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Aphicidal activity of *Bacillus amyloliquefaciens* strains in the peach-potato aphid (*Myzus persicae*)

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ABSTRACT

Myzus persicae Sulzer (Hemiptera: Aphididae), is a generalist cosmopolitan insect that infests more than 400 plant species of 40 different families and is one of the major pests infesting potato crops. It causes direct damage and also spread plant viruses. The intensive use of synthetic insecticide to control aphids has led to resistant populations. Therefore, there is a need to develop biopesticides for effective control that minimizes environmental hazards. The bacteria *Bacillus amyloliquefaciens* is recognized as a producer of a variety of bioactive compounds. The aim here was to evaluate the aphicidal effect of *B. amyloliquefaciens* strains, CBMDDrag3, PGPBacCA2, and CBMDLO3, and their metabolites on the mortality and fecundity of *M. persicae.* Cells suspensions, heat-killed cell suspensions, cell-free supernatants, or isolated lipopeptide fractions from *B. amyloliquefaciens* strains were offered to aphids through artificial diets. The isolated lipopeptide fractions composed mainly of kurstakins, surfactins, iturins, and fengycins, when were administrated through diets, had no aphicidal effect against *M. persicae.* However, aphids fed on diets with whole cell suspensions and its cell-free supernatant of all three bacteria strains resulted in 100% mortality of adult aphids and nymphs. Specially, *B. amyloliquefaciens* CBMDLO3, has an effective aphicidal effect on *M. persicae,* used both bacterial cells and their metabolites. Moreover, heat-killed cells of *B. amyloliquefaciens* CBMDLO3 also had aphicidal action, although the aphid mortality was lower than on diet with living bacteria. Therefore, these results propose that *B. amyloliquefaciens,* could function as a novel eco-friendly biopesticide for the control of *M. persicae.*

1. Introduction

The peach-potato aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), is a generalist cosmopolitan insect that infests more than 400 plant species of 40 different families such as Solanaceae, Asteraceae, Malvaceae, Brasicaceae, Amaranthaceae, Rosaceae, Curcubitaceae, among others (Blackman, 1974a,b) and is one of the mayor pests infesting potato crops (Hill, 2008; Kuroli and Lantos, 2006). In potato crops, *M. persicae* causes damage by removing nutrients from the plant, by secreting honeydew which causes a secondary growth of fungi that inhibits photosynthesis, and by inoculating plant viruses (Castle and Berger, 1993; Salazar, 1996).

Myzus persicae is a piercing–sucking insect that ingests plant phloem fluid through the stylets, modified mouthparts that are used to penetrate the plant tissues (Tjallingii and Hogen Esch, 1993). Their feeding behavior, made aphids the most efficient vector of plant viruses (Loebenstein et al., 2001; Radcliffe and Ragsdale, 2002). Particularly, in vegetatively propagated crops, such as potato crops, virus dispersion is significantly higher in the presence of aphids, which reduces the performance of crops by up to 90% (Jeffries, 1998).

Potato growers, in order to keep potato crops virus-free, apply an intensive aphid control scheme that requires multiple insecticide applications, which frequently results in the development of resistant populations; in particular *M. persicae* display several types of resistance to insecticides (Devonshire and Field, 1991; Devonshire et al., 1998; Moores et al., 1994; Simon and Peccoud, 2018; van Toor et al., 2008). Seed potato crops are commonly treated with neonicotinoids, specially imidacloprid (van Toor et al., 2008), although *M. persicae* was

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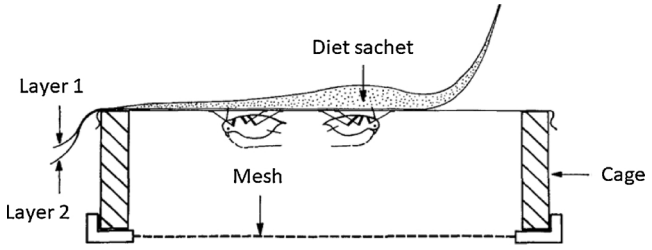

Fig. 1. Artificial diet cages: a plastic cylinder of 3 cm height × 379 2 cm wide, with a mesh on the bottom and the top opened. On this opened side the diet sachet was 380 applied (diet solution between two layers of Parafilm) (Machado-Assefh and Alvarez, 2018).

