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Local *Bacillus* species as potential biocontrol agents for *Meloidogyne enterolobii* in melon (*Cucumis melo* L.)

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ABSTRACT

Biological control is an environmentally safe alternative in the management of nematodes. The selection of new biocontrol agents has focused on the potential of *Bacillus* species in *in vitro* and *in vivo* studies. In this study, twenty-three local *Bacillus* isolates were characterised and evaluated for metabolisable carbon source, secondary metabolite production and nematicidal action. The *in vitro* antagonism studies were evaluated after direct confrontation between infecting juveniles of *M. enterolobii* and bacterial isolates. In a greenhouse, nematicidal activity was evaluated 51 days after nematode inoculation in melon plants previously inoculated with the bacteria and cultivated in pots. The numbers of egg masses, galls, J2 and eggs in the root, J2 in the soil, the reproduction factor and the biomass yield of the plants were evaluated. From the results obtained in the laboratory experiments, it was observed that five carbon sources (D-glucose, N-acetyl-glucosamine, D-maltose, malic acid and trisodium citrate) were common to all isolates, with variation in the production of secondary metabolites. The sum of nematostatic and nematicidal effects was above 90%. In the pot experiment, a significant reduction in the number of galls was observed with the treatments LCB 03, LCB 40, LCB 45, LCB 47, LCB 51, LCB 56 and LCB 5(3) and there was no increase in plant biomass. Root protection may be associated with effective colonisation through biofilm formation and the action of metabolites with nematicide and disorientation action. However, it is necessary to carry out further studies.

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Introduction

The occurrence and spread of root-knot nematode (*Meloidogyne* spp.) in irrigated areas in Northeastern Brazil is a limiting factor for melon crops (*Cucumis melo* L.) (Pinheiro & Amaro, 2010). Among the species, *M. enterolobii* (= *M. mayaguensis*) has drawn attention due to its aggressiveness and polyphagy, overcoming genes that confer resistance

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against other species and races of *Meloidogyne* (Carneiro et al., 2001; Carneiro et al., 2006; Castro, 2019; Rosa et al., 2015). Since there are no resistant melon genotypes available in the country, the use of nematicides has been the main control tool, as they are not specific to soil microorganisms and have high toxicity (Dong & Zhang, 2006). Measures based on cultural practices often provide insufficient control (Marques et al., 2012). A smart, environmentally safe alternative deploys microbes, such as the rhizobacteria of the *Bacillus* genus, which has been reported to have nematicidal effect in seed treatment (Fernandes et al., 2014), seedlings (Araújo et al., 2018) and soil application (Niu et al., 2016; Zhou et al., 2016).

The process of biological control depends on multiple interactions between the plant, the nematode and the bacteria (Mhlongo et al., 2018; Schirawski & Perlin, 2018). Plants continually release root exudates to attract and select microbial communities to cope with stresses caused by biotic and abiotic factors (Haichar et al., 2014). The various carbon sources present in these compounds favour the proliferation of rhizobacteria inside, on the surface and outside the roots (Canarini et al., 2019). Citric and fumaric acids from root exudates have been found to attract and stimulate biofilm formation in *B. amyloliquefaciens* and *B. subtilis* (Zhang et al., 2014). The response of nematodes to chemotaxis depends on the plant species, and there may be attraction, repellency or no response (Bell et al., 2019; Sikder & Vestergård, 2020). In general, root exudates of *Cucumis* species are attractive to root-knot nematodes (Bitencourt & Silva, 2010).

The ability to form biofilm is an important characteristic of *Bacillus* species and is a prerequisite for the biocontrol of nematodes, because it works as a protective barrier that reduces the arrival and penetration of infecting segund-stage juveniles (J2) in the host's roots (Pinho et al., 2009). Furthermore, secondary metabolites produced and released by *Bacillus* into the rhizosphere can directly affect the nematode or egg masses (Singh et al., 2017). Indirectly, these compounds can induce systemic resistance in the host, as well as alter root exudates and confuse J2 regarding the location of roots (Ferraz et al., 2010). Some of these compounds are similar to phytohormones and stimulate root emission and development (Diaz et al., 2019), while others may have chelation action, such as siderophores, that help the rhizobacteria with the acquisition of metallic ions and making them unavailable to pathogens (Dimkpa et al., 2008; Vacheron et al., 2013).

Research carried out by Embrapa Semiarido (Research Unit of the Brazilian Agricultural Research Corporation) with *Bacillus* isolates native to the Northeast region of Brazil has enabled the selection of bioagents with potential control of different phytopathogens (Gava et al., 2017; Gava et al., 2018; Gava & Menezes, 2012; Gava & Pinto, 2016). However, the nematicidal potential of these isolates against any species of root-knot nematode is still unknown. Thus, the present work aimed to characterise and evaluate the nematicidal potential of local *Bacillus* species against *M. enterolobii* under greenhouse conditions.

