

Effect of oral administration of lactic acid bacteria on colony performance and gut microbiota in indoor-reared bumblebees (*Bombus terrestris*)

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Table S1. Composition of the agar used for the cultivation of lactobacilli and bifidobacteria.

Lactobacilli: MRS agar	Bifidobacteria
10 g Peptone	23 g 'special peptone'
8 g Lab-Lemco Powder	1 g Soluble starch
4 g Yeast extract	5 g NaCl
20 g Glucose	0.3 g Cysteine hydrochloride
1 mL Tween 80	5 g Glucose
2 g Tri-ammonium citrate	15 g Agar
5 g Sodium acetate x 3H ₂ O	1 L Distilled water
0.2 g MgSO ₄ x 7H ₂ O	
0.05 g MnSO ₄ x 4H ₂ O	
2 g K ₂ HPO ₄	
15 g Agar	
Up to 1 L Distilled water	

Table S2. Developmental parameters of the continuous supplementation of several *Lactobacillus* and *Bifidobacterium* strains to low nutritional pollen A, the single supplementation of *Lactobacillus kunkeei* LMG 18925 to pollen B and pollen C and the three time supplementation of *Bifidobacterium actinocoloniiforme* R-53049 to a pollen substitute.

Treatment	Days until first egg	Days until first pupa	Days until first drone	Number of drones per microcolony	Total drone mass per microcolony (g)	Average drone mass (mg)
Control with pollen A	7.2 ± 0.1	23.2 ± 0.85	36.0 ± 0.84	18.20 ± 2.80	5.27 ± 0.44	292.4 ± 1.6
<i>Bifidobacterium asteroides</i> LMG 10735 + pollen A	7.0 ± 0.0	23.2 ± 0.79	36.0 ± 0.71	19.80 ± 3.39	5.48 ± 0.82	279.2 ± 9.1
<i>Bifidobacterium asteroides</i> LMG 11581 + pollen A	7.2 ± 0.1	23.4 ± 0.88	36.7 ± 0.96	18.80 ± 2.84	5.16 ± 0.75	269.5 ± 4.8
<i>Bifidobacterium coryneforme</i> LMG 18911 + pollen A	7.0 ± 0.0	23.0 ± 1.18	36.7 ± 1.51	23.10 ± 2.23	6.18 ± 0.67	267.1 ± 12.1
<i>Lactobacillus acidophilus</i> LMG 11430 + pollen A	7.1 ± 0.1	24.7 ± 1.67	38.6 ± 1.89	16.89 ± 2.20	5.48 ± 0.84	315.4 ± 11.1
<i>Lactobacillus crispatus</i> LMG 9479 + pollen A	7.0 ± 0.0	23.6 ± 1.59	35.8 ± 1.28	28.11 ± 1.24	7.96 ± 0.57	289.8 ± 6.9
<i>Lactobacillus kunkeei</i> LMG 18925 + pollen A	7.0 ± 0.0	22.5 ± 0.76	35.2 ± 0.36	27.10 ± 3.05	7.74 ± 0.87	286.0 ± 5.0
Control with pollen B	7.0 ± 0.0	21.4 ± 0.4	32.3 ± 0.4	44.1 ± 4.17	13.13 ± 0.84	283.2 ± 14.8
<i>Lactobacillus kunkeei</i> LMG 18925 + pollen B	7.2 ± 0.1	21.2 ± 0.4	32.4 ± 0.2	33.8 ± 3.04	9.54 ± 0.75	285.6 ± 6.9
Control with pollen C	7.3 ± 0.2	21.7 ± 0.4	33.2 ± 0.5	27.9 ± 2.0	8.95 ± 0.55	323.8 ± 8.9
<i>Lactobacillus kunkeei</i> LMG 18925 + pollen C	7.1 ± 0.1	21.6 ± 0.3	32.6 ± 0.3	32.9 ± 3.5	9.54 ± 1.01	290.9 ± 5.3
Control with pollen substitute	7.8 ± 0.4	23.0 ± 0.0	33.1 ± 0.1	17.7 ± 1.08	--	323.5 ± 9.7
<i>Bifidobacterium actinocoloniiforme</i> R-53049 + pollen substitute	6.8 ± 0.1	22.7 ± 0.2	33.1 ± 0.5	18.7 ± 2.28	--	325.6 ± 4.0

Table S3. Taxonomic identification of the OTUs and their closest match in GenBank or EzBioCloud.

