

Supplementary materials

Identification of ϵ -Poly-L-lysine as an Antimicrobial Product from an Epichloë Endophyte and Isolation of Fungal ϵ -PL Synthetase Gene

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Figure S9. Fungal growth inhibition by ϵ -PL against *Drechslera erythrospila* and *Phytophthora capsici*.

Table S1 | Antifungal activity of ϵ -PL^a

Fungi	MID (μ g/disk)		
	Standard ϵ -PL (DP = 25–35)	ϵ -PL (DP = 8–25 and 26–34) ^b	ϵ -PL (DP = 8–23) ^c
<i>D. erythrospila</i>	300	300	300
<i>P. capsici</i>	300	300	300
<i>C. orbiculare</i>	>300	>300	>300
<i>F. oxysporum</i>	>300	>300	>300
<i>B. cinerea</i>	>100	>100	>100
<i>M. oryzae</i>	>100	>100	>100
<i>A. alternata</i>	>100	>100	>100
<i>A. niger</i>	>100	>100	>100

^a Expressed as minimum inhibitory dose (MID) that induces a definite inhibitory zone between paper disk and pathogen colony.

^b Isolated from *Ptef::Epls* (E437) transformant. Degree of polymerization (DP) is indicated in parenthesis.

^c Isolated from *Ptef::Epls* (F11) transformant.

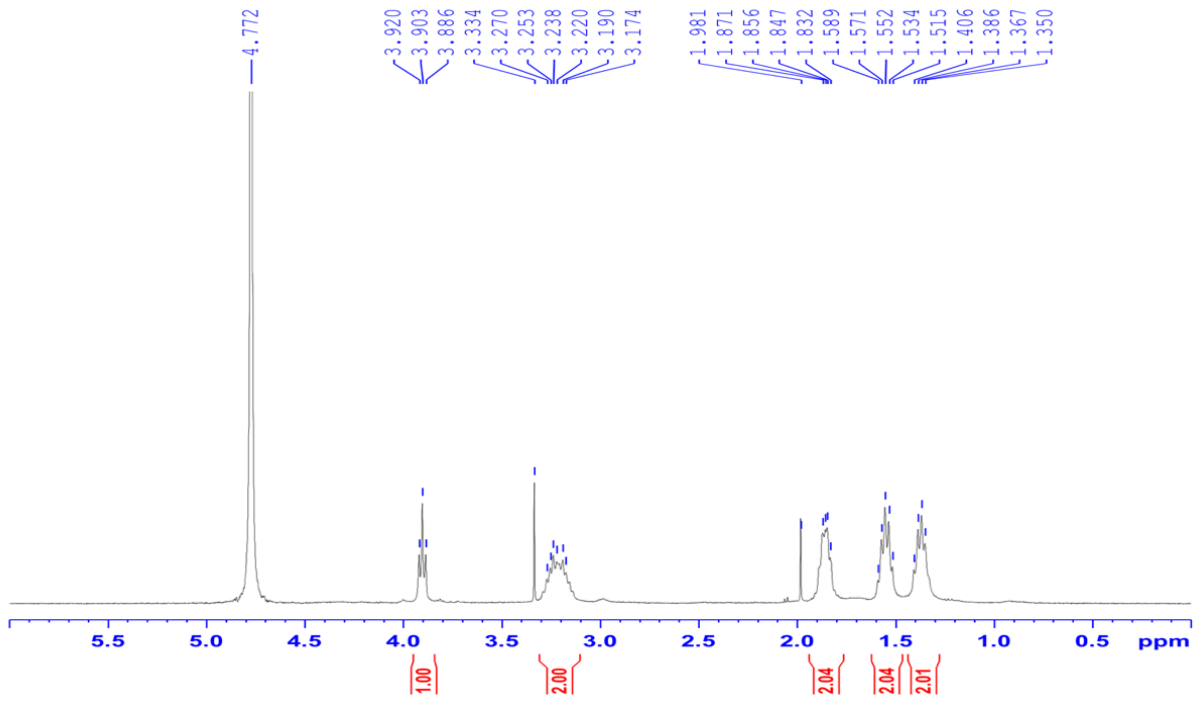


Figure S1 | ¹H-NMR spectrum of ε-PL from *Ptef::VibA* (E437) (400 Hz, D₂O)

