



Supporting Information

for

Secondary metabolites of *Bacillus subtilis* impact the assembly of soil-derived semisynthetic bacterial communities

Heiko T. Kiesevalter, Carlos N. Lozano-Andrade, Mikael L. Strube and Ákos T. Kovács

Beilstein J. Org. Chem. **2020**, *16*, 2983–2998. [doi:10.3762/bjoc.16.248](https://doi.org/10.3762/bjoc.16.248)

Bacterial strains used in this study, 16S rRNA V3-V4 primer list, number of sequencing reads per sample, and supporting figures

Supporting tables

Table S1. Bacterial strains used in this study.

Strains	Characteristics	Reference
P5_B1	<i>B. subtilis</i> soil isolate from sampling site 55.788800, 12.558300	[1]
DTUB55	P5_B1 <i>sfp</i> :: <i>mls</i>	[2]
DTUB79	P5_B1 <i>srfAC</i> :: <i>Tn10</i> (Spec ^R)	[1]
DTUB102	P5_B1 Δ <i>pksL</i> (Chl ^R)	[2]
DTUB125	P5_B1 Δ <i>ppsC</i> (Tet ^R)	[2]
M5	<i>Lysinibacillus fusiformis</i>	[3]

REFERENCES

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2. Kiesevalter, H. T.; Lozano-Andrade, C. N.; Wibowo, M.; Strube, M. L.; Maróti, G.; Snyder, D.; Jørgensen, T. S.; Larsen, T. O.; Cooper, V. S.; Weber, T.; Kovács, Á. T. *bioRxiv* **2020**, 2020.08.05.238063.
3. Gallegos-Monterrosa, R.; Kankel, S.; Götze, S.; Barnett, R.; Stallforth, P.; Kovács, Á. T. *J. Bacteriol.* **2017**, *199*, e00204-17.

Table S2. 16S rRNA V3-V4 primer used in this study

Name	Barcode	Primer
V3V4_forw_1	TTTTAATC	CCTACGGGNGGCWGCAG
V3V4_rev_1	TTTTAATC	GACTACHVGGGTATCTAATCC
V3V4_forw_2	ATAATTAG	CCTACGGGNGGCWGCAG
V3V4_rev_2	ATAATTAG	GACTACHVGGGTATCTAATCC
V3V4_forw_3	ACCAAATT	CCTACGGGNGGCWGCAG
V3V4_rev_3	ACCAAATT	GACTACHVGGGTATCTAATCC
V3V4_forw_4	CTTATCAA	CCTACGGGNGGCWGCAG
V3V4_rev_4	CTTATCAA	GACTACHVGGGTATCTAATCC
V3V4_forw_5	TGATCATT	CCTACGGGNGGCWGCAG
V3V4_rev_5	TGATCATT	GACTACHVGGGTATCTAATCC
V3V4_forw_6	AGAATCTA	CCTACGGGNGGCWGCAG
V3V4_rev_6	AGAATCTA	GACTACHVGGGTATCTAATCC
V3V4_forw_7	TCAAGAAA	CCTACGGGNGGCWGCAG
V3V4_rev_7	TCAAGAAA	GACTACHVGGGTATCTAATCC
V3V4_forw_8	ATCGAAAT	CCTACGGGNGGCWGCAG
V3V4_rev_8	ATCGAAAT	GACTACHVGGGTATCTAATCC
V3V4_forw_9	ACATTTAC	CCTACGGGNGGCWGCAG
V3V4_rev_9	ACATTTAC	GACTACHVGGGTATCTAATCC
V3V4_forw_10	TAGAAAAC	CCTACGGGNGGCWGCAG
V3V4_rev_10	TAGAAAAC	GACTACHVGGGTATCTAATCC
V3V4_forw_11	TTATCACC	CCTACGGGNGGCWGCAG
V3V4_rev_11	TTATCACC	GACTACHVGGGTATCTAATCC
V3V4_forw_12	AATAGGGT	CCTACGGGNGGCWGCAG
V3V4_rev_12	AATAGGGT	GACTACHVGGGTATCTAATCC
V3V4_forw_13	ATTGCTGA	CCTACGGGNGGCWGCAG
V3V4_rev_13	ATTGCTGA	GACTACHVGGGTATCTAATCC
V3V4_forw_14	TGAGTTCT	CCTACGGGNGGCWGCAG
V3V4_rev_14	TGAGTTCT	GACTACHVGGGTATCTAATCC
V3V4_forw_15	GGCTATTT	CCTACGGGNGGCWGCAG
V3V4_rev_15	GGCTATTT	GACTACHVGGGTATCTAATCC
V3V4_forw_16	CAAGAGAT	CCTACGGGNGGCWGCAG
V3V4_rev_16	CAAGAGAT	GACTACHVGGGTATCTAATCC
V3V4_forw_17	GGAATACA	CCTACGGGNGGCWGCAG
V3V4_rev_17	GGAATACA	GACTACHVGGGTATCTAATCC
V3V4_forw_18	AAGGCAAT	CCTACGGGNGGCWGCAG
V3V4_rev_18	AAGGCAAT	GACTACHVGGGTATCTAATCC
V3V4_forw_19	ACAAAACG	CCTACGGGNGGCWGCAG
V3V4_rev_19	ACAAAACG	GACTACHVGGGTATCTAATCC
V3V4_forw_21	TTGAGTGA	CCTACGGGNGGCWGCAG
V3V4_rev_21	TTGAGTGA	GACTACHVGGGTATCTAATCC
V3V4_forw_22	GCTTCTGA	CCTACGGGNGGCWGCAG
V3V4_rev_22	GCTTCTGA	GACTACHVGGGTATCTAATCC
V3V4_forw_23	GGCAAGAT	CCTACGGGNGGCWGCAG
V3V4_rev_23	GGCAAGAT	GACTACHVGGGTATCTAATCC