occasionally registered as resistant to neonicotinoids (Bass et al., 2011; Garzo et al., 2015; van Toor et al., 2013). Also use of synthetic insecticides produce environmental impacts, including poisoning of humans; destruction of wildlife; disruption of natural biological control and pollination (Atanasova and Leather, 2018); soil and groundwater contamination, and the evolution of resistance to pesticides in other pest populations (Ekström and Ekbom, 2011; Isman, 2006). Hence, there is an urgent need to develop biopesticides for effective control of agricultural pests without causing serious harm to the environment (Carlini and Grossi-de-Sá, 2002; Mnif and Ghribi, 2015).

A biopesticide is a mass-produced agent manufactured from a living microorganism or a natural product, and sold for the control of plant pests (Mnif and Ghribi, 2015). In recent years, the use of biopesticides has increased, and it is estimated that 50% of the biopesticides active substances registered in EU and USA come from microorganisms, and are referred to as microbial pesticides (Chandler et al., 2011). Among the beneficial bacteria used as biocontrol agents are several species of *Bacillus,* Gram-positive and spore-forming bacteria capable of colonizing the roots and the rhizosphere with beneficial effects on plants, and therefore are designated as plant growth-promoting rhizobacteria (PGPR) (Zouari et al., 2016). In addition, the genus *Bacillus* has been studied due to its capacity to produce a diversity of secondary metabolites with interesting biological activities, especially non-ribosomal synthesized cyclic lipopeptides (LPs) such as, surfactin, iturin and fengycin (Mnif and Ghribi, 2015; Ongena and Jacques, 2007). These compounds have a hydrophilic peptide part and a hydrophobic alkyl chain, and may differ on the amino acid sequence and fatty acid branching. They are characterized by a low toxicity, high biodegradability, and for being environmentally friendly; but they exhibit significant inhibitory activity on viruses, bacteria, fungi, oomycetes and mosquitoes (Kim et al., 2004; Ongena et al., 2005; Porrini et al., 2010; Sabaté et al., 2009). *Bacillus amyloliquefaciens* is a bacteria recognized for producing microbial pesticide, with an array of bioactive compounds with fungicide effect (Alvarez et al., 2012; Torres et al., 2017; Zouari et al., 2016), aphicidal effect against *M. persicae* (Yun et al., 2013), and biosurfactants with mosquitocidal effect against the mosquitoes *Aedes stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (Geetha et al., 2011) and insecticidal activity against larvae of *Spodoptera littoralis* and *Tuta absoluta* (Ben Khedher et al., 2017, 2015). In addition, *B. amyloliquefaciens* has a protective antiviral effect on tomato against TSWV and PVY viruses (Beris et al., 2018). The objective of this work was to assess the effect of *B. amyloliquefaciens* strains and their metabolites on the mortality and fecundity of *M. persicae*.

The aphicidal effect of *B. amyloliquefaciens* was evaluated in bacterial cells suspensions, heat-killed cell suspensions, cell-free supernatants, and isolated lipopeptide fractions. This work aims to contribute in the search for new environmentally friendly products of bacteria origin to control aphids.

2. Materials and methods

2.1. Aphids

Myzus persicae Sulzer was reared on radish (*Raphanus sativus* L). Aphids used in the experiments came from a colony maintained at the Faculty of Natural Science (National University of Salta, Salta, Argentina). This colony was initiated from a single virginoparous apterous individual collected in field in 2009. The colony was reared in a climate chamber at 22 ± 2 °C, 30–40% R.H., and 16/8 h light/ dark photoperiod to induce parthenogenesis. A new colony was started every week, and newly moulted apterae adult aphids were used for the experiments.

Microorganisms, suspensions of bacterial cells (CS), heat-killed cell suspension (hkCS), cell-free supernatants (CFS), and isolated lipopeptide fractions (LF)

Bacillus amyloliquefaciens strain CBMDDrag3 (GenBank access code JX120510) *B. amyloliquefaciens* PGPBacCA2 (GenBank access code JX120521), and *B. amyloliquefaciens* CBMDLO3 (GenBank access code JX12506) isolated from a honey sample and from an air sample, were cultivated at the Laboratory of Applied Bacteriology of INIQUICONICET. These strains were grown in Luria Bertani (LB) (Britania, Argentina) at 37 °C.

