



Engineered live bacteria suppress *Pseudomonas aeruginosa* infection in mouse lung and dissolve endotracheal-tube biofilms

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SUPPLEMENTARY TABLES:

Supplementary Table 1. Histopathologic evaluation of the lung lobes (left, right cranial and right medium) of mice inoculated with 1×10^7 CFU of the WT or CV2 *M. pneumoniae* strains or with PBS at 2, 14 or 45 days post-infection (dpi). Data represent the average and the SD of the evaluations from five mice per group. The scoring system is detailed in the Methods section.

Time post-infection	Group	Peribronchiolar/bronchial infiltrate (%)			Quality of peribronchiolar/bronchial infiltrates			Bronchiolar/bronchial luminal exudate			Perivasular infiltrate (%)			Parenchymal pneumonia			Total score (0-26)			
		Left	RCr	RM	Left	RCr	RM	Left	RCr	RM	Left	RCr	RM	Left	RCr	RM	Left	RCr	RM	
2 dpi	PBS	Average	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.2	0.2	0.0	
	PBS	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.0	0.0	0.0	0.0	0.4	0.4	0.0	
	WT	Average	0.8	0.4	1.0	0.8	0.4	1.0	2.0	1.2	1.6	1.8	1.8	1.6	2.2	2.8	3.0	13. 2	9.8	13.4
	WT	SD	0.4	0.5	0.0	0.4	0.5	0.0	0.0	1.0	0.8	0.4	0.4	0.5	1.9	1.6	0.0	2.1	4.5	2.7
	CV2	Average	0.8	0.2	0.4	0.8	0.2	0.4	0.8	0.8	0.4	1.6	1.0	1.4	0.6	1.2	0.0	7.8	5.4	4.2
	CV2	SD	0.4	0.4	0.5	0.4	0.4	0.5	1.0	1.0	0.8	0.5	0.0	0.5	1.2	1.5	0.0	4.0	3.0	3.9
	PBS	Average	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.2	0.2	0.0	
	PBS	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.0	0.0	0.0	0.0	0.4	0.4	0.0	
14 dpi	WT	Average	0.8	0.4	1.0	0.8	0.8	0.8	2.0	1.2	1.6	1.8	1.8	1.6	2.2	2.8	3.0	13. 2	9.8	13.4
	WT	SD	0.4	0.5	0.0	0.4	0.4	0.4	0.0	1.0	0.8	0.4	0.4	0.5	1.9	1.6	0.0	2.1	4.5	2.7
	CV2	Average	0.8	0.2	0.4	0.0	0.0	0.0	0.8	0.8	0.4	1.6	1.0	1.4	0.6	1.2	0.0	7.8	5.4	4.2
	CV2	SD	0.4	0.4	0.5	0.0	0.0	0.0	1.0	1.0	0.8	0.5	0.0	0.5	1.2	1.5	0.0	4.0	3.0	3.9
45 dpi	PBS	Average	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.2	0.2	0.0	
	PBS	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.0	0.0	0.0	0.0	0.4	0.4	0.0	
	WT	Average	0.8	0.4	1.0	0.0	0.0	0.0	2.0	1.2	1.6	1.8	1.8	1.6	2.2	2.8	3.0	13. 2	9.8	13.4
	WT	SD	0.4	0.5	0.0	0.0	0.0	0.0	0.0	1.0	0.8	0.4	0.4	0.5	1.9	1.6	0.0	2.1	4.5	2.7
	CV2	Average	0.8	0.2	0.4	0.0	0.0	0.0	0.8	0.8	0.4	1.6	1.0	1.4	0.6	1.2	0.0	7.8	5.4	4.2
	CV2	SD	0.4	0.4	0.5	0.0	0.0	0.0	1.0	1.0	0.8	0.5	0.0	0.5	1.2	1.5	0.0	4.0	3.0	3.9

Supplementary Table 2. Gene expression of cytokines analyzed in lungs of mice inoculated with WT or CV2 *M. pneumoniae* or with PBS. Analysis was performed in three major lung lobes (RCr, right cranial; RM, right middle; and L, left). Values are presented as mean \pm SD; n = 5 mice per group.

