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Scientific Article

Fatty acid composition of lipopeptides with antifungal activity synthesized by *Bacillus amyloliquefaciens*

Nancy Ley-López[1], José Basilio-Heredia[2], Isabel Cruz-Lachica[2], Isidro Márquez-Zequera[2], Raymundo Medina-López[1], Cesar San Martín-Hernández[3], Raymundo Saúl García-Estrada[2*], Facultad de Agronomía, Universidad Autónoma de Sinaloa. Carretera Culiacán-Eldorado Km 17.5, Aparatado Postal 25, CP. 80000, Culiacán, Sinaloa, México[1], Centro de Investigación en Alimentación y Desarrollo, A.C. Coordinación Culiacán (CIAD). Km 5.5 Carretera a Eldorado, Campo El Diez, CP. 80110. Culiacán, Sinaloa, México[2], Colegio de Postgraduados, Campus Montecillo, Km 36.5 Carretera México-Texcoco, Montecillo, CP 56264, Estado de México, México[3].

***Corresponding Author:**
Raymundo Saúl García-Estrada
rsgarcia@ciad.mx

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ABSTRACT

Background/Objective. Some *Bacillus* species synthesize biologically active lipopeptides containing fatty acids of varying length. Fatty acid composition is key to their potential activity as surfactants. The objective was to determine the composition and quantification of fatty acids in the structures of lipopeptides synthesized by *Bacillus amyloliquefaciens* KX953161.1 and to observe the effect of lipopeptides on the mycelial growth of *Fusarium oxysporum* MG557870 and *Sclerotium rolfsii* OM510466.

Materials and Methods. The lipid profile of the extract with lipopeptides synthesized by *B. amyloliquefaciens* KX953161.1 was performed by gas chromatography with a flame ionization detector (GC-FID). The quantification of the detected fatty acids was performed using the area standardization method, and the fat determination was carried out using the 920.39 AOAC method. In addition, scanning electron microscopy (SEM) was used to observe the inhibitory effect of lipopeptides on the mycelial growth of *F. oxysporum* MG557870 and *S. rolfsii* OM510466. The data obtained (determination of fatty acids and MIC) were analyzed by analysis of variance (ANOVA), and the comparison of means was performed using the Tukey test ($p \leq 0.05$).

Results. Variability was observed in C_{13} to C_{18} β-hydroxylated fatty acids. The C_{14} β-hydroxylated fatty acid (tetradecanoic acid) had the highest content, with 8.254 ± 0.031 ng mg^{-1} of sample, followed by the C_{13} β-hydroxylated fatty acids (tridecanoic acid) and C_{16} fatty acids (palmitic acid), with 4.304 ± 0.064 and 4.100 ± 0.120 ng mg^{-1}, respectively. The minimum inhibitory concentration (MIC) of the lipopeptides on *F. oxysporum* MG557870 and *S. rolfsii* OM510466 was 20 and 15 µg mL^{-1}. Damage to fungal cells was evident in their structure and morphology.

Conclusion. Lipopeptide synthesis by *B. amyloliquefaciens* KX953161.1 exhibits variation in the length of the fatty acid chains, which are primarily composed of C_{14} (tetradecanoic) fatty acids. This variability could be related to the biological activity of

the lipopeptides, and the C_{14} (tetradecanoic) fatty acid could be important in their antagonism against phytopathogenic fungi.

Keywords: Surfactant activity, β-hydroxylated, Tetradecanoic, *Fusarium oxysporum*, *Sclerotium rolfsii*

INTRODUCTION

The bacterial species *Bacillus amyloliquefaciens* can secrete secondary metabolites with biological activity as a response to the stimuli of the external microbiota (Gimenez *et al*., 2021; Bai *et al*., 2023; Ley-López *et al*., 2023). These compounds are an important source of novel chemical structures for biotechnology, and a large number of secondary microbial metabolites have been identified (Yan *et al*., 2020; Posa *et al*., 2023). Likewise, they are characterized by having surfactant properties, including the non-ribosomal cyclic lipopeptides of the iturin, surfactin and fengycin families (Carolin *et al*., 2021; Bai *et al*., 2023). These metabolites have different biological abilities, such as antitumor, hemolytic and antimicrobial properties; the latter is associated with activity against a variety of phytopathogenic microorganisms (bacteria, fungi and oomycetes), causing cell lysis and leakage through binding to the plasma membrane (Zhou *et al*., 2020), a main mechanism which helps the biological activities of the lipopeptides to take place. Such is the case of the inhibition of the mycelial growth of *Sclerotium rolfsii* by the lipopeptides synthesized by *B. subtilis* (Abdel-Gayed *et al*., 2019; Zhao *et al*., 2017). The antifungal activity of the fungicidal lipopeptid on *Fusarium oxysporum* inhibits the mycelial growth of the phytopathogen (Nam *et al*., 2015) and the fungicidal lipopeptide is said to affect the structural and morphological characteristics of the cell membrane (Zhao *et al*., 2017). It also induces apoptosis at low concentrations and necrosis at high concentrations when the fungal cells are treated with fengycin (Gimenez *et al*., 2021).