To obtain the CS, LB broth was inoculated with a pure culture of each bacterial strain in a percentage of 2% (v/v) and incubated for 1, 4 and 6 days, at 37 °C, without agitation. Concentrations of about 1×10^8 cells/ mL were obtained. Then, 50 µL of each culture were suspended on 5 mL of peptone water. The number of cells was determined by counting serial dilutions in peptone water on LB agar plates.

The CFS fractions were obtained from CS by centrifugation at 10,000 *g*, 10 min at 4 °C, and filtrated with a sterilized cellulose acetate filter of 0.22 µm pore-size and kept at 4 °C until further analysis.

The LF fractions were obtained from CFS of strains CBMDDrag3 and CBMDLO3. CFS were centrifuged and acidified to pH 2 with

Table 1
Aphicidal effect in *M. persicae* of cell suspension (CS) of *B. amyloliquefaciens* strains, CBMDDrag3, PGPBacCA2, and CBMDLO3, incubated 1, 4 and 6 days. The effect was observed after 4 days of feeding on artificial diets containing 10 µL of CS per 100 µL of diet, Numbers are means (± SE) of aphids, adults and nymphs.

Treatment	Diet control n = 8	Diet + peptone water n = 9	CBMDDrag3 n = 10			PGPBacCA2 n = 10			CBMDLO3 n = 10			H	P
			1 day	4 days	6 days	1 day	4 days	6 days	1 day	4 days	6 days		
Adults alive	4.25 ± 0.37 a	4.6 ± 0.16 a	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	44.6	< 0.0001
Nymphs alive	37.88 ± 5.87 a	47.7 ± 1.99 a	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	44.65	< 0.0001

Values in a row with different letters indicate significant differences at $p \leq 0.0009$, Kruskal-Wallis non-parametric ANOVA, followed by of pair comparisons between means of treatments with Bonferroni correction.

Table 2
Aphicidal effect in *M. persicae* of cell-free supernatants (CFS) of *B. amyloliquefaciens* strains, CBMDDrag3, PGPBacCA2, and CBMDLO3, incubated 1, 4 and 6 days. The effect was observed after 4 days of feeding on artificial diets containing 10 µL of CFS per 100 µL of diet, Numbers are means (± SE) of aphids, adults and nymphs.

	Diet control n = 15	Diet + LB medium n = 14	CBMDDrag3			PGPBacCA2			CBMDLO3			H	P
			1 day n = 15	4 days n = 15	6 days n = 15	1day n = 15	4 days n = 10	6 days n = 15	1 day n = 14	4 days n = 14	6 days n = 15		
Adults alive	4.27 ± 0.36 a	4.07 ± 0.44 a	0.07 ± 0.07 b	0 b	0.07 ± 0.07 b	0 b	0 b	0 b	0 b	0 b	0 b	63.48	< 0.0001
Nymphs alive	54.4 ± 7.35 b	53.53 ± 6.7 b	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	63.45	< 0.0001
Total nymphs	57.6 ± 6.42 c	54.00 ± 6.44 c	11.67 ± 1.38 bc	2.2 ± 0.83 a	2.4 ± 0.56 a	8.73 ± 1.42 b	1.7 ± 0.84 a	3.07 ± 1.25 a	6.86 ± 2.00 ab	1.93 ± 0.76 a	2.27 ± 0.67 a	99.23	< 0.0001

Values in a row with different letters indicate significant differences at $p \leq 0.0009$, Kruskal-Wallis non-parametric ANOVA, followed by of pair comparisons between means of treatments with Bonferroni correction.

concentrated HCl, the precipitated lipopeptides were recovered by centrifugation at 14,000 *g,* 25 min at 4 °C, and extracted with 10 mL of methanol. Then, the solvent was evaporated and the precipitate were dissolved in sterile distilled water and adjusted to pH 8–9 with 0.5 M NaOH. The LF final concentration were, 5.8 mg/mL for CBMDDrag3, and 5 mg/mL for CBMDLO3.

A heat-killed cell suspension (hkCS) of the strain CBMDLO3, was obtained from a 4-day culture by autoclaving at 121 °C, 15 min.

