrates, Raman-SIP with reverse labelling has been applied to detect initially ^{13}C-labelled bands of ADP1 SCRS reverting back to ^{12}C positions in the presence of ^{12}C citrate. Raman-SIP with D_2O labelling has been employed to probe metabolic activity of single cells without the need of cell replication. Our results show that *E. coli* alone in minimal medium with citrate as the sole carbon source had no metabolic activity, but became metabolically active in the presence of ADP1. Mass spectrometry-based metabolite footprint analysis suggests that putrescine and phenylalanine excreted by ADP1 cells may support the metabolic activity of *E. coli*. This study demonstrates that Raman-SIP with reverse labelling would be useful tool to probe metabolism of any carbon substrate, overcoming limitations when stable isotopic substrates are not readily available. Raman-SIP with D_2O labelling is a sensitive and reliable approach, which distinguishes metabolically active cells but not quiescent cells. This novel approach extends the application of Raman-SIP and demonstrates its potential application as a powerful tool for probing cellular metabolism and their metabolic interactions at the single cell level in microbial communities.

Table S1. List of the metabolites detected and identified by footprint GC-MS analysis

Name of compound	RI	RT	MSI ID level	chEBI code
Tridecane	958.02	332.728	1	35998
Myristoleic acid	968.91	336.428	1	27781
Undecane	981.85	340.828	1	46342
Pyridine	1081.7	374.778	1	-
Decane	1201	415.328	1	41808
Oxalic acid	1231.8	425.828	2	16995
Glycerol	1284.5	443.728	1	17754
Phosphate	1418.9	489.428	1	18367
Unknown	1500.5	517.228	4	-
Unknown	1500.9	517.428	4	-
Unknown	1535.8	534.078	4	-
Cystathionine	1619.3	573.978	3	17755
Putrescine	1649	588.128	1	17148
Phenylalanine	1730.6	622.328	1	28044
Citric acid	1842.4	680.478	1	30769
Unknown	1901.9	708.528	4	-