The structural composition of the lipopeptides consists of a variable hydrophilic peptide macrocycle, joined to a lateral chain of fatty acid residue (Zhao *et al*., 2017). In addition, these lipopeptide families display counterparts, similar structures or identical molecular weights that differ in the length in the fatty acid chains and in the composition of amino acids of the peptide ring (Biniarz *et al*., 2017; Ley-López *et al*., 2023). The biosynthesis of lipopeptides by *Bacillus* spp. begins with the assembly by successive addition of both proteinogenic and non-proteinogenic amino acids with the synthesis of non-ribosomal peptide synthetases (NRPS), as well as by the attachment of fatty acids to the NRPS in the form of acetyl-CoA for straight chain fatty acids and isobutyryl-CoA, isovaleryl-CoA and methyl butyryl-CoA for branched fatty acid (Chooi *et al*., 2010; Zhu *et al*., 2021). The members of the family of the surfactin lipopeptide have been reported to present a length in the fatty acid chain from C_{12} to C_{16} (de Faria *et al*., 2011; Jajor *et al*., 2016), whereas the iturin family presents an acid chain length of C_{14} a C_{17} (Jin *et al*., 2020) and the fengicin family has chain lengths from C_{14} to C_{18}, which may be saturated or unsaturated (Sa *et al*., 2018). There is a basic structural diversity of bacterial cyclic lipopeptides, in which the carboxyl group of the fatty acid can form a peptide bond with an amino acid residue located in the side chain of the lipopeptide (Urajová *et al*., 2016; Biniarz *et al*., 2017). Likewise, the fatty acid can be incorporated into the peptide structure through cyclization, frequently at carbon C_2 or C_3 via *β*-amino or *β*-hydroxy groups (Duitman *et al*., 1999; Mareš *et al*., 2014). This diversity in the structure of the peptide

macrocycle and the length of the fraction of the fatty acids of the lipopeptides present amphipatic properties that play a decisive role in their mode of action and can act as antibiotics, anti-adhesives, cleaning and foaming agents, but their action on living cells is not always clear (Tomek *et al*., 2015; Biniarz *et al*., 2017). Youssef *et al*. (2005) observed that the fatty acid composition is fundamental to the biosurfactant activity of the lipopeptides, displaying a significantly positive correlation between the mass percentage of iso-3-OH-C_{14} fatty acid and the specific activity of the lipopeptide. Therefore, the composition of the fatty acids is important for the biological properties of the lipopeptides such a the surfactin, iturin and fengycin familis (Chooi *et al*., 2010; Yan *et al*., 2020). Bai *et al*. (2023) analyzed lipopeptides with GC-MS and described the main differences between the lipopeptide counterparts, which arise from the fatty acid chain length and the amino acids in the peptide chain. Likewise, Qian *et al*. (2020) reported that the bacillomycin D counterparts with C_{14} and C_{15} fatty acids presented antifungal activity; in both studies, the highest fatty acid mass content corresponded to carbon chain lengths of C_{14} and C_{15}. However, new structural types of lipopeptide composition are still being reported, highlighting their rich chemical diversity and variability in fatty acid chain length.

Recently, in the *Bacillus amyloliquefaciens* KX953161.1 bacterial strain with oomycidal activity on *Phytophthora capsici* (Ley-López *et al*., 2018), several counterparts were identified in the families of (fengycin, surfactin and bacillomycin) with differences in molecular weight, due to the presence of fatty acid chains with varying lengths. However, the lipid profile of the lipopeptide extract synthesized by this bacterium is unknown, as is the quantity of the fatty acids detected (Ley-López *et al*., 2023). Due to this, interest has been placed on the composition of the fatty acids in the structure of the lipopeptides produced by this bacterium. The aim of this study was to determine the composition and quantification of the fatty acids in the structures of the lipopeptides synthesized by the bacterium *B. amyloliquefaciens* KX953161.1, using gas chromatography with flame detection (GC-FID) and determine the effect of the lipopeptides produced by the bacteria in this study on the microscopic morphology of the *F. oxysporum* MG557870 and *S. rolfsii* OM510466 mycelia.

MATERIALS AND METHODS

Biological material. The strains evaluated (bacteria and fungi) for this study were provided by the strain collection of the Phytopathology Laboratory at the Research Center for Food and Development, Culiacán Unit, Mexico. *B. amyloliquefaciens* B17 (KX953161.1) isolated from the tomato plant rhizosphere, was reactivated on nutrient agar (NA) at 27 °C. *F. oxysporum* MG557870 (Isaac *et al*., 2018) and *S. rolfsii* OM510466 (García-León *et al*., 2022) were isolated from roots of vegetable crops (tomato and chili). Moth microorganisms were identified based on morphological characteristics, using specific taxonomic keys, and pathogenicity tests had previously been performed on them. They were reactivated on potato dextrose agar (PDA) for 10 d at 28-30 °C. The culture media for microbial growth were purchased from Sigma-Aldrich®, USA.

