Supplementary data

Ovomucin and its hydrolysates differentially influenced colitis severity in *Citrobacter rodentium*-infected mice

Xiaoyu Bao[1], Tingting Ju[1,2], Stephanie Tollenaar[1], Consolato Sergi[3], Benjamin P. Willing[1], and Jianping Wu[1]

1. *Department of Agricultural, Food and Nutritional Science, Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, Alberta, Canada*
2. *Department of Animal Sciences, Purdue University, West Lafayette, IN, USA*
3. *Division of Anatomic Pathology, Children's Hospital of Eastern Ontario (CHEO), Ottawa, Ontario, Canada*

Figure captions

Fig S1. Expression levels of tight junction protein occludin and ZO-1 in mouse colon tissues at 7 dpi. Data are expressed as mean ± SEM (n=8). Ctrl, untreated control group; OVM, ovomucin group; OP, ovomucin-protex 26L hydrolysate group; OPP, ovomucin-pepsin/pancreatin hydrolysate group.

Fig S2. Comparison of relative abundance of bacterial taxonomies before *C. rodentium* infection. *$P < 0.05$, **$P < 0.01$, #$P < 0.1$, according to the Kruskal-Wallis test combined with Dunn's test.

Fig S3. Comparison of relative abundance of bacterial taxonomies at 7dpi. *$P < 0.05$, **$P < 0.01$, #$P < 0.1$, according to the Kruskal-Wallis test combined with Dunn's test.

Fig S4. Growth of *C. rodentium* in M9 minimal media supplemented with 2.5% of OP and OPP. (A) Optical density at 600nm of *C. rodentium* after 6 h culture in M9 media. (B) *C. rodentium* quantification after cultivation in M9 media for different time periods. Data are expressed as mean ± SEM (n=3-4). Groups that do not share a letter are significantly different (one-way ANOVA and Tukey's test) ($\alpha = 0.05$).

Fig S5. Effects of OP and OPP on CMT-93 cell viability. Cells grown on 96-well plates were treated with OP or OPP at concentrations ranging from 0.1% to 2.5% for 24 h before being incubated with the alamarBlue solution in dark for 4. Fluorescence densities were detected with the excitation and emission wavelengths being 560 nm and 590 nm, respectively. Data are indicated by mean ± SEM.

Fig S6. *C. rodentium* adhesion to CMT-93 cells after 5 h incubation in DMEM supplemented with 2.5% of OP and OPP. Data are expressed as mean ± SEM. Groups that do not share a letter are significantly different (Kruskal-Wallis test with Dunn's test) ($\alpha = 0.05$).

Table S1. Primers for real-time PCR analysis

Fig. S1

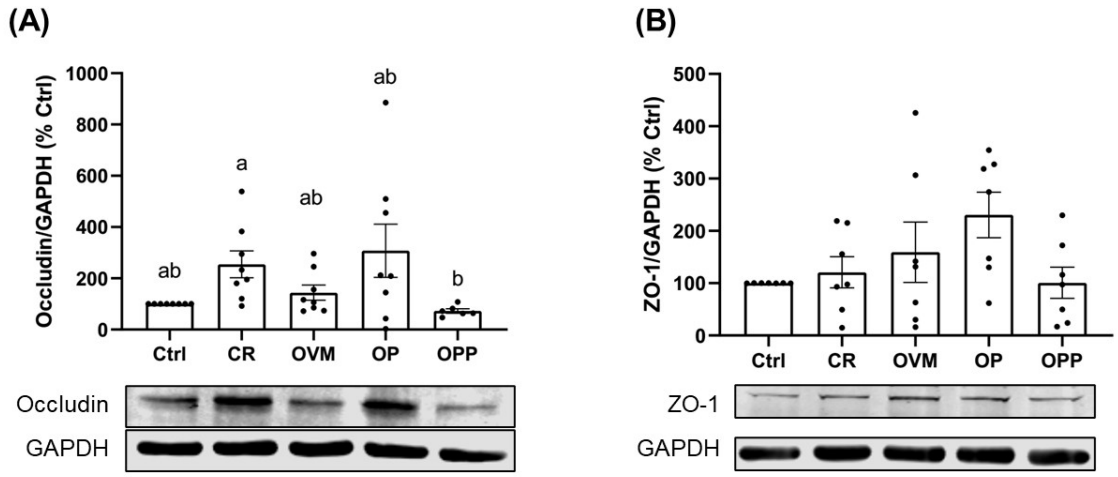
(A)
Occludin/GAPDH (% Ctrl)
1000
800
600
400
200
0
ab
a
ab
ab
b
Ctrl
CR
OVM
OP
OPP
Occludin
GAPDH
(B)
ZO-1/GAPDH (% Ctrl)
500
400
300
200
100
0
Ctrl
CR
OVM
OP
OPP
ZO-1
GAPDH