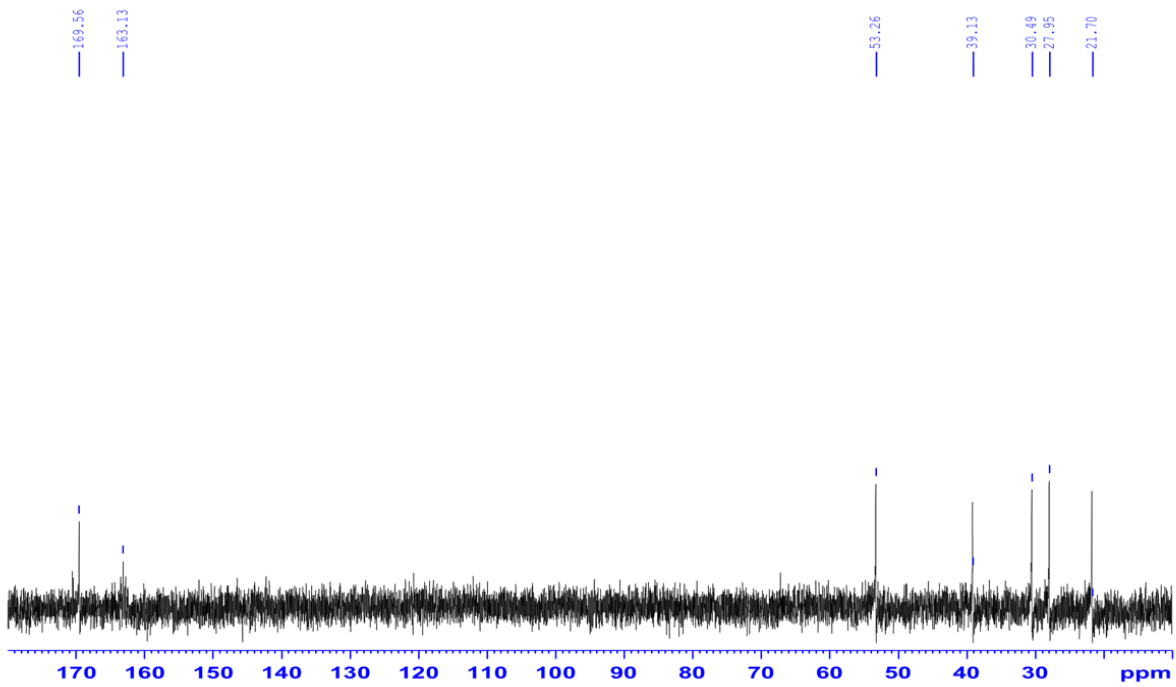


Figure S2 | ¹³C-NMR spectrum of ε-PL from *Ptef::VibA* (E437) (100 Hz, D₂O)