Production of lipopeptides. For the synthesis of lipopeptides by the bacterium *B. amyloliquefaciens* KX953161.1, a bacterial inoculum was used in 50 mL of Luria-Bertani

(LB) liquid culture medium (casein peptone 10.0 g L^{-1}, yeast extract 5.0 g L^{-1}, and sodium chloride10.0 g L^{-1} with a pH of 7.0). It was incubated at 30±1 °C in an orbital shaker at 150 rpm for 18-20 h, reaching a bacterial cell concentration of $3x10^8$ CFU, according to the McFarland scale. The second culture medium consisted of 500 mL of Landy medium (glucose 20 g L^{-1}, L-glutamic acid 5 g L^{-1}, yeast extract 1 g L^{-1}, K_2HPO_4 1 g L^{-1}, $MgSO_4$ 0.5 g L^{-1}, KCl 0.5 g L^{-1}, $CuSO_4$ 1.6 mg L^{-1}, $Fe_2(SO_4)_3$ 0.4 mg L^{-1}, $MnSO_4$ 1.2 mg L^{-1}) with an initial pH of 7.0 (Landy *et al*., 1948). Both culture media were sterilized at 121 °C for 15 min. Later, 30 mL of the bacterial inoculum were transferred to the Landy medium and incubated for 6 d at 30±1 °C, stirred at 180 rpm.

The extraction of bacterial cells was performed by centrifuging (HERMLE® Z 36 HK, Germany) at 10,000 rpm for 12 min at 4 °C. The extraction of the lipopeptides was performed with the method of acid precipitation of the supernatant (Unás *et al*., 2018). The supernatant without bacterial cells was acidified by adding HCl 6N, until a pH of 2.0 was obtained pH=2.0; it was incubated for 24 h at 4 °C, and to obtain the pellet with the lipopeptides, it was centrifuged at 12,000 rpm for 20 min a 4 °C. Finally, it was lyophilized and stored until use (FreeZone Triad Benchtop Freeze Dryer, LABCONCO®, USA).

Analysis of fatty acids with CG-FID. The process involves the hydrolytic fragmentation of the bond between the peptide/protein part and the lipid portions. Therefore, the resulting fatty acid chains were derivatized to fatty acid methyl esters (FAME) and their subsequent analysis with GC-FID. For the derivatization, the MAK174 extraction kit (Sigma®), was used, following the protocols by Smyth *et al*. (2014). In the analysis of the lipid part of the material, 25 mg of the purified and lyophilized material was used, which was washed with hexane to remove the free fatty acids. The product was collected in an amber glass vial for subsequent gas chromatography analysis.

An Agilent 7890B gas chromatograph was mounted onto a flame ionization detector (FID). The samples were injected using an Agilent GC Automatic Sampler 80 with split/splitless injection ports with a VF-5ms capillary column, 30 m x 0.25 mm x 0.25 µm, under the conditions shown in Table 1. The temperature of the injector was 250 °C, the carrying gas was helium with a flow of 1.0 µL min^{-1}, Pulsed Splitless mode. To determine the possible compound detected, a spectral comparison was performed in the NIST Library (NIST 2011b Mass Spectral Library using NIST MS Search Rev. 2011b or the Probability Based Matching (PBM) Search Format as a part of Agilent Technologies MS WorkStation Software Version 7.0).

Table 1. Conditions of injection by Gas Chromatography (GC).

Stage	Temperature	Speed (°C/min)	Duration (min)	Total (min)
Initial	70	-	5	5
1	180	20	6	15
2	230	15	5	30.5
3	300	10	3	40

Quantification of fatty acids. Fatty acids detected were quantified using the area standardization method; this was done directly from the chromatographic run of the

sample, adding the areas of all the peaks eluted in the sample run. The total represents 100% of the area of each peak identified as fatty acids. The fat determination was carried out using the AOAC method 920.39. This parameter was obtained with 3 g of lyophilized lipopeptides in a Goldfish extraction thimble (LABCONCO®, USA). The extraction was performed using anhydrous petroleum ether for 4 h. The extract obtained with the solvents was evaporated until dry in an oven at 105 °C. Finally, the calculation of fat was carried out using the following equation.

$$\%\text{EE} = \frac{P_e \text{ x } 100}{Pm}$$

%EE= Percentage of ethereal extract.
P_e= Weight of extract (weight of the dry cup at the end of the extraction and its constant weight before extraction).
P_m= Weight of sample.

Inhibiting activity by extraction with lipopeptides. The minimum inhibiting concentration (MIC) of the extract with the lipopeptides synthetized by *B. amyloliquefaciens* KX953161.1 on *F. oxysporum* MG557870 and *S. rolfsii* OM510466 was determined using the diffusion method on dishes. This consisted in placing discs, 6 in diameter, containing fungal mycelia, in the middle of a Petri dish with PDA and placing Whatman paper discs, 6 mm in diameter, impregnated with the concentrations of 5, 10, 15, 20, 25, 30 and 40 µg mL^{-1} of lipopeptides, incubated at 26±1 °C for 6 d. The MIC was determined visually, based on the minimum concentration of sample solution required to guarantee that no fungal growth was observed. All analyses were performed in triplicate. The MIC was considered for the ultrastructural observation samples of the fungi through scanning electron microscopy (SEM).