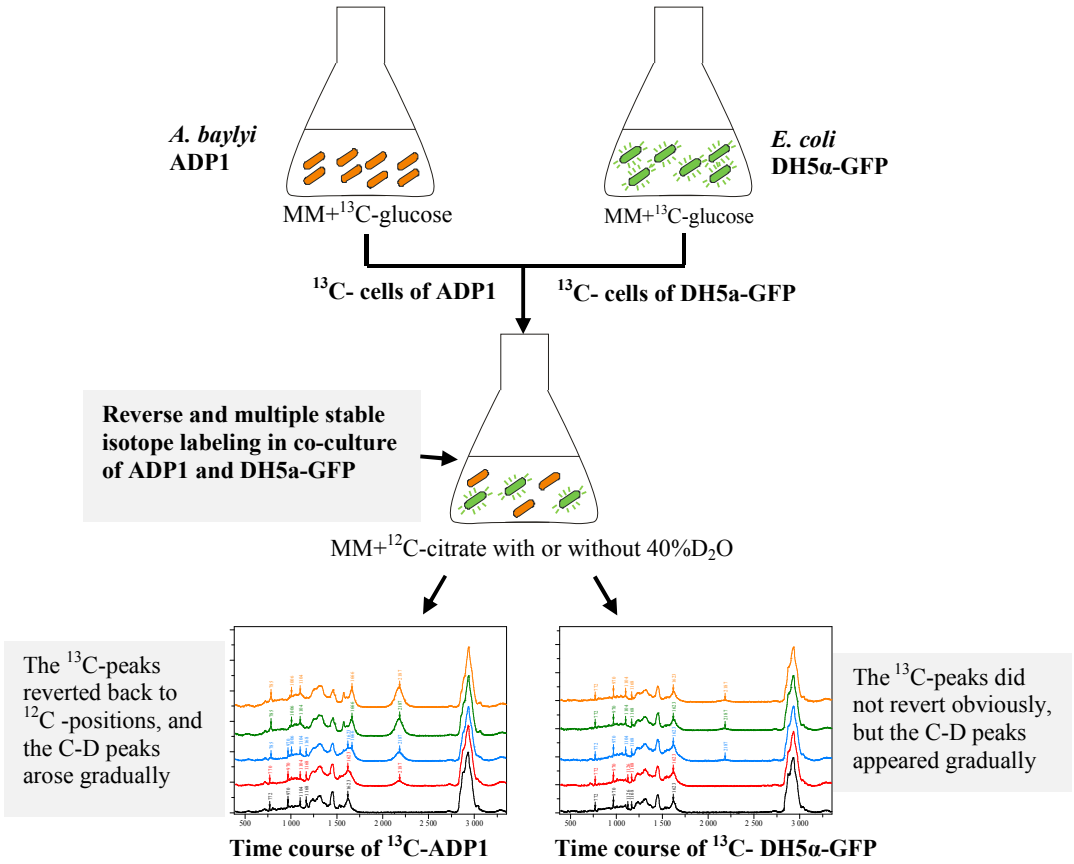

Figure S1. Illustration of the reverse and multiple stable isotope labeling processes

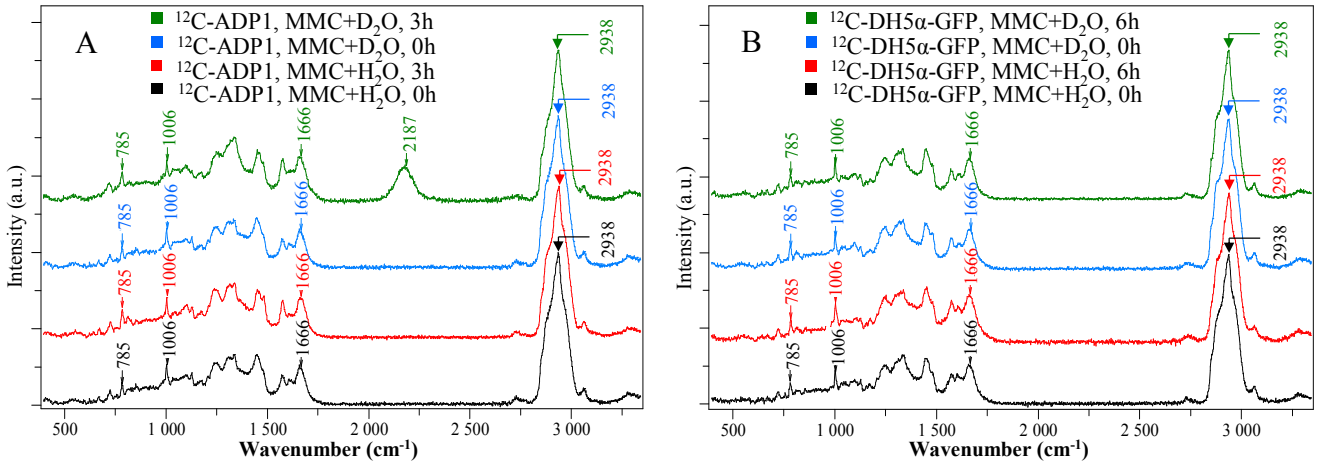

Figure S2 (A). SCRS of *A. baylyi* ADP1 pure culture cells grown in MMC with/without 40% D_2O. After 3 h of incubation, there was no observable change of ^{12}C-SCRS positions, and the C-D band appeared around 2187 cm^{-1} in the presence of D_2O. **S2 (B).** SCRS of ^{13}C-labeled *E. coli* DH5α-GFP pure culture cells grown in MMC with/without 40% D_2O. After 7 h of incubation, there was no observable change of ^{12}C-SCRS positions and the C-D band did not appear around 2187 cm^{-1} in the presence of D_2O.