Materials and methods

M. enterolobii inoculum used in the experiments

Nematode inoculum was obtained from infested guava plant and the multiplication took place in Santa Cruz 'Kada Gigante' tomato plants. Identification was performed by

isoenzyme electrophoresis with alpha-esterase staining according to Alfenas et al. (1991). Infected tomato roots were crushed in a blender (model NL-26 / Mondial Company) in a sodium hypochlorite solution (0.5%) as proposed by Boneti and Ferraz (1981). The eggs obtained were then placed in hatching chamber for 24 h for calibration of the suspension (30–37 J2/100 µL) that was used in the *in vitro* experiments. In the pot experiment 5,000 eggs and/or J2 per plant were inoculated.

Bacillus isolates and laboratory experiments

Twenty-three *Bacillus* isolates from the Embrapa microorganisms collection were evaluated [LCB 5 (1), LCB 40, LCB 08, LCB 51, LCB 47, LCB 21, LCB 55, CLB 57, LCB 06, LCB 03, LCB 45, LCB 28, LCB 53, I 27, RAB 7, LCB 56, LCB 8 (1), LCB 5 (3), LCB 5 (2), LCB 38, LCB 33 (1), LCB 30 (1), LCB 04] for the source of metabolisable carbon, production of biofilm, biosurfactant molecules, indolacetic acid, siderophore and *in vitro* antagonism against J2 *M. enterolobii*. References used were: FMC Quartzo® [*B. subtilis* FMC H002 (1×10^{11} UFC g^{-1}) + *B. licheniformis* FMC H001 (1×10^{11} UFC g^{-1})], Biovar from Biotrop [*B. subtilis* CNPSo2657 (1×10^{12} UFC L^{-1})] and a negative control. *In vitro* experiments were conducted in a completely randomised design, with three replicates per treatment. Each experiment was conducted twice.

Ability to metabolise different carbon sources

Rapid tests were performed using the commercial API® 20 NE kit (BioMérieux). The bacterial suspensions were calibrated (1×10^{9} UFC/mL) and placed in vats containing carbon compounds (D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium glucanate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid). The interpretation of the observed results followed the manufacturer's recommendations.

Biofilm production

Bacterial growth occurred in polypropylene tubes containing a Luria Bertani medium with potassium chloride as a mineral source (10 g of tryptone, 5 g of yeast extract and 5 g of KCl^{-1}) at 28 °C for 24 h. The contents were then dismissed and bacterial cells adhered to the tube walls were stained with a crystal violet solution (0.08% v/v) for 15 min and solubilised in ethanol. Aliquots of 100 µL were transferred into polystyrene microplate wells (capacity 400 µL) and read at a wavelength of 590 nm in a Multiskan GO reader (Thermo Scientific), as described by Fall et al. (2004). The results were expressed as optical density (OD). Bacteria-free tubes and filled with sterile distilled water were used as a negative control.

Production of biosurfactants

Luria Bernati medium (10 g of tryptone, 5 g of yeast extract and 5 g of $NaCl^{-1}$) was used for bacterial growth at 28°C for 24 h. After centrifugation at 10,000 rpm for five minutes, 2 mL aliquots of the supernatant of each bacterium were transferred to test tubes along

with 1 mL of vegetable oil, stirred and left to rest for 24 h, as described by Iqbal et al. (1995). The emulsification index (E24%) represents the ratio between the height of the emulsion layer and the height of the volume of the solution in the tube. Triton (1% v/v) was used as a negative control. 25 µL aliquots of each supernatant were placed on a flat, hydrophobic and millimetric surface for evaluation of the surfactant activity, as an adaptation of the method described by Youssef et al. (2004), and the scattering was quantified through image analysis (Motic Images Plus softwere, v. 2.0) using sterile water as a control.

Production of indolacetic acid (IAA)

The production of indole compounds, including IAA, was evaluated in the presence and absence of tryptophan, using a polystyrene microplate (96-walls) following the method described by Sarwar and Kremer (1995). The concentration of bacteria-synthesised IAA was obtained using an IAA calibration curve, with concentrations ranging from 0 to 1000 µM. Bacterial growth occurred in an erlenmeyer containing a S.D.Y. medium (1 g of tryptone, 2 g of yeast extract and 4 g of dextrose^{-1}) under constant stir (120 rpm) for 48 h. After centrifugation at 10,000 rpm for 10 min, aliquots of 100 µL were transferred to microplate wells (capacity 400 µL) along with 100 µL of Salkowski's reagent (Gordon & Weber, 1951). The reaction was held in the absence of light at room temperature for 30 min and the spectrophotometric reading taken at 540 nm.