Ultrastructural analysis via MEB. For the structural observation of the fungi with SEM analysis, *F. oxysporum* MG557870 spores and hyphae were taken, along with *S. rolfsii* OM510466 mycelia, aged 6 and 3 days, respectively. They were placed in 2 mL Eppendorf tubes and the lipopeptide suspension, with the concentration obtained from the MIC test for each fungus was added; they were then let to stand for 10 min. Subsequently, they were fixated in 2.5% glutaraldehyde (prepared in 0.1 M sodium phosphate buffer) at 4 °C for 24 h, then rinsed three times with phosphate buffer (0.02 M) and later fixated with 2% osmium tetroxide for 2 h at 20 ºC. Dehydration was carried out with a gradually ascending series of ethanol (30, 50, 75 and 95%) for10 minutes each, dried with CO_2 and covered using Nanotech (ES-2030 HITACHI®, Japan) cathodic pulverization. The samples were kept in a drier until the test with a scanning electron microscope (Philips, SEM-505, Netherlands) run at 30 kV.

Statistical analysis. The data obtained from the determination of fatty acids and MIC were analyzed using an analysis of variance (ANOVA), and the means comparison was carried out using Tukey's test ($p \leq 0.05$) using the Minitab 19 software.

RESULTS

GC-FID analysis. The fatty acids found in the lipopeptides synthetized by the bacterium *B. amyloliquefaciens* KX953161.1 were analyzed after derivatization with GC-FID. The chromatogram obtained shows 10 main peaks with a retention time of 12.82, 13.30, 14.58, 15.04, 16.43, 17.13, 18.25, 19.49, 19.97 and 21.33 min, each of which corresponds to the fatty acids detected (Figure 1). *β*-hydroxylated C_{13} to C_{18} fatty acids were detected, and myristic (C_{14}) and palmitic (C_{16}) fatty acids were identified (Table 2). In the analysis of the total content of each fatty acid identified, *β*-hydroxylated C_{14} (tetradecanoic) fatty acid (Figure 2) was observed to have the highest content, with 8.254±0.031 ng mg^{-1} of sample, followed by the *β*-hydroxylated C_{13} (tridecanoic) and C_{16} (palmitic) fatty acids, with 4.304±0.064 and 4.100±0.120 ng mg^{-1}, respectively (Table 2).

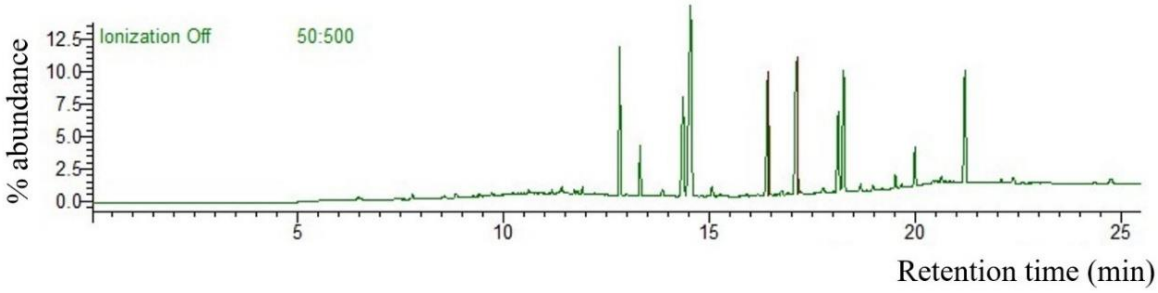

Figure 1. Chromatogram of the lipid profile of the lipopeptides synthesized by *Bacillus amyloliquefaciens* KX953161.1, analyzed by CG-MS.

Table 2. Profile of fatty acids of the lipopeptides biosynthesized by *Bacillus amyloliquefaciens* KX953161.1, identified using GC -MS.

Compound	Retention time	Carbon	Total (%)	ng mg^{-1} off sample
1	12.827	3-OH-C_{13}	14.348	4.304±0.064 b*
2	13.306	C_{14} (Mirístico)	4.158	1.247±0.138 d
3	14.588	3-OH-C_{14}	27.516	8.254±0.031 a
4	15.048	C_{15}	1.042	0.312±0.056 ef
5	16.434	3-OH-C_{15}	12.482	3.744±0.088 c
6	17.135	C_{16} (Palmítico)	13.667	4.100±0.120 b
7	18.256	3-OH-C_{16}	12.118	3.635±0.089 c
8	19.499	3-OH-C_{17}	0.914	0.274±0.071 f
9	19.974	C_{18}	1.788	0.536±0.033 e
10	21.330	3-OH-C_{18}	0.140	0.042±0.024 g

*In the column, different letters indicate significant differences between samples according to Tukey's test ($p \leq 0.05$).