Gene	Group	2 dpi			14 dpi			45 dpi		
		RCr	RM	L	RCr	RM	L	RCr	RM	L
<i>tnf</i>	PBS	1 \pm 1.2	1 \pm 0.4	1 \pm 0.9	1 \pm 1.0	1 \pm 1.0	1 \pm 0.8	1 \pm 0.7	1 \pm 0.8	1 \pm 0.0
	WT	2.8 \pm 2.4	3.1 \pm 2.4	3.3 \pm 0.2	3.4 \pm 2.5	1.9 \pm 0.2	2.2 \pm 0.0	1.4 \pm 0.5	2.11 \pm 1.6	2.2 \pm 1.4
	CV2	1.1 \pm 0.3	1.8 \pm 0.6	1.5 \pm 0.8	0.5 \pm 0.4	0.4 \pm 0.7	0.4 \pm 0.1	1.2 \pm 0.4	1.7 \pm 0.3	1.9 \pm 0.3
<i>cxcl1</i>	PBS	1 \pm 0.3	1 \pm 0.3	1 \pm 0.9	1 \pm 0.5	1 \pm 0.4	1 \pm 0.6	1 \pm 0.2	1 \pm 0.3	1 \pm 0.8
	WT	3.8 \pm 1.6	3.5 \pm 1.8	4.4 \pm 0.1	0.8 \pm 0.6	0.7 \pm 0.2	0.1 \pm 0.0	1.2 \pm 0.6	1.2 \pm 0.8	1.9 \pm 0.0
	CV2	1.3 \pm 0.7	0.94 \pm 0.4	1.3 \pm 0.7	0.4 \pm 0.4	0.3 \pm 0.1	0.2 \pm 0.1	0.7 \pm 0.3	0.7 \pm 0.6	0.9 \pm 0.2
<i>ccl3</i>	PBS	1 \pm 0.3	1 \pm 0.5	1 \pm 0.6	1 \pm 0.4	1 \pm 0.7	1 \pm 0.5	1 \pm 0.6	1 \pm 0.7	1 \pm 0.5
	WT	4.0 \pm 0.7	3.2 \pm 2.4	4.4 \pm 0.1	2.7 \pm 1.3	1.6 \pm 0.4	1 \pm 0.0	0.7 \pm 0.7	1.5 \pm 0.8	1 \pm 0.5
	CV2	1.9 \pm 0.1	1.2 \pm 0.5	2.2 \pm 0.8	1.5 \pm 0.6	1.5 \pm 1.4	1.7 \pm 0.4	0.8 \pm 0.5	1.0 \pm 0.4	1.6 \pm 0.2
<i>ccl2</i>	PBS	1 \pm 0.3	1 \pm 0.2	1 \pm 0.5	1 \pm 0.5	1 \pm 0.4	1 \pm 0.5	1 \pm 0.4	1 \pm 0.3	1 \pm 0.0
	WT	3.8 \pm 0.7	2.8 \pm 1.1	2.4 \pm 0.3	2.4 \pm 1.3	1.9 \pm 0.5	2.2 \pm 0.0	2.3 \pm 3.4	1.3 \pm 0.4	1.5 \pm 0.9
	CV2	1.4 \pm 0.3	0.8 \pm 0.2	0.9 \pm 0.6	1.1 \pm 0.2	1.3 \pm 0.7	1.8 \pm 0.3	0.8 \pm 0.5	0.6 \pm 1.1	1.3 \pm 0.6
<i>ifng</i>	PBS	1 \pm 0.3	1 \pm 0.6	1 \pm 0.6	1 \pm 0.6	1 \pm 0.6	1 \pm 0.1	1 \pm 0.1	1 \pm 0.6	1 \pm 0.5
	WT	4.5 \pm 2.2	5.8 \pm 3.4	5.7 \pm 3.0	2.6 \pm 1.4	1.1 \pm 0.9	0.6 \pm 0.0	1.7 \pm 0.5	2.54 \pm 1.2	1.8 \pm 0.6
	CV2	1.0 \pm 0.5	0.5 \pm 0.1	1.3 \pm 0.6	1.1 \pm 0.7	0.9 \pm 0.6	0.7 \pm 0.1	2.0 \pm 0.7	1.45 \pm 1.7	1.7 \pm 1.7
<i>il1b</i>	PBS	1 \pm 0.5	1 \pm 0.3	1 \pm 0.7	1 \pm 0.4	1 \pm 0.4	1 \pm 0.3	1 \pm 0.3	1 \pm 0.2	1 \pm 0.2
	WT	3.3 \pm 1.5	2.8 \pm 1.5	2.9 \pm 1.7	1.4 \pm 0.1	1.0 \pm 0.4	0.9 \pm 0.0	0.9 \pm 0.2	1.0 \pm 0.4	1.3 \pm 0.1
	CV2	1.6 \pm 0.7	1.2 \pm 0.1	1.4 \pm 0.9	1.1 \pm 0.8	0.6 \pm 0.1	1.1 \pm 0.4	0.7 \pm 0.3	0.5 \pm 0.5	1.4 \pm 0.9
<i>il6</i>	PBS	1 \pm 0.3	1 \pm 0.1	1 \pm 0.8	1 \pm 0.3	1 \pm 0.6	1 \pm 0.8	1 \pm 0.8	1 \pm 0.0	1 \pm 0.1
	WT	0.7 \pm 0.5	0.4 \pm 0.2	0.5 \pm 0.2	2.3 \pm 0.7	2.1 \pm 0.2	1.9 \pm 0.0	1.5 \pm 0.3	1.1 \pm 0.2	0.8 \pm 0.0
	CV2	0.4 \pm 0.1	0.8 \pm 0.5	0.7 \pm 0.3	0.9 \pm 0.3	0.9 \pm 0.4	1.2 \pm 0.2	0.7 \pm 0.2	0.5 \pm 0.4	1.1 \pm 0.5
<i>il12a</i>	PBS	1 \pm 0.3	1 \pm 0.3	1 \pm 0.5	1 \pm 0.5	1 \pm 0.5	1 \pm 0.3	1 \pm 0.3	1 \pm 0.4	1 \pm 0.1
	WT	3.3 \pm 1.5	4.2 \pm 1.6	2.7 \pm 1.6	2.1 \pm 0.8	0.9 \pm 0.4	1.2 \pm 0.0	1.4 \pm 0.9	1.7 \pm 0.9	2.2 \pm 1.2
	CV2	1.7 \pm 1.0	1.7 \pm 0.9	1.5 \pm 1.2	1.3 \pm 0.6	0.5 \pm 0.2	1.6 \pm 0.4	1.6 \pm 1.2	1.0 \pm 0.7	1.5 \pm 0.7
<i>il23a</i>	PBS	1 \pm 0.6	1 \pm 0.3	1 \pm 0.5	1 \pm 0.5	1 \pm 0.5	1 \pm 0.3	1 \pm 0.4	1 \pm 0.4	1 \pm 0.5
	WT	1.3 \pm 0.2	1.0 \pm 0.1	0.9 \pm 0.5	0.7 \pm 0.4	0.9 \pm 0.5	0.3 \pm 0.0	1.0 \pm 2.2	0.9 \pm 1.2	1.0 \pm 0.4
	CV2	1.1 \pm 0.6	1.2 \pm 0.5	0.5 \pm 0.3	0.8 \pm 0.6	1.0 \pm 0.7	0.9 \pm 0.6	0.7 \pm 0.4	0.6 \pm 0.3	1 \pm 0.6