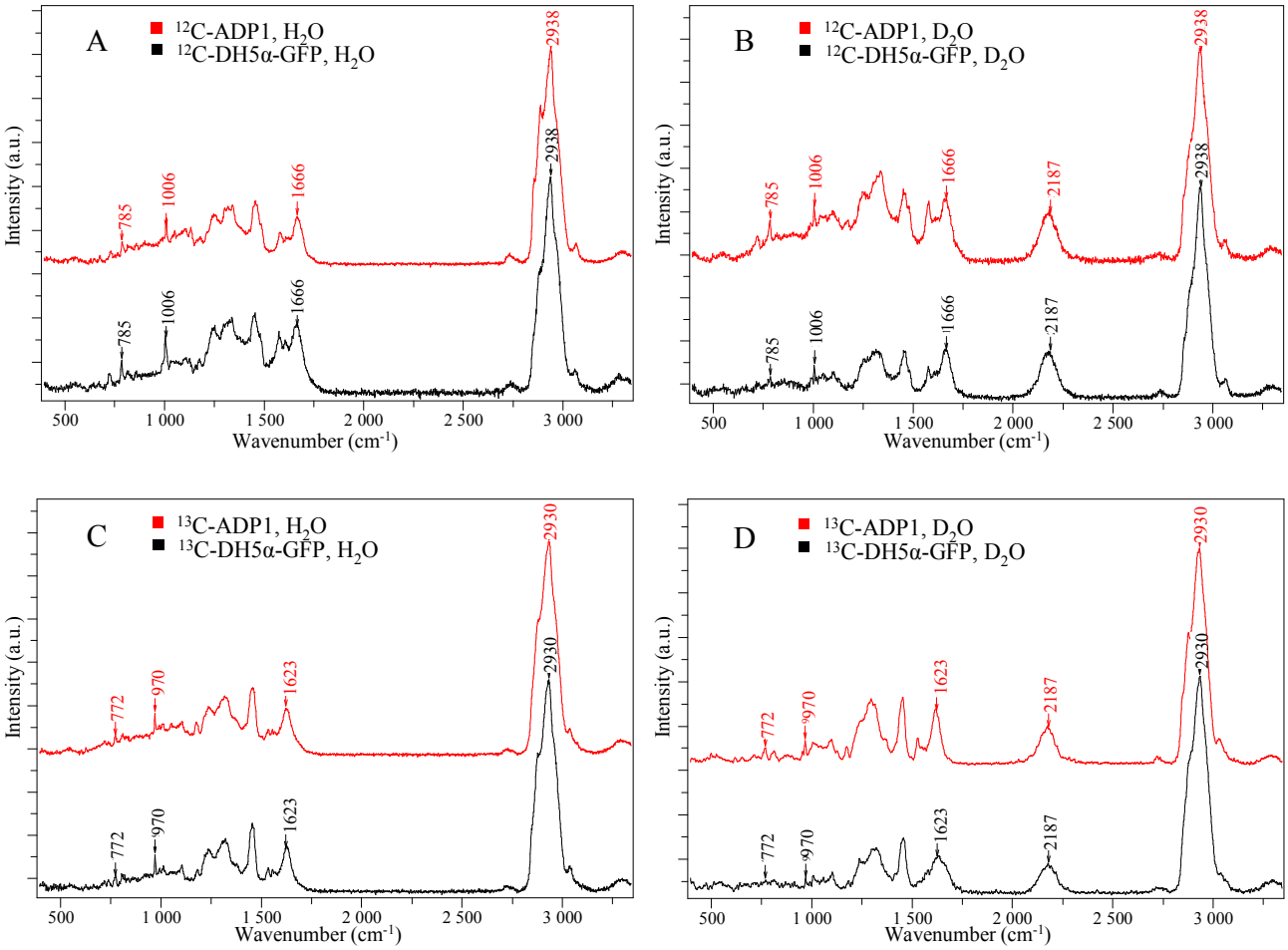

Figure S3. Main features of Raman spectra of *A. baylyi* ADP1 and *E. coli* DH5α-GFP pure cultures under no labelling (A), D_2O labelling (B), ^{13}C labelling (C) and co-labelling of D_2O and ^{13}C (D).

