

Supplementary Information

Difficidin and bacilysin from *Bacillus amyloliquefaciens* FZB42 have antibacterial activity against *Xanthomonas oryzae* rice pathogens

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Table S1. Bacterial strains used in this study.

Strain	Description	Source or reference
<i>B. amyloliquefaciens</i>		
FZB42	Wild type, producer of lipopeptides, polyketides and bacilysin	8
AK1	$\Delta bmyA::Em^r$, deficient in bacillomycin D synthesis	8
AK2	$\Delta fenA::Cm^r$, deficient in fengycin synthesis	8
CH1	$\Delta srfA::Em^r$, deficient in surfactin synthesis	8
CH3	$\Delta sfp::Em^r$, deficient in lipopeptides and polyketides synthesis	10
CH6	$\Delta bae::Cm^r$, no synthesis of bacillaene	10
CH7	$\Delta mln::Cm^r$, no synthesis of macrolactin	10
CH8	$\Delta dfn::Em^r$, no synthesis of difficidin	10
RS2	$\Delta bac::Cm^r \Delta dfn::Em^r$, deficient in bacilysin and difficidin	26
RS6	$\Delta sfp::Em^r \Delta bac::Cm^r$, no lipopeptides, polyketides and bacilysin	21
Rice pathogenic bacteria		
PXO99 ^A	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> , causal agent of bacterial blight	27
RS105	<i>Xanthomonas oryzae</i> pv. <i>oryzicola</i> , causal agent of bacterial leaf streak,	2
	Rif ^r	

Table S2. DNA primers used in this study.

<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>		<i>Xanthomonas oryzae</i> pv. <i>oryzicola</i>	
Name	Sequence (5'→3')	Name	Sequence (5'→3')
16S-F	TGGCAACTAAGGACAAGGG	16S-F	AATGGGCGCAAGCCTGATC
16S-R	AAGCGGTGGAGTATGTGG	16S-R	AACCACCACCTACGCACGC
rpfF-F	GTTGGTCTGATAGCGGGTGA	rpfF-F	TAGCGGGTGATGTCGTCG
rpfF-R	TGAAGAACCGCAGCGTGAG	rpfF-R	TGAAGAACCGCAGCGTGAG
gumD-F	TTTCATAGTGC GTGTTGTGCT	gumD-F	AACGACGCACGCATCACG
gumD-R	AACGACGCACGCATCACG	gumD-R	ATCCAGCCACAGCGACCAAC
ftsZ-F	CTGCTGGACGATGTGAACCTG	ftsZ-F	CCGTGATGTCGGAAATGGG
ftsZ-R	ATAATCGTTGGGCAGGTCAGC	ftsZ-R	GCCAGGTTACATCGTCCAG
glmS-F	CCCCAGCGATTTGGCGTAC	glmS-F	ACGATCAGATGCTGTGGGTTC
glmS-R	GCGTATGCGAACCCACAGC	glmS-R	GCCAAGGGTTTCCCGTCAT
rrlA-F	ACAGGGTGGTATTTCAAGG	rrlA-F	GCAAACCTCCGAATACCAG
rrlA-R	GCCGCTGCGACTGTTTAT	rrlA-R	CAACCTCCTGGCTGTCTAT