Production of siderophore

The methodology proposed by Cattelan (1999) was deployed using a polystyrene microplate (96-walls). Bacterial growth occurred in an erlenmeyer containing a S.D.Y. medium (1 g of tryptone, 2 g of yeast extract and 4 g of dextrose^{-1}) under constant stir (120 rpm) for 48 h. After centrifugation at 10,000 rpm for 10 min, aliquots 100 µL were transferred to microplate wells (capacity 400 µL) along with 100 µL of 2 mM chromium azurol S (CAS) reagent. The reaction occurred in the absence of light, at room temperature, for 15 min and the spectrophotometric reading was taken at 640 nm. The results were expressed as optical density (OD). The EDTA reagent was used as a control treatment.

In vitro nematicidal activity of Bacillus isolates against M. enterolobii

We adapted the methodology proposed by Chinheya et al. (2017) by using the crude extract of the bacterial growth S.D.Y. medium (1 g of peptone, 2 g of yeast extract and 4 g of dextrose^{-1}) at 28 °C for 48 h. The bacterial suspension was adjusted (1×10^9 UFC/mL) before 100 µL along with 100 µL of the nematode suspension (30 a 37 J2/100 µL) were transferred into polystyrene microplate wells (capacity 400 µL) and incubated under the same conditions for 24 h. Total mortality was attributed from the proportion of immobile J2 in relation to the total number of J2 in each well. Subsequently, the nematicide effect was evaluated under similar conditions and obtained by the ratio between the number of dead J2 (with completely straight and immobile bodies) and the total number of J2 in each well. Confirmation of the specimens' death was performed by adding 50 µL of sodium hydroxide (1 N) to the suspension contained in each well and

waiting three minutes before counting, according to the methodology proposed by Chen and Dickson (2000). The nematostatic effect, in turn, was obtained indirectly from the difference between total mortality and the nematicidal effect.

Bacillus isolates in the in-pot biocontrol of M. enterolobii

Nematicidal potential of 23 *Bacillus* isolates was assessed in comparison to Quartzo® from FMC [*B. subtilis* FMC H002 (1×10^{11} CFU.g^{-1}) + *B. licheniformis* FMC H001 (1×10^{11} CFU.g^{-1})], Biovar from Biotrop [*B. subtilis* CNPSo2657 (1×10^{12} UFC.L^{-1})] and a positive control. The experiment was conducted in completely randomised design, with four plants per treatment and one per pot. The pots (3 L) were filled with autoclaved soil and received F1 hybrid melon seeds (Gladial) previously disinfected on the surface (immersion in 70% ethanol for 30 s, then in sodium hypochlorite 1% for 30 s and twice in distilled water for one minute). Uniform irrigation and fertilisation with NPK and micronutrients were maintained throughout the plant development. The bacterial growth occurred as described for the *in vitro* nematicidal activity experiment. Bacterial suspensions were adjusted (1×10^{9} UFC/mL) and applied onto the plant's lap 15 days after the sowing. After six days, the nematodes were inoculated into two holes (3–5 cm deep) around the plant's lap.

The study was completed 51 days after the inoculation of the nematode. The fresh and dry weights of the plant were evaluated. The roots were carefully washed and stained with acid fuchsin to count the numbers of egg masses and galls. The number of J2 and root eggs were assessed after the extraction as proposed by Boneti and Ferraz (1981). Portions of 100 cm^3 of soil from each pot were used to extract and count the J2, according to the method described by Jenkins (1964). The reproduction factor was calculated by the ratio between the final population (Pf) (number of J2 found in the soil + number of J2 and eggs extracted from the roots) and the initial (Pi) (5,000 eggs + J2), according to the methodology proposed by Oostenbrink (1966).

Statistical analysis

Data from the *in vitro* and *in vivo* experiments were transformed using the equations $[(X - \overline{X}) / s^{2}]$ and $\sqrt{X+1}$, respectively. The variance analysis was performed and the averages were compared using the Tukey test at 5% significance through the software Statistica for Windows (v. 12, Stat Soft Inc.).

Results

Metabolisation of different carbon sources

The isolates in the study were able to metabolise more than 50% of the compounds, especially LCB 55 and the *B. subtilis* + *B. licheniformes*, which used ten of the twelve carbon sources (83.3%) (Table 1). Five compounds were common to all isolates, namely: D-glucose, N-acetyl-glucosamine, D-maltose, malic acid and trisodium citrate. Only LCB 55 and *B. subtilis* + *B. licheniformes* metabolised adipic acid and none treatment used capric and phenyl-acetic acids as a carbon source.

Table 1. Characterization of 23 *Bacillus* isolates and two commercial products regarding the ability to metabolise carbon compounds.