Fig. S2

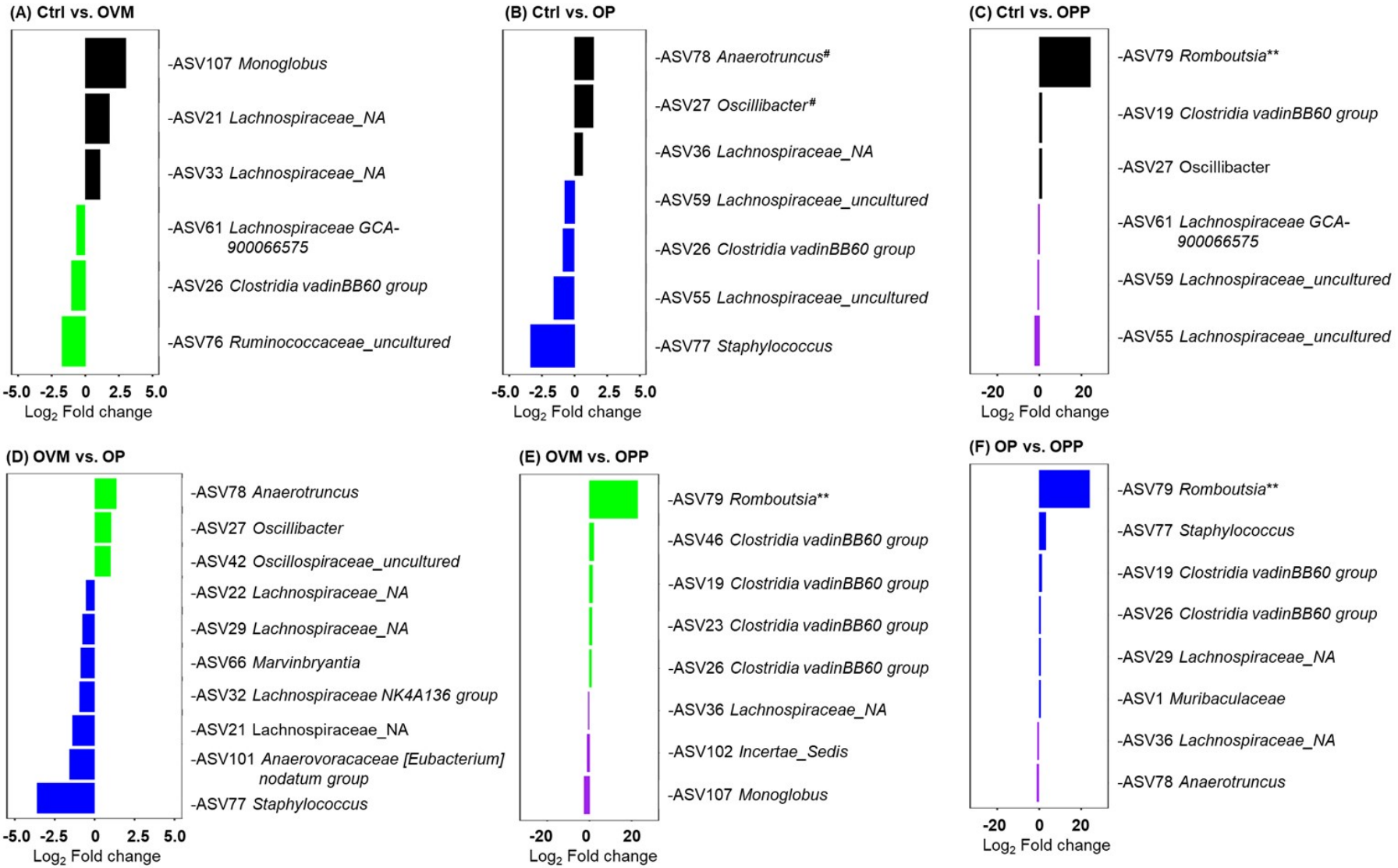
(A) Ctrl vs. OVM
-ASV107 Monoglobus
-ASV21 Lachnospiraceae_NA
-ASV33 Lachnospiraceae_NA
-ASV61 Lachnospiraceae GCA-900066575
-ASV26 Clostridia vadinBB60 group
-ASV76 Ruminococcaceae_uncultured
-5.0 -2.5 0 2.5 5.0
Log2 Fold change
(B) Ctrl vs. OP
-ASV78 Anaerotruncus#
-ASV27 Oscillibacter#
-ASV36 Lachnospiraceae_NA
-ASV59 Lachnospiraceae_uncultured
-ASV26 Clostridia vadinBB60 group
-ASV55 Lachnospiraceae_uncultured
-ASV77 Staphylococcus
-5.0 -2.5 0 2.5 5.0
Log2 Fold change
(C) Ctrl vs. OPP
-ASV79 Romboutsia**
-ASV19 Clostridia vadinBB60 group
-ASV27 Oscillibacter
-ASV61 Lachnospiraceae GCA-900066575
-ASV59 Lachnospiraceae_uncultured
-ASV55 Lachnospiraceae_uncultured
-20 0 20
Log2 Fold change
(D) OVM vs. OP
-ASV78 Anaerotruncus
-ASV27 Oscillibacter