V3V4_forw_24	GTGCTTTC	CCTACGGGNGGCWGCAG
V3V4_rev_24	GTGCTTTC	GACTACHVGGGTATCTAATCC
V3V4_forw_25	ACACACTG	CCTACGGGNGGCWGCAG
V3V4_rev_25	ACACACTG	GACTACHVGGGTATCTAATCC
V3V4_forw_26	CGATTCTG	CCTACGGGNGGCWGCAG
V3V4_rev_26	CGATTCTG	GACTACHVGGGTATCTAATCC
V3V4_forw_27	GCAGAGTT	CCTACGGGNGGCWGCAG
V3V4_rev_27	GCAGAGTT	GACTACHVGGGTATCTAATCC
V3V4_forw_28	CGTCCTAT	CCTACGGGNGGCWGCAG
V3V4_rev_28	CGTCCTAT	GACTACHVGGGTATCTAATCC
V3V4_forw_30	GCTTGGTT	CCTACGGGNGGCWGCAG
V3V4_rev_30	GCTTGGTT	GACTACHVGGGTATCTAATCC
V3V4_forw_31	ACAGGCTT	CCTACGGGNGGCWGCAG
V3V4_rev_31	ACAGGCTT	GACTACHVGGGTATCTAATCC
V3V4_forw_33	TGACGCTT	CCTACGGGNGGCWGCAG
V3V4_rev_33	TGACGCTT	GACTACHVGGGTATCTAATCC

Table S3. Number of reads per sample.

