Effect of marine bacterial polysaccharides on the germination of *Hemileia vastatrix* spores †

[Efecto de polisacáridos de bacterias marinas en la germinación de esporas de *Hemileia vastatrix*]

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SUMMARY

Background. As a result of intensive agricultural practices, the loss of agrobiodiversity, climate change and global trade, increasingly virulent strains and races of phytopathogens are emerging. These factors also promote their spread and increase crops susceptibility. Coffee, cultivated in more than 50 countries, has recently suffered significant damage caused by coffee leaf rust (*Hemileia vastatrix*). In Latin America, many plantations were replaced with leaf rust-resistant varieties, however these have lost their effectiveness within a few years. In this context, biotechnological tools based on the use of microorganisms, including their metabolites, represent sustainable alternatives for pathogen control. Marine microorganisms are promising sources of biologically active products. **Objective.** To evaluate *in vitro* the effect of crude polysaccharides extracts produced by marine bacteria on the germination of *H. vastatrix* spores, and to explore their potential for developing field application strategies. **Methodology.** Seven bacterial strains capable of producing capsules on nutrient agar supplemented with sodium chloride were selected from 12 samples of marine algae collected at La Mancha beach, Veracruz, Mexico. Crude polysaccharide extracts were obtained from the selected strains, and their activity on *H. vastatrix* spores was assessed *in vitro*. **Results.** Polysaccharide extracts of *Leucobacter albus*, *Bacillus cereus*, and *Alcaligenes* sp., isolated from the algae *Caulerpa webbiana* f. *disticha*, *Fucus serratus*, and *Padina pavonica*, respectively, inhibited *H. vastatrix* spore germination by 50–75%. **Implications.** The inhibitory effect of *B. cereus* extracts was observed across all tested dilutions, suggesting that this is primarily determined by the chemical composition of the extract. In contrast, the effects of extracts from *L. albus* and *Alcaligenes* sp. appeared to depend on the concentration of their components. **Conclusions.** Crude polysaccharide extracts from marine bacteria, particularly *B. cereus*, *L. albus*, and *Alcaligenes* sp. have potential for the development of biocontrol strategies against *H. vastatrix* through inhibition of spore germination.
Key words: Encapsulated bacteria; coffee leaf rust biocontrol; seaweed.

RESUMEN

Antecedentes. Como consecuencia de las prácticas agrícolas intensivas, la pérdida de agrobiodiversidad, el cambio climático y el comercio mundial, están surgiendo cepas y razas de fitopatógenos cada vez más virulentas. Estos factores favorecen también su dispersión y aumentan la susceptibilidad de los cultivos. El café se cultiva en más de 50 países y, en años recientes, ha sufrido daños considerables causados por la roya anaranjada del café (*Hemileia vastatrix*). En América Latina, muchas plantaciones han sido remplazadas por variedades resistentes; sin embargo, estas han perdido su efectividad en pocos años. En este contexto, herramientas biotecnológicas basadas en el uso de microorganismos o sus componentes químicos representan alternativas sostenibles para el control de fitopatógenos. Los microorganismos marinos son fuentes prometedoras de un gran número de productos biológicamente activos. **Objetivo.** Evaluar *in vitro* el efecto de extractos crudos de polisacáridos producidos por bacterias marinas sobre la germinación de esporas de *H. vastatrix*, con el fin de explorar su potencial en el desarrollo de estrategias de aplicación en campo. **Metodología.** Se

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seleccionaron siete cepas bacterianas capaces de producir cápsulas en agar nutritivo suplementado con cloruro de sodio obtenidas a partir de 12 muestras de algas marinas recolectadas en la playa La Mancha, Veracruz. Se obtuvieron extractos de polisacáridos producidos por las bacterias seleccionadas y se evaluó *in vitro* su actividad sobre esporas de *H. vastatrix*. **Resultados.** Los extractos de polisacáridos de *Leucobacter albus*, *Bacillus cereus* y *Alcaligenes* sp., aislados de las algas *Caulerpa webbiana* f. *disticha*, *Fucus serratus* y *Padina pavonica*, respectivamente, inhibieron entre 50 % y 75 % la germinación de esporas de *H. vastatrix*. **Implicaciones.** Dado que el efecto inhibitorio de los polisacáridos de *B. cereus* se observó en todas las diluciones analizadas, se infiere que este depende principalmente de la composición química del extracto. En contraste, en los extractos obtenidos de *L. albus* y *Alcaligenes* sp. el efecto parece estar determinado por la concentración de sus componentes. **Conclusiones.** Los extractos crudos de polisacáridos de bacterias marinas, particularmente *B. cereus*, *L. albus* y *Alcaligenes* sp. tienen potencial para el desarrollo de métodos de biocontrol de *H. vastatrix*, por su capacidad para inhibir la germinación de sus esporas.
Palabras clave: Bacterias encapsuladas; control biológico de la roya del café; algas marinas.