-ASV42 Oscillospiraceae_uncultured
-ASV22 Lachnospiraceae_NA
-ASV29 Lachnospiraceae_NA
-ASV66 Marvinbryantia
-ASV32 Lachnospiraceae NK4A136 group
-ASV21 Lachnospiraceae_NA
-ASV101 Anaerovoracaceae [Eubacterium] nodatum group
-ASV77 Staphylococcus
-5.0 -2.5 0 2.5 5.0
Log2 Fold change
(E) OVM vs. OPP
-ASV79 Romboutsia**
-ASV46 Clostridia vadinBB60 group
-ASV19 Clostridia vadinBB60 group
-ASV23 Clostridia vadinBB60 group
-ASV26 Clostridia vadinBB60 group
-ASV36 Lachnospiraceae_NA
-ASV102 Incertae_Sedis
-ASV107 Monoglobus
-20 0 20
Log2 Fold change
(F) OP vs. OPP
-ASV79 Romboutsia**
-ASV77 Staphylococcus
-ASV19 Clostridia vadinBB60 group
-ASV26 Clostridia vadinBB60 group
-ASV29 Lachnospiraceae_NA
-ASV1 Muribaculaceae
-ASV36 Lachnospiraceae_NA
-ASV78 Anaerotruncus
-20 0 20
Log2 Fold change