Sample	Number of reads
Soil 1	751
Soil 2	12,660
Pre 1	20,440
Pre 2	17,787
Pre 3	15,471
Pre 4	34,802
Control 1	4,649
Control 2	20,846
Control 3	16,229
Control 4	16,556
WT 1	12,436
WT 2	18,554
WT 3	18,182
WT 4	11,964
<i>sfp</i> 1	13,354
<i>sfp</i> 2	16,737
<i>sfp</i> 3	12,472
<i>sfp</i> 4	21,421
<i>srfAC</i> 1	7,047
<i>srfAC</i> 2	1,184
<i>srfAC</i> 3	3,563
<i>srfAC</i> 4	7,663
Δ <i>pksL</i> 1	11,017
Δ <i>pksL</i> 2	2,115
Δ <i>pksL</i> 3	7,063
Δ <i>pksL</i> 4	5,187
Δ <i>ppsC</i> 1	9,443
Δ <i>ppsC</i> 2	10,823
Δ <i>ppsC</i> 3	9,947
Δ <i>ppsC</i> 4	2,112

Supporting figures

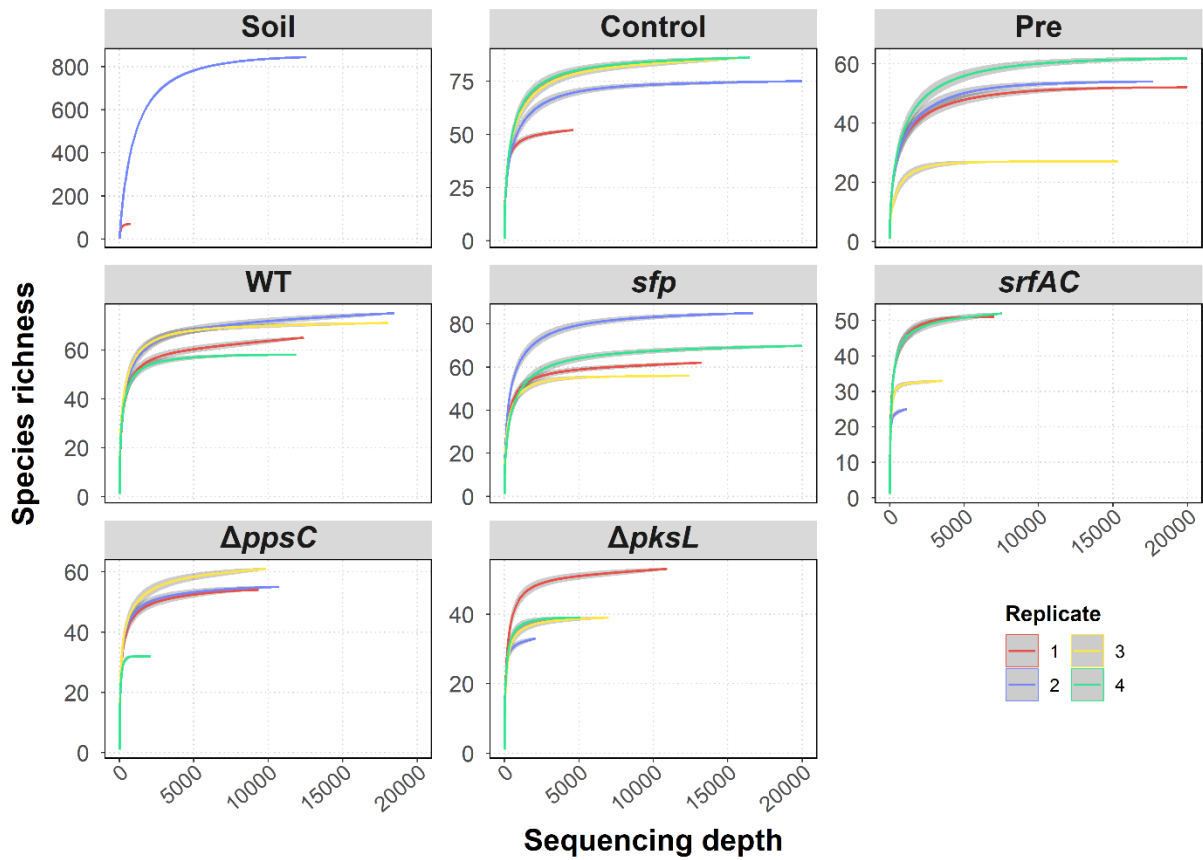


Figure S1. Rarefaction curves for each assay and replicate. Species richness is visualised for sampling depths from 0 to 20,000.