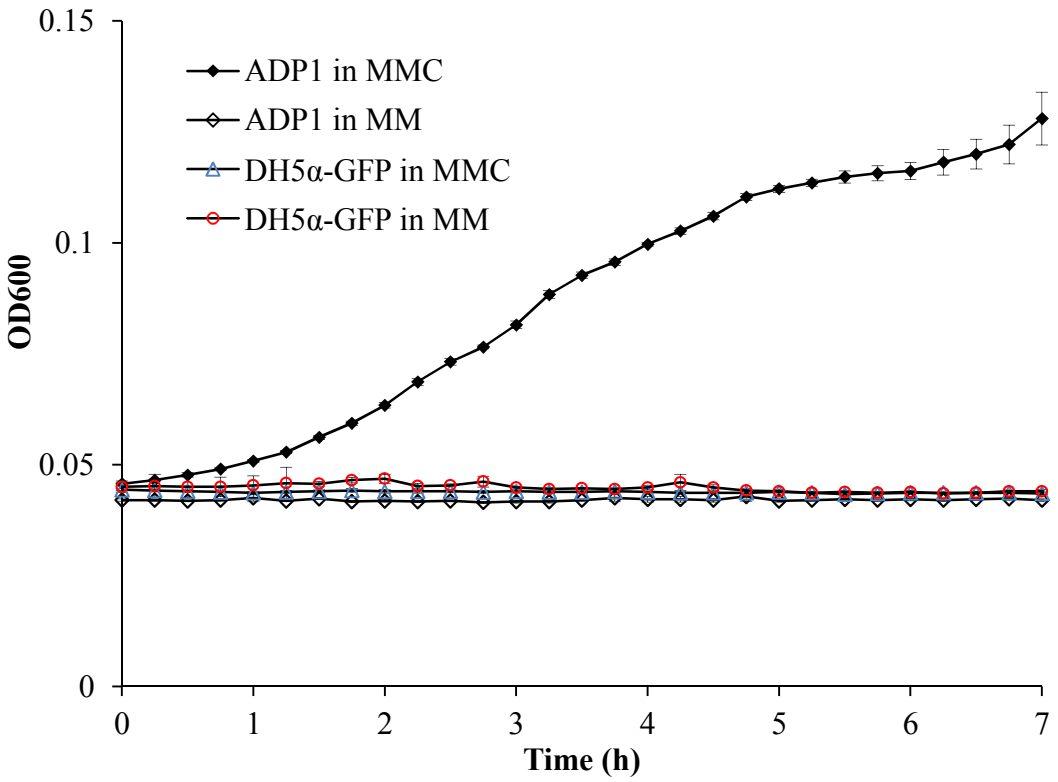

Figure S4. Growth curves of *A. baylyi* ADP1 and *E. coli* DH5a-GFP in MMC and MM medium under aerobic conditions. *A. baylyi* ADP1 could grow by using citrate as the sole carbon source but *E. coli* DH5a-GFP was unable to use citrate.