INTRODUCTION

Coffee plays an important role in the global economy and employs more than 125 million people, mainly smallholders and their families from tropical countries (Siles *et al.*, 2022). The main producing countries are Brazil, Colombia, Vietnam, Indonesia, Mexico, India, and Guatemala, whose economies derive considerable foreign exchange from coffee exports. However, this crop may be threatened by changing climatic conditions and fungal infections (Parada *et al*., 2020). *Hemileia vastatrix* is a fungus that causes the disease known as coffee rust, producing gradual localized damage to the leaves, which reduces the photosynthetic capacity and production of the plantations; it mainly attacks plantations with nutritional deficiencies, susceptible varieties, and poor plantation management (Avelino *et al*., 2015).

To control it, the use of agrochemicals, mainly copper-based, has raised questions about inappropriate practices (Avelino *et al*., 2004), which could have an impact on the environment and generate resistance in pathogens, limitations currently associated with the chemical control of crop diseases. The importance of implementing good cultural practices through thinning and pruning to ensure a less humid microclimate and reduce the period of leaf wetness has been highlighted, along with adequate fertilization to provide effective and balanced nutrient levels to make plants less susceptible to disease (Avelino *et al*., 2006). On the other hand, the generation of rust-resistant coffee hybrids does not represent definitive control, as they are susceptible to losing this quality due to the adaptation processes of the pathogen, which has given rise to new, more severe and aggressive strains, reducing their effectiveness (Cressey, 2013).

Fungi of the genus *Trichoderma* have been among the most studied as a biocontrol agent for rust, due to the feasibility of their foliar application (del Carmen *et al*., 2021), based on their ability to produce a wide variety of metabolites (Saravanakumar *et al*., 2016; Al-Ani, 2019; Khan *et al*., 2020; Pakora *et al*., 2021; Guo *et al*., 2023). For their part, bacteria of the genus Bacillus have managed to reduce the damage caused by coffee rust by up to 70%, compared to other biological alternatives. These bacteria produce hydrolytic enzymes that degrade polysaccharides, such as chitin, which make up the cell walls of fungi (Shiomi *et al*., 2006) and synthesize lipopeptides (e.g., iturin A, surfactins) that act as antagonists against viruses, mycoplasmas, other bacteria, yeasts, fungi, and nematodes (Zeriouh *et al*., 2014).

In aquatic environments, marine organisms produce a variety of bioactive compounds with unique chemical structures (cyclic peptides, halogenated alkaloids, etc.) that have the potential to be developed into new fungicides (Li *et al*., 2021). Marine bacteria are widely distributed in the oceans and can produce exopolysaccharides (EPS). EPS forms a physiological barrier around the bacterial cell and confers functions of desiccation prevention, protection against environmental stress, adhesion to substances, and symbiosis with other cells (Netrusov *et al*., 2023). According to Stadnik and Freitas (2014), EPS show great potential for pathogen control in crops and as new resistance inducers for use in agriculture. However, their role has not been extensively explored (Saha *et al*., 2020) and most of them remain poorly understood (Nichols *et al*., 2005).

The control of plant pathogens has become an important task in ensuring food security, coupled with the need to seek alternatives that contribute to agrochemical-free food production and sustainable agriculture. Likewise, in recent years there has been growing interest in isolating new EPS-producing bacteria from marine environments (García *et al.,* 2022) in search of new areas of application. Therefore, the objective of the study was to evaluate *in vitro* the effect of marine bacterial polysaccharides on the germination of *Hemileia vastatrix* spores, with a view to innovating strategies for pathogen control.

MATERIALS AND METHODS

Collection and identification of marine algae

Specimens of 12 species of algae were collected on La Mancha beach, located in the municipality of Actopan

in the state of Veracruz, Mexico (latitude 19° 35' 36" north and longitude 96° 22' 42" west), in May 2022. The algae were identified in the laboratory by observation under a stereoscopic microscope and comparative taxonomic characterization with the information recorded in AlgaeBase (https://www.algaebase.org/). The selection of algal samples was based on their availability, abundance, and physiological condition in the study area. Samples were collected at shallow depths, between approximately 0.5 and 2 m, depending on the distribution of each species along the beach.

Isolation of capsule-producing bacteria associated with algae

For the isolation of bacteria, 4 g of plant tissue from each algae specimen was used. Each sample was macerated in 3 mL of sterile deionized water in a sterile mortar at room temperature in a laminar flow cabinet. The suspension was allowed to settle for 20 minutes, and the supernatant was used to prepare six serial dilutions (1:9 mL) in sterile distilled water. Next, 500 µL of dilutions 10^5 and 10^6 of the suspension plus 20 mL of nutrient agar (NA) supplemented with 3% NaCl were inoculated into Petri dishes (Bueno and García-Cruz, 2006). The cultures were incubated at 28 ± 1 °C for four days.