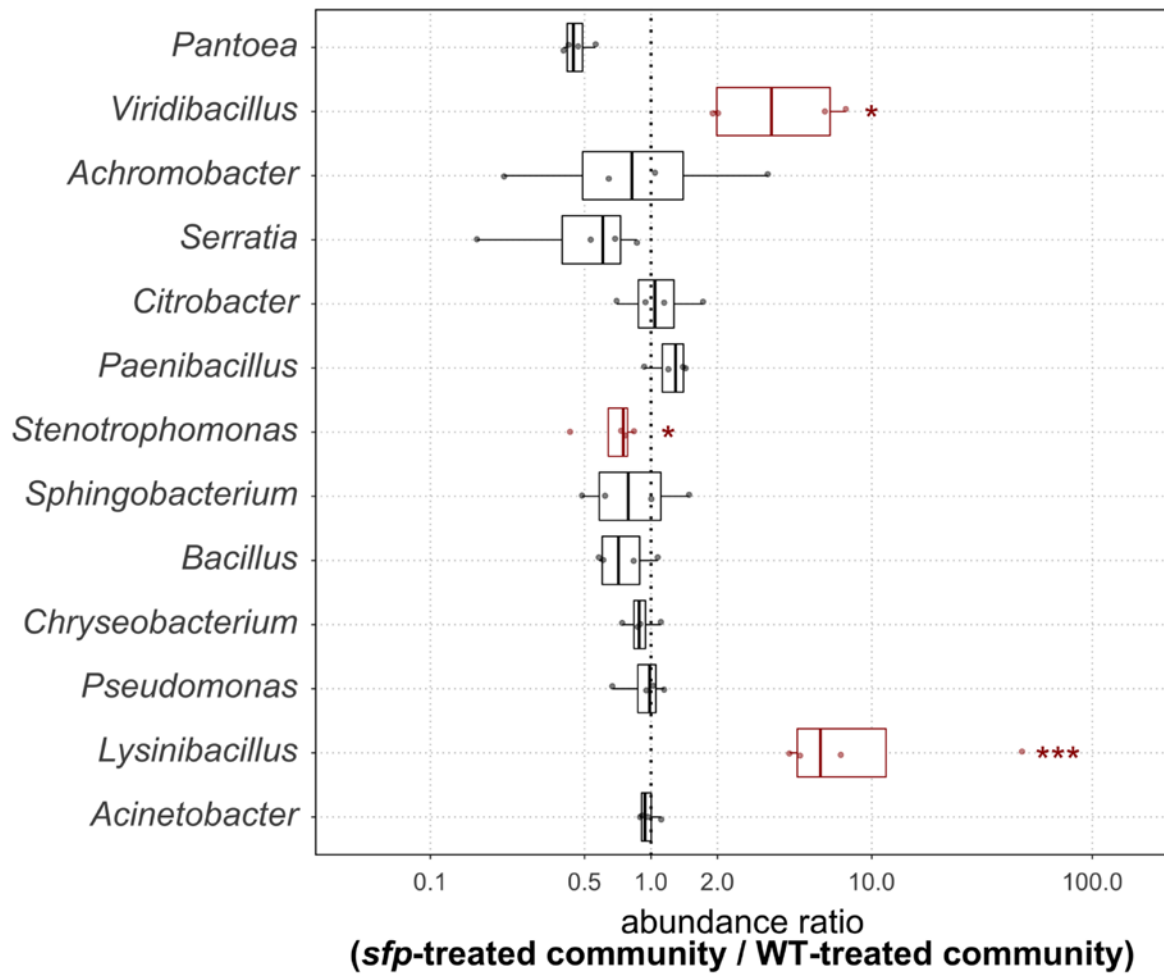


Figure S2. Abundance ratios for each genus and replicate (points) in the *sfp*-treated community compared to the WT-treated community. Statistical significance is defined as $P \leq 0.05$ (*) and $P \leq 0.001$ (***)