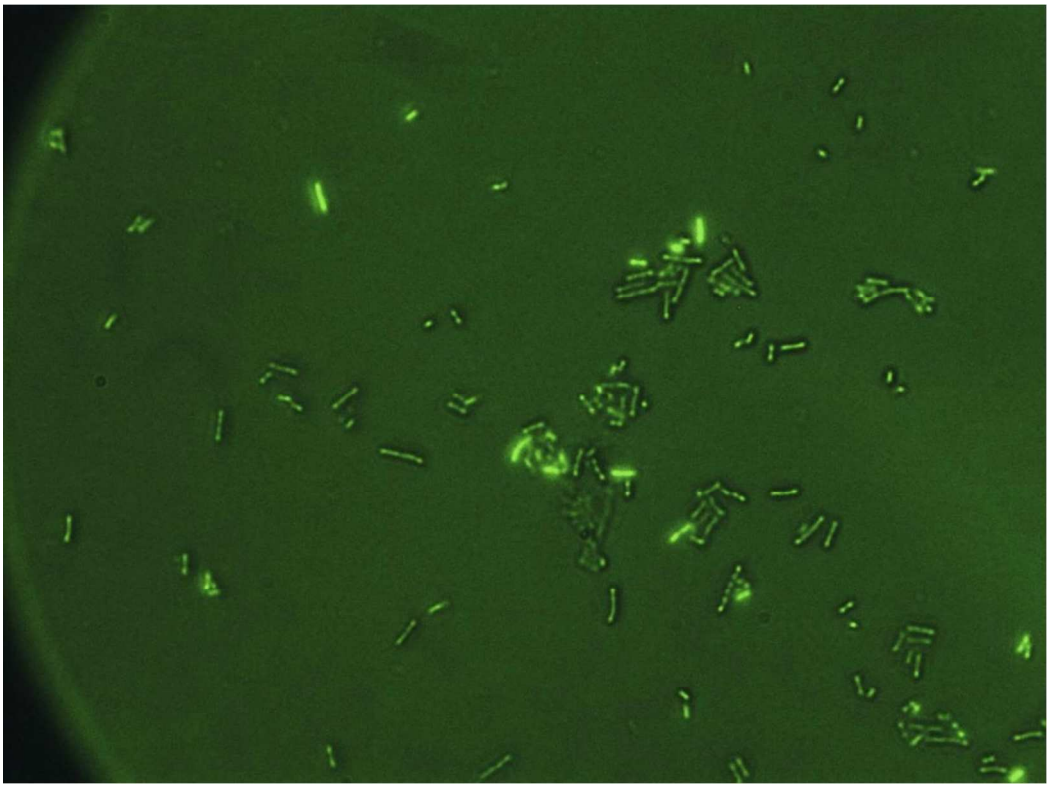

Figure S5. Combined image of the co-culture of DH5α-GFP & ADP1 cells

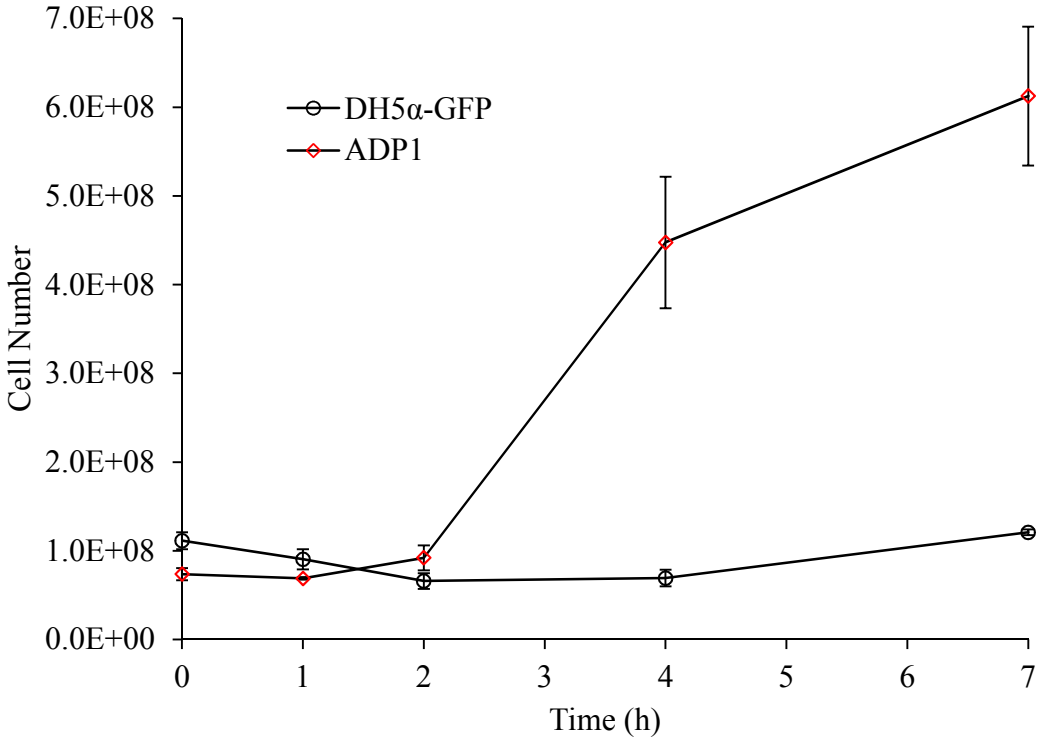

Figure S6. Cell counting results of *A. baylyi* ADP1 and *E. coli* DH5a-GFP in MMC with 40% D_2O. *A. baylyi* ADP1 could grow by using citrate as the sole carbon source. *E. coli* DH5a-GFP was able to be labeled by D_2O, but did not grow obviously.