Fig. S3

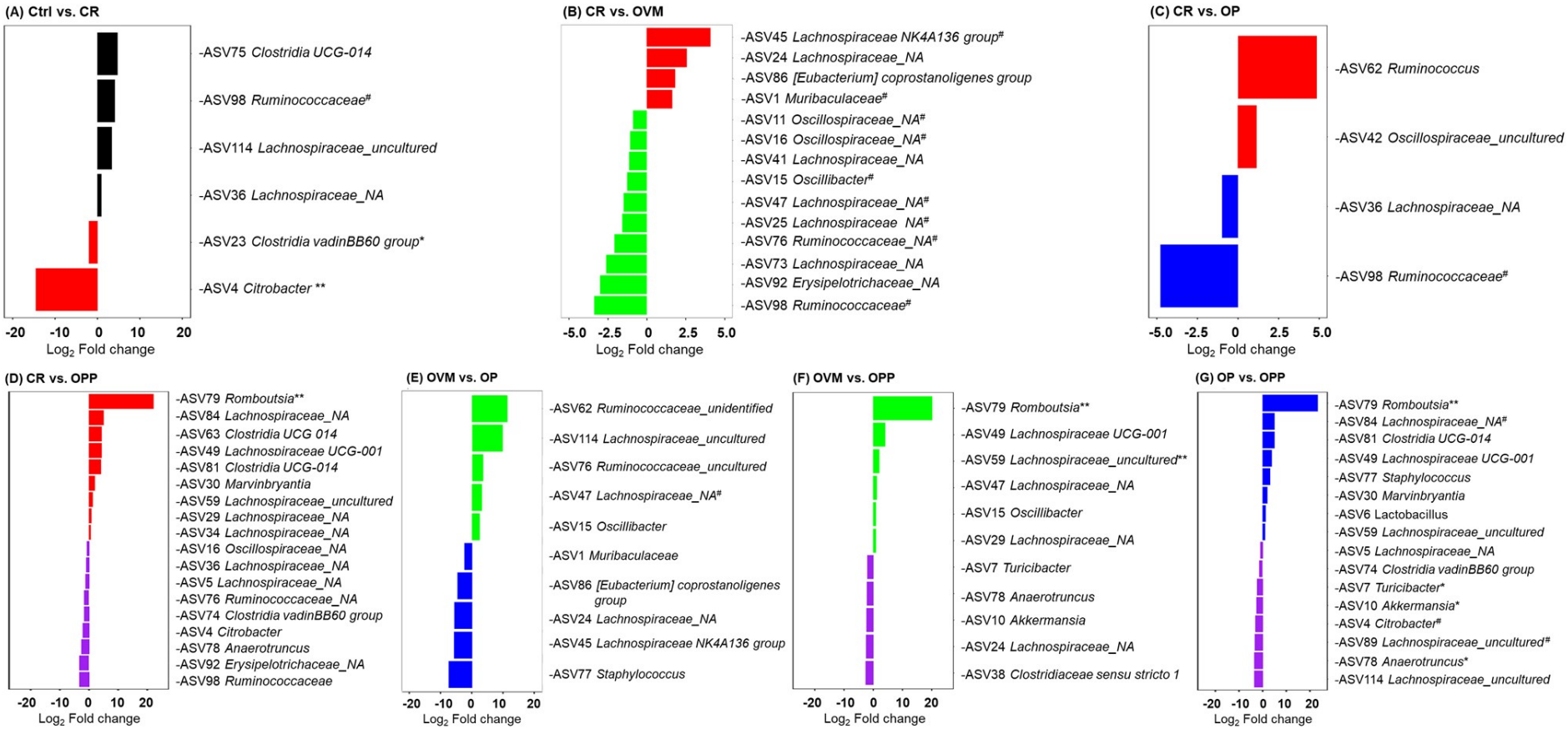
(A) Ctrl vs. CR
-ASV75 Clostridia UCG-014
-ASV98 Ruminococcaceae#
-ASV114 Lachnospiraceae_uncultured
-ASV36 Lachnospiraceae_NA
-ASV23 Clostridia vadinBB60 group*
-ASV4 Citrobacter **
-20 -10 0 10 20
Log2 Fold change
(B) CR vs. OVM
-ASV45 Lachnospiraceae NK4A136 group#
-ASV24 Lachnospiraceae_NA
-ASV86 [Eubacterium] coprostanoligenes group
-ASV1 Muribaculaceae#
-ASV11 Oscillospiraceae_NA#
-ASV16 Oscillospiraceae_NA#
-ASV41 Lachnospiraceae_NA
-ASV15 Oscillibacter#
-ASV47 Lachnospiraceae_NA#
-ASV25 Lachnospiraceae NA#
-ASV76 Ruminococcaceae_NA#
-ASV73 Lachnospiraceae_NA
-ASV92 Erysipelotrichaceae_NA
-ASV98 Ruminococcaceae#
-5.0 -2.5 0 2.5 5.0
Log2 Fold change
(C) CR vs. OP
-ASV62 Ruminococcus
-ASV42 Oscillospiraceae_uncultured
-ASV36 Lachnospiraceae_NA
-ASV98 Ruminococcaceae#
-5.0 -2.5 0 2.5 5.0
Log2 Fold change
(D) CR vs. OPP
-ASV79 Romboutsia**
-ASV84 Lachnospiraceae_NA
-ASV63 Clostridia UCG 014
-ASV49 Lachnospiraceae UCG-001
-ASV81 Clostridia UCG-014
-ASV30 Marvinbryantia
-ASV59 Lachnospiraceae_uncultured
-ASV29 Lachnospiraceae_NA
-ASV34 Lachnospiraceae_NA
-ASV16 Oscillospiraceae_NA
-ASV36 Lachnospiraceae_NA
-ASV5 Lachnospiraceae_NA
-ASV76 Ruminococcaceae_NA
-ASV74 Clostridia vadinBB60 group
