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Research article

Characterization of *Lysobacter enzymogenes* B25, a potential biological control agent of plant-parasitic nematodes, and its mode of action

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Supplemental

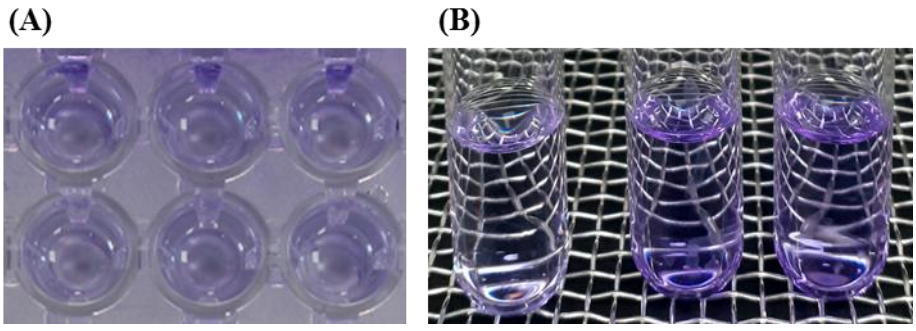

Figure S1. *Lysobacter enzymogenes* B25 biofilm formation on different surfaces: (A) polystyrene plate and (B) glass tubes. After B25 was grown in wells and tubes, they were stained with crystal violet 0.1 % and filled with ethanol. Violet color indicates cell adhesion to the surface and biofilm formation.

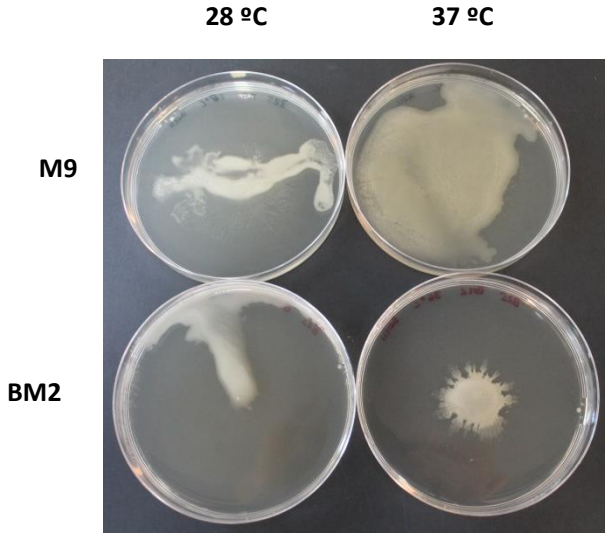

Figure S2. Swarming motility of B25 was measured on modified M9 medium and BM2 medium solidified with 0.5% Noble agar after 24 hours incubation at 28 ℃ or 37 ℃.