Supplementary Table 3. Description of different vectors used to obtain the different engineered strains.

Vector name	Vector description	Transformed strain	Obtained strain name
pMTnCmLox_EfTu_MPN142(OPT)_AI-II'	Minitransposon vector used to obtain strains that express and secrete AI-II'	WT and CV2	WT_A; CV2_A
pMTnTclox_EfTu_MPN142(OPT)_PeIAh_Flag	Minitransposon vector used to obtain strains that express and secrete PeIAh	WT and CV2	WT_PeIAh; CV2_PeIAh
pMTnTclox_Eftu_MPN142(OPT)_PslGh_Flag	Minitransposon vector used to obtain strains that express and secrete PslGh	WT and CV2	WT_PslGh; CV2_PslGh
pMTnTclox_EfTu_MPN142(OPT)_PeIAh_Flag_Eftu_MPN142(OPT)_PslGh_Flag	Minitransposon vector used to obtain strains that express and secrete PeIAh and PslGh	WT and CV2; WT_A and CV2_A	WT_H; CV2_H; WT_HA; CV2_HA
pMTnGmLox_P3_MPN142(OPT2)_pyocin_L1	Minitransposon vector used to express and secrete of PyocinL1 without Flag	WT; CV2 WT_HA and CV2_HA	WT_PL1; CV2_PL1; WT_HA_PL1 and CV2_HA_PL1
pMTnGmLox_P3_MPN142(OPT2)_pyocin_S5	Vector to test secretion of PyocinS5 without Flag. Expression of gene under P3 promoter	WT	WT_PS5

