SUPPLEMENTARY INFORMATION

Insights into the ecological role of Pseudomonas spp. in an ant-plant symbiosis

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Figure S1. Isolation of the phytopathogen *Pestalotiopsis clavispora* from *Cecropia* leaves. (A) *Cecropia* sp. leaf contaminated with a fungal pathogen. (B) *P. clavispora* FB1 isolated from *Cecropia* sp.



Figure S2. Heatmap of the biological activity of *Cecropia-Azteca* microbial strains against human pathogens, phytopathogens and an entomopathogen.



Figure S3. Antifungal activity of *Pseudomonas* sp. ICBG1870 against *Pestalotiopsis clavispora* FB1. (A) Inhibition zone of *Pseudomonas* sp. ICBG1870 against *P. clavispora*. (B) *Methylobacterium* sp. ICBG1884 against *P. clavispora*, negative control.



Figure S4. Phylogenetic analysis based on the 16S rRNA gene of viscosin-producers. The bootstrap support values are based on 1000 bootstrap replicates. The phylogeny was reconstructed using IQ-TREE, using the TPM3+F+R2 model of nucleotide substitution and including *P. aeruginosa* PAO1 as outgroup.



Figure S5. Phylogeny of condensation domains. The phylogeny was reconstructed using IQ-TREE, using the LG+F+R10 amino acid substitution model. Support values are based on 1000 bootstrap replicas. Blue: clade that comprises $C_{starter}$ modules; orange: Cyc; yellow: LCL; green: DCL; pink: C_{dual} . The clade that comprises the condensation domains of module 1 viscosins-producers is highlighted in bold. Other clades were collapsed.



Figure S6. Phylogeny of condensation domains. The phylogeny was reconstructed using IQ-TREE, using the LG+F+R10 amino acid substitution model. Support values are based on 1000 bootstrap replicas. Blue: clade that comprises $C_{starter}$ modules; orange: Cyc; yellow: LCL; green: DCL; pink: C_{dual} . The clade that comprises the condensation domains of module 8 viscosins-producers is highlighted in bold. Other clades were collapsed.



Figure S7. Phylogeny of condensation domains. The phylogeny was reconstructed using IQ-TREE, using the LG+F+R10 amino acid substitution model. Support values are based on 1000 bootstrap replicas. Blue: clade that comprises C_{starter} modules; orange: Cyc; yellow: LCL; green: DCL; pink: C_{dual} . The clade that comprises the C_{dual} domains of viscosins-producers is highlighted in bold. Other clades were collapsed.



Figure S8. Phylogeny of adenylation domains. The phylogeny was reconstructed using IQ-TREE, using the LG+F+R10 amino acid substitution model. Support values are based on 1000 bootstrap replicas. In pink are domains responsible for recruiting Glu/Gln; in blue, Ser; in green, Leu; in yellow, Val; in lilac, Ile; in orange, Thr. Other clades were collapsed.



Figure S9. HRESI-MS spectrum of viscosinamide.



Figure S10. MALDI-MS/MS spectrum of viscosinamide.



Figure S11. ¹H NMR spectrum of viscosinamide (CD₃COCD₃, 500 MHz).



Figure S12. ¹H,¹H-COSY spectrum of viscosinamide (CD₃COCD₃, 500 MHz).



Figure S13. gHSQC spectrum of viscosinamide (CD₃COCD₃, 500 MHz).



Figure S14. gHMBC spectrum of viscosinamide (CD₃COCD₃, 500 MHz).

Amino acid	Position	δC	δH	Multiplicity (Hz)	COSY	НМВС
Fatty acid	1	174.7				
	2	447	2.61	dd (14.23; 4.04)	3	1, 3
	2	44./	2.53	d (9.47)	3	1, 3, 4, 5
	3	69.7	4.11	m	4	
	4	38.3	1.55	m	5	
	5	26.3	1.48	m		
	6	n.a.	n.a.			
	7	n.a.	n.a.			
	8	n.a.	n.a.			
	9	n.a.	n.a.			
	10	n.a.	n.a.			
	11	n.a.	n.a.			
	12		8.74	d (5.88)	13	13, 14
	13	53.4	3.99	m	12, 15	15,14
T 4.4	14	17/4.9			12.14	
Leu1*	15	38.9	1.87	m	13, 16	16, 17, 13,
	16	25.3	1.77	m	15, 17	13, 17
	17	22.3	0.91	m	16	16, 13
	18	21.3	0.91	<u>m</u>	16	16, 13
	19		8.25	d (7.10)	20	20
	20	58.3	4.15	m	22	23, 21,
C1-2	21	1/6.6			20.22	22 20 24
GIn2	22	28.7	2.02	m 14 (11 10, 5 02)	20, 23	23,20,24
	23	31./	2.48	dt (11.10; 5.92)	22	24, 20,
	24	1/6.5	 7 24/6 61	c.		
	25		0.22	5 hr a	27	27.20
	20	57.0	9.25	or s	27	27, 20
Tha?	27	37.0 174.5	4.1/	111	29	28, 21, 50, 29
1111.5	20	174.5	5 36	da(12.00.5.89)	27 30	28 63
	30	17.9	1 32	d (6 00)	27, 50	28,05
Val4	31		7.95	$\frac{d(0.00)}{d(5.21)}$	32	32 34 33
	32	65.8	3 44	dd(11.08.5.63)	34	35 34
	33	174.1		uu (11.00, 5.05)	51	55, 51,
	34	29.7	2.19	m	35.36	33.35.32
	35	20.3	1.01	m	34	36
	36	19.5	0.96	m	34	35.34.32
Leu5*	37		8.47	d (6.75)	38	38, 33, 39,
	38	54	3.67	m	40	41, 39, 33
	39	171.1				, ,
	40	38.8	1.77	m	38,41	38, 40, 43
	41	25.6	1.62	m	42,43	43
	42	21.3	0.92	m	41	43, 41
	43	21.8	0.94	m	41	41, 40
Ser6 Leu7*	44		07.08	d (5.69)	45	45, 47
	45	56.4	4.4	m	44,47	46,47
	46	171.9				
	47	64.5	3.79	m	45,48	46
	48		5.11	dd (8.98; 5.59)	47	47
	49		7.47	d (8.45)	50	46, 52
	50	56.2	4.16	dt (8.42; 2.43)	49	52, 51, 46

Table S1. NMR spectroscopic data of viscosinamide (CD₃COCD₃).

	51	173.6				
	52	41.6	1.60	m	53	50
	53	36.1	1.98	m		50, 51
	54	16	0.84	m		50
	55	16	0.85	m		50
Ser8	56		8.02	d (9.14)	57	57, 51,59
	57	56.7	4.50	dt (8.88; 4.45)	56,59	59,58, 51
	58	171.7				
	59	62.4	3.65	m	60, 57	58
	60		4.04	m	59	59
Ile9	61		6.61	d (9.99)	62	62,58,63
	62	56.7	4.57	dd (10.15; 3.03)	61,64	64,65,66,63,5 8
	63	170.1				
	64	36.1	1.98	m	62, 65,66	66,67
	65	16	0.85	m	64	64, 66, 67, 62
	66	25.1	1.6	m	67,64	62
	67	21.3	0.94	m	66	66

* Interchangeable.



Figure S15. Chromatograms of viscosinamide products after hydrolysis and advanced Marfey derivatization. In blue, the chromatograms of the product of the reaction with L-FDLA are represented, and in pink, with L, D-FDLA. (A) EIC m/z 426.45, corresponding to L-Leu. (B) EIC m/z 412.43, corresponding to D-Val. (C) EIC m/z 400.37, corresponding to D-Ser. (D) EIC m/z 414.4, corresponding to D-Thr. (E) EIC m/z 442.41, corresponding to D-Glu.





Figure S16. Chemical structures of viscosin, WLIP, viscosinamide A and massetolide A.