Bacteria	GLU	ARA	MNE	MAN	NAG	MAL	GNT	CAP	ADI	MLT	CIT	PAC
I 27	+	+	+	+	+	+	+	–	–	+	+	–
B. S[a]	+	+	+	+	+	+	+	–	–	+	+	–
B. S. + B. L[b]	+	+	+	+	+	+	+	–	+	+	+	–
CLB 57	+	–	+	+	+	+	+	–	–	+	+	–
LCB 03	+	+	+	+	+	+	–	–	–	+	+	–
LCB 04	+	+	+	+	+	+	+	–	–	+	+	–
LCB 06	+	+	+	+	+	+	+	–	–	+	+	–
LCB 08	+	+	+	+	+	+	+	–	–	+	+	–
LCB 21	+	+	+	+	+	+	+	–	–	+	+	–
LCB 28	+	+	+	+	+	+	+	–	–	+	+	–
LCB 30 (1)	+	–	+	+	+	+	+	–	–	+	+	–
LCB 33 (1)	+	+	+	+	+	+	+	–	–	+	+	–
LCB 38	+	+	+	+	+	+	+	–	–	+	+	–
LCB 40	+	–	–	–	+	+	+	–	–	+	+	–
LCB 45	+	+	+	+	+	+	+	–	–	+	+	–
LCB 47	+	+	+	+	+	+	+	–	–	+	+	–
LCB 5 (1)	+	+	+	+	+	+	+	–	–	+	+	–
LCB 5 (2)	+	+	+	+	+	+	+	–	–	+	+	–
LCB 5 (3)	+	+	+	+	+	+	+	–	–	+	+	–
LCB 51	+	+	+	+	+	+	+	–	–	+	+	–
LCB 53	+	–	+	+	+	+	+	–	–	+	+	–
LCB 55	+	+	+	+	+	+	+	–	+	+	+	–
LCB 56	+	+	+	+	+	+	+	–	–	+	+	–
LCB 8 (1)	+	+	+	+	+	+	–	–	–	+	+	–
RAB 7	+	–	+	–	+	+	+	–	–	+	+	–

[a]*B. subtilis* CNPSo26571 (Biovar), [b]*B. subtilis* + *B. licheniformes* (Quartzo®); GLU: D-glucose; ARA: L-arabinose; MNE: D-mannose; MAN: D-mannitol; NAG: N-acetyl-glucosamine; BAD: D-maltose; GNT: potassium glucanate; CAP: capric acid; ADI: adipic acid; MLT: malic acid; CIT: trisodium citrate; CAP: phenyl-acetic acid

Biofilm production

The isolates produced biofilm in variable proportions ($GL_{25:51}$ = 18.29; P <0.001). Seven isolates stood out among the largest biofilm producers, with results similar to the *B. subtilis* + *B. licheniformes*, namely: LCB 03, LCB 08, LCB 21, LCB 40, LCB 45, LCB 5 (3), LCB 51 (Table 2).

Production of biosurfactants

The emulsifying activity of the supernatant of the isolates varied ($GL_{25:51}$ = 59.3; P <0.001). The production of compounds with emulsifying action of the LCB 56 isolate was 96 and 213% higher compared to *B. subtilis* + *B. licheniformes* and *B. subtilis*, respectively (Table 2). Other isolates with remarkable E24% were LCB 5 (3), LCB 21 and LCB 30 (1), which did not differ statistically from the LCB 56 isolate. There was significant variation between the isolates in terms of production of surfactant metabolites ($GL_{25:51}$ = 91.15; p <0.001), with the highest scattering areas observed for the LCB 04 and RAB 7 isolates (Table 2).

Production of indolacetic acid (IAA)

In the presence of tryptophan, the concentration of indolic compounds from isolates LCB 51 (81.41 µM) and LCB 57 (79.2 µM) was significantly higher compared to

Table 2. Production of secondary metabolites and nematicidal activity of 23 *Bacillus* isolates and two commercial products in in vitro experiments.