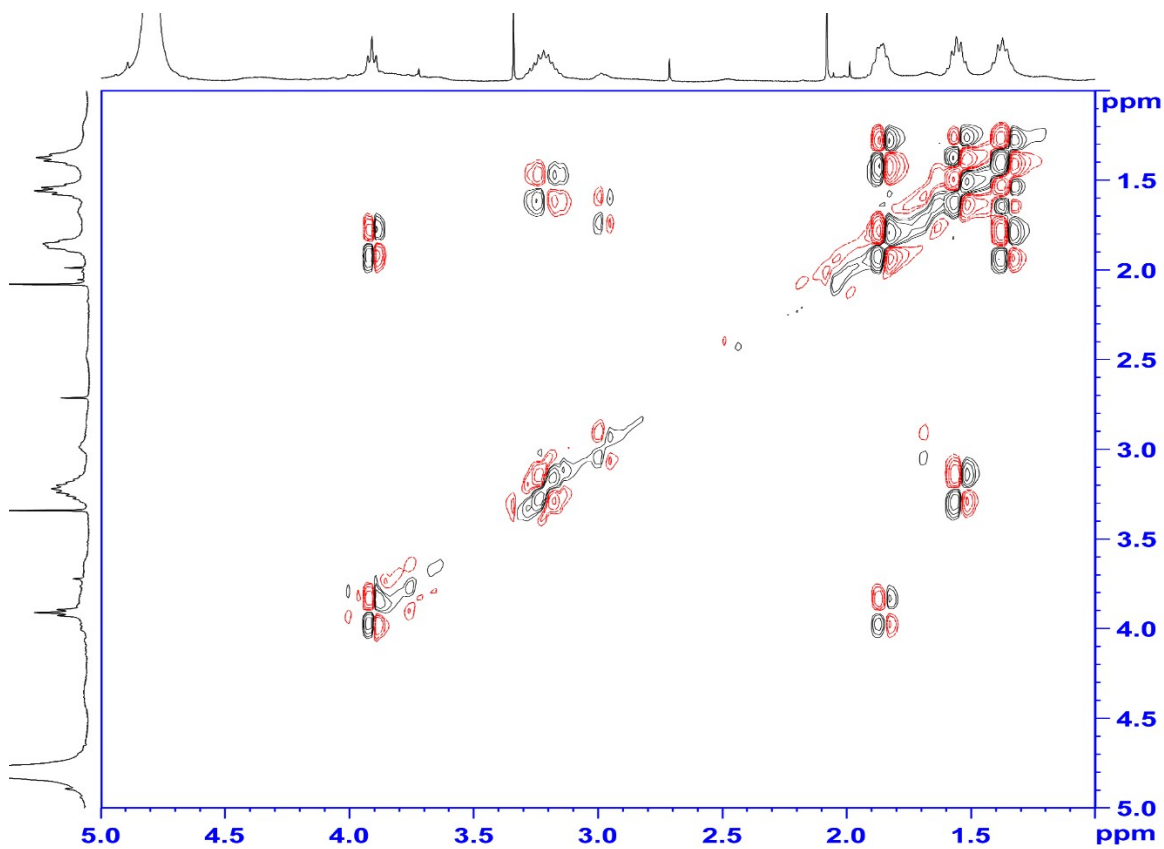


Figure S3 | DQF-COSY spectrum of ϵ -PL from *Ptef::VibA* (E437) (400 Hz, D₂O)

