

# **Microbial consortia: An engineering tool to suppress clubroot of Chinese cabbage by changing the rhizosphere bacterial community composition**

Jinshao Zhang<sup>1,2,†</sup>, Waqar Ahmed<sup>1,2,†</sup>, Zhenlin Dai<sup>1,2,†</sup>, Xinghai Zhou<sup>1,2</sup>, Zulei He<sup>1,2</sup>, Lanfang Wei<sup>1,3\*</sup> and Guanghai Ji<sup>1,2\*</sup>

<sup>1</sup>State Key Laboratory for Conservation and Utilization of Bio-resources in Yunnan, Yunnan Agricultural University, Kunming 650201, Yunnan, China

<sup>2</sup>Key Laboratory of Agro-Biodiversity and Pest Management of Ministry of Education, Yunnan Agricultural University, Kunming 650201, Yunnan, China

<sup>3</sup>Agricultural Foundation Experiment Teaching Center, Yunnan Agricultural University, Kunming 650201, Yunnan, China

<sup>†</sup>These authors contributed equally to this work.

## **\*Correspondence:**

Guanghai Ji

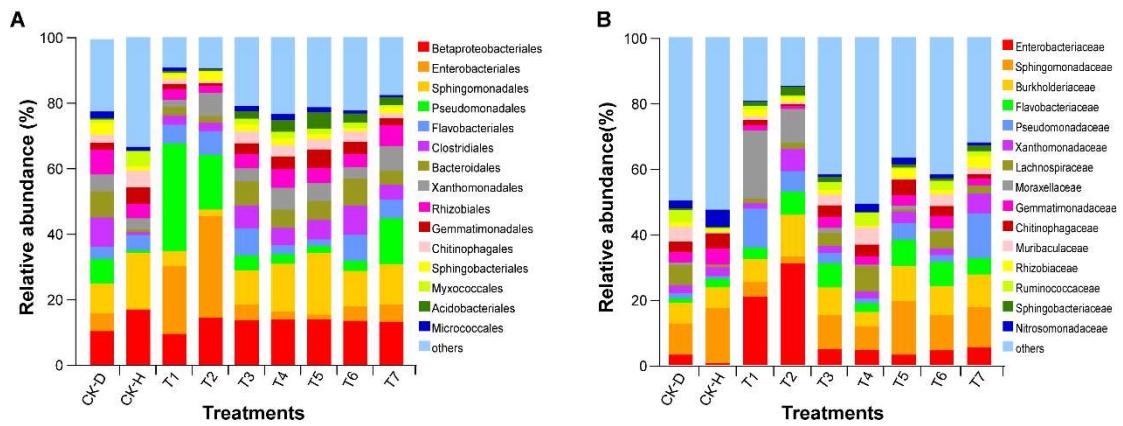
jghai001@163.com

Lanfang Wei

wlfang2000@aliyun.com



**Figure S1.** Effect of bacterial biocontrol strains as single strains, inter-/intra-genus, and microbial consortia on clubroot disease. Disease control (**CK-D**), healthy control (**CK-H**), single strain *Lysobacter antibioticus* 13-6 (**T1**), single strain *L. capsici* ZST1-2 (**T2**), single strain *Bacillus cereus* BT-23 (**T3**), intra-genus co-culture of *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T4**), inter-genus co-culture of *L. antibioticus* 13-6 + *B. cereus* BT-23 (**T5**), microbial consortium *B. cereus* BT-23 + *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T6**), and commercial fungicide Kejia (**T7**).



**Figure S2.** Relative abundance bar plots for top 15 most abundant bacterial communities at order (A) and family (B) level. Disease control (**CK-D**), healthy control (**CK-H**), single strain *Lysobacter antibioticus* 13-6 (**T1**), single strain *L. capsici* ZST1-2 (**T2**), single strain *Bacillus cereus* BT-23 (**T3**), intra-genus co-culture of *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T4**), inter-genus co-culture of *L. antibioticus* 13-6 + *B. cereus* BT-23 (**T5**), microbial consortia *B. cereus* BT-23 + *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T6**), and commercial fungicide Kejia (**T7**).