Bacteria	Biofilm (DO 590 nm)	Biosurfactant E24 (%)[c]	Biosurfactant Scattering area (mm[b])	IAA-T (µM)[d]	IAA+T (µM)[e]	Siderophore (DO 640 nm)	Nematostatic effect (%)	Nematicidal effect (%)
Control	0.140±0,01 fi*	46.39 ± 1 a	13.66 ± 1.0 dg	2.93 ± 0.5 dg	3.87 ± 0.4 fh	0.659 ± 0.01 a	5.23 ± 4.5 ef	0
B. S.[a]	0.180 ± 0.0 bf	7.48 ± 1.7 eg	15.79 ± 0.8 cd	7.66 ± 0.1 b	4.45 ± 0.9 eg	0.269±0.01 eg	17.30 ± 5.3 df	82.11 ± 4.4 a
I 27	0.117 ± 0.0 gi	11.43 ± 2.5 df	13.64 ± 0.4 dg	2.12 ± 0.1 eg	1.4 ± 0.1 io	0.170 ± 0.01 g	19.91 ± 4.5 cf	80.10 ± 4.5 ab
LCB 04	0.118 ± 0.01 gi	9.05 ± 1.7 eg	24.27 ± 1.1 a	5.78 ± 0.2 be	6.77 ± 1.0 cd	0.188 ± 0.0 g	36.66 ± 6.5 ae	63.34 ± 6.5 af
LCB 08	0.228±0.01 ab	0 h	13.21 ± 0.6 g	3.23 ± 0.3 dg	1.15 ± 0.2 jo	0.393 ± 0.04 bf	57.48 ± 18.8 a	42.52±18.8 dh
LCB 21	0.205±0.01 ad	17.14 ± 3.3 bd	12.39 ± 1.6 fh	2.34 ± 0.3 eg	0.07 ± 0.3 o	0.508± 0.04 ab	45.75±13.8 ad	53.05±14.9 ah
LCB 28	0.147 ± 0.01 ei	16.57 ± 4.8 cd	14.78 ± 0.8 df	3.51 ± 0.6 cg	1.60 ± 0.7 io	0.460 ± 0.02 d	51.58 ± 15.8 ac	48.42±15.8 ch
LCB 03	0.257 ± 0.02 a	11.43 ± 2.5 df	13.44 ± 0.7 dg	4.92 ± 1.8 bg	2.34 ± 0.4 hm	0.443 ± 0.0 be	0.60 ± 0.6 f	36.98±11.7 eh
LCB 30 (1)	0.138 ± 0.00 fi	19.00 ± 0.9 bc	12.17 ± 0.4 gh	7.30 ± 0.5 bc	8.74 ± 0.3 c	0.419 ± 0.02 bf	47.14 ± 4.2 ad	52.86 ± 4.2 ah
LCB 33 (1)	0.139 ± 0.01 fi	8.10 ± 2.2 eg	17.28 ± 0.5 c	8.13 ± 0.2 b	3.84 ± 0.6 fh	0.171 ± 0.02 g	34.59 ± 5.2 ae	65.41 ± 5.2 ae
LCB 38	0.123 ± 0.0 gi	7.14 ± 1.4 eg	7.01 ± 0.7 i	1.82 ± 0.4 fg	6.11 ± 0.8 de	0.232 ± 0.02 fg	42.22 ± 3.6 ad	57.78 ± 3.6 ah
LCB 40	0.207±0.0 ad	6.67 ± 0.8 eg	14.76 ± 0.4 df	2.70 ± 0.5 eg	2.20 ± 0.1 hn	0.485 ± 0.05 ac	47.76±17.1 ad	52.24±17.1 bh
LCB 45	0.219 ± 0.03 ac	7.14 ± 2.5 eg	15.93 ± 1.2 cd	5.67 ± 0.5 bf	2.54 ± 0.1 gl	0.434±0.04 be	54.23±22.0 ab	33.65±11.0 gh
LCB 47	0.100 ± 0.0 i	15.71 ± 0.7 cd	6.57 ± 0.7 i	5.50 ± 0.9 bf	0.54 ± 0.2 lo	0.435±0.03 be	59.08 ± 15.6 a	38.96±12.3 dh
LCB 5 (1)	0.143 ± 0.02 fi	0 h	10.68 ± 0.0 h	1.35 ± 0.5 g	3.17 ± 0.4 fi	0.432 ± 0.1 be	57.48 ± 10.1 a	41.64±11.4 dh
LCB 5 (2)	0.190 ± 0.02 bf	5.71±0.0 fh	15.21 ± 0.7 ce	3.34 ± 0.6 dg	0.41 ± 0.1 mo	0.4233±0.1 be	43.74 ± 7.0 ad	56.26 ± 7.0 ah
LCB 5 (3)	0.201 ± 0.07 ae	17.14±3.8 bd	14.76 ± 0.4 df	5.47 ± 5.1 bf	2.92 ± 0.5 fj	0.355 ± 0.1 bg	33.29 ± 7.5 af	66.71 ± 7.5 ad
LCB 51	0.207±0.01 ad	4.76±0.8 gh	6.35 ± 0.6 i	3.42 ± 0.2 cg	81.41 ± 1.9 a	0.447 ± 0.1 be	52.58 ± 8.7 ac	39.02 ± 1.6 dh
LCB 53	0.146 ± 0.01 ei	11.43±0.7 df	13.25 ± 0.6 eg	3.53 ± 0.9 cg	4.97 ± 0.3 df	0.313±0.02 cg	64.08 ± 3.1 a	30.58 ± 4.2 h
LCB 55	0.138 ± 0.0 fi	0 h	20.19 ± 1.7 b	6.75 ± 1.6 bd	5.03 ± 0.5 de	0.658 ± 0.1 a	57.51 ± 5.0 a	42.49 ± 5.0 dh
LCB 56	0.169±0.01 dg	23.43±1.7 b	13.64 ± 0.4 dg	7,74 ± 0.5 b	1.87 ± 0.3 ho	0.287±0.07 dg	20.47 ± 3.0 cf	79.53 ± 3.0 ab
LCB 57	0.159 ±0.01 dh	12.57±4.7 ce	7.27 ± 0.8 i	3.2 ± 1.5 dg	79.2 ± 1.1 b	0.384±0.01 bg	40.18±16.9 ad	36.24 ± 9.6 eh
LCB 06	0.182 ± 0.01 bf	6.19±0.8 eh	12.62 ± 0.6 fh	1.4 ± 0.5 g	6.44 ± 0.6 de	0.403 ± 0.01 bf	64.64 ± 7.6 a	35.36 ± 7.6 fh
LCB 8 (1)	0.171± 0.01 dg	8.57±1.4 eg	14.31 ± 0.4 dg	3.65 ± 0.3 cg	0.24 ± 0.8 no	0.298±0.02 cg	39.50±10.2 ad	60.50 ± 10.2 af
B. S. + B. L.[b]	0.254 ± 0.02 a	11.90±1.7 df	15.69 ± 0.8 ce	2.62 ± 0.2 eg	0.52 ± 0.2 mo	0.441±0.09 be	56.15 ± 10.2 a	43.85±10.2 dh
RAB 7	0.110 ± 0.0 hi	6.20±0.8 eh	22.99 ± 0.8 a	14.42 ± 1.6 a	1.51 ± 0.1 io	0.488 ± 0.07 ac	22.25 ± 6.9 bf	77.75 ± 6.9 ac
Minimum	0.100	0.00	6.35	1.35	0.07	0.170	0.60	0.00
Maximum	0.257	46.39	24.27	14.42	81.41	0.659	64.64	82.11
Average	0.169	11.20	13.92	4.64	8.97	0.392	41.21	50.67