Supplementary Table 4. Average values of areas under curve (AUC) for the three best peptides obtained by MS for the heterologous proteins identified in cells and supernatants (SPN) of different *M. pneumoniae* strains. (ND, Not Detected).

Protein	CV2_HA (Cells)	CV2_HA (SPN)	CV2_A1 -II' (Cells)	CV2_A1-II' (SPN)	CV2_PelAh- PslGh (Cells)	CV2_PelAh- PslGh (SPN)	CV2_HA_ P1 (Cells)	CV2_HA_ P1 (SPN)
AI-II'	8.27E+07	9.87E+07	5.87E+07	3.83E+07	ND	ND	6.97E+07	2.17E+08
PelAh	1.43E+08	2.40E+07	ND	ND	1.43E+08	8.53E+07	1.40E+08	6.30E+07
PslGh	8.87E+08	4.10E+07	ND	ND	8.27E+08	9.97E+07	9.00E+08	6.40E+07
Pyocin- L1	ND	ND	ND	ND	ND	ND	7.27E+08	7.85E+07

Supplementary Table 5. Histopathologic evaluation of the lung lobes (left, right cranial and right medium) of mice inoculated with 1×10^8 CFU of the WT or CV2_HA_P1 *M. pneumoniae* strains or with PBS at 2-, 14- and 45 days post-infection (dpi). The indicated numbers represent the average and the SD of the evaluations from five mice per group. The scoring system is detailed in the Methods section.

		Peribronchial/bronchial infiltrate (%)			Quality of peribronchial/bronchial infiltrates			Bronchiolar/bronchial luminal exudate			Perivasculat infiltrate (%)			Parenchymal pneumonia			Total score (0-26)		
Time post-infection	Group	Left	RCr	RM	Left	RCr	RM	Left	RCr	RM	Left	RCr	RM	Left	RCr	RM	Left	RCr	RM
2 dpi	PBS	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.2	0.2	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.0	0.0	0.0	0.0	0.4	0.4	0.0
	WT	Mean	1.4	1.6	1.4	1.4	1.6	1.4	2.0	1.8	1.2	2.0	1.4	1.4	3.2	2.8	1.6	16.8	16.0
		SD	0.5	0.5	0.5	0.8	0.5	0.5	0.0	0.4	0.4	0.6	0.5	0.5	1.8	1.6	2.1	4.2	4.7
	CV2_HA_P1	Mean	0.4	0.6	0.2	0.4	0.6	0.2	1.6	1.4	1.6	1.6	1.8	2.0	0.6	1.2	0.6	8.6	9.6
		SD	0.5	0.5	0.4	0.5	0.5	0.4	0.5	0.5	0.5	0.8	0.4	0.6	1.2	1.5	1.2	3.2	1.6
	PBS	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.2	0.2	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.0	0.0	0.0	0.0	0.4	0.4	0.0
14 dpi	WT	Mean	0.2	0.4	0.2	0.4	0.4	0.2	0.8	0.6	0.4	1.6	2.0	1.6	1.2	0.6	0.6	6.6	6.0
		SD	0.4	0.5	0.4	0.8	0.5	0.4	0.4	0.5	0.5	0.0	0.5	1.5	1.2	1.2	1.7	2.7	2.8
	CV2_HA_P1	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	1.0	1.2	1.2	0.6	0.6	2.6	1.6	1.8
		SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.6	0.4	1.5	1.2	1.2	1.6	1.7	1.2
	PBS	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.2	0.2	0.0
45 dpi	WT	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.0	0.0	0.0	0.0	0.4	0.4	0.0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	0.6	1.2	0.6	1.6	2.2	1.6
	CV2_HA_P1	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	1.2	1.5	1.2	1.2	2.3	1.2	
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.0	0.0	0.0	0.2	0.2	0.2	
		SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.4	0.0	0.0	0.0	0.4	0.4	0.4	

Supplementary Table 6. Gene expression of cytokines analyzed in lungs of mice inoculated with 1×10^8 CFU of WT or CV2_HA_P1 *M. pneumoniae* or with PBS. Analysis was performed on the left lung and values are presented as mean \pm SD ($n = 5$ mice per group).