**Table S1.** Effect of bacterial biocontrol strains on the disease incidence, disease index, control effect, and soil pH of cabbage clubroot.

Treatment	Disease incidence (%)	Disease index	Control effect (%)	pH
CK-D	93.33±3.33a	76.28±9.43a	-	4.20±0.06b
CK-H	8.89±1.92e	14.29±2.57f	81.27±3.67a	4.45±0.02ab
T1	77.78±11.70b	37.96±12.57cd	50.23±9.09d	4.42±0.34ab
T2	75.56±6.94bc	31.51±3.13de	58.69±1.67c	4.46±0.04ab
T3	77.78±8.39b	50.20±9.43b	34.19±2.63f	4.27±0.16ab
T4	72.22±6.94bc	41.54±3.15c	45.54±3.29e	4.63±0.58ab
T5	68.89±6.69bcd	31.79±10.99de	58.32±8.07c	4.65±0.06ab
T6	56.67±8.82d	26.10±7.12e	65.78±5.83b	4.73±0.12a
T7	63.33±8.82cd	32.30±7.85d	57.66±6.65c	4.73±0.08a

Disease control (**CK-D**), healthy control (**CK-H**), single strain *Lysobacter antibioticus* 13-6 (**T1**), single strain *L. capsici* ZST1-2 (**T2**), single strain *Bacillus cereus* BT-23 (**T3**), intra-genus co-culture of *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T4**), inter-genus co-culture of *L. antibioticus* 13-6 + *B. cereus* BT-23 (**T5**), microbial consortia *B. cereus* BT-23 + *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T6**), and commercial fungicide Kejia (**T7**). Significant difference among treatments is shown by different small letters within a column according to Duncan's multiple range test at  $p < 0.05$ .

**Table S2.** Effect of bacterial biocontrol strains on yield of Chinese cabbage.

Treatment	Yield (Kg/Acre)	Rate of growth (%)
CK-D	7165.45±1068.49c	-
CK-H	16875.33±929.91a	135.51
T1	13167.53±899.23b	83.76
T2	14015.27±545.21b	95.66
T3	12748.88±174.78b	77.92
T4	13020.15±871.22b	81.71
T5	16070.65±764.12a	124.28
T6	17662.49±1545.05a	146.50
T7	16097.64±1746.36a	124.66

Disease control (**CK-D**), healthy control (**CK-H**), single strain *Lysobacter antibioticus* 13-6 (**T1**), single strain *L. capsici* ZST1-2 (**T2**), single strain *Bacillus cereus* BT-23 (**T3**), intra-genus co-culture of *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T4**), inter-genus co-culture of *L. antibioticus* 13-6 + *B. cereus* BT-23 (**T5**), microbial consortia *B. cereus* BT-23 + *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T6**), and commercial fungicide Kejia (**T7**). Significant difference among treatments is shown by different small letters within a column according to Duncan's multiple range test at  $p < 0.05$ .

**Table S3.** Effect of single strains, intra/inter-genus co-culture, and microbial consortia on alpha diversity of bacterial community associated with the rhizosphere of Chinese cabbage ( $\pm$ SEM, n = 3/treatment).