Variables transformed by the formula[$(X - \bar{X}) / s^2$]. *Groups of means (± standard deviation) followed by the same letter in the column do not differ at 5% significance, using the Tukey test; when there were more than two letters, we used an abbreviation with the first and last letters. [a]*B. subtilis* CNPSo26571 (Biovar), [b]*B. subtilis* + *B. licheniformes* (Quartzo®); [c]E24: emulsification index; [d]IAA-T: indolacetic acid without tryptophan; [e]IAA+T: indolacetic acid with tryptophan.

commercial strains ($GL_{25:51}$=3,334.85; P<0.001). However, there was higher variability in the synthesis of such compounds in the absence of the amino acid ($GL_{25:51}$ = 15.93; p<0.001). The RAB 7 isolate stood out with a mean concentration (14.42 µM) significantly higher than the one observed for the commercial strains (Table 2).

Production of siderophore

LCB 55 isolate showed the highest capacity for synthesising molecules with chelation action of Fe^{+3} in the CAS reagent ($GL_{25:51}$ = 13.27; P <0.001), with better performance than the supernatants of commercial strains (Table 2). Other isolates with high chelation potential were LCB 21, RAB 7 and LCB 40.

In vitro nematicidal activity of Bacillus isolates against M. enterolobii

The nematostatic effect was significant for 69.56% of the isolates when compared to control ($GL_{25:51}$ = 8.71; P<0.001). The performance of the isolates LCB 08, LCB 47, LCB 5 (1), LCB 53, LCB 55 and LCB 06 was better than that observed for *B. subtilis* (Table 2). All isolates showed a significantly higher nematicidal effect comparison to control ($GL_{25:51}$ = 12.17; p<0.001), particularly I 27 and LCB 56, which presented a control efficiency higher than 80% in comparison to the commercial product *B. subtilis*. + *B. licheniformes* (Table 2). In general, the total mortality, or the sum of the effects on the tested J2, was over 90%.

Bacillus isolates in the in-pot biocontrol of M. enterolobii

No isolate or commercial product significantly altered (P > 0.05) the weight of the aerial part and roots of the treated plants in relation to the control (Table 3). Among the variables related to the attack of the pathogen, a significant reduction (P <0.05) was observed only for the number of galls.

All evaluated plants showed galls. There was a significant reduction when the plants were treated with the isolate LCB 5 (3). This reduction was 81.2 and 76.9% in relation to plants not treated and treated with *B. subtilis* + *B. licheniformes*, respectively (Table 3). This performance did not differ statistically from treatments with LCB 03, LCB 40, LCB 45, LCB 47, LCB 51, LCB 56 and with *B. subtilis*. The number of galls for most of the isolates was fewer than 31, except of the isolates LCB 04, LCB 33 (1), LCB 38 and LCB 53 that exceeded 40 galls, which did not differ statistically from the control treatment.

Discussion

Biological control is an intelligent, environmentally safe alternative in the management of nematodes (Abd-Algawad, 2016). The selection of new biocontrol agents has focused on the potential of *Bacillus* species against *Meloidogyne* in *in vitro* and *in vivo* experiments (Abd-El-Khair et al., 2019; Lee & Kim, 2016; Wei et al., 2014). Research has proven the interference of nematophagous fungi in the reproduction and survival of *M. enterolobii* (Carneiro et al., 2011; Jindapunnapat et al., 2013), but data on the nematicidal potential

Table 3. Effect of 23 *Bacillus* isolates and two commercial products on nematological and plant variables 51 days after inoculation with 5,000 eggs +J2 of *M. enterolobii*, in an experiment in a greenhouse.