-ASV4 Citrobacter
-ASV78 Anaerotruncus
-ASV92 Erysipelotrichaceae_NA
-ASV98 Ruminococcaceae
-20 -10 0 10 20
Log2 Fold change
(E) OVM vs. OP
-ASV62 Ruminococcaceae_unidentified
-ASV114 Lachnospiraceae_uncultured
-ASV76 Ruminococcaceae_uncultured
-ASV47 Lachnospiraceae_NA#
-ASV15 Oscillibacter
-ASV1 Muribaculaceae
-ASV86 [Eubacterium] coprostanoligenes group
-ASV24 Lachnospiraceae_NA
-ASV45 Lachnospiraceae NK4A136 group
-ASV77 Staphylococcus
-20 -10 0 10 20
Log2 Fold change
(F) OVM vs. OPP
-ASV79 Romboutsia**
-ASV49 Lachnospiraceae UCG-001
-ASV59 Lachnospiraceae_uncultured**
-ASV47 Lachnospiraceae_NA
-ASV15 Oscillibacter
-ASV29 Lachnospiraceae_NA
-ASV7 Turicibacter
-ASV78 Anaerotruncus
-ASV10 Akkermansia
-ASV24 Lachnospiraceae_NA
-ASV38 Clostridiaceae sensu stricto 1
-20 -10 0 10 20
Log2 Fold change
(G) OP vs. OPP
-ASV79 Romboutsia**
-ASV84 Lachnospiraceae_NA#
-ASV81 Clostridia UCG-014
-ASV49 Lachnospiraceae UCG-001
-ASV77 Staphylococcus
-ASV30 Marvinbryantia
-ASV6 Lactobacillus
-ASV59 Lachnospiraceae_uncultured
-ASV5 Lachnospiraceae_NA
-ASV74 Clostridia vadinBB60 group
-ASV7 Turicibacter*
-ASV10 Akkermansia*
-ASV4 Citrobacter#
-ASV89 Lachnospiraceae_uncultured#
-ASV78 Anaerotruncus*
-ASV114 Lachnospiraceae_uncultured
-20 -10 0 10 20
Log2 Fold change