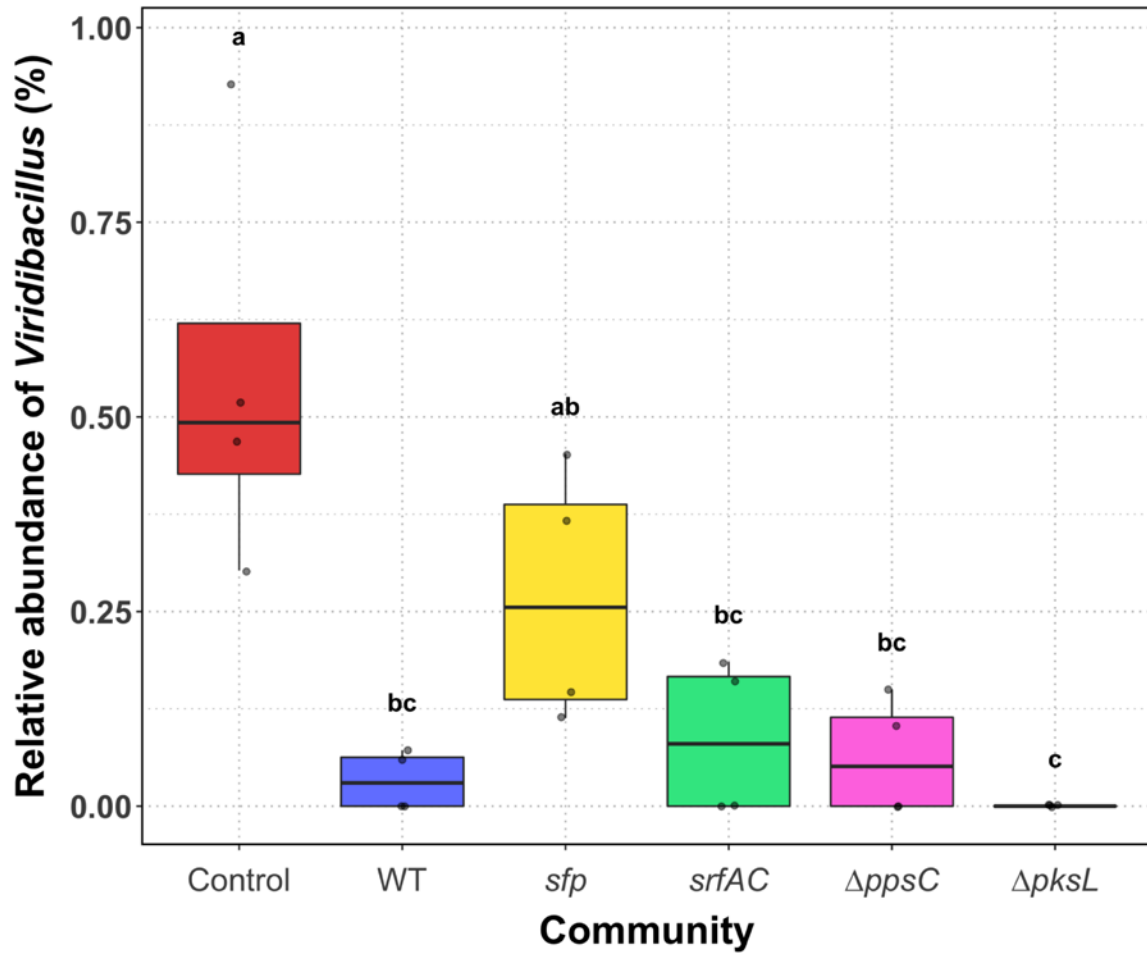


Figure S3. Abundance of *Viridibacillus* in the untreated mock communities (Control) and treated mock communities with either *B. subtilis* wild type (WT), NRPs deficient strain (*sfp*), surfactin mutant (*srfAC*), plipastatin mutant ($\Delta ppsC$) or bacillaene mutant ($\Delta pksL$) and co-cultivated for 48 h. Points represent the abundance in each replicate. Treatments with varying letters are significantly different ($P < 0.05$).