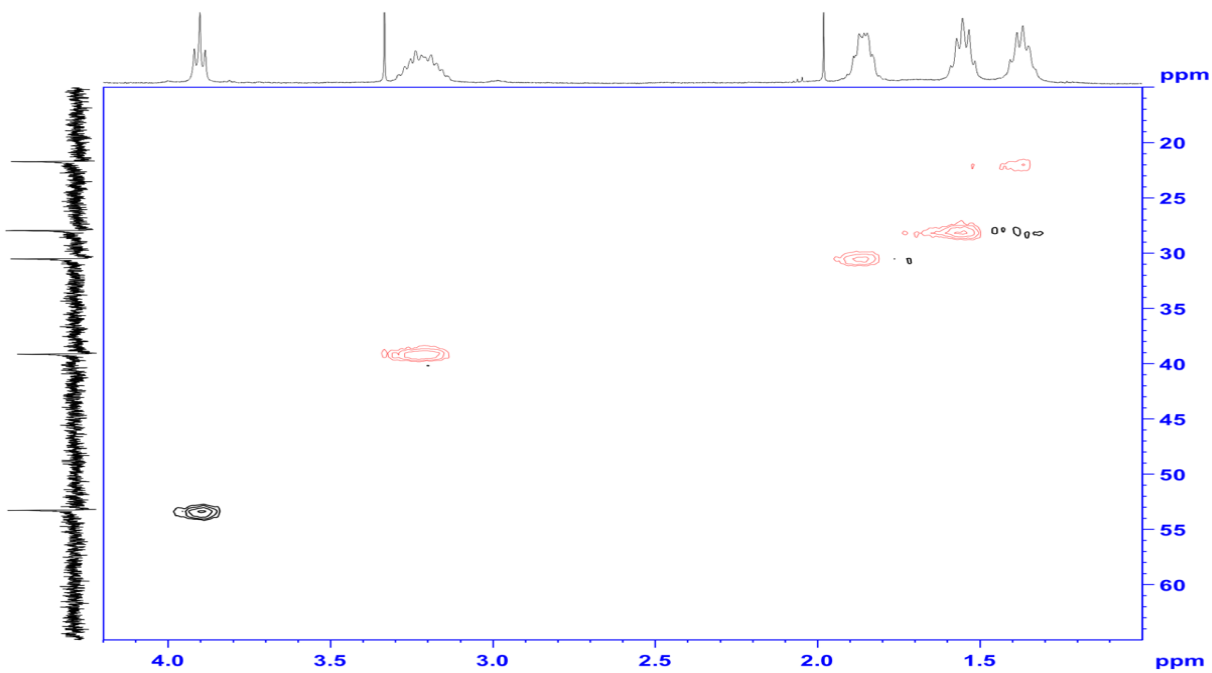


Figure S4 | HSQC spectrum of ϵ -PL from *Ptef::VibA* (E437) (400 MHz, D₂O)

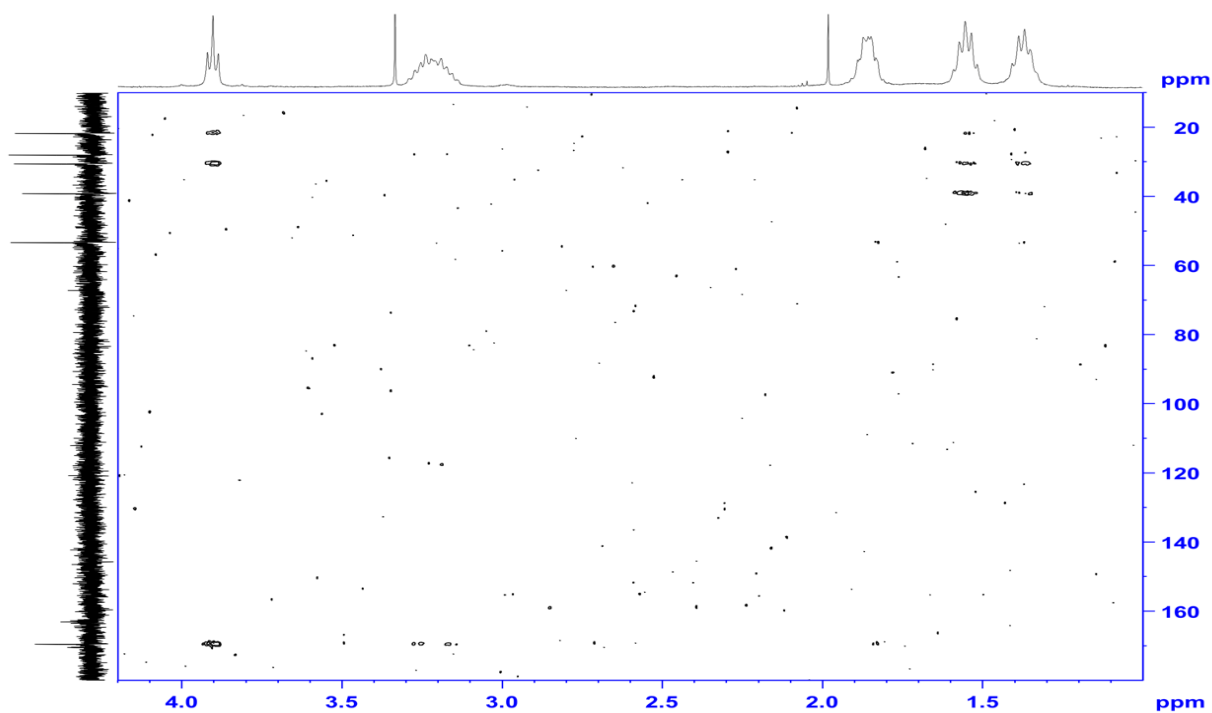


Figure S51| HMBC spectrum of ϵ -PL from *Ptef::VibA* (E437) (400 Hz, D₂O)