Fig. S4

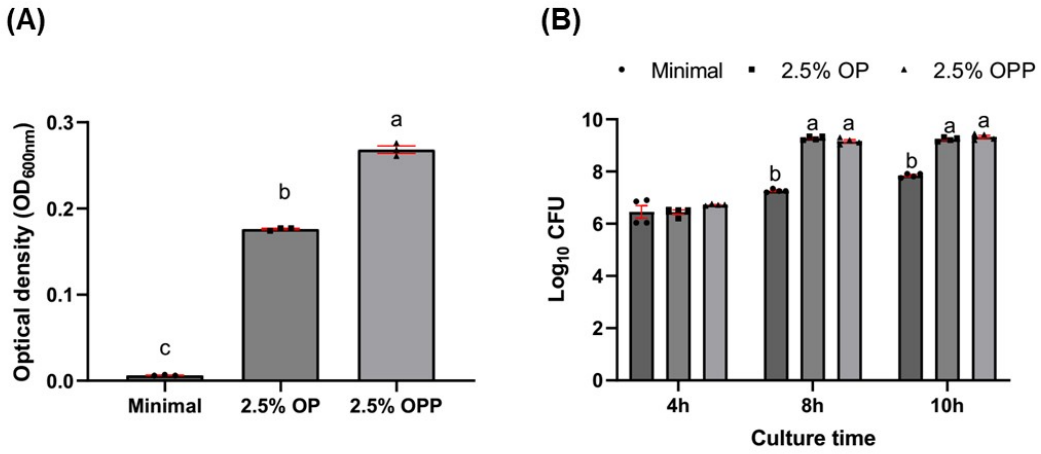
(A)
Optical density (OD_{600nm})
0.3
0.2
0.1
0.0
a
b
c
Minimal
2.5% OP
2.5% OPP
(B)
Minimal
2.5% OP
2.5% OPP
Log_{10} CFU
10
8
6
4
2
0
a
a
b
a
a
b
4h
8h
10h
Culture time

Fig. S5

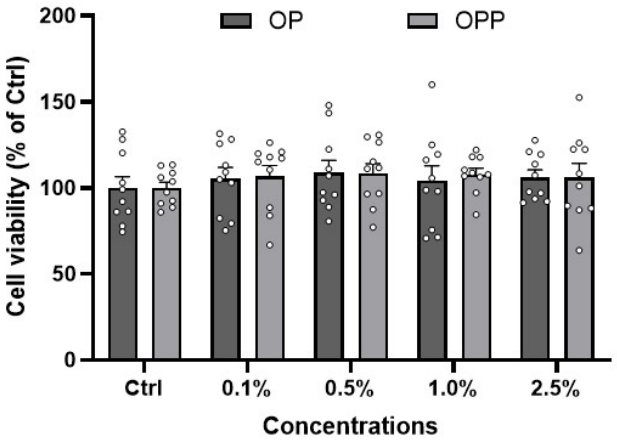
OP
OPP
Cell viability (% of Ctrl)
200
150
100
50
0
Ctrl
0.1%
0.5%
1.0%
2.5%
Concentrations

Fig. S6

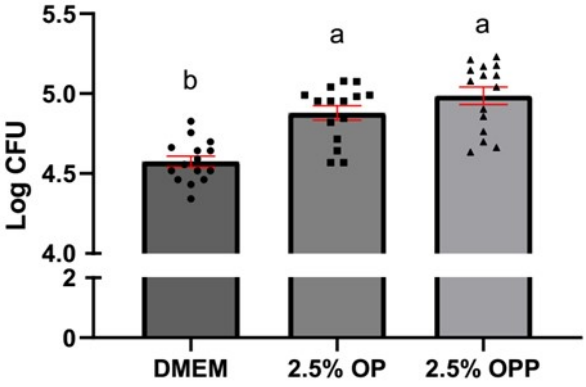

Table S1

Targeted gene	Oligonucleotide sequence (5'–3')	Reference
EspB	Forward: ATGCCGCAGATGAGACAGTTG	[1]
	Reverse: CGTCAGCAGCCTTTTCAGCTA	
Total bacteria	Forward: AAACTCAAAKGAATTGACGG	[2]
	Reverse: CTCACRRCACGAGCTGAC	

Reference

1. S. Sagaidak, A. Taibi, B. Wen and E. M. Comelli, Development of a real-time PCR assay for quantification of Citrobacter rodentium, *J. Microbiol. Methods*, 2016, **126**, 76-77.
2. T. B. De Gregoris, N. Aldred, A. S. Clare and J. G. Burgess, Improvement of phylum-and class-specific primers for real-time PCR quantification of bacterial taxa, *J. Microbiol. Methods*, 2011, **86**, 351-356.