Treatment	Chao1	Goods_coverage	Shannon	Observed_species	Simpson	PD_whole_tree
CK-D	2694.36 $\pm$ 159.32c	0.98 $\pm$ 0a	6.32 $\pm$ 0.24d	1862.87 $\pm$ 108.13e	0.95 $\pm$ 0.01b	72.13 $\pm$ 3.26e
CK-H	3995.20 $\pm$ 169.91a	0.98 $\pm$ 0a	9.53 $\pm$ 0.27a	3235.27 $\pm$ 211.87a	1.00 $\pm$ 0a	108.94 $\pm$ 7.04a
T1	3433.88 $\pm$ 189.53ab	0.98 $\pm$ 0a	8.70 $\pm$ 1.08a	2632.20 $\pm$ 201.51c	0.98 $\pm$ 0.02a	92.95 $\pm$ 5.00a
T2	3453.39 $\pm$ 143.99ab	0.98 $\pm$ 0a	8.84 $\pm$ 0.33ab	2602.90 $\pm$ 126.72c	0.99 $\pm$ 0a	91.84 $\pm$ 3.61bc
T3	3089.13 $\pm$ 255.56b	0.98 $\pm$ 0a	8.06 $\pm$ 0.57c	2310.70 $\pm$ 233.38d	0.98 $\pm$ 0.01a	83.70 $\pm$ 5.85d
T4	3619.18 $\pm$ 207.86a	0.98 $\pm$ 0a	9.46 $\pm$ 0.18a	2858.70 $\pm$ 139.39b	1.00 $\pm$ 0a	100.42 $\pm$ 3.76a
T5	3411.68 $\pm$ 112.85ab	0.98 $\pm$ 0a	9.13 $\pm$ 0.27ab	2684.27 $\pm$ 117.31c	0.99 $\pm$ 0.01a	94.43 $\pm$ 2.49b
T6	3487.64 $\pm$ 127.3ab	0.98 $\pm$ 0a	9.23 $\pm$ 0.06a	2655.10 $\pm$ 87.67c	0.99 $\pm$ 0a	94.33 $\pm$ 2.58b
T7	3467.79 $\pm$ 257.75ab	0.98 $\pm$ 0a	8.50 $\pm$ 0.46b	2580.17 $\pm$ 214.62cd	0.99 $\pm$ 0a	90.37 $\pm$ 5.30c

Disease control (**CK-D**), healthy control (**CK-H**), single strain *Lysobacter antibioticus* 13-6 (**T1**), single strain *L. capsici* ZST1-2 (**T2**), single strain *Bacillus cereus* BT-23 (**T3**), intra-genus co-culture of *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T4**), inter-genus co-culture of *L. antibioticus* 13-6 + *B. cereus* BT-23 (**T5**), microbial consortia *B. cereus* BT-23 + *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T6**), and commercial fungicide Kejia (**T7**). Significant difference among treatments is shown by different small letters within a column according to *t*-test at  $p < 0.05$ .

**Table S4.** Relative abundance of 10 most dominant phyla under different experimental conditions ( $\pm$ SEM, n = 3/treatment).