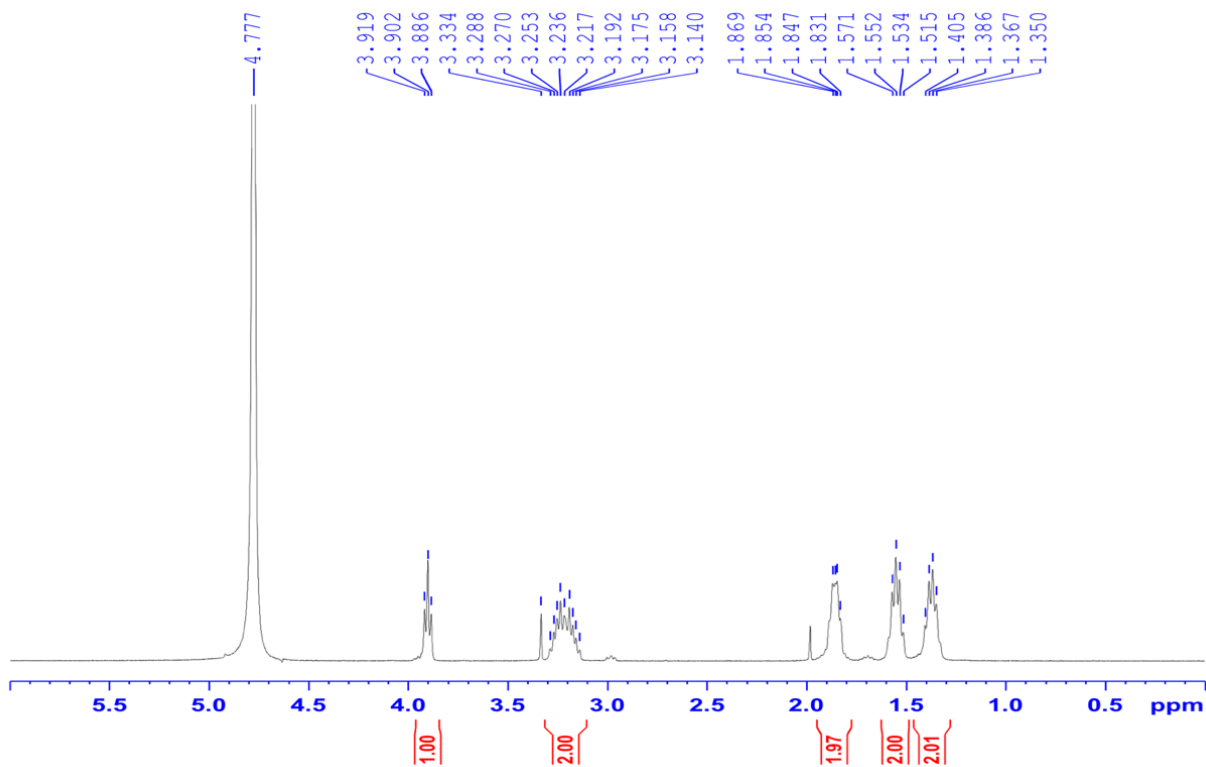


Figure S6| ¹H-NMR spectrum of standard ϵ -PL from (400 Hz, D₂O)

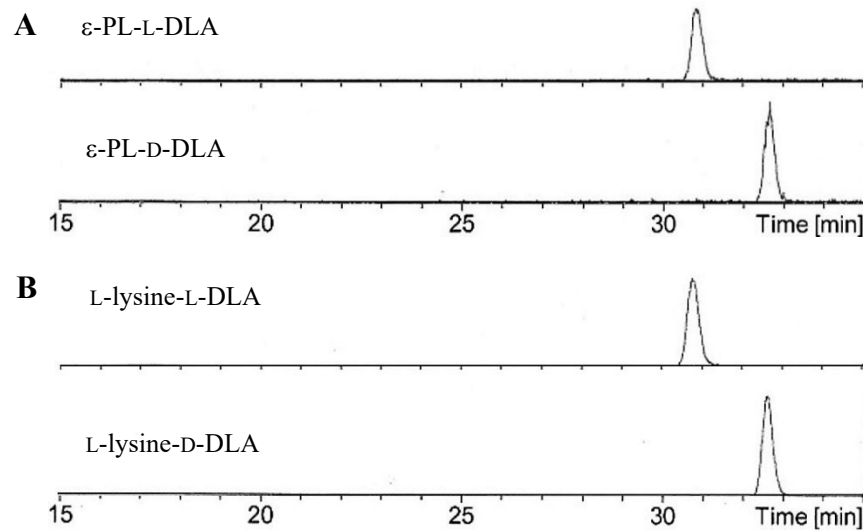


Figure S7 | Determination of absolute configuration of ϵ -PL by the advanced Marfey's method. Extracted ion chromatograms obtained by LC/MS analysis of L- and D-DLA derivatives of acid hydrolysate of ϵ -PL from *Ptef::VibA* (E437) (A) and L-lysine (B).