First, bacterial colonies that showed good development with a mucoid appearance were selected. Subsequently, to select capsule-forming bacteria, each bacterial colony selected was reinoculated using the streak depletion technique on NA medium supplemented with 3% NaCl in Petri dishes. The cultures were incubated at 28 ± 1 °C for 48 hours. Capsule formation was verified by cell staining tests with strong phenol fuchsin, Muir mordant, and crystal violet, according to conventional procedures for microscopic observation. The selected colonies were subculture using the streak depletion technique in Petri dishes with NA supplemented with 1.5% NaCl, as many times as necessary to obtain pure cultures.

Preparation of crude polysaccharide extracts from bacterial strains

Seven of a total of 14 bacterial isolates were selected. The selection criterion was capsule production capacity. The seven isolates were identified as Strain A, B, D, E, F, G, and I. Each strain was cultured in Petri dishes containing NA + 1.5% NaCl and incubated for 48 hours. Subsequently, the procedure described by Franken *et al*. (2013) was applied. To do this, the bacterial culture generated in a Petri dish was added with a spatula to two 1.5 mL tubes containing 1 mL of sterile distilled water; subsequently, a glass bead was placed in each tube and were vortexed at maximum speed for 10 minutes. Once homogenized, the glass bead was removed and centrifuged at 12,000 rpm for one minute. The supernatant was transferred to 1.5 mL tubes and incubated at 55 °C until completely dry. The precipitate was resuspended in 1 mL of sterile double-distilled water and stored at 4 °C for 24 hours. Each sample was treated with 10 µL of proteinase K (30 U/mg) and incubated at 37 °C for one hour. The samples were then centrifuged at 10,000 rpm for 10 minutes, and the supernatant was transferred to a sterile 1.5 mL tube. Next, 500 µL of cold absolute ethanol was added and allowed to precipitate at 4 °C overnight. The carbohydrates were collected by centrifugation at 12,000 rpm for 10 minutes. The supernatant was removed, and the precipitate was dried at 55 °C. Each extract was resuspended in sterile distilled water to a final concentration of 8.5 $mg.mL^{-1}$.

Effects of crude polysaccharides extracts on spore germination

Uredospores of *H. vastatrix* were collected from the 2022 and 2023 cycles. The spores were extracted by scraping them directly from the pustules on the leaves and collected in a 1.5 mL tube with 0.01% Tween 20, using a stereoscope (Gómez-De la Cruz *et al*., 2018). Before the bioassays, the viability of the spores was determined with a solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.25 mg/mL^{-1}) 1% at 27 °C and 80% relative humidity (RH), according to the procedure described by An *et al*. (1998).

The effect of the crude polysaccharide extracts from strains A, B, D, E, F, G, and I on the germination of *H. vastatrix* spores was evaluated. The bioassays were performed in 96-well plates, considering two columns of wells as replicates and six dilutions for each evaluation time. For each crude polysaccharide extract evaluated, 50 µL of sterile distilled water was placed in six wells of each column. Next, 50 µL of the evaluated crude polysaccharide extract was applied to the first well and mixed by pipetting. Then, 50 µL of the mixture was taken and transferred to the second well, and the same procedure was repeated until reaching well 6. Finally, 10 µL of the spore suspension adjusted to $1x10^8$ $spores.mL^{-1}$ was applied to each well of the extract dilution column. For each bioassay, a control treatment was placed (100 µL of sterile distilled water + 10 µL of the spore suspension adjusted to $1x10^8$ $spores.mL^{-1}$). The tests were incubated for 48 hours at rest at 26 °C. The percentage of spore germination was evaluated based on 100 spores per treatment (Kefialew and Ayalew, 2008). The depigmentation and spore deterioration were evaluated by comparative visual analysis with a control treatment without considering any value scale, only positive or negative. The observations were made using a compound microscope. The bioassays were performed at three different times over a one-year period.

A factorial design was used in which the effects of the levels of two quantitative factors and their interactions on spore germination percentage, spore depigmentation, and cell wall deterioration were studied. The variables representing the factors to be studied were crude polysaccharide extracts and dilutions; the levels of the factors were defined as: seven crude polysaccharide extracts and six dilution levels (10^1 to 10^6). Statistical analysis of the data was performed using the STATGRAPHICS Centurion Version 15.2.06 (2007) statistical package. The normality of the data was verified using the Kolmogorov-Smirnov test and the homogeneity of variance using the Levene test. A two-way factorial analysis of variance (ANOVA) was applied in the comparison of several samples for a 5% probability of error ($P<0.05$). In cases in which there was a statistically significant difference, a comparison of means was performed using Tukey's parametric test for a 5% probability of error ($P<0.05$).

Extraction and sequencing of bacterial DNA

Cultures of the strains under study were obtained in AN medium incubated for 48 h. For DNA extraction, the procedure proposed by Adame-García *et al*. (2016) was applied. TE buffer (Tris-EDTA, 800 µL) was added directly to the bacterial culture in the Petri dish. A volume of 500 µL of cell suspension was collected with a microdosing pipette and placed in sterile mortars. Each sample was crushed until it reached a homogeneous form. Tris-saturated phenol (400 µL) was added to the homogenate. Next, 500 µL of the homogenate was collected and deposited in a sterile 1.5 mL tube. The samples were centrifuged at 9000 rpm for 5 minutes. An aliquot of 160 µL of the supernatant was transferred to a sterile 1.5 mL tube, avoiding carryover of interphase material. Subsequently, 40 µL of TE buffer plus 100 µL of chloroform were added. Again, the samples were centrifuged at 9000 rpm for 5 minutes, and the procedure was repeated twice until no interface was observed. Sample were then centrifuged at 13,000 rpm for 5 minutes. An aliquot of 160 µL of the supernatant from each sample was transferred to sterile microcentrifuge, followed by the addition of 300 µL of cold absolute ethanol. The sample were incubated at 4 °C for 24 hours and subsequently centrifuged at 13,000 rpm for 8 minutes to obtain a pellet. When necessary, 100 µL of cold absolute ethanol was added, followed by an additional centrifugation step under the same conditions. The supernatant was carefully decanted, and the pellet was resuspended in 40 µL of TE buffer and allowed to stand for 5 minutes. Subsequently, 300 µL of cold absolute ethanol was added, and the sample were centrifuged at 13,000 rpm for 8 minutes. The supernatant was decanted, and the pellet was air-dried at room temperature. The pellet was subsequently resuspended in 60 µL of nuclease-free water. Finally, the DNA quality analysis was performed on 0.8% (w/v) agarose gel stained with ethidium bromide (2 µg/mL) with 1X TBE buffer for 35 minutes at 90 V. The gel was observed in a photo-documenter (Gel Doc XR system (BioRad)).

Sequencing of the 16S rRNA gene was performed with primers 27F 5' AGAGTTTGATCMTGGCTCAG 3' and 1492R 5' TACGGYTACCTTGTTACGACTT 3' by Macrogen Inc.

RESULTS

Identification of algae

Twelve species of algae were identified (Table 1). These species have been reported within the group of tropical marine algae of the Veracruz coast (León *et al*., 2017; León and Nuñez, 2017; León *et al*., 2019).

Selection of marine bacteria producing exopolysaccharides

Initially, 12 bacterial isolates were selected for their ability to grow in Nutrient Agar (NA) supplemented with 3% NaCl. Of these, seven were able to grow in NA supplemented with 1.5% NaCl during subculturing. The bacterial isolates were obtained from the algae *Colpomenia peregrina* (isolate A), *Asparagopsis armata* (isolate B), *Caulerpa webbiana* f. *disticha* (isolate D), *Fucus serratus* (isolate E), *Padina pavonica* (isolate F), *Boodlea composita* (isolate G), and *Rosenvingea orientalis* (isolate I) (Figure 1). All isolates presented colonies with a mucoid appearance, which was indicative of the ability to produce EPS (Bueno and García-Cruz, 2006), corroborated by the capsule staining test.

Table 1. Algae species collected at La Mancha beach, Veracruz, Mexico, used for the bacteria isolation.

Class	Species
Phaeophyta	*Colpomenia peregrina* (*Sauvageau*)
Rhodophyta	*Asparagopsis armata*
Phaeophyta	*Sargassum sp.*
Chlorophyta	*Caulerpa webbiana* f. *disticha*
Phaeophyta	*Fucus serratus*
Phaeophyta	*Padina pavonica*
Chlorophyta	*Boodlea composita*
Phaeophyta	*Liagora viscida*
Phaeophyta	*Rosenvingea orientalis*
Phaeophyta	*Dictyota dichotoma*
Phaeophyta	*Laurencia sp.*
Chlorophyta	*Ulva lactuca L.*

Figure 1. Appearance of algae *Colpomenia peregrina*, *Asparagopsis armata*, *Caulerpa webbiana* f. *disticha*, *Fucus serratus*, *Padina pavonica*, *Boodlea composita*, and *Rosenvingea orientalis*.

Effect of polysaccharides on the germination of *Hemileia vastatrix* spores

For the first and second tests, *H. vastatrix* spores obtained from adult plants established on a productive farm in the town of San Marcos in the municipality of Coatepec, Veracruz, were used, while for the third test, spores obtained from a young plant in its second production cycle established on a farm in the town of Xalapa, Veracruz, were used.

Figure 2 shows the appearance of germination, depigmentation and deterioration of *Hemileia vastatrix* spores after application of crude polysaccharide extracts.

First test

Significant differences were found in the germination of *H. vastatrix* spores as result of the effect of polysaccharides, as well as in the number of depigmented spores. No significant differences were found for the wall deterioration variable.

Consistently, the lowest spore germination percentages were obtained with all dilutions of the crude polysaccharide extracts from strains D and E, followed by the extracts from strains B and F. Crude polysaccharide extracts A and I stimulated germination at the first dilution; however, as the dilutions increased, an inhibitory response was observed (Figure 3a). Extracts from strain F showed a decreasing effect as the dilution increased, but the inhibitory effect was significantly different from the control.

The depigmentation variable was influenced by both the polysaccharide extracts and their dilutions (Figure 3b). The highest depigmentation values were obtained with the polysaccharide treatments from strains D, E, and F, followed by the highest dilutions of extracts from strain I (Figure 3b). Pigmentation in the spores of the control treatment did not change.

Second test

In this phase, only the variables of spore germination and pigmentation were evaluated. As in the first test, the lowest spore germination percentages were obtained in all dilutions of the polysaccharide extracts from strain E, followed by the extracts from strains D and F. The extracts from strains G and I significantly stimulated spore germination in all dilutions (Figure 4a). The extracts from strain E generated the highest percentages of spore depigmentation with significant differences starting from the second dilution (Figure 4b).

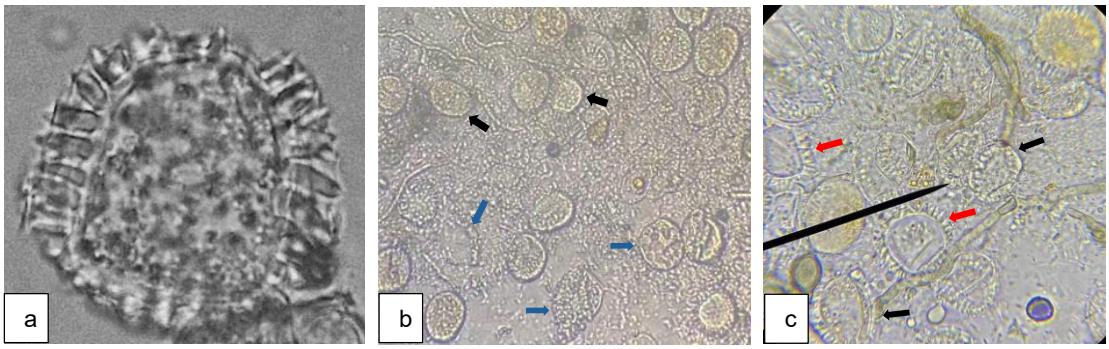

Figure 2. Appearance of germination, depigmentation and deterioration of *Hemileia vastatrix* spores after application of crude polysaccharide extracts. a) normal appearance of the spore (100 x); b and c) germinated spores (black arrows), depigmented spores (red arrows) and deteriorated spores (blue arrows) (40 x).

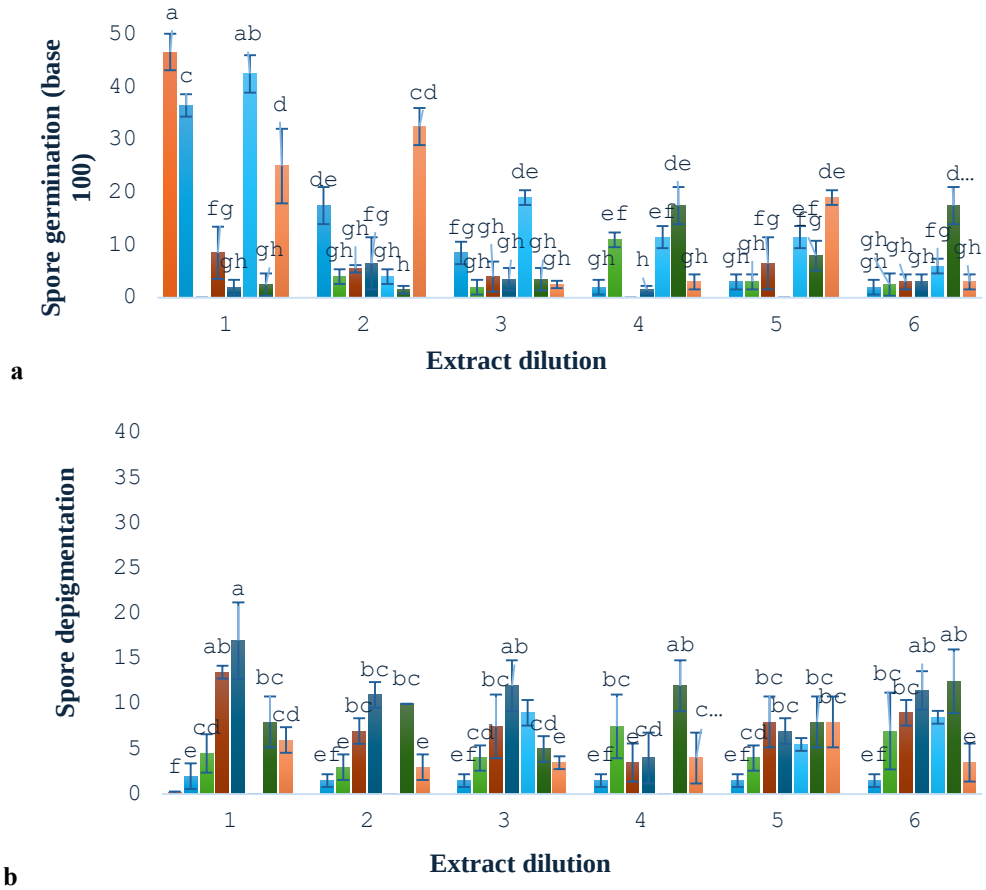

Figure 3. Significant interactions between the crude polysaccharide extracts used and the dilutions applied. a) Spore germination: First bar in the histogram = control ( ); polysaccharide A ( ), polysaccharide B ( ), polysaccharide D ( ), polysaccharide E ( ), polysaccharide F ( ), polysaccharide G ( ), polysaccharide I ( ); b) Spore depigmentation: First bar in the histogram = control ( ); polysaccharide A ( ), polysaccharide B ( ), polysaccharide D ( ), polysaccharide E ( ), polysaccharide F ( ), polysaccharide G ( ), polysaccharide I ( ). The error bars represent the standard deviation (± SD) of three independent repetitions. A significant difference was observed between the dilutions of the polysaccharide extracts, with a $P < 0.05$ according to Tukey's HSD test.

Third test

Based on the results of the first and second tests, a third test was carried out considering only crude polysaccharide extracts from strains D, E, and F generated from new subcultures of the strains. The lowest spore germination percentages were obtained with dilutions 10^4, 10^5, and 10^6 of the extracts from strain E, which showed significant differences compared to the control. Although no significant differences were found for dilutions 10^1, 10^2, and 10^4 of this extract, a trend toward germination inhibition was observed. The extract from strain D decreased spore germination only in dilutions 10^4 and 10^6 (Figure 5a). Coincidentally, the highest percentages of depigmentation were achieved with extract E in dilutions 10^4, 10^5, and 10^6, statistically exceeding the control (Figure 5b). For this variable, there was only a statistically significant difference compared to the control in dilutions 10^3 and 10^4 of extracts from strains D and F.

Molecular identification of bacteria isolated from marine algae

DNA was extracted from strains D, E, and F using the protocol described by Adame-García *et al*. (2016). Genomic DNA samples were sent to Macrogen, Inc. for sequencing of the 16S rRNA gene. The strain identification results are presented in Table 2. The bacterial isolates were obtained from the algae *Caulerpa webbiana* f. *disticha* (strain D: *Leucobacter albus*), *Fucus serratus* (strain E: *Bacillus cereus*), and *Padina pavonica* (strain F: *Alcaligenes* sp.).

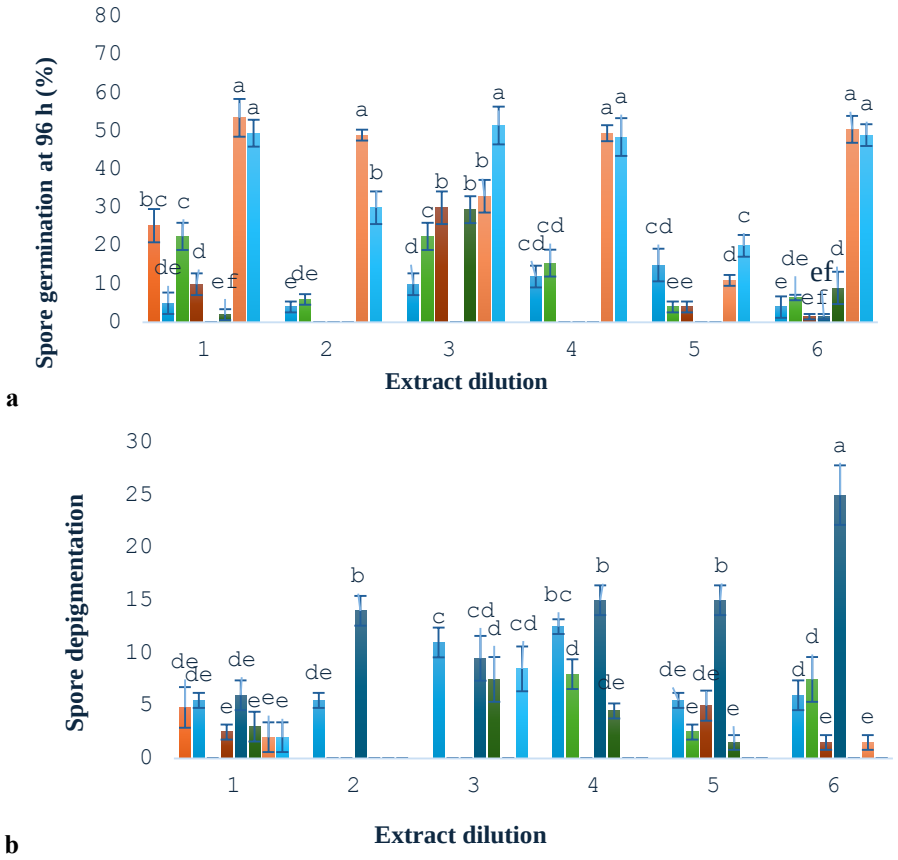

Figure 4. Effect of dilutions of bacterial crude polysaccharide extracts on *Hemileia vastatrix* spores (bioassay at 3 months). a) Spore germination: First bar in the histogram = control ( ); polysaccharide A ( ), polysaccharide B ( ), polysaccharide D ( ), polysaccharide E ( ), polysaccharide F ( ), polysaccharide G ( ), polysaccharide I ( ). b) Spore depigmentation: First bar in the histogram = control ( ); polysaccharide A ( ), polysaccharide B ( ), polysaccharide D ( ), polysaccharide E ( ), polysaccharide F ( ), polysaccharide G ( ), polysaccharide I ( ). The error bars represent the standard deviation (± SD) of three independent repetitions. A significant difference was observed between the dilutions of the polysaccharide extracts, with a $P < 0.05$ according to Tukey's HSD test.

DISCUSSION

In this study, the crude polysaccharide extracts evaluated differentially influenced spore germination, either by stimulation or inhibition, depending on the bacterial strain of origin and, in some cases, on the concentration. Previous studies have indicated that the effects of polysaccharides can be stimulatory or inhibitory, affecting spore morphology, metabolic activity, and germination rate. It has been indicated that several nutrients, including carbon sources (D-glucose and D-fructose) (Jia *et al*., 2024), amino acids (L-phenylalanine and β-alanine) (Yang *et al*., 2024), and metal chelates such as calcium, magnesium, and zinc (Lorenzetti *et al*., 2020), play crucial roles in improving the germination of spores from different fungal species. The uredospores of *H. vastatrix* contain significant fractions of polysaccharides, mainly mannose and glucose, which play an important role in germination, as they are a primary source of carbon (Leal *et al*., 1983). Therefore, the stimulation of germination observed with extracts of bacterial isolates G and I could be attributed to the presence of nutrients for *H. vastatrix* spores or to stimulation caused by a response to cell signaling. Further research is needed into the reasons strains G and I stimulated germination.

In this regard, Kou and Naqvi (2016) and Sharma and Gautam (2019) mention that pathogenic fungi have developed a wide variety of strategies to invade and infect their hosts, using specific receptors to detect and respond to different surface signals, such as topographical features (epidermic striations and ridges, stomata, trichomes, and cell junctions), hydrophobicity, hardness, plant lipids, phytohormones, and/or secreted enzymes, activating various signaling components, such as G proteins, cyclic AMP/protein kinase A, and MAP kinases, to enable the differentiation of infection structures.

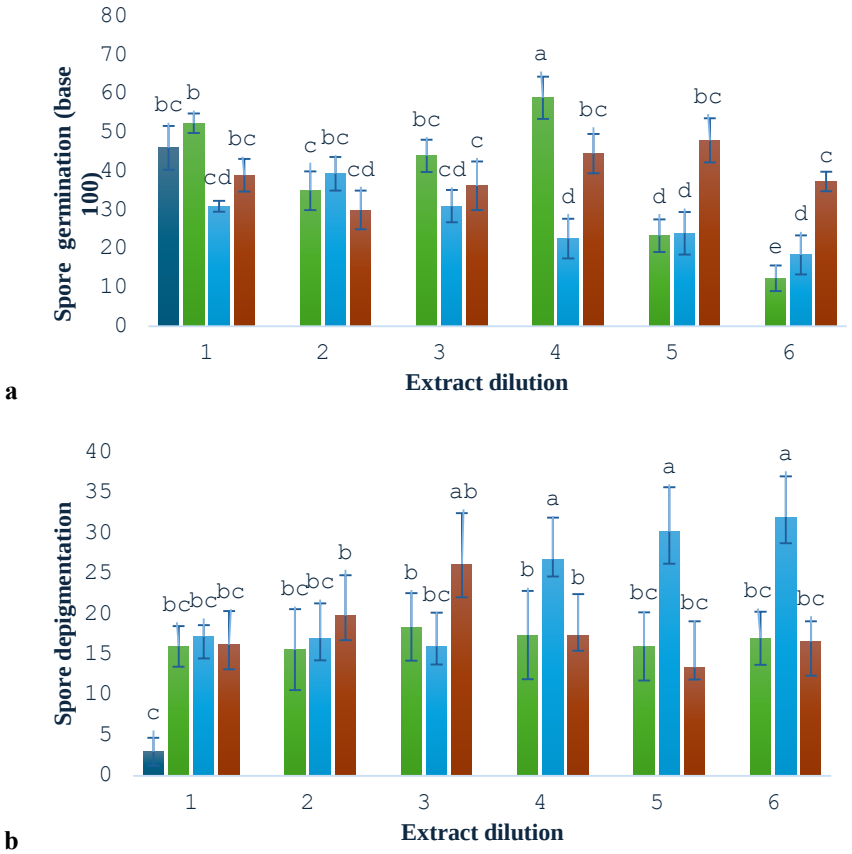

Figure 5. Effect of dilutions of crude polysaccharide extracts D, E, and F on *Hemileia vastatrix* spores six months after bioassays one and two. a) Spore germination: control (■), polysaccharide D (■), polysaccharide E (■), polysaccharide F (■); b): Spore depigmentation: control (■), polysaccharide D (■), polysaccharide E (■), polysaccharide F (■). The error bars represent the standard deviation (± SD) of three independent repetitions. A significant difference was observed between the dilutions of the polysaccharide extracts, with a P < 0.05 according to Tukey's HSD test.

Table 2. Identification of marine bacterial strains

Strain	Silva Accession	Identity	Organism
D	JQ680438.1.1371	99.3%	*Leucobacter albus*
E	PIYQ01000021.4158.5712	92.1%	*Bacillus cereus* group
F	KX289402.1.1394	97.7%	*Alcaligenes* sp. CSA4

Hemileia vastatrix is an example of stigmotropism, where the first contact between uredospores and host is essential for establishing the signal chain through transmembrane proteins (Salazar-Navarro *et al*., 2024). In this regard, it has been indicated that the germination of *H. vastatrix* spores, although dependent on the presence of nutrients, is equally dependent on the perception of these environmental signals and, with them, the activation of intracellular signaling cascades, through which it can receive and process extracellular information in transcription factors, activating protein synthesis pathways to maintain cell wall thickness and morphogenesis (Voegele *et al*., 2009; Castillo *et al*., 2022).

Several studies have reported similarities in the chemical composition of the extracellular polysaccharides of *B. cereus*. Li *et al*. (2016) reported the presence of N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), and D-glucose, while Abdelhamid *et al*. (2025) reported mannuronic acid, xylose, fructose, and glucuronic acid. According to He *et al*. (2024), although D-glucose acts as a primary carbon source, promoting growth and biofilm formation in various fungi, its interaction with amino sugars such as GlcNAc can inhibit their growth.

Additionally, it was observed that the crude polysaccharides extract of *B. cereus* caused the loss of pigmentation in the spores of *H. vastatrix*. Alvarado-

Castillo *et al.* (2017) indicates that depigmented spores with external damage are unable to germinate. According to Carvalho *et al*. (2011), *H. vastatrix* spores have a thickened, highly ornamented or warty upper wall containing carotenoid lipid guttules that give them an orange-yellow color, while Salazar-Navarro *et al*. (2024) reported that the surface of *H. vastatrix* spores has a mucilaginous matrix whose oil confers hydrophobicity, which could help prevent dehydration, facilitate adhesion to the leaf surface, or repel fungicides while allowing germination and infection of the host. Excess reactive oxygen species (ROS) promote irreversible damage to cellular lipids and prevent them from performing their original functions (Juan *et al*., 2021), such as altering the structural integrity of the walls and, consequently, increasing osmotic stress due to high permeability and loss of viability (Madkour, 2020).

The genus *Bacillus* is widely distributed in agrosystems and has broad applications in agriculture, due to its ability to directly stimulate plant growth or control a variety of phytopathogens, including bacteria, fungi, nematodes, and protozoa (Villarreal-Delgado *et al*., 2018; Kulkova *et al*., 2023). Among the various ecosystems that harbor organisms of the genus *Bacillus* are marine environments, thanks to the production of EPS, which gives them the ability to adhere to solid surfaces and overcome extreme conditions (Díaz-Cornejo *et al*., 2023), such as the strain *B. cereus* AG3, isolated from the Red Sea coast