CFS of CBMDDrag3 and CBMDLO3 were analysed by UV-MALDI TOF. The main lipopeptides were identified, and spectra were recorded on a Bruker Ultraflex II TOF/TOF (Bruker Daltonics, Bremen, Germany) using 9 Hpyrido[3,4b]-indole (norharmane, nHo) as a matrix in positive ion mode. Two spots (technical replicates) were analyzed from each sample.

2.2. Aphicidal assays with B. amyloliquefaciens strains

The biological effect of *B. amyloliquefaciens* strains on *M. persicae* were assessed by offering the insects with artificial diets supplemented with CS, CFS, hkCS or FL, and after 4 days the adults mortality and the number of nymphs, alive and dead, were counted. The aphid's artificial diets contained 150 mM amino acids, 500 mM sucrose, vitamins, and minerals and were administrated through parafilm sachets on diet cages as previously described (Fig. 1) (Machado-Assefh et al., 2015; Prosser and Douglas, 1991). Diet cages consisted on plastic cylinders (3 cm diameter and 2 cm high) sealed on top with a diet sachet containing 100 µL of diet solution between two layers of Parafilm, and sealed at the bottom with a mesh (Koga et al., 2007; Prosser and Douglas, 1991). Five recently moulted apterae adult aphids were placed on the diet cages and were maintained in a climate chamber at 22 ± 2 °C, 30–40% R.H., in darkness. After 4 days of treatment, the number of adults and offspring, dead or alive, were counted per cage.

Five assays were carried using 100 µL of artificial diet, each with an added portion of the different *B. amyloliquefaciens* strain derivatives, as follows: 1) 10 µL of CS from strains CBMDDrag3, PGPBacCA2, or CBMDLO3, on days 1, 4, and 6 after incubation; controls were plain diet and diet with 10 µL of peptone water; 2) 10 µL of CFS from strains CBMDDrag3, PGPBacCA2, or CBMDLO3, on days 1, 4, and 6 days after incubation; controls were plain diet and diet with 10 µL of LB medium; 3) 1, 5 and 10 µL CFS from strains CBMDDrag3 and CBMDLO3 on day 4 day after incubation; control was only plain diet; 4) 10 µL of CS and hkCS from strain CBMDLO3 on day 4 after incubation; controls were plain diet and diet with 10 µL of LB medium; 5) LF from CBMDDrag3 at a concentration of 5.8, 14.5, 29 and 58 µg of LF on 100 µL of diet, and LF from CBMDLO3 at 5, 12.5, 25 and 50 µg/ 100 µL of diet; controls were plain diet and diet plus 10 µL of distilled water at pH 8.

Assay 1) was repeated twice, and assays 2), 3), 4) and 5), were run in triplicate; in all cases, they were carried out under the same conditions and using the same solutions of CS, CFS, hkCS, and LF. The final number of replicates per assay 1) was n = 10, whereas for assays 2), 3), 4) and 5) the number of replicates varied between n = 10-15.

2.3. Statistics analysis

The aphicidal effect of *B. amyloliquefaciens* strains on *M. persicae*, based on mortality of adults, number of nymphs, and total number of nymphs alive and dead, was analyzed by the Kruskal–Wallis non-parametric analysis of variance at one way of classification, followed by multiple comparisons between means of treatments with Bonferroni correction (Weisstein, 1999). Analysis were conducted with InfoStat 2015 (Di Rienzo et al., 2015).

3. Results

The potential aphicidal effect of the CS, CFS and LF of *B. amyloliquefaciens* CBMDDrag3, PGPBacCA2 and CBMDLO3 strains, was

Table 3
Aphicidal effect in *M. persicae* of cell-free supernatants (CFS) of *B. amyloliquefaciens* strains, CBMDDrag3 and CBMDLO3, after 4 days of feeding on artificial diets containing 1, 5 and 10 µL of CFS per 100 µL of diet. Numbers are means (± SE) of aphids, adults and nymphs.