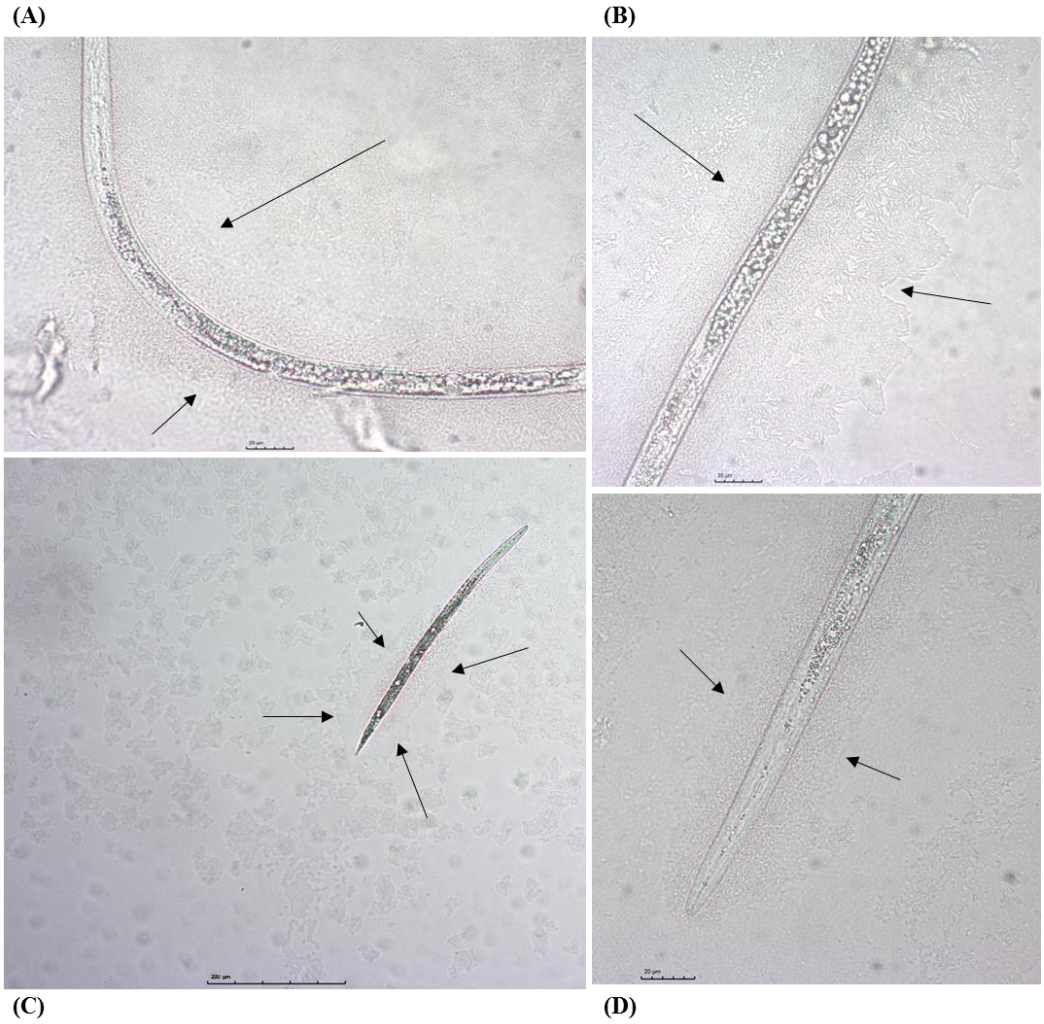

Figure S3. Twitching motility of B25 in the presence *of Meloidogyne incognita*. (A, B and D) Magnification x400. (C) Magnification x100. Arrows indicate the high-density cell envelope around the *M. incognita.*