Identification of OTUs Phylum Class <u>Family</u> Genus	Matching basepairs to best match in <i>Bombus</i> or <i>Apis</i>	Name used here
Proteobacteria Betaproteobacteria		
<u>Neisseriaceae</u> <i>Snodgrassella</i>	253/253 JQ746649 <i>Snodgrassella alvi</i> strain wkB29	<i>Snodgrassella</i>
<u>Neisseriaceae</u>	251/253 HM215015 Uncultured Betaproteobacterium	<i>Neisseriaceae</i> sp.
Proteobacteria Gammaproteobacteria		
<u>Orbaceae</u> <i>Gilliamella</i>	253/253 JQ936676 <i>Gilliamella apicola</i> strain wkB30	<i>Gilliamella</i>
<u>Orbaceae</u> <i>Schmidhempelia</i>	253/253 HM215025 <i>Schmidhempelia</i> strain D08049A2	<i>Schmidhempelia</i>
Firmicutes Bacilli		
<u>Lactobacillaceae</u> <i>Lactobacillus</i>	253/253 LK054485 <i>Lactobacillus bombicola</i> LMG 28288 ^T	<i>L. bombicola</i>
<u>Lactobacillaceae</u> <i>Lactobacillus</i>	253/253 KJ078643 <i>Lactobacillus bombi</i> BTLCH M 1/2 ^T	<i>L. bombi</i>
<u>Lactobacillaceae</u> <i>Lactobacillus</i>	253/253 Y11374 <i>Lactobacillus kunkeei</i> YH-15 ^T	<i>L. kunkeei</i>
<u>Lactobacillaceae</u> <i>Lactobacillus</i>	253/253 Y17362 <i>Lactobacillus crispatus</i> DSM 20584 ^T	<i>L. crispatus</i>
<u>Lactobacillaceae</u> <i>Lactobacillus</i>	250/254 KJ078643 <i>Lactobacillus bombi</i> BTLCH M 1/2 ^T	<i>Lactobacillus</i> sp.
Actinobacteria Actinobacteria		
<u>Bifidobacteriaceae</u>	253/253 FJ858733 <i>Bombiscardovia coagulans</i> LISPASI-P3	<i>B. coagulans</i> LISPASI-P3
<u>Bifidobacteriaceae</u> <i>Bifidobacterium</i>	253/253 LK054489 <i>Bifidobacterium commune</i> LMG 28292 ^T	<i>B. commune</i>
<u>Bifidobacteriaceae</u> <i>Bifidobacterium</i>	252/253 AB437355 <i>Bifidobacterium asteroides</i> YIT 11866 ^T	<i>Bifidobacterium</i> sp.
<u>Bifidobacteriaceae</u> <i>Bifidobacterium</i>	253/253 JDUR01000035 <i>Bifidobacterium actinocolonii</i> forme DSM 22766 ^T	<i>B. actinocolonii</i> forme
Bacteroidetes Flavobacteria		
<u>Flavobacteriaceae</u>	253/253 HM215036 Uncultured Bacteroidetes	Bacteroidetes

Figure S1. The genetic distance of the OTUs with their closest bacterial family members for *Lactobacillaceae*. Species *L. crispatus* and *L. acidophilus* cannot be distinguished, based on the 254 bp sequence. We named OTU.88 *L. crispatus*, because this OTU appeared only once in the *L. crispatus* treatment, while *L. acidophilus* was never before observed in the gut of bumblebees. Also *L. kunkeei* and *L. apinorum* cannot be distinguished. Both were originally found in the honeybee crop (Olofsson and Vasquez 2008; Olofsson et al. 2014), but only *L. kunkeei* has before been detected in the gut of *Bombus* with culture-dependent techniques (unpublished data). We therefore assigned OTU.16 to *L. kunkeei*.