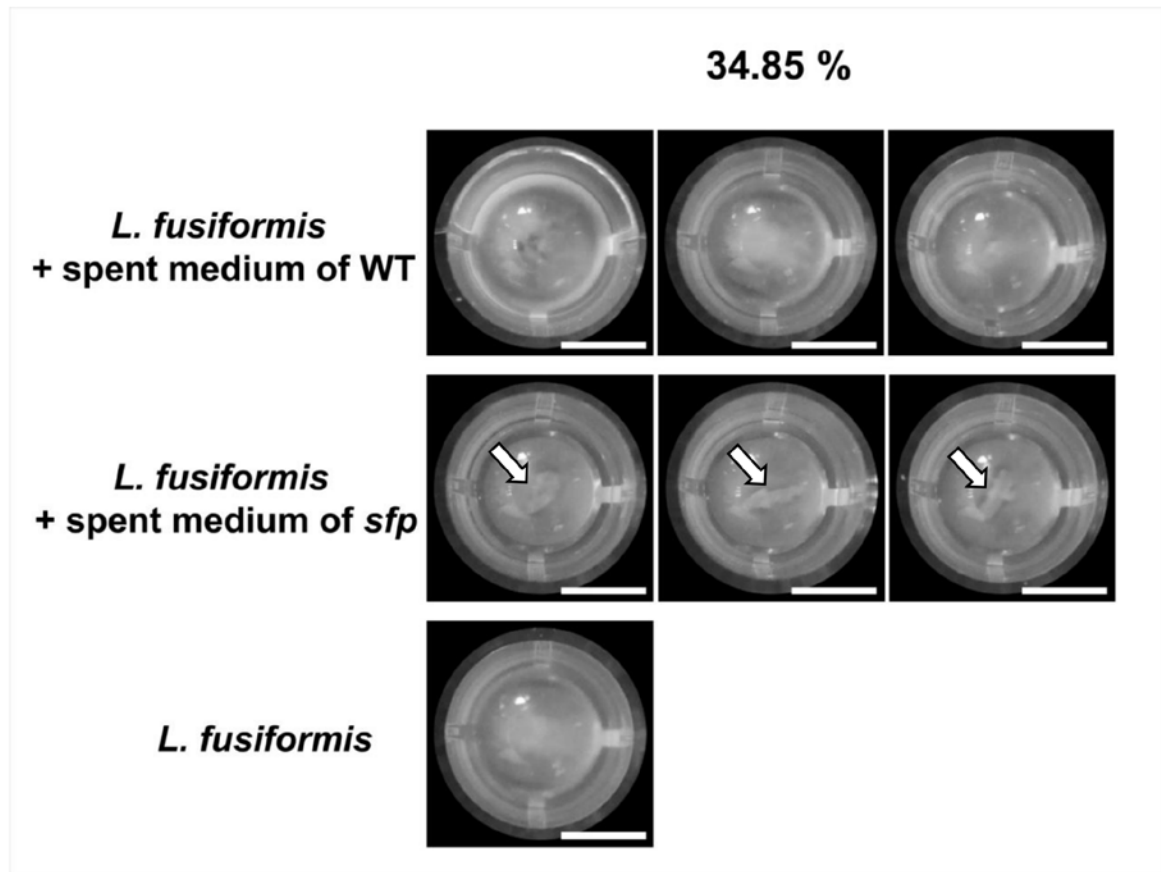


Figure S4. Different levels of aggregation of *L. fusiformis* M5 after exposure to spent medium of *B. subtilis* WT and *sfp* or without treatment. Solid aggregates in assays supplemented with spent medium of *sfp* are marked with arrows. Scale bars indicate 3 mm.