Bacteria	Number of gall / g of root	Number of J2 and egg / g of root	RF (Pf/Pi)[c]	MDA (g)[d]	FRM (g)[e]
Control	38.25 ± 22.2 a	1,485 ± 1,090 bc	1.13 ± 0.7 ab	6.97 ± 1.4 ab	4.35 ± 2.5 ab
B. S.[a]	12.72 ± 9.9 bc	1,650 ± 83 bc	1.69 ± 1.3 ab	6.52 ± 1.5 ab	4.85 ± 3.8 ab
I 27	20.59 ± 5.7 b	2,430 ± 1,599 ab	2.71 ± 1.6 a	6.89 ± 0.8 ab	6.76 ± 3.2 a
LCB 04	41.63 ± 33 a	2,602 ± 2,464 ab	1.25 ± 1.1 ab	6.7 ± 0.5 ab	3.48 ± 3.2 b
LCB 08	34.91 ± 48 a	5,065 ± 3,795 ab	1.55 ± 0.4 ab	7.70 ± 1.7 a	2.86 ± 2.7 b
LCB 21	24.04 ±25.8 b	1,668 ± 1,587 bc	1.16 ± 1.1 ab	6.12 ± 1.7 ab	3.47 ± 2.7 b
LCB 28	25.71±16.1 ab	1,559 ± 282 bc	1.86 ± 1.3 ab	5.96 ± 0.4 ab	5.28 ± 3.4 ab
LCB 03	13.51 ± 9.6 bc	2,120 ± 2,202 ab	1.79 ± 2 ab	6.26 ± 0.8 ab	3.97 ± 2.4 ab
LCB 30 (1)	26.95 ± 10.5 ab	1,357 ± 1,569 bc	0.85 ± 0.4 b	6.01 ± 0.8 ab	4.32 ± 1.8 ab
LCB 33 (1)	54.36 ± 23.8 a	7,914 ± 6,138 a	3.55 ± 2.1 a	4.39 ± 2.2 b	2.49 ± 1.8 b
LCB 38	42.79 ± 44.3 a	1,443 ± 1,862 bc	1.06 ± 1 ab	5.88 ± 2.6 b	3.74 ± 4.5 ab
LCB 40	16.10 ±11.1 bc	5,413 ± 6,939 a	2.4 ± 1.1 a	6.75 ± 1.4 ab	4.17 ± 3 ab
LCB 45	14.7 ± 10.6 bc	1,522 ± 1,056 bc	2.15 ± 1.5 a	7.20 ± 2.1 a	5.50 ± 3.4 a
LCB 47	13.66 ± 10.7 bc	1,782 ± 1,112 b	1.78 ± 0.9 ab	7.42 ± 0.8 a	5.17 ± 1.9 ab
LCB 5 (1)	23.83 ± 20.1 b	1,590 ± 1,418 bc	0.90 ± 0.7 b	6.01 ± 0.7 ab	3.64 ± 1.6 ab
LCB 5 (2)	27.74 ± 20.4 ab	2,752 ± 2,074 ab	1.94 ± 1.5 ab	6.32 ± 0.4 ab	5.16 ± 4.3 ab
LCB 5 (3)	7.17 ± 5.9 c	482 ± 440 c	0.77 ± 0.9 b	6.67 ± 1.6 ab	5.25 ± 3.9 ab
LCB 51	17.43 ±15.7 bc	1,960 ± 1,327 b	3.03 ± 1.4 a	7.93 ± 2 a	9.7 ± 7.1 a
LCB 53	44.02 ± 54.1 a	1,163 ± 1,181 c	0.93 ± 0.7 b	7.50 ± 0.9 a	4.23 ± 3.6 ab
LCB 55	27.57 ± 24.4 ab	3,436 ± 2,923 ab	1.74 ± 0.4 ab	5.21 ± 0.8 b	3.62 ± 2.4 ab
LCB 56	18.16 ±14.2 bc	5,695 ± 5,560 a	3.08 ± 3.3 a	6.0 ± 1.5 ab	2.77 ± 1 b
LCB 57	20.41 ± 15.8 b	2,458 ± 2,419 ab	2.1 ± 0.7 a	6.98 ± 1.5 ab	6.22 ± 2.9 a
LCB 06	19.36 ± 14.2 b	3,663 ± 3,900 ab	2.1 ± 0.9 a	6.45 ± 0.4 ab	7.14 ± 6.6 a
LCB 8 (1)	25.15 ± 25.1 ab	1,214 ± 1,185 bc	1.06 ± 0.8 ab	7.13 ± 0.9 a	4.57 ± 4.5 ab
B. S. + B. L[b]	31.11 ± 6.4 ab	1,114 ± 1,132 c	0.98 ± 0.8 b	5.12 ± 2.2 b	4.87 ± 1.1 ab
RAB7	19.21 ± 16.7 b	2,341 ± 1,346 ab	1.69 ± 0.6 ab	6.35 ± 0.5 ab	4.37 ± 2.5 ab
Minimum	7.17	482	0.77	4.39	2.49
Maximum	54.36	7,914	3.55	7.93	9.7
Average	25.43	2,534	1.74	6.48	4.68