Phylum	CK-D	CK-H	T1	T2	T3	T4	T5	T6	T7	Average (%)
Proteobacteria	61.04 $\pm$ 1.38abc	71.75 $\pm$ 9.83a	48.74 $\pm$ 3.10d	57.17 $\pm$ 5.83bcd	64.38 $\pm$ 3.87ab	48.48 $\pm$ 4.65d	53.69 $\pm$ 2.5bcd	50.94 $\pm$ 3.46cd	64.01 $\pm$ 3.81ab	57.80
Bacteroidetes	16.84 $\pm$ 2.39a	17.10 $\pm$ 9.35a	20.64 $\pm$ 1.64a	18.78 $\pm$ 3.70a	18.64 $\pm$ 2.46a	21.03 $\pm$ 2.18a	16.85 $\pm$ 4.09a	18.17 $\pm$ 2.38a	19.23 $\pm$ 2.66a	18.59
Firmicutes	1.73 $\pm$ 0.25d	4.19 $\pm$ 0.28cd	11.99 $\pm$ 1.08a	7.61 $\pm$ 1.37bc	6.26 $\pm$ 0.41c	13.73 $\pm$ 1.94a	7.88 $\pm$ 0.94bc	10.80 $\pm$ 0.64ab	4.95 $\pm$ 2.63cd	7.68
Actinobacteria	8.89 $\pm$ 1.14a	3.49 $\pm$ 0.55c	9.64 $\pm$ 2.43a	7.55 $\pm$ 0.46ab	5.01 $\pm$ 1.18bc	7.62 $\pm$ 1.12ab	8.85 $\pm$ 0.74a	8.87 $\pm$ 1.65a	5.72 $\pm$ 0.39bc	7.29
Gemmatimonadetes	5.83 $\pm$ 1.23a	1.38 $\pm$ 0.41d	3.68 $\pm$ 0.88bc	3.25 $\pm$ 0.76bc	2.05 $\pm$ 0.59cd	3.60 $\pm$ 0.30bc	4.55 $\pm$ 0.96ab	3.73 $\pm$ 0.47bc	2.88 $\pm$ 0.32bcd	3.44
Acidobacteria	3.08 $\pm$ 0.82bc	0.99 $\pm$ 0.28d	1.96 $\pm$ 0.43cd	3.53 $\pm$ 0.63bc	2.24 $\pm$ 1.4cd	1.98 $\pm$ 0.23cd	5.71 $\pm$ 1.74a	4.48 $\pm$ 0.28ab	1.77 $\pm$ 0.57cd	2.86
Cyanobacteria	0.14 $\pm$ 0.03e	0.38 $\pm$ 0.01de	1.60 $\pm$ 0.07a	0.86 $\pm$ 0.15bc	0.63 $\pm$ 0.12bcd	1.67 $\pm$ 0.34a	0.96 $\pm$ 0.14b	1.36 $\pm$ 0.12a	0.50 $\pm$ 0.24cde	0.90
Nitrospirae	1.62 $\pm$ 0.44a	0.15 $\pm$ 0.06c	0.40 $\pm$ 0.26bc	0.51 $\pm$ 0.1bc	0.26 $\pm$ 0.05bc	0.59 $\pm$ 0.13bc	0.59 $\pm$ 0.19bc	0.63 $\pm$ 0.12b	0.40 $\pm$ 0.11bc	0.57
Verrucomicrobia	0.14 $\pm$ 0.04cd	0.04 $\pm$ 0.01d	0.31 $\pm$ 0.05ab	0.16 $\pm$ 0.05c	0.11 $\pm$ 0.02cd	0.34 $\pm$ 0.09a	0.19 $\pm$ 0.02c	0.21 $\pm$ 0.05bc	0.10 $\pm$ 0.05cd	0.18
Low Abundance	0.69 $\pm$ 0.26abc	0.53 $\pm$ 0.24c	1.04 $\pm$ 0.18a	0.59 $\pm$ 0.15bc	0.42 $\pm$ 0.06c	0.95 $\pm$ 0.12ab	0.73 $\pm$ 0.05abc	0.8 $\pm$ 0.12abc	0.45 $\pm$ 0.18c	0.69

Disease control (**CK-D**), healthy control (**CK-H**), single strain *Lysobacter antibioticus* 13-6 (**T1**), single strain *L. capsici* ZST1-2 (**T2**), single strain *Bacillus cereus* BT-23 (**T3**), intra-genus co-culture of *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T4**), inter-genus co-culture of *L. antibioticus* 13-6 + *B. cereus* BT-23 (**T5**), microbial consortia *B. cereus* BT-23 + *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T6**), and commercial fungicide Kejia (**T7**). Significant difference among treatments is shown by different small letters within a column according to t-test at  $p < 0.05$ .

**Table S5.** Relative abundance of 20 most dominant genera in rhizosphere soil under different experimental conditions ( $\pm$ SEM, n = 3/treatment).