EfEpls	1	DAW	I	F	T	S	G	S	T	G	K	P	K	G	V	A	V	T	H	R	S	A	A	F	V	D	A	E	S	K	I	F	L	P	N	K	P	L	A	P	G	D	R	V	L	A	G	L	S	V	A	F	D	A	S	C	E	**	*	
BIBacB	1	L	A	V	I	V	T	S	G	S	S	G	R	P	K	G	V	M	T	H	R	N	V	V	H	Y	V	D	A	F	T	K	R	---	I	P	L	S	E	H	D	T	V	L	Q	V	V	S	F	S	F	D	A	F	S	E				
EfEpls	61	E	M	W	L	A	R	Y	G	A	C	L	V	P	A	P	R	S	L	V	K	A	G	A	D	L	G	A	F	L	T	A	Q	S	I	S	V	V	S	T	V	P	T	L	A	A	M	W	P	A	E	A	L	Q	G	L	R	---		
BIBacB	57	E	V	P	I	L	A	C	S	G	R	L	V	I	S	R	K	V	S	D	L	N	I	D	E	L	V	K	T	I	G	K	Y	R	V	T	L	V	S	C	S	P	L	L	L	N	E	I	D	K	N	Q	H	L	T	F	H	P	Q	M
EfEpls	118	L	L	*	L	*	G	G	E	A	C	P	P	E	L	A	T	R	L	A	N	T	V	D	S	V	W	N	T	Y	G	P	T	E	A	T	V	V	A	C	A	P	L	V	A	G	---	Q	P	V	A	I	G	L	P	L	A	G		
BIBacB	117	K	F	I	S	G	G	D	V	L	K	F	E	Y	V	E	N	I	I	K	G	A	D	-	V	Y	N	S	Y	G	P	T	E	A	T	V	C	A	T	Y	Q	L	S	S	A	D	R	K	K	T	S	I	P	I	G	K	P	L	S	N
EfEpls	174	W	K	L	A	V	V	D	A	E	G	N	T	V	R	W	G	E	E	G	E	L	V	I	G	G	V	G	M	A	R	-	Y	L																										
BIBacB	176	Y	K	V	Y	I	A	D	Q	Y	G	R	P	Q	P	V	G	V	P	G	E	L	I	G	G	E	G	V	A	R	G	Y	L																											

Figure S8 | Alignment of the deduced amino acid sequences of adenylation domain of *E. festucae* ϵ -PL synthetase (EfEpls) and the first adenylation domain of *Bacillus licheniformis* bacitracin synthetase 2 (BIBacB, accession No. AAC06347). Amino acid residues for the lysine-binding pocket in the first A domain of BIBacB are indicated by asterisks.