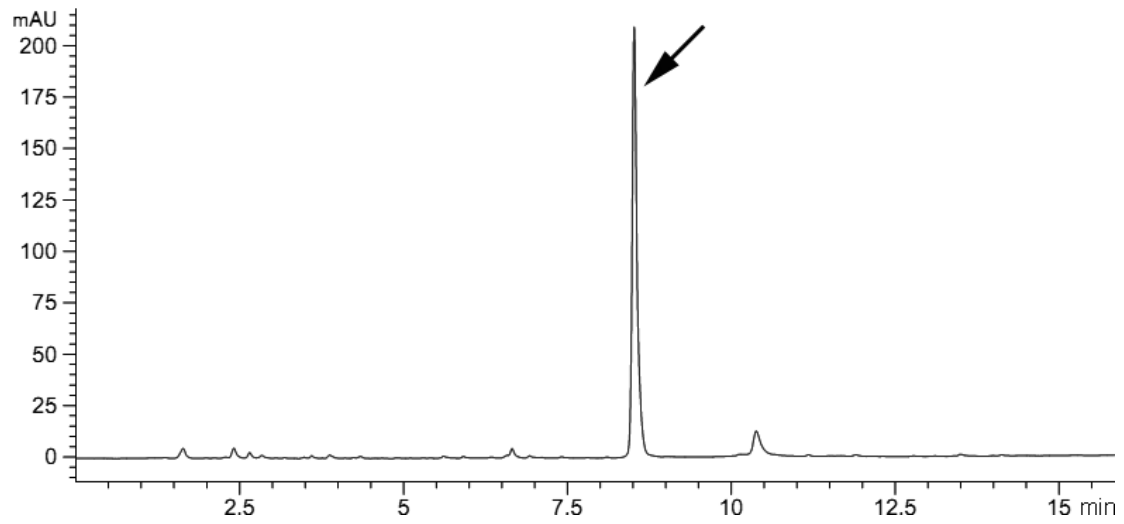


Fig. S1. HPLC analysis of difficidin from *B. amyloliquefaciens* FZB42. The retention time of difficidin is 8.574 min

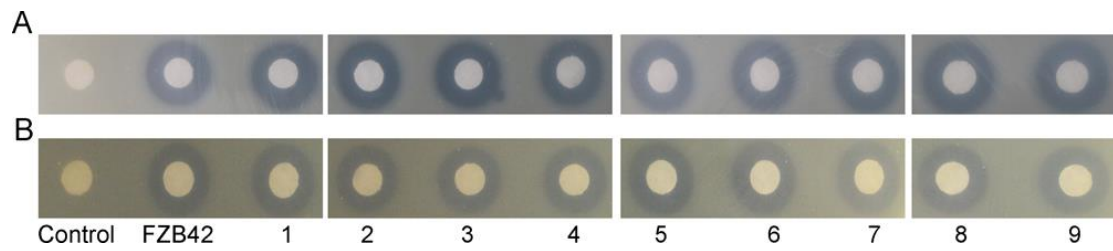


Fig. S2. Detection of antagonistic action of *B. amyloliquefaciens* mutants strains against *Xanthomonas oryzae* pv. *oryzae* (A) and *Xanthomonas oryzae* pv. *oryzicola* (B) by paper-disc agar diffusion assay. 1, complemented FZB42 Δ *sfp* Δ *amyE::sfp*; 2, complemented FZB42 Δ *bmyA* Δ *amyE::bmyA*; 3, complemented FZB42 Δ *fenA* Δ *amyE::fenA*; 4, complemented FZB42 Δ *srfA* Δ *amyE::srfA*; 5, complemented FZB42 Δ *bae* Δ *amyE::bae*; 6, complemented FZB42 Δ *mln* Δ *amyE::mln*; 7, complemented FZB42 Δ *dfn* Δ *amyE::dfn*; 8, complementation of RS6; 9, complementation of RS2.