Genus	CK-D	CK-H	T1	T2	T3	T4	T5	T6	T7	Average (%)
<i>Sphingomonas</i>	14.02 $\pm$ 0.10a	3.70 $\pm$ 0.83e	8.28 $\pm$ 1.44cd	10.67 $\pm$ 1.25abc	5.66 $\pm$ 1.77de	6.73 $\pm$ 1.17cde	13.54 $\pm$ 3.61ab	10.94 $\pm$ 0.72abc	9.36 $\pm$ 3.15bcd	9.21
<i>Pseudomonas</i>	2.24 $\pm$ 2.20c	12.7 $\pm$ 3.90a	4.14 $\pm$ 1.94bc	8.53 $\pm$ 4.14ab	10.89 $\pm$ 1.03a	3.90 $\pm$ 2.87bc	2.53 $\pm$ 0.54c	2.70 $\pm$ 0.64c	11.07 $\pm$ 2.69a	6.52
<i>Flavobacterium</i>	5.31 $\pm$ 2.41a	8.71 $\pm$ 6.42a	3.45 $\pm$ 1.92a	6.09 $\pm$ 3.00a	7.38 $\pm$ 4.00a	2.59 $\pm$ 0.20a	3.61 $\pm$ 3.05a	4.53 $\pm$ 2.40a	7.49 $\pm$ 4.48a	5.46
<i>Acinetobacter</i>	0.38 $\pm$ 0.46b	11.41 $\pm$ 8.38a	0.93 $\pm$ 0.47b	0.97 $\pm$ 0.58b	4.42 $\pm$ 3.48b	1.24 $\pm$ 0.67b	0.79 $\pm$ 0.52b	0.70 $\pm$ 0.28b	2.25 $\pm$ 1.52b	2.57
<i>Citrobacter</i>	0.06 $\pm$ 0.03b	5.62 $\pm$ 3.05a	0.47 $\pm$ 0.11b	0.92 $\pm$ 0.99b	3.56 $\pm$ 3.46ab	1.01 $\pm$ 0.56b	0.23 $\pm$ 0.08b	0.34 $\pm$ 0.08b	1.97 $\pm$ 1.02b	1.58
<i>Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium</i>	1.02 $\pm$ 0.86b	2.06 $\pm$ 0.72ab	1.92 $\pm$ 0.95ab	1.48 $\pm$ 0.42ab	1.23 $\pm$ 0.2ab	0.79 $\pm$ 0.46b	0.96 $\pm$ 0.27b	1.09 $\pm$ 0.26ab	2.48 $\pm$ 0.67a	1.45
<i>Massilia</i>	1.05 $\pm$ 0.31ab	1.07 $\pm$ 0.09ab	0.88 $\pm$ 0.32b	1.87 $\pm$ 1.29ab	0.92 $\pm$ 0.48b	0.79 $\pm$ 0.32b	1.62 $\pm$ 0.5ab	2.02 $\pm$ 0.83ab	2.58 $\pm$ 1.01a	1.42
<i>Stenotrophomonas</i>	0.31 $\pm$ 0.30a	4.45 $\pm$ 4.89a	0.84 $\pm$ 0.64a	1.86 $\pm$ 1.31a	1.75 $\pm$ 0.20a	0.90 $\pm$ 0.69a	0.39 $\pm$ 0.23a	0.60 $\pm$ 0.22a	1.65 $\pm$ 0.38a	1.42
<i>Gemmimonas</i>	1.14 $\pm$ 0.06bcd	0.68 $\pm$ 0.18d	1.13 $\pm$ 0.29bcd	1.55 $\pm$ 0.39bc	1.04 $\pm$ 0.38cd	1.07 $\pm$ 0.19bcd	2.51 $\pm$ 0.52a	1.76 $\pm$ 0.28b	1.26 $\pm$ 0.08bcd	1.35
<i>Lysobacter</i>	0.46 $\pm$ 0.07c	1.80 $\pm$ 0.12a	1.49 $\pm$ 0.13ab	1.19 $\pm$ 0.18bc	1.18 $\pm$ 0.09bc	1.37 $\pm$ 0.11abc	1.47 $\pm$ 0.51ab	1.79 $\pm$ 1.21a	1.38 $\pm$ 0.27ab	1.35
<i>Bacteroides</i>	0.21 $\pm$ 0.08e	0.53 $\pm$ 0.02de	1.62 $\pm$ 0.20ab	1.18 $\pm$ 0.29abc	0.86 $\pm$ 0.1bcd	2.08 $\pm$ 0.42a	1.26 $\pm$ 0.07abc	1.37 $\pm$ 0.04ab	0.72 $\pm$ 0.42cde	1.09