Treatment	Diet control	CBMDDrag3			CBMDLO3			H	P
	n = 12	1 µL n = 15	5 µL n = 14	10 µL n = 15	1 µL n = 15	5 µL n = 15	10 µL n = 15		
Adults alive	4.67 ± 0.19 c	3.29 ± 0.46 bc	1.93 ± 0.44 b	2.53 ± 0.49 b	0.07 ± 0.07 a	0 a	0.07 ± 0.07 a	56.2	< 0.0001
Nymphs alive	63.08 ± 2.02 c	33.36 ± 8.36 b	16.8 ± 4.63 b	16.93 ± 4.87 b	0.07 ± 0.07 a	0.87 ± 0.64 a	0 a	51.63	< 0.0001
Total nymphs	63.08 ± 2.02 c	44.64 ± 5.58 bc	29.93 ± 3.62 b	30.87 ± 4.18 b	16.27 ± 2.66 a	13.2 ± 1.65 a	8.6 ± 1.59 a	63.58	< 0.0001

Values in a row with different letters indicate significant differences at $p \leq 0.002$, Kruskal-Wallis non-parametric ANOVA, followed by of pair comparisons between means of treatments with Bonferroni correction.

Table 4
Aphicidal effect in *M. persicae* of cell suspension (CS) and heat-killed cell suspension (hkCS) of *B. amyloliquefaciens* strain CBMDLO3, after 4 days of feeding on artificial diets containing 10 µL of CS or hkCS per 100 µL of diet. Numbers are means (± SE) of aphids, adults and nymphs.

Treatment	Diet control	Diet + LB	CBMDLO3		H	P
	n = 14	n = 14	n = 15			
			CS	hkC		
Adults alive	3.07 ± 0.43 a	3.21 ± 0.42 a	0 c	1.27 ± 0.32 b	44.41	< 0.0001
Nymphs alive	23.93 ± 3.94 a	28.86 ± 5.17 a	0 c	6.20 ± 1.86 b	49.06	< 0.0001

Values in a row with different letters indicate significant differences at $p \leq 0.0083$, Kruskal-Wallis non-parametric ANOVA, followed by of pair comparisons between means of treatments with Bonferroni correction.

evaluated on *M. persicae* after 4 days of feeding on artificial diet.

Aphids fed on diets with CS of *B. amyloliquefaciens* CBMDDrag3, PGPBacCA2, and CBMDLO3 at 1, 4, and 6 days post incubation, caused 100% adult mortality and no nymphs were found, alive or dead (Table 1). Aphids fed on diets with CFS from strain PGPBacCA2 and CBMDLO3 at 1, 4, and 6 days, caused 100% mortality of adults and nymphs. Although in diets with CFS, several nymphs were found, all were dead and the numbers were significantly lower than in the control at all incubation times (Table 2). On diets supplemented with CFS from CBMDDrag3 strain, after 1 day of incubation, the number of total nymphs, although dead, did not differ from the controls (Table 2). Aphids fed on diets in different concentrations of CFS, with an incubation period of 4 days, had a significant mortality of adults and nymphs at 5 and 10 µL/100 µL of diet of CFS of strain CBMDDrag3, and at 1, 5 and 10 µL/ 100 µL of diet of CFS of strain CBMDLO3 (Table 3). Aphids fed on diets with hkCS of strain CBMDLO3, had significant more mortality of adults and nymphs than aphids in control diet and in diet LB control, although the mortality of adults and nymphs is significantly lower than on diet with CS (Table 4).

Finally, the LFs extracted from the CFS of *B. amyloliquefaciens* CBMDDrag3 and CBMDLO3 and, in all of the concentrations tested, had no aphicidal effect on *M. persicae* (Table 5).

The lipopeptides produced by *B. amyloliquefaciens* CBMDDrag3 and CBMDLO3 incubated for 4 days were analyzed in cell-free supernatant of each strain, respectively, using UV-MALDI TOF. The compounds found fell into two m/z ranges 850–1200 (Fig. 2), which includes kurstakins, surfactins and iturins and m/z 1400–1700 (Fig. 3), which includes fengycins. Lipopeptide homologues were observed as protonated, sodiated and/ or potasiated adducts (Table 6). Cyclic homologues from kurstakin were detected as potasiated adducts at m/z 890.65, 902.88 and 917.21. Additionally, surfactin homologues were observed as adduct of sodium at m/z 1015.06, 1031.11, 1045.28 and 1059.34 (Fig. 2, Table 6). These peaks revealed differences of 14 m/z unities, suggesting a series of homologous molecules having different lengths of fatty acid chains (i.e., CH2 = 14 Da). Mass spectra profiles were similar for both strains under the study CBMDLO3 (Fig. 2a) and CBMDDrag3 (Fig. 2b). Fengycin A and fengycin B homologues were also detected in CBMDLO3 and CBMDDrag3 samples (Fig. 3). However, the intensity of the signals was higher in CBMDLO3 than in CBMDDrag3.