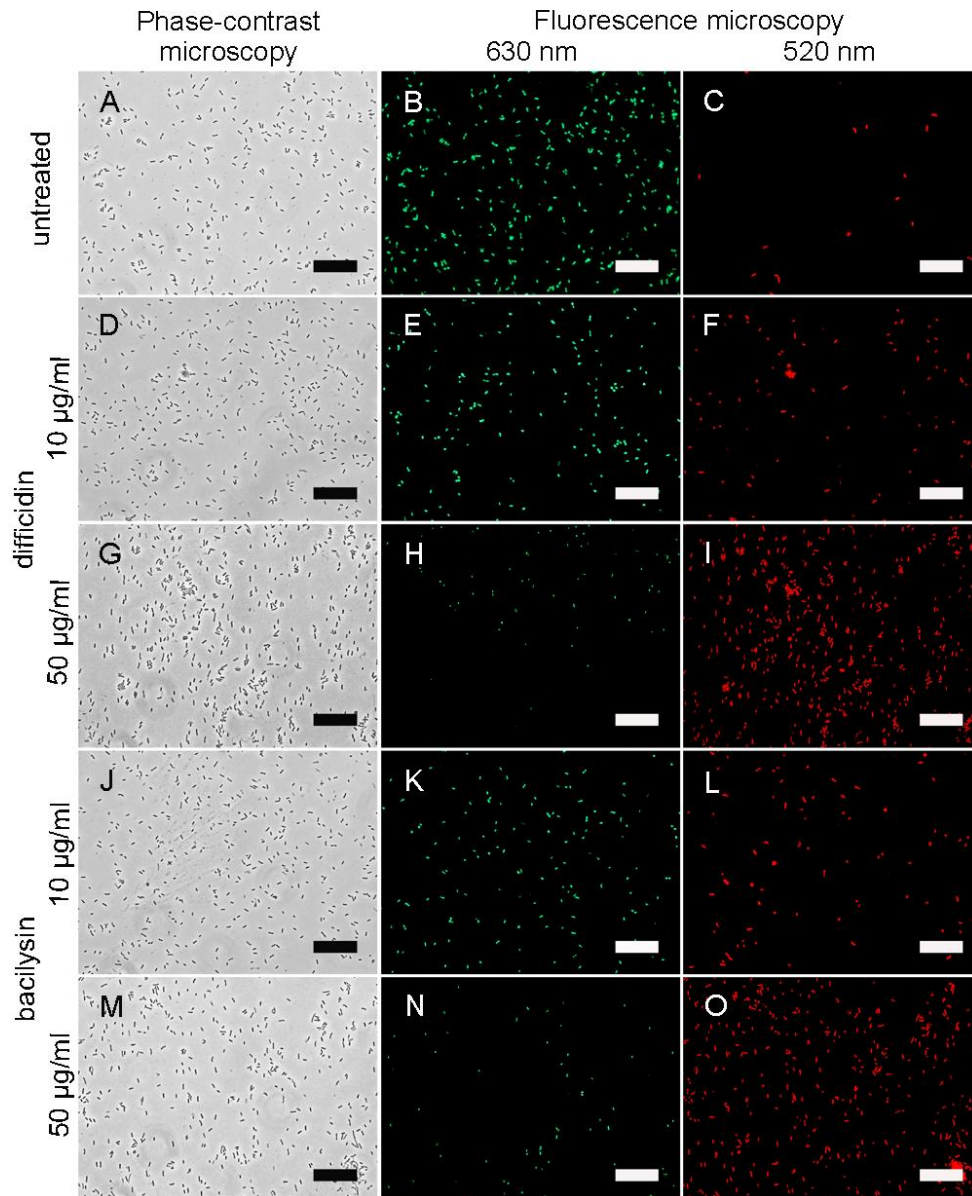


Fig. S3. Phase-contrast and fluorescence microscope images of *Xanthomonas oryzae* pv.

***oryzae* cells in the absence and presence of diffcidin or bacilysin after 12 h of cultivation.**

Staining using the LIVE/DEAD BacLight bacterial viability kit assessed bacterial viability: green areas indicate live cells; red areas indicate dead cells. Untreated *X. oryzae* pv. *oryzae* cells were observed by A, phase-contrast microscopy and B-C, fluorescence microscopy. *X. oryzae* pv. *oryzae* cells after treatments with diffcidin (10 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$) were examined by D and G, phase-contrast microscopy and E-F and H-I, fluorescence microscopy. Pictures were taken after treatments with bacilysin (10 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$) from the J to L and M to O. Bars indicate 20 μm .