Variables transformed by the formula $\sqrt{X+1}$.* Groups of means (± standard deviation) followed by the same letter in the column do not differ at 5% significance, using the Tukey test; [a]*B. subtilis* CNPSo26571 (Biovar), [b]*B. subtilis* + *B. licheniformes* (Quartzo®); [c]RF: reproduction factor; [d]MDA: dry matter of the aerial part; [5]FRM: fresh root matter.

of rhizobacteria against this nematode is still scarce. Moghaddam et al. (2014) proved the nematicidal potential of native *Bacillus* isolates in the control of *M. javanica*, which provides useful information for the exploration of new isolates.

In this study, it was observed that seven *Bacillus* isolates [LCB 03, LCB 40, LCB 45, LCB 47, LCB 51, LCB 56 and LCB 5 (3)] native to the Northeast region of Brazil reduced the severity of the attack of *M. enterolobii* when applied to the rhizosphere of melons. This performance was similar to commercial products used as reference. This approach shows in a practical way the potential of the isolates and reinforces the importance of this research, since the available works use only positive control (Chinheya et al., 2017; Sohrabi et al., 2018; Zhao et al., 2018).

The performance of these isolated can be associated with a set of characteristics. The versatility in the use of carbon compounds in root exudates as an energy source may have provided greater capacity for root colonisation through biofilm formation (Beauregard et al., 2013; Pelzer et al., 2011). This protective barrier reduced the arrival and penetration of infecting juveniles into the roots, decreasing the number of galls (Cao et al., 2011; Li et al., 2013). Furthermore, it may have potentiated the synthesis of metabolites with nematicidal and disorienting action (Cao et al., 2019). The best known nematicidal

compounds are surfactins, iturins, phengycin and lytic enzymes (Kavitha et al., 2012). These compounds can partially or totally affect the motility of juveniles, compromising the diffusion of water, gases and metabolites into and out of their bodies and their internal transport, which can lead to death (Lambert & Bekal, 2002). They can also have ovicidal action, causing disintegration of the egg mass and reduction in hatching of juveniles (Niu et al., 2006; Norabadi et al., 2013).

The findings by Kravchenko et al. (2003) demonstrated that the growth and ability to control *Pseudomonas* pathogens rely on acids and sugars in tomato root exudates. Wang et al. (2019) highlighted the importance of root exudates in the biocontrole mediated by *Bacillus cereus* against *Rastonia solanacearum*. In another study, Xiong et al. (2015) evidenced the importance of nematicidal metabolites synthesised by *B. firmus* in suppressing the attack of *M. incognita*.

Although rhizobacteria are known to promote plant growth (Kannahi & Senbagam, 2014), in this study, as it was reported by Fernandes et al. (2014), no increase was observed in the weight of the aerial part and the roots. However, in spite of no increase in plant biomass as reported in other studies (Araújo & Marchesi, 2009; Silva et al., 2017) there was no undesirable phytotoxic action. Other mechanisms deployed by *Bacillus* in the suppression of phytonematodes involve the production of volatile organic compounds (VOCs) and the activation of the plant's defense system. The first can cause the death, repellency or disorientation of infecting juveniles, preventing their arrival at the host's roots (Xu et al., 2015) and the second makes the plant resistant to nematode attack (Adam et al., 2014).

Several commercial *Bacillus*-based products are available worldwide (Abd-Elgawad & Askary, 2018). However, the application of biological agents poorly adapted to the whether conditions of Brazil may restrict their effectiveness. Hence, the need to study native microorganisms as to the ability to suppress nematodes and other phytopathogens, offering new approaches for the integrated management of plant diseases (Ludwuig et al., 2013). As a result, the seven isolates demonstrated potential as biocontrol agents by reducing the severity of the attack of *M. enterolobii* when applied to the rhizosphere of melons. However, it is necessary to carry out complementary studies, with regard to the persistence in the rhizosphere, interaction with other soil microorganisms, effectiveness and consistency of results of field experiments. Lastly, we seek to obtain one or more isolates to recommended in the integrated management of root-knot nematodes in infested areas. To our best knowledge, this is the first study that reports nematicidal activity of *Bacillus* isolates native to Brazil against *M. enterolobii*.

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No potential conflict of interest was reported by the author(s).

Declaration of interesti

The authors declare that there is no conflict of interest.

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