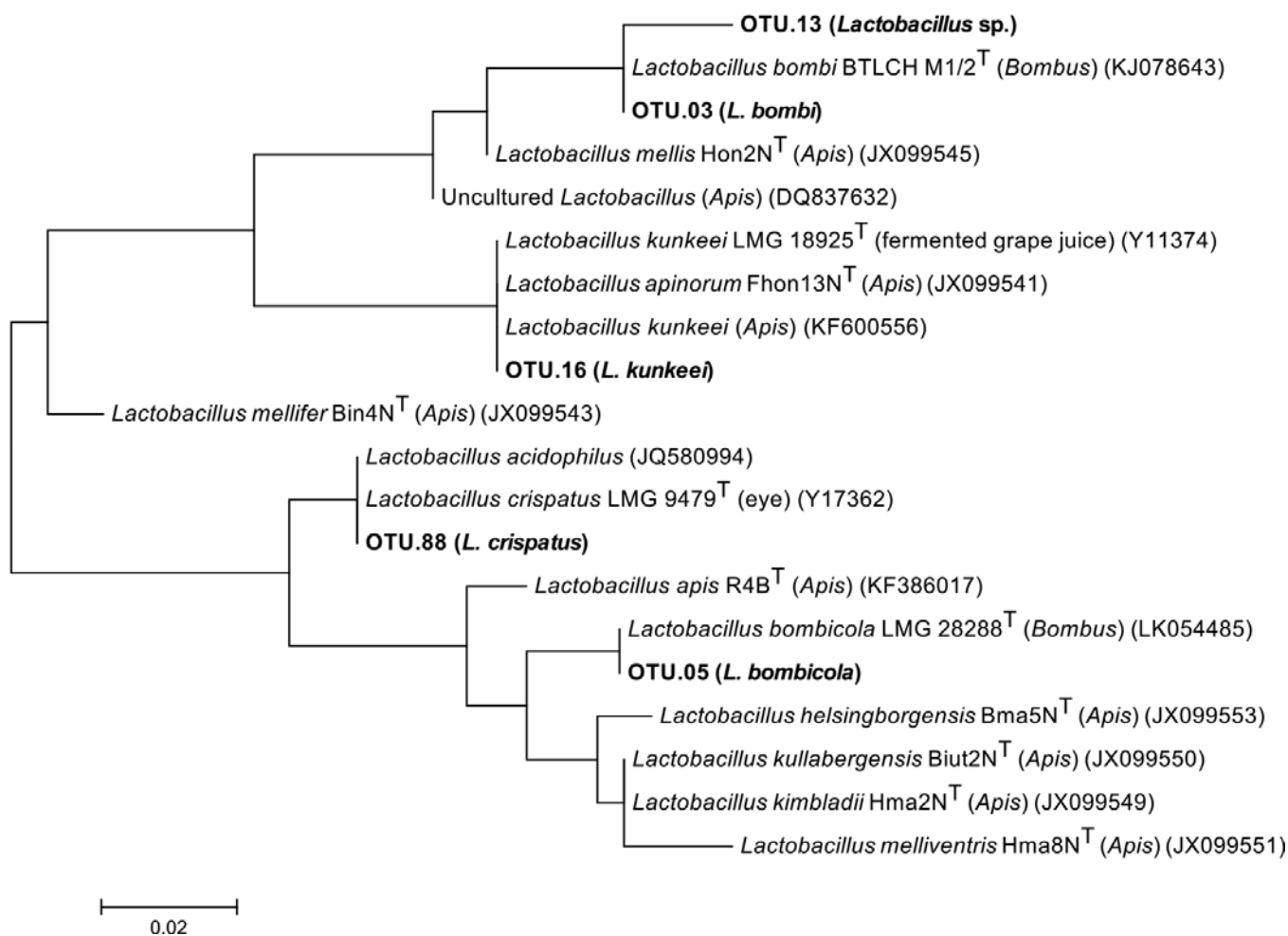


Figure S2. The genetic distance of the OTUs with their closest bacterial family members is shown for *Bifidobacteriaceae*, based on the 254 bp sequences.