4. Discussion

Our results revealed that all strains of *B. amyloliquefaciens* have aphicidal activity against *M. persicae*. Our findings also suggest that *B. amyloliquefaciens* could function as a biocontroller in three ways: (a) as a bacterial cellular suspension (CS) containing the viable bacterial cells, spores, and their metabolites; (b) as heat-killed cell suspension (hkCS); and (c) as cell-free supernatant (CSF) containing only the bacteria metabolites. We found that mortality time (before the appearance of nymphs) varied between CS and hkCS, or CSF. All evaluated *B. amyloliquefaciens* elicited some lethal action on *M. persicae*, but mortality was strain- and treatment-dependent. No live or dead nymphs were found in diets with CS but some dead and alive nymphs were found in diets containing CFS or hkCS. The presence of nymphs (dead or alive) could indicate that adult aphids took longer time to die on CFS or hkCS than on diets containing the living bacteria (CS). The presence of living cells of *B. amyloliquefaciens* inside aphids may also have an inhibitory effect on their fertility. Our results suggest that viable bacterial cells and their active enzymes are key in their aphicide activity on *M. persicae*. Stavrinides et al. (2009) found that the plant pathogenic bacteria *Pseudomonas syringae* could be ingested by the aphid *Acyrthosiphon pisum* and migrate through the digestive tract, establish in the gut, and multiply. High titers of these bacteria are lethal for aphids, which stop feeding and begin to wander, continually depositing infected honeydew over plant surfaces.

Among the possible cause of mortality in aphids, an effect on the aphid endosymbionts *Buchnera aphidicola* should also be considered. *B.*

Table 5
Aphicidal effect in *M. persicae* of lipopeptide fraction (LF) extracted from *B. amyloliquefaciens* strains, CBMDDrag3 and CBMDLO3, after 4 days of feeding on artificial diets containing different concentrations per 100 µL of diet. Numbers are means (± SE) of aphids, adults and nymphs.

Treatment	Diet	Diet +	CBMDDrag3			CBMDLO3				H	P
	control n = 14	water pH8 n = 14	5.8 µg n = 14	14,5 µg n = 11	29 µg n = 12	58 µg n= 12	5 µg n = 15	12,5 µg n = 14	25 µg n = 14	50 µg n = 13		
N° adults alive	3.87 ± 0.27	3.85 ± 0.32	4.36 ± 0.23	4.00 ± 0.33	4.25 ± 0.28	4.15 ± 0.27	4.47 ± 0.19	3.14 ± 0.36	4.43 ± 0.2	3.31 ± 0.33	17.06	0.0245
N° nymphs alive	34.47 ± 3.7	38.15 ± 3.75	40.71 ± 3.55	35.91 ± 4.96	40.5 ± 4.59	35.15 ± 3.75	40.27 ± 3.3	31.5 ± 4.5	38.93 ± 4.29	29.46 ± 3.52	9.49	0.3923

Values in a row with different letters indicate significant differences at $p \leq 0.002$, Kruskal-Wallis non-parametric ANOVA, followed by of pair comparisons between means of treatments with Bonferroni correction.

aphidicola is a Proteobacteria and an obligatory endosymbiont, which produces essential amino acids that supplement aphid diet (Douglas, 1996). When *B. aphidicola* are eliminated by chemical or antibiotic treatments, the aposymbiotic aphids are sterile, show reduced performance and increased mortality (Douglas, 1998), and a constrained feeding behavior (Machado-Assefh and Alvarez, 2018). Here, we hypothesized that once *B. amyloliquefaciens* cells are ingested by *M. persicae*, the insect succumbs, either due to a competition with the endosymbiont *B. aphidicola* or due to a bacterial sepsis. The final mechanism/s involved must be further evaluated.