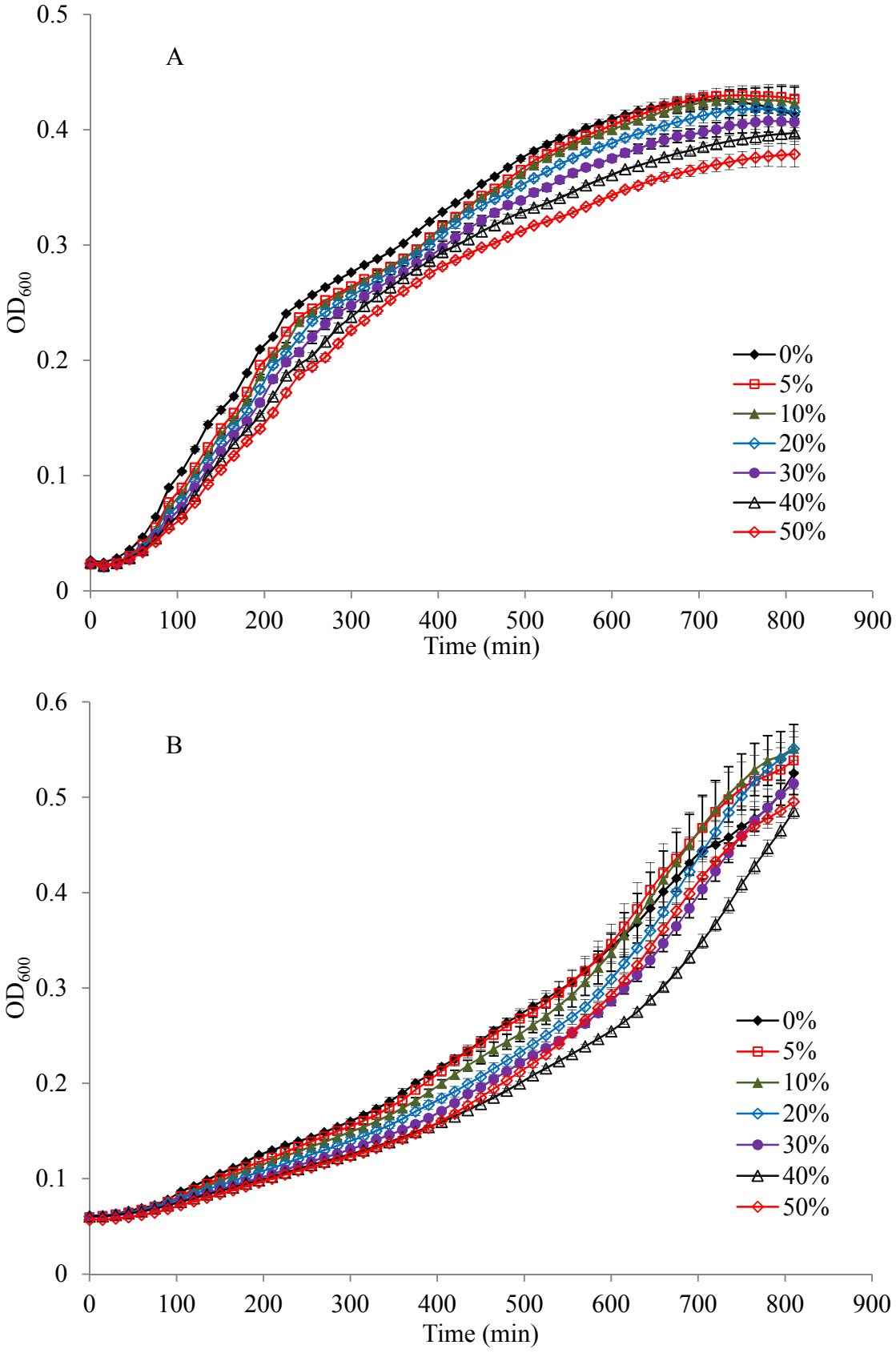

Figure S7. (A) Growth curve of *E. coli* DH5a-GFP under different concentrations of D_2O. (B) Growth curve of *A. baylyi* ADP1 under different concentrations of D_2O.