Inhibiting activity and ultrastructural observation of the fungi. In the MIC analysis fir the antifungal effect with the lipopeptides synthesized by *B. amyloliquefaciens* KX953161.1, with the minimum concentration of 20 µg mL^{-1} of lipopeptides, an inhibiting halo of 0.95 mm was observed in the mycelial growth of *F. oxysporum*

MG557870 and for *S. rolfsii* OM510466 with 15 µg mL^{-1} of lipopeptides, an inhibiting halo of 1.5 mm was observed (Table 3).

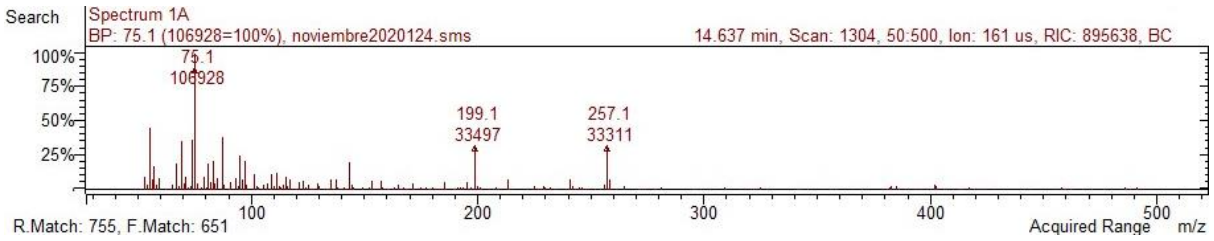

Figure 2. Mass spectrum of fatty acid C_{14} (Tetradecanoic) of the extract with lipopeptides synthesized by *B. amyloliquefaciens* KX953161.1.

Table 3. Antifungal effect of the lipopeptides synthesized by *B. amyloliquefaciens* KX953161.1 with the CMI on the mycelial growth of *F. oxysporum* MG557870 and *S. rolfsii* OM510466.

lipopeptides concentration (µg mL^{-1})	**Inhibition of mycelial growth (mm)**	
	***F. oxysporum* MG557870**	***S. rolfsii* OM510466**
C/1[z]	0 b[y]	0 e
5	0 b	0 e
10	0 b	0 e
15	0 b	1.50±0.13 d
20	0.95±0.06 a	1.60±0.15 c
25	1.03±0.09 a	2.00±0.08 b
30	1.26±0.15 a	2.20±0.08 b
40	1.08±0.15 a	2.52±0.09 a

[y]Different letters indicate significant differences, according to Tukey's test ($p \geq 0.05$).
[z]Control (lipopeptide-free).

The minimal inhibition concentrations with lipopeptides of 15 and 20 µg mL^{-1} on *S. rolfsii* OM510466 and *F. oxysporum* MG557870 respectively were used for the SEM observation analysis of spores and the mycelia of fungi related to diseases, in order to try to understand the action mechanism of lipopeptides. The *F. oxysporum* MG557870 hyphae, not treated with lipopeptides, displayed smooth surfaces, without any damage or cracks (Figure 3B). Normal growth and germination were observed in the spores, with a relatively smooth surface (Figure 3D), whereas in the hyphae treated with lipopeptides, deformities, cracks and wrinkles were found on the surface (Figure 3A). In the spores, it was found that most were unable to germinate successfully; some of the ones that did germinate were abnormal and deformed, with twists, distortions and shrinkage (Figure 3C). In the untreated *S. rolfsii* OM510466 mycelium, a similar pattern to the one in *F. oxysporum* MG557870 was observed, with no disturbances on the surface, and it displayed normal development (Figure 4B and D), whereas in pore formation was observed on the fungal cell surface, leading to rupture, deformation and a rough texture (Figure 4A and C).