Regarding the incubation time of *B. amyloliquefaciens* that is needed to get an aphicide effect (measured as the total number of nymphs found dead) on the CFS of the three strains, the incubation time of 4 and 6 days had stronger aphicide effects than that of CFS incubated only one day. Although in diet with CFS of strain CBMDDrag3 the total number of nymphs did not differ from that found in the controls, all nymphs were found dead, which suggest that, even one day of incubation is enough to get aphicide effect. Since the three *B. amyloliquefaciens* strains, CBMDDrag3, PGPBacCA2, and CBMDLO3, were toxic to adults and nymphs of *M. persicae*, the strains CBMDDrag3 and CBMDLO3 at 4 days of incubation were selected for dose evaluation since they showed insecticidal effect on larvae and adults of *Musca domestica* (Torres, 2014). Finally, the CFS of CBMDLO3 strain had stronger aphicide effect over adults and nymphs of *M. persicae* than that of CBMDDrag3 at the three concentrations evaluated.

Aphids are hemimetabolous insects, with a complex life cycle that varies according to the environment. Parthenogenetic females generated on spring and summer are combined with a single annual sexual generation produced in late summer to overwinter as eggs (holocycle). *M. persicae* characteristic feature is its life-cycle variation, since as long as favorable conditions exist, the colony is built of parthenogenetic females that produce offspring in a viviparous manner. Newborn nymphs feed immediately and become adults in one week, resulting in a telescopic population growth. In tropical, sub-tropical, and temperate climates *M. persicae* can reproduce parthenogenetically throughout the year (anholocycle) and increase their pest potential (Blackman, 1974a,b). In temperate zones, potato crops sown in spring are a secondary host for *M. persicae* and are therefore threatened by the build-up of aphid populations. Under these circumstances, adult aphids must be quickly and effectively controlled to avoid an outbreak. Insecticides should kill adults fast to prevent the appearance of nymphs.

The composition of the lipopeptides produced by the strains CBMDDrag3 and CBMDLO3 of *B. amyloliquefaciens* was very diverse and depended on each strain. The lipopeptides produced by both strains were identified as homologs of kurstakins, surfactin, iturines, and fengicines (Table 6). Isolated lipopeptide fractions (LF) extracted from CFS of strains CBMDDrag3 and CBMDLO3 were not active against *M. persicae*, contrary to what was reported by Yun et al. (2013), who reported aphicidal activity by surfactins isolated from *B. amyloliquefaciens* applied topically to the dorsum of *M. persicae*. Yun et al. (2013) exposed aphids to purified surfactin applied topically while we exposed aphids to a blend of lipopeptides provided through the diet. According to our results, isolated lipopeptide fractions are not responsible for aphicidal activity present in CS and CFS, at least not when ingested by aphids. However, the negative effect of lipopeptides cannot be completely disregarded since they may need the presence of other bacterial metabolites that were not identified.

Overall, the finding that CS, hkCS, and CSF of the *B. amyloliquefaciens* acts on *M. persicae* in a short time with a high aphicidal effect, makes it a promising alternative to the development of a bacterial biopesticide. In potato crops, *M. persicae* is responsible for the spread of viruses that cause important economic losses. This aphid is also prone to develop resistance to synthetic pesticides. A microbiological pesticide made of a blend of compounds that act synergistically can minimize the development of such resistance.

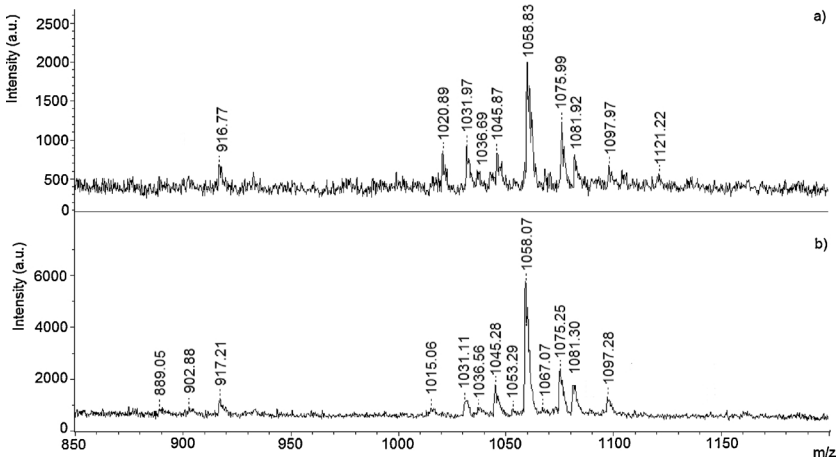

Fig. 2. UV-MALDI mass spectra of cell-free supernatant from *B. amyloliquefaciens* a) CBMDLO3, b) CBMDDrag3 in positive ion mode. Matrix: nHo; m/z range: 850 to 1200.