Gene	Group	2 dpi	14 dpi
<i>tnf</i>	PBS	1.0 ± 0.5	1.0 ± 0.5
	WT	18.0 ± 1.8	3.0 ± 0.7
	CV2_HA_P1	5.1 ± 2.2	9.5 ± 3.5
<i>cxcl1</i>	PBS	1.0 ± 0.5	1.0 ± 0.5
	WT	715.4 ± 246.0	3.4 ± 1.5
	CV2_HA_P1	343.3 ± 281.9	1.25 ± 0.3
<i>ccl3</i>	PBS	1.0 ± 0.67	9.4 ± 3.5
	WT	85.4 ± 62.9	3.0 ± 0.7
	CV2_HA_P1	31.6 ± 32.6	1.0 ± 0.5
<i>ccl2</i>	PBS	1.0 ± 0.5	1.0 ± 0.5
	WT	145.4 ± 18.4	3.4 ± 1.5
	CV2_HA_P1	115.2 ± 42.0	1.3 ± 0.3
<i>Ifng</i>	PBS	1.0 ± 0.3	1.0 ± 0.8
	WT	55.8 ± 7.0	0.9 ± 0.2
	CV2_HA_P1	26.6 ± 17.7	0.7 ± 0.1
<i>il1b</i>	PBS	1.0 ± 0.44	1.0 ± 0.4
	WT	40.3 ± 1.9	2.9 ± 1.0
	CV2_HA_P1	12.5 ± 1.4	1.7 ± 0.9
<i>il6</i>	PBS	1.0 ± 0.5	1.0 ± 0.9
	WT	65.9 ± 18.9	0.5 ± 0.1
	CV2_HA_P1	26.4 ± 22.7	0.4 ± 0.1
<i>il12a</i>	PBS	1.0 ± 0.5	1.0 ± 0.7
	WT	3.1 ± 0.9	1.5 ± 0.5
	CV2_HA_P1	2.1 ± 0.9	1.2 ± 0.4
<i>il23a</i>	PBS	1.0 ± 0.5	1.0 ± 0.5
	WT	13.4 ± 2.9	2.1 ± 0.3
	CV2_HA_P1	5.7 ± 0.9	1.4 ± 0.2

Supplementary Table 7. Oligonucleotides used in the qPCR to analyze the inflammation markers in lungs.

Gene	Forward (F)/reverse (R)	Sequence (5'-3')
<i>gapdh</i>	F	CCCACTAACATCAAATGGGG
	R	CCTTCCACAATGCCAAAGTT
<i>tnf</i>	F	AGGCACACTCCCCAAAAGA
	R	GCTCCTCCACTGGTGGTTT
<i>cxcl1</i>	F	GACAG ACTGCTCTGATGGCA
	R	TGCAC TTCTTCG CACAAC
<i>ccl3</i>	F	CCCAGCCAGGTGTCATTTC
	R	GCATT CAGTTCCAGGT CAGTG
<i>ccl2</i>	F	CTTCTGGGCCTGCTGTTCA
	R	CCAGCCTACTCATTGGGATCA
<i>ifn</i>	F	CTGGCAAAGGATGGTGAC
	R	GCTGATGGCCTGATTGTCTT
<i>il1b</i>	F	GCTGCTTCAAACCTTGAC
	R	AGCTTCTCCACAGCCACAAT
<i>il6</i>	F	TGATGCACTTGCAGAAAACA
	R	GGTCTTGGTCCTTAGCCACTC
<i>il12a</i>	F	CAGCACCAAGCTTCTCATCAG
	R	TCTTCAAAGGCTTCATCTGCAA
<i>il23a</i>	F	TGCTGGATTGCAGAGCAGTA
	R	GCATGCAGAGATTCCGAGAGA