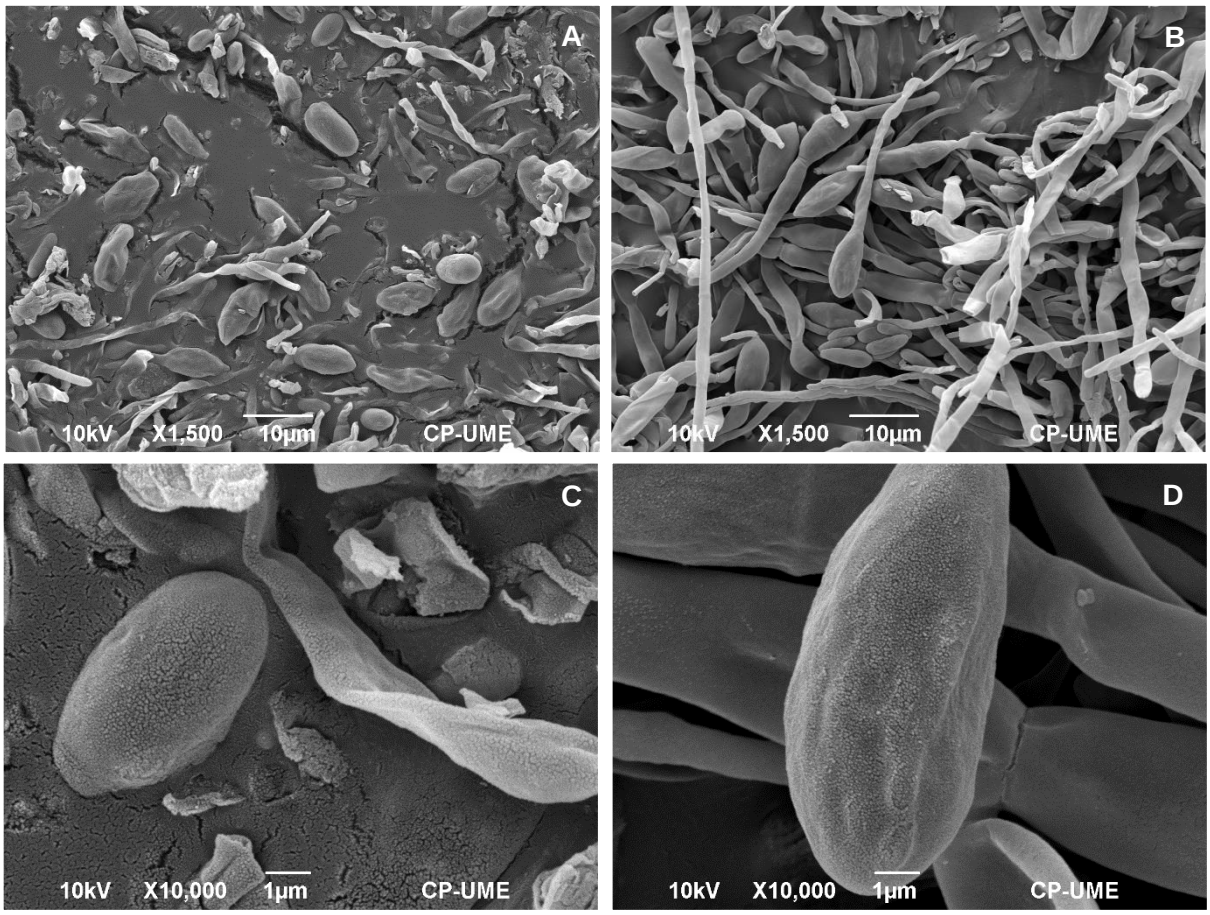

Figure 3. Micrographs (MEB) of spores and hyphae from *Fusarium oxysporum* MG557870 after the treatment with lipopeptides synthesized by *Bacillus amyloliquefaciens* KX953161.1. A and C: spores and hyphae treated with lipopeptides (1,500x, bar= 10 µm and 10,000x, bar= 1 µm, respectively); B and D: spores and hyphae without lipopeptides (1,500x, bar= 10 µm y 10,000x, bar= 1 µm, respectively).