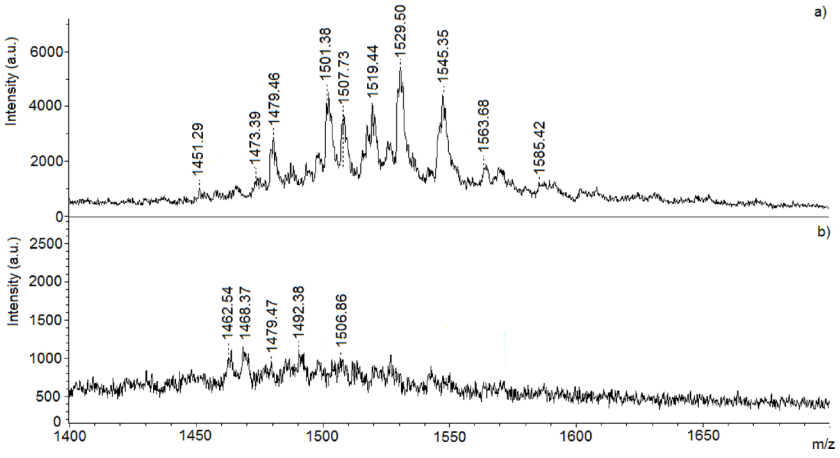

Fig. 3. UV-MALDI mass spectra of cell-free supernatant from *B. amyloliquefaciens* a) CBMDLO3, b) CBMDDrag3 in positive ion mode. Matrix: nHo; m/z range: 1400 to 1700.

Table 6
Mass spectrometry analysis of lipopeptides produced by *Bacillus amyloliquefaciens* in Luria Bertani media at 37 °C. Experiments were conducted on two spots (duplicate) prepared with each individual sample.

m/z	Lipopeptide assignment		CBMDLO3	CBMDDrag3
	Name	Chemical formula		
888	Kurstakins	C9[M + K]$^+$	–	+
902	Kurstakins	C10[M + K]$^+$	–	+
916	Kurstakins	C11[M + K]$^+$	+	+
1014	Surfactin	C12 [M + Na]$^+$	–	+
1021	Not assigned		+	–
1030	Surfactin	C13 [M + Na]$^+$	+	+
1036	Surfactin	C15 [M+H]$^+$	+	+
1044	Surfactin	C14 [M + Na]$^+$	+	+
1052	Bacillomycin D	C14 [M + Na]$^+$	+	+
1058	Surfactin	C15[M + Na]$^+$	+	+
1066	Bacillomycin D/iturin A	C15[M + Na]$^+$/C14 [M + Na]$^+$	–	+
1074	Surfactin	C15 [M + K]$^+$	+	+
1080	Bacillomycin D/iturin A	C16[M + Na]$^+$/C14 [M + K]$^+$	+	+
1096	Bacillomycin D/iturin A	C16[M + K]$^+$/C18[M+H]$^+$	+	–
1120	Iturin A	C18[M + Na]$^+$	+	+
1450	Fengycin	Ala-6-C15[M+H]$^+$	+	–
1462	Fengycin	Ala-6-C16[M+H]$^+$	–	+
1472	Fengycin	Ala-6-C15[M + Na]$^+$	+	–
1478	Fengycin	Ala-6-C17[M+H]$^+$	+	+
1492	Fengycin	Val-6-C16[M+H]$^+$	–	+
1500	Fengycin	Ala-6-C17[M + Na]$^+$	+	–
1506	Fengycin	Val-6-C17[M+H]$^+$	+	+
1528	Fengycin	Val-6-C16[M + K]$^+$	+	–
1544	Fengycin	Val-6-C17[M + K]$^+$	+	–
1562	Fengycin	Val-6-C21[M+H]$^+$	+	–
1584	Fengycin	Val-6-C21[M + Na]$^+$	+	–

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