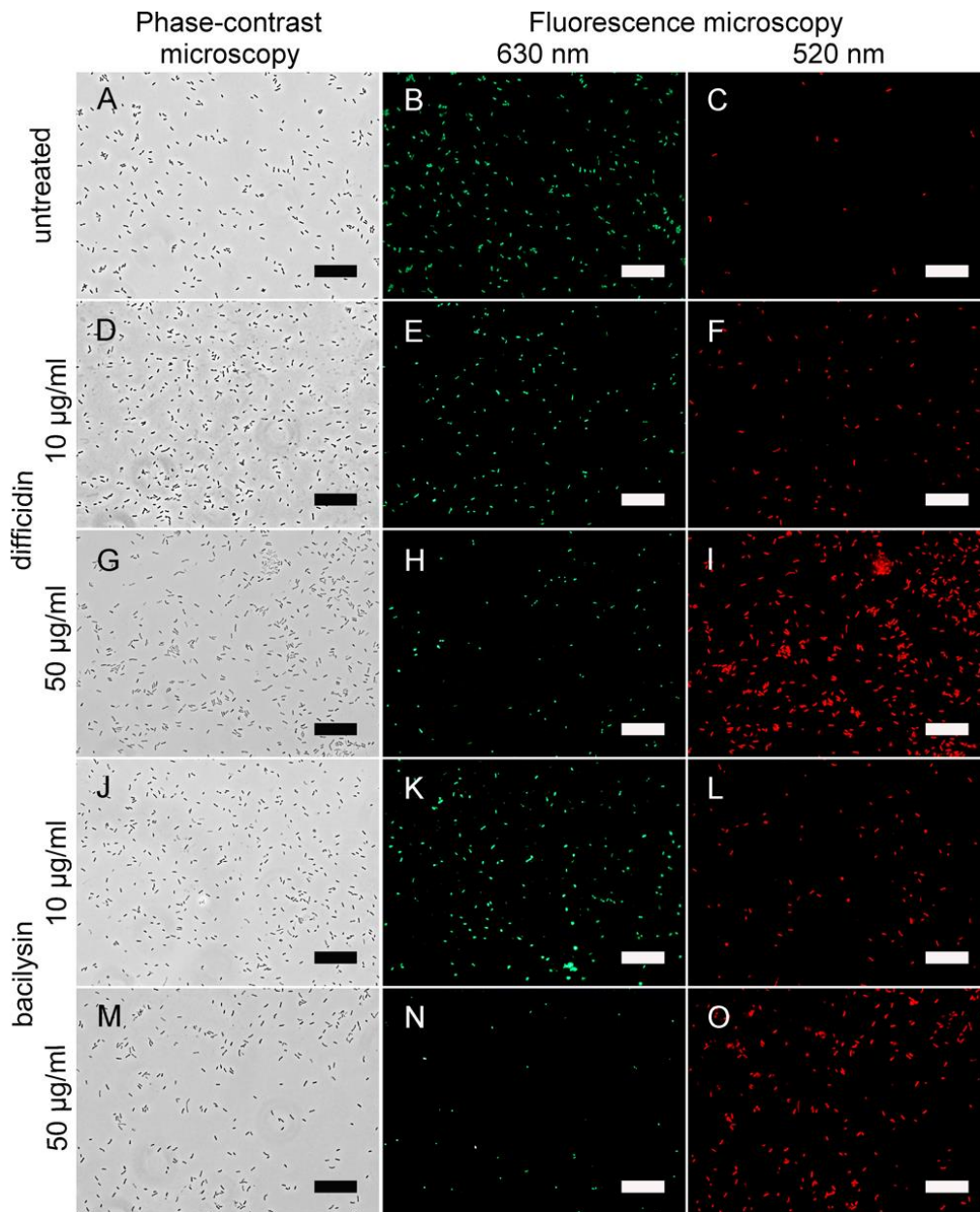


Fig. S4. The phase-contrast and fluorescence microscope images of *Xanthomonas oryzae* pv. *oryricola* cells in the absence and presence of diffcidin and bacilysin after 12 h of cultivation. Staining using the LIVE/DEAD BacLight bacterial viability kit assessed bacterial viability: green areas indicate live cells; red areas indicate dead cells. Bars: 20 μm .