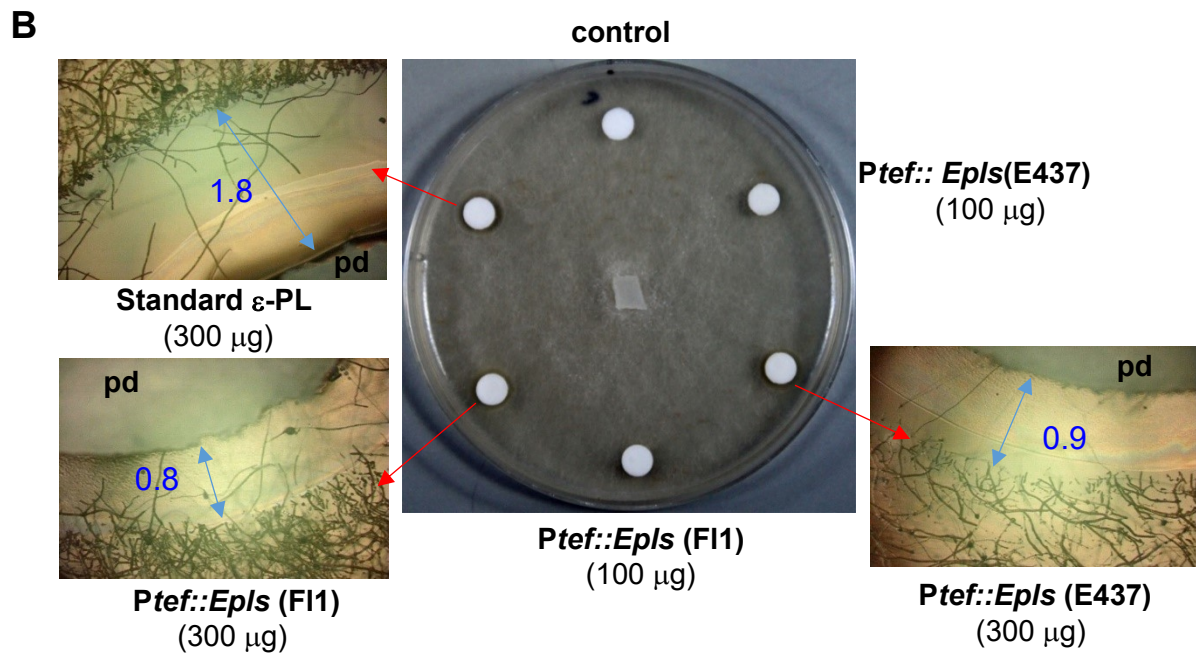
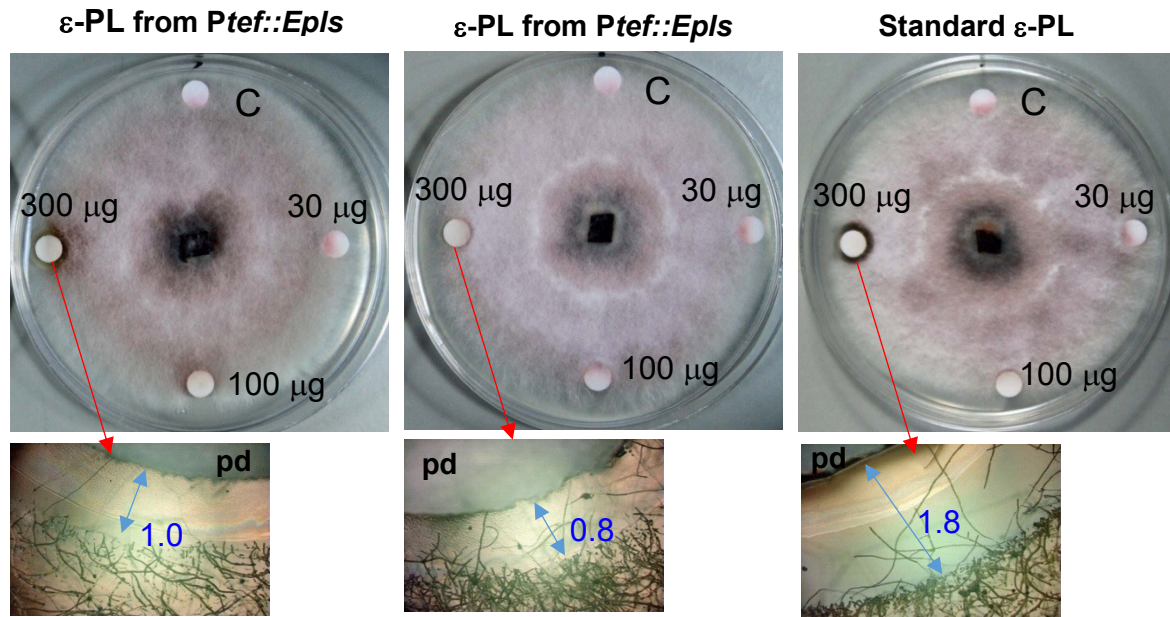


Figure S9 | Fungal growth inhibition by ϵ -PL against (A) *Drechslera erythrospila* and (B) *Phytophthora capsici*. Effects of the ϵ -PL was observed 3 (A) or 5 days (B) after applying sample-containing disks. Inhibition zones between paper disk edge and colony front (in mm) around the paper disk (“pd”) were observed through a stereo microscope.