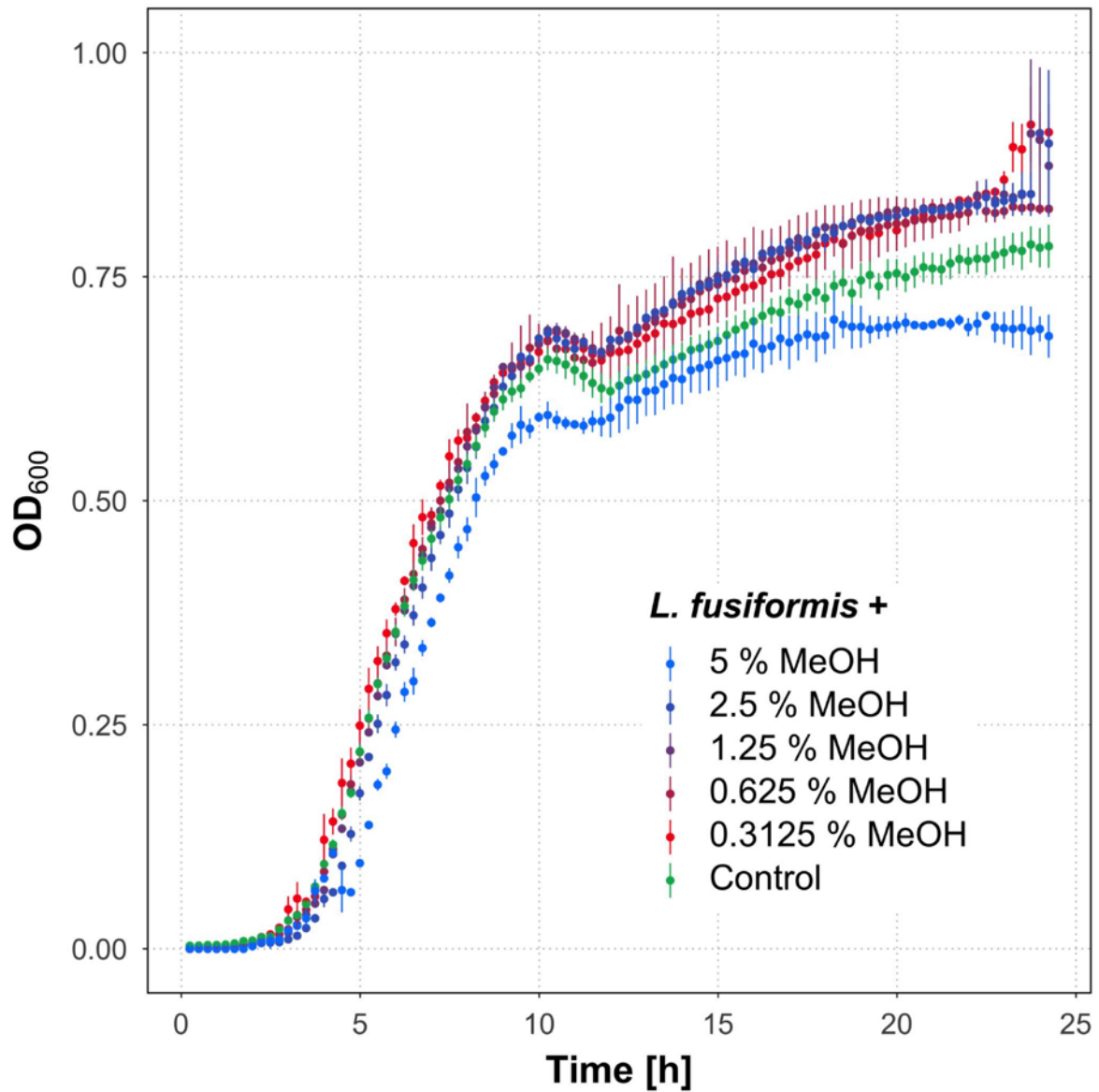


Figure S5. Growth curves of *L. fusiformis* M5 exposed to different concentrations of the solvent MeOH and without treatment (control). Error bars represent the standard error. N = 6 (control assay), N = 2 (MeOH-treated assays)