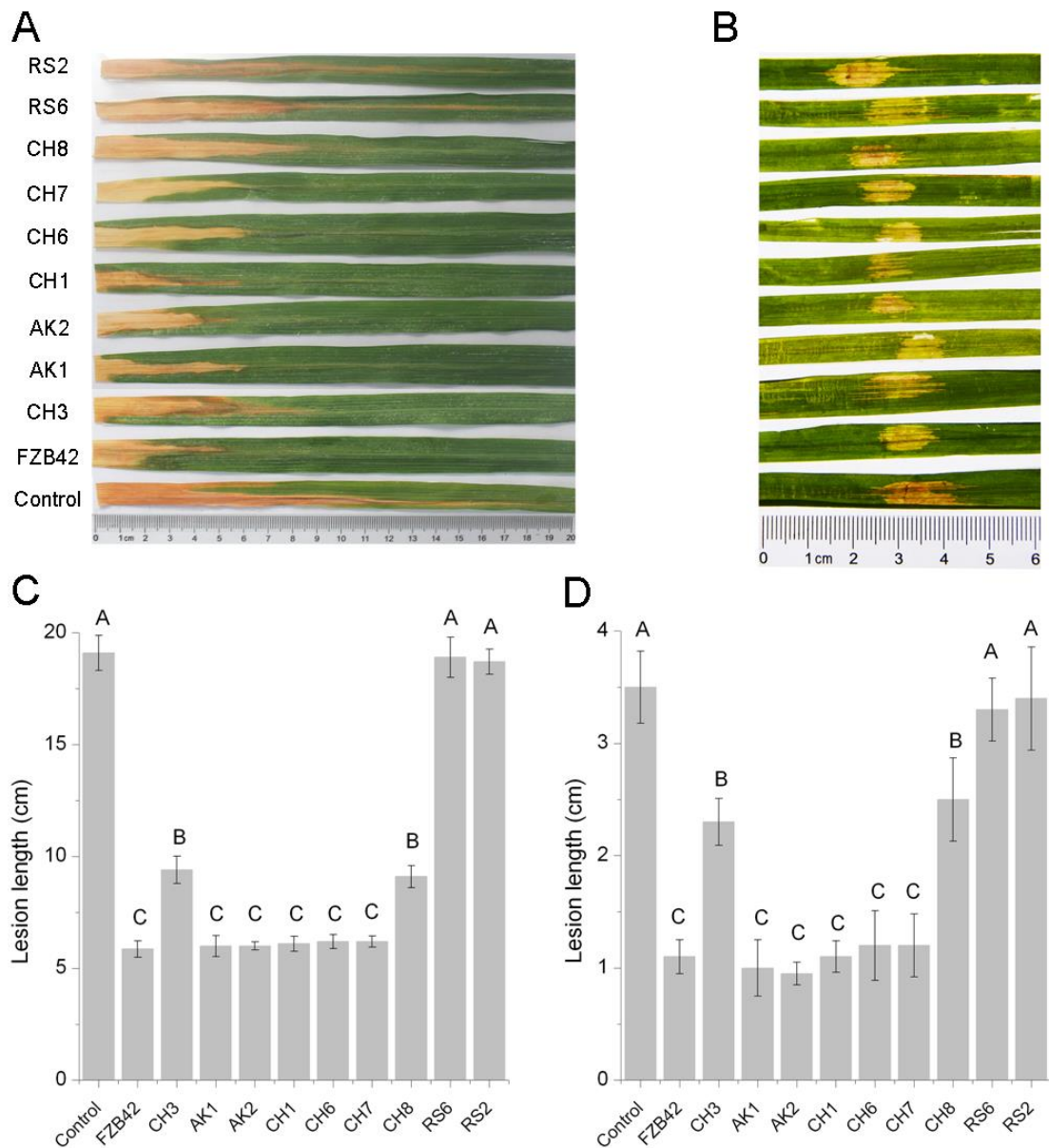


Fig. S5. Pathogenicity test of *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *orycolica* strains on rice. A. Representative result of lesion length symptom tests on the leaves of adult susceptible rice after treatment with *B. amyloliquifaciens* FZB42 mutants. B. Representative result of water-soaking lesion length tests on rice seedling leaves after infiltration with *B. amyloliquifaciens* FZB42 mutants. C. Calculated lesion lengths on the leaves of susceptible adult rice. D. Calculated water-soaking lesion lengths on the leaves of rice seedlings. Data are expressed as means \pm standard deviation (SD); Different letters indicate means that differ statistically at $P < 0.01$.

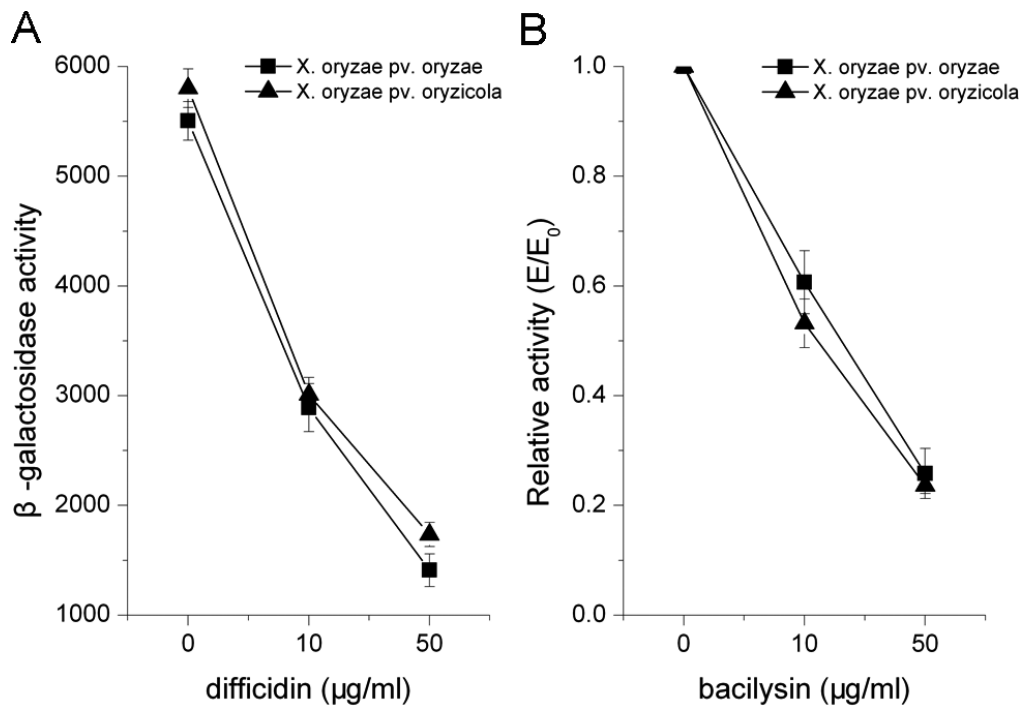


Fig. S6. The level of gene *rrlA* and of glucosamine synthase activity (encoded by *glmS*) in *Xanthomonas* spp. A. The *rrlA* activity in *Xanthomonas* spp. treated with 10 µg/ml and 50 µg/ml difficidin for 30 min. β-galactosidase activity was assayed using o-nitrophenyl-β-D-galactopyranoside as the substrate and is reported in Miller units. B. The glucosamine synthase activity of *Xanthomonas* spp. treated with 10 µg/ml and 50 µg/ml bacilysin for 30 min. The measured activity in the sample containing no bacilysin (E_0) was 0.501 units for *X. oryzae* pv. *oryzae* and 0.489 units for *X. oryzae* pv. *oryzicola*. E represents the corresponding relative residual activities in the presence of bacilysin.