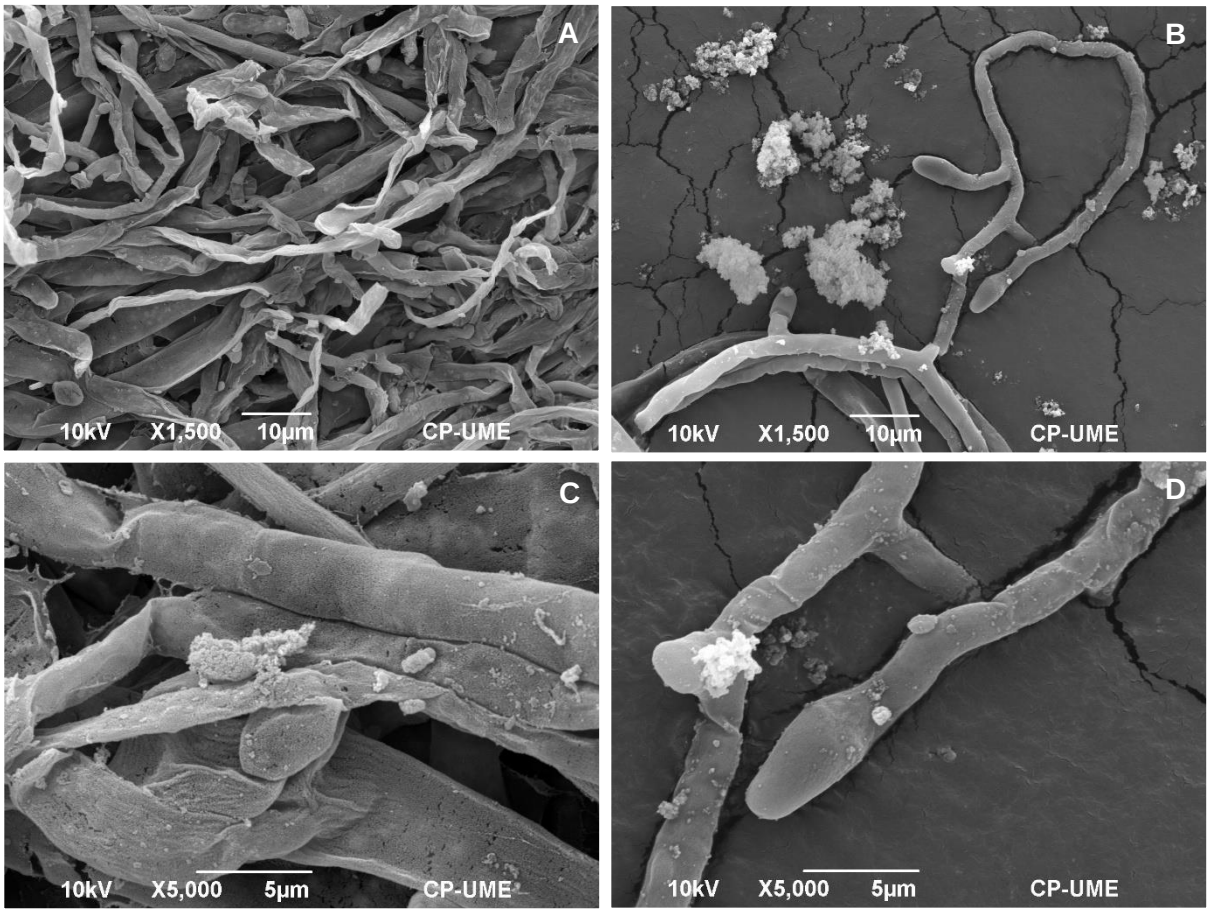

Figure 4. Micrographs (MEB) of mycelia from *Sclerotium rolfsii* OM510466 after the treatment with lipopeptides synthesized by *Bacillus amyloliquefaciens* KX953161.1. A and C: mycelia treated with lipopeptides (1,500x, bar= 10 µm and 5,000x, bar= 5 µm, respectively); B y D: micelium without lipopeptides (1,500x, bar= 10 µm y 5,000x, bar= 5 µm, respectively).

DISCUSSION

Some species of the *Bacillus* genus produce a variety of antimicrobial compounds, such as *B. subtilis* and *B. amyloliquefaciens*, which produce various lipopeptide families (bacillomycin, fengycin and surfactin), as well as diverse counterparts of these families (Carolin *et al*., 2021; Qian *et al*., 2020; Ley-López *et al*., 2023).

Microbial lipopeptides are a type of molecules with a structural composition that consists of a variable hydrophillic peptide macrocycle, joined to a lateral fatty acid residue chain (Zhao *et al*., 2017). These lipopeptides can reduce surface and interfacial tension of the biofilms, and can eventually alter the membrane structure, reported as the main mechanism of lipopeptides, which helps biological activities take place (Zhao *et al*., 2017). Many of these molecules with active membrane properties play an important part in the formation of pores on the membrane, causing leakage of the cytoplasm to the outside of the cell (Liu *et al*., 2010), the formation of ionic channels, acting as cation transporters (Sheppard *et al*., 1991) and presenting detergent-like effects (Heerklotz and Seelig, 2007), which can lead to cytotoxic effects on the vegetative and reproductive

structures of competing fungi. The lipopeptide surfactin presents surface activity effects; it inserts itself into lipid bilayers, solubilizes the fluid phase of the phospholipids, chelates monovalent and divalent cations, and alters membrane permeability through the formation of channels or membrane solubilization via a detergent-like mechanism (Deleu *et al*., 2013). This lipopeptide can form channels, independent to voltage in biofilms and disrupt membrane integrity and the permeability of ions, including Ca^{2+} and K^{+}, which can lead to membrane rupture (Inès and Dhouha 2015; Ostroumova *et al*., 2010). The lipopeptide bacillomyxin L presents a strong antifungal activity and it is believed to cause membrane rupture; this antifungal activity is associates with its interaction with intracellular targets (Zhang *et al*., 2013). Likewise, fengycin has been reported to affect the structural and morphological characteristics of the biological membranes, and in high concentrations, it can completely disintegrate the lipid layers (Deleu *et al*., 2005).

This study identified different *β*-hydroxylated fatty acids, with different C_{13} to C_{18} chain lengths (Table 2), which can be attached to the cyclic lipopeptides, which were identified as counterparts of the lipopeptides fengycin, surfactin and bacillomycin, synthetized by this bacterium, in other studies (Ley-López *et al*., 2023). This diversity in the chain length of fatty acids provide the shape and composition of lipopeptide isomers. The variation of the position of the functional groups in small organic molecules is responsible for the biological activity (Routhu *et al*., 2021). The lipid effect of the lipopeptides synthetized by *B. subtilis* and *B. mojavensis* reported by Yousset *et al*. (2005) displayed variability in the C_{13} to C_{16} fatty acid chain, which were present as mixtures of isomers and Bai *et al*. (2023) reported that the synthesis of fatty acids by *B. amyloliquefaciens* covered the chain of fatty acids from C_{14} to C_{18}. They also mention that, in some cases, fatty acids 3-OH-C_{14} (tetradecanoic) and 3-OH-C_{15} (pentadecanoic) together make the majority of the lipopeptide fatty acids. Thus, for some *Bacillus* species, tetradecanoic fatty acid has been the main isomer (Qian *et al*., 2020: Besson *et al*., 1992; Youssef *et al*., 2005). In addition, a positive relation was proven to exist between the percentage content of tetradecanoic fatty acid and specific surfactant activity. It was proposed that the branched-chain fatty acid (in this case, iso-C_{14}) could provide the optimal hydrophilic-lipophilic balance required for an optimum surface activity (Youssef *et al*., 2005). Besson *et al*. (1992) were the first to report *β*-hydroxylated tetradecanoic fatty acid as a constituent found in a surfactant lipopeptide synthesized by *Bacillus subtilis* and that the percentages of *β*- hydroxylated fatty acids vary, depending on the antibiotic-producing strain and can be influenced by the addition of amino acids to the growth medium of the producing bacterium. It has also been reported that under pressure (presence of competing organisms) the *B. subtilis* bacterium increases the production of lipopeptides, with a powerful antimicrobial activity (both antifungal and antibacterial); furthermore, they confirmed a substantial accumulation of fatty acids in response to the high pressure (Kumar *et al*., 2020; Ley-López *et al*., 2023). Due to this, an appropriate induction with amino acids should be carried out to increase the production of the *β*-hydroxylated tetradecanoic fatty acid in the lipopeptide and purify this compound to carry out specific biological activity studies.

According to Youssef *et al*. (2005) and the results from this study, in which a higher content of the *β*- hydroxylated tetradecanoic was obtained, along with an inhibiting effect on the fungi *F. oxysporum* MG557870 and *S. rolfsii* OM510466 (Figure 3 and 4), it can be inferred that this fatty acid, a component of lipopeptides, is related to the compound's biological activity. Antifungal compounds synthesized by bacteria of the *Bacillus* genus

have been reported to cause damage to the structure and morphology of *F. oxysporum*, *S. rolfsii* and *Aspergillus* cells (Xu *et al*., 2020; Abdel-Gayed *et al*., 2019; Gong *et al*., 2014). Additionally, the number of carbon atoms in the fatty acid chain is another important factor that determines the biological capacity of some lipopeptides—greater hydrophobicity of the fatty acids enhances their biological capacity (de Faria *et al*., 2011). The results of this study indicate that the composition and variation in the chain length *β*-hydroxylated fatty acids in biosurfactant lipopeptides are important for their antifungal activity.

For future studies related to biological activity, it is recommended to purify the C_{14} fatty acid together with the lipopeptide, as well as to induce the bacterium to produce specific fatty acids and enhance the biological effect of the lipopeptides.

CONCLUSIONS

In the lipid profile of the sample with lipopeptides synthesized by the bacterium *Bacillus amyloliquefaciens* KX953161.1, there is a variation in the length of the fatty acid chain from C_{13} to C_{18} *β*-hydroxylated. These fatty acids found in the lipopeptides are composed mainly of the fatty acid *β*-hydroxylate C_{14} (tetradecanoic) with 27.5% of the total of fatty acids, which increases its probability with the biological activity of the lipopeptide. These compounds synthesized by *B. amyloliquefaciens* KX953161.1 displayed an antifungal effect on *F. oxysporum* MG557870 and *S. rolfsii* OM510466. The effect of the lipopeptides was observed through ultrastructural analysis of the fungal cell wall, which revealed damages to the structure and morphology of the fungal cells.

Limitations

A limitation for the follow up of this study is the induction to increase lipopeptide synthesis with a higher number of fatty acids, particularly *β*-hydroxylated C_{14} (tetradecanoic) and to carry out biological effectiveness tests.

Conflict of interest

The authors declare they have no conflict of interest.

Contributions of authors

"Conception, N.L.-L. and R.S.G.-E.; conceptualization, C.S.M.-H. and R.M.-L.; methodology, N.L.-L., J.B.H. and R.S.G.-E; software, N.L.-L., C.S.M.-H. and I.C.-L; validation, N.L.-L., R.S.G.-E., J.B.H. and R.M.-L.; formal analysis, N.L.-L., R.S.G.-E., J.B.H. and I.M.-Z.; investigation, N.L.-L., R.S.G.-E. and J.B.H.; resources, N.L.-L., R.S.G.-E., and I.M.-Z.; data curation, N.L.-L., R.S.G.-E., J.B.H. and R.M.-L.; writing-original draft preparation, N.L.-L., R.S.G.-E. and J.B.H.; writing-review and editing, N.L.-L., R.S.G.-E. and J.B.H.; visualization, N.L.-L. and R.S.G.-E.; supervision, N.L.-L., R.S.G.-E. and J.B.H.; project administration, N.L.-L. and R.S.G.-E.; funding acquisition, N.L.-L. and R.S.G.-E. All authors have read and agreed to the published version of the manuscript."

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