<i>Bifidobacterium</i>	0.14 $\pm$ 0.03d	0.64 $\pm$ 0.01cd	1.71 $\pm$ 0.17a	0.88 $\pm$ 0.16bc	1.10 $\pm$ 0.51bc	1.82 $\pm$ 0.31a	0.86 $\pm$ 0.29bc	1.42 $\pm$ 0.06ab	0.55 $\pm$ 0.27cd	1.01
<i>Variovorax</i>	0.91 $\pm$ 0.46a	0.95 $\pm$ 0.07a	0.77 $\pm$ 0.20a	1.17 $\pm$ 0.20a	0.91 $\pm$ 0.36a	0.55 $\pm$ 0.22a	1.30 $\pm$ 0.52a	1.29 $\pm$ 0.23a	1.20 $\pm$ 0.47a	1.01
<i>Chryseobacterium</i>	0.31 $\pm$ 0.37b	1.91 $\pm$ 1.11a	0.59 $\pm$ 0.38b	0.85 $\pm$ 0.31ab	1.50 $\pm$ 0.09ab	1.02 $\pm$ 0.92ab	0.62 $\pm$ 0.13b	0.75 $\pm$ 0.37ab	1.26 $\pm$ 0.14ab	0.98
<i>Solanum_torvum</i>	0.08 $\pm$ 0.02e	0.41 $\pm$ 0.03de	1.62 $\pm$ 0.07a	0.87 $\pm$ 0.15bc	0.67 $\pm$ 0.15bcd	1.76 $\pm$ 0.36a	0.97 $\pm$ 0.15b	1.42 $\pm$ 0.11a	0.51 $\pm$ 0.26cd	0.92
<i>Altererythrobacter</i>	1.78 $\pm$ 0.12a	0.37 $\pm$ 0.00d	1.13 $\pm$ 0.29b	0.95 $\pm$ 0.08b	0.45 $\pm$ 0.12cd	0.83 $\pm$ 0.16bc	0.95 $\pm$ 0.16b	0.79 $\pm$ 0.16bcd	0.84 $\pm$ 0.41bc	0.90
<i>Escherichia-Shigella</i>	0.22 $\pm$ 0.08d	0.39 $\pm$ 0.15cd	1.41 $\pm$ 0.19a	0.88 $\pm$ 0.21b	0.59 $\pm$ 0.02c	1.69 $\pm$ 0.23a	0.77 $\pm$ 0.07b	1.43 $\pm$ 0.06a	0.49 $\pm$ 0.18c	0.87
<i>Acidovorax</i>	0.72 $\pm$ 0.62c	1.57 $\pm$ 0.63b	0.55 $\pm$ 0.32cd	0.49 $\pm$ 0.32cd	0.75 $\pm$ 0.40c	0.46 $\pm$ 0.29cd	0.18 $\pm$ 0.09d	0.35 $\pm$ 0.17d	2.58 $\pm$ 0.72a	0.85
<i>Sphingobacterium</i>	0.13 $\pm$ 0.12d	1.35 $\pm$ 0.64ab	0.96 $\pm$ 1.20c	0.78 $\pm$ 0.61b	1.78 $\pm$ 0.51a	0.24 $\pm$ 0.19d	0.22 $\pm$ 0.14d	0.21 $\pm$ 0.05d	1.35 $\pm$ 0.20ab	0.78
<i>Bacillus</i>	0.18 $\pm$ 0.08a	0.17 $\pm$ 0.14a	0.13 $\pm$ 0.07a	0.17 $\pm$ 0.06a	0.19 $\pm$ 0.12a	0.11 $\pm$ 0.01a	0.17 $\pm$ 0.03a	0.20 $\pm$ 0.08a	0.14 $\pm$ 0.04a	0.16

Disease control (CK-D), healthy control (CK-H), single strain *Lysobacter antibioticus* 13-6 (T1), single strain *L. capsici* ZST1-2 (T2), single strain *Bacillus cereus* BT-23 (T3), intra-genus co-culture of *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (T4), inter-genus co-culture of *L. antibioticus* 13-6 + *B. cereus* BT-23 (T5), microbial consortia *B. cereus* BT-23 + *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (T6), and commercial fungicide Kejia (T7). Significant difference among treatments is shown by different small letters within a column according to t-test at  $p < 0.05$ .