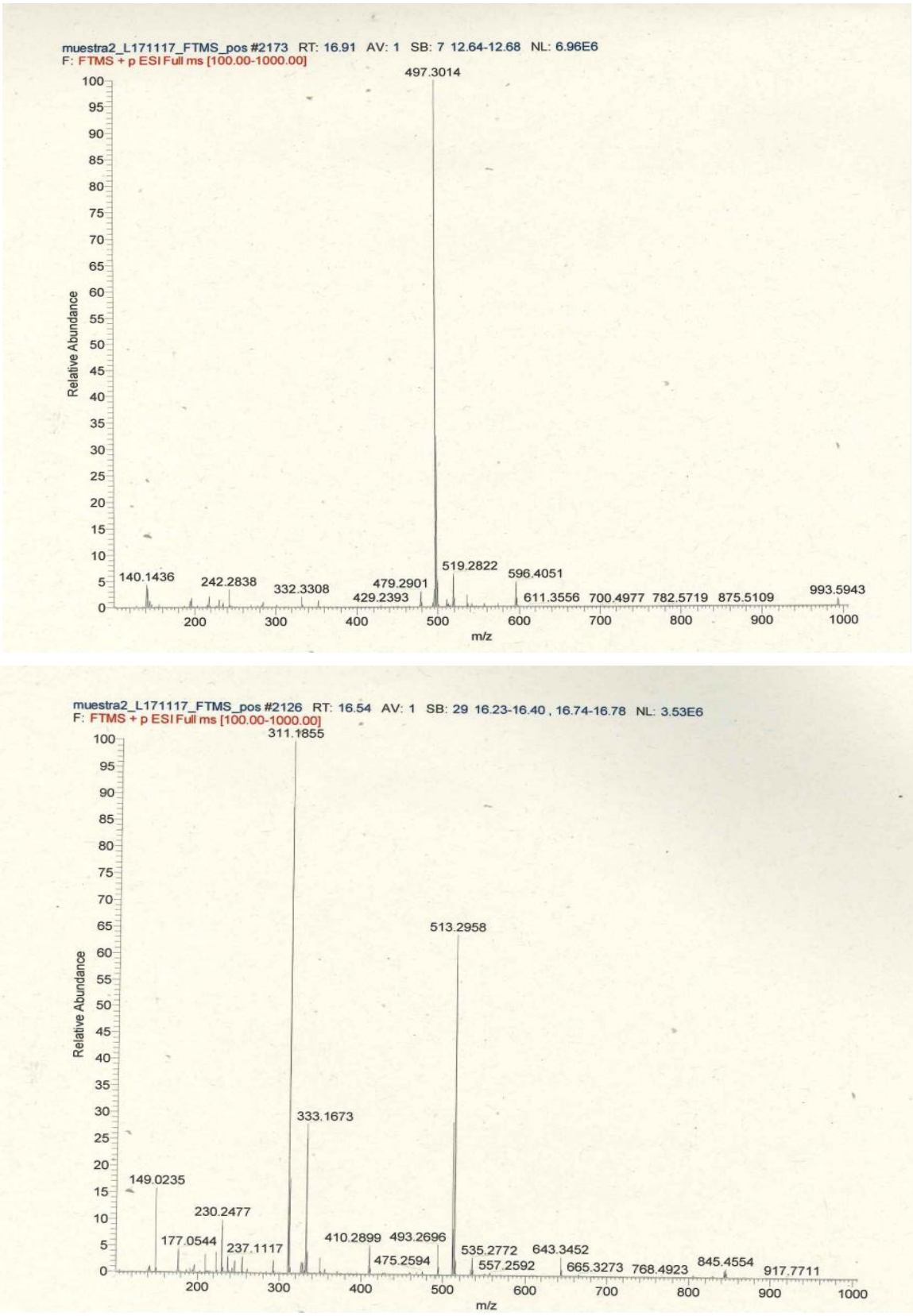

Figure S4: FTMS spectrum of identified peaks by HPLC-HRMS analysis.

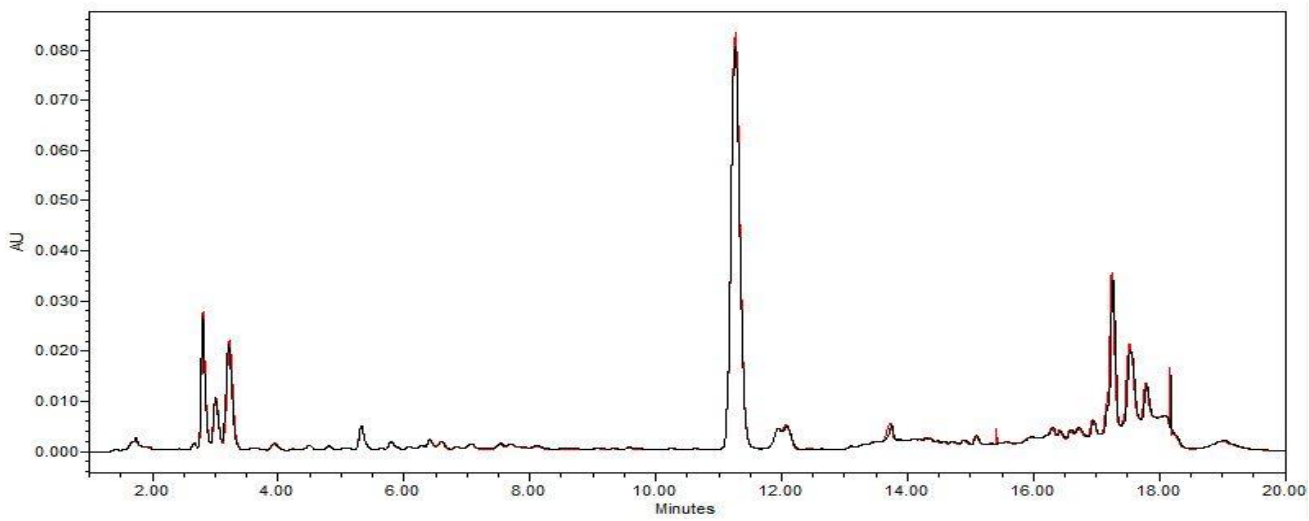

Figure S5. HPLC chromatograms of cell-free culture supernatant from B25 after growing in TSB 1/10 medium (black) and after heat treatment at 80 ℃ for 15 min (red). Chromatograms were obtained by injection of 10 µL of sample and detected by UV at 254 nm.

Table S1. Results of 4 greenhouse assays performed at Futureco Bioscience S.A. facilities on tomato cv. Durinta. Strain B25 was applied 4 times in 7–14 days intervals as described in Materials and Methods section.

Trial code	Target	Dose (CFU's/mL)	Pathogen pressure in control plants Eggs/g of root	% Efficacy of B25 Eggs/g of root	% Efficacy chemical reference	% Efficacy biological reference 2
160314 IN MT	*Meloidogyne javanica* + *Meloidogyne incognita*	3,03E + 08	4239	46.98%	(Oxamyl 10%) 96.98%	-
160307 IN MT	*Meloidogyne javanica* + *Meloidogyne incognita*	2,67E + 08	5828	60.96%	(Oxamyl 10%) 98.25%	-
181211 IN MT	*Meloidogyne hapla*	1,74E + 08	2274	90.09%	(Flupyram 40%) 85.49%	(*Bacillus firmus* 5%) 6.19%
190122 IN MT	*Meloidogyne hapla*	1,05E + 08	4138	94.59%	(Flupyram 40%) 81.56%	(*Bacillus firmus* 5%) 0%

Table S2. Primers used in this study.

Gene	Genbank Accession	Cultivar	Primers (forward/reverse; 5'-3')	Product size (nt)[a]	E
PR1a	DQ159948	Jin Bao/ Castlemart	CCAAGCTATAACTACGCTACCAAC GCAAGAAATGAACCACCATCC	139	1.981
PR3b	Z15140	Jin Bao/ Castlemart	AACTATGGGCCATGTGGAAGA GGCTTTGGGGATTGAGGAG	128	2.043
RCR3	NM_001320315.1	Money Maker	TACAAGCCGTAACTAAACAG TCATATACCCATTCTCACCC	209	2.053
PINII	X94946	Jin Bao/ Castlemart	AATTATCCATCATGGCTGTTCAC CCTTTTTGGATCAGATTCTCCTT	254	1.997

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Gene	Genbank Accession	Cultivar	Primers (forward/reverse; 5'-3')	Product size (nt)[a]	E
PR2b	LOC543986	Jin Bao	GGACACCCTTCCGCTACTCTT	81	2.052
			TGTTCCTGCCCCTCCTTTC		
MTS1	AY840091	Money Maker	TTTGGGGACATCTTCGGATGAA	309	2.042
		/Castlemart	CTACTCGAGTTACTTGAGAGCGAATGCAAC		
4CL	XM_004235822	Pausa	ACACACAAAGGCTTAGTCACGA	103	1.999
			AACAGAGGCAACACACACATCA		
EF1α	XM_004240531	M82/	GATTGGTGGTATTGGAACTGTC	129	1.977
		Heinz 1706	AGCTTCGTGGTGCATCTC		

[a]Product sizes may vary among tomato cultivars.

Table S3. Raw Ct values of qPCR analysis.

Target_Pos	Target_treat	Target_Cp	Target	Ref_Pos	Ref_Treat	Ref_Cp	Ref
A1	T1	23,77	4CL	A9	T1	21,67	EF1a
B1	T1	23,34	4CL	B9	T1	21,62	EF1a
C1	T1	23,52	4CL	C9	T1	21,67	EF1a
B2	T4	22,73	4CL	B10	T4	21,4	EF1a
C2	T4	22,75	4CL	C10	T4	21,6	EF1a
D2	T4	22,9	4CL	D10	T4	21,48	EF1a
A9	T1	21,94	MTS1	A9	T1	21,67	EF1a
B9	T1	21,96	MTS1	B9	T1	21,62	EF1a
C9	T1	21,82	MTS1	C9	T1	21,67	EF1a
B10	T4	21,45	MTS1	B10	T4	21,4	EF1a
C10	T4	21,7	MTS1	C10	T4	21,6	EF1a
D10	T4	21,42	MTS1	D10	T4	21,48	EF1a
A5	T1	17,96	PINII	A9	T1	21,67	EF1a
B5	T1	18	PINII	B9	T1	21,62	EF1a
C5	T1	18,36	PINII	C9	T1	21,67	EF1a
B6	T4	16,15	PINII	B10	T4	21,4	EF1a
C6	T4	16,14	PINII	C10	T4	21,6	EF1a
D6	T4	15,95	PINII	D10	T4	21,48	EF1a
A1	T1	16,72	PR1a	A9	T1	21,67	EF1a
B1	T1	16,44	PR1a	B9	T1	21,62	EF1a
C1	T1	16,52	PR1a	C9	T1	21,67	EF1a
B2	T4	14,7	PR1a	B10	T4	21,4	EF1a
C2	T4	14,79	PR1a	C10	T4	21,6	EF1a
D2	T4	14,64	PR1a	D10	T4	21,48	EF1a
A5	T1	24,68	PR2b	A1	T1	21,77	EF1a_PR2
B5	T1	24,29	PR2b	B1	T1	21,5	EF1a_PR2
C5	T1	25,44	PR2b	C1	T1	14,97	EF1a_PR2
B6	T4	22,64	PR2b	B2	T4	15,03	EF1a_PR2
C6	T4	22,96	PR2b	C2	T4	14,55	EF1a_PR2
D6	T4	22,64	PR2b	D2	T4	14,64	EF1a_PR2
A5	T1	19,56	PR3b	A9	T1	21,67	EF1a
B5	T1	19,59	PR3b	B9	T1	21,62	EF1a
C5	T1	19,61	PR3b	C9	T1	21,67	EF1a
B6	T4	19,65	PR3b	B10	T4	21,4	EF1a
C6	T4	19,67	PR3b	C10	T4	21,6	EF1a
D6	T4	19,62	PR3b	D10	T4	21,48	EF1a
A1	T1	26,69	RCR3	A9	T1	21,67	EF1a
B1	T1	26,63	RCR3	B9	T1	21,62	EF1a
C1	T1	26,59	RCR3	C9	T1	21,67	EF1a
B2	T4	24,67	RCR3	B10	T4	21,4	EF1a

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Target_Pos	Target_treat	Target_Cp	Target	Ref_Pos	Ref_Treat	Ref_Cp	Ref
C2	T4	24,71	RCR3	C10	T4	21,6	EF1a
D2	T4	24,88	RCR3	D10	T4	21,48	EF1a

The Cp of NTCs (no-template controls) was in all cases > 30

PR2 required different qPCR conditions so the reference gene was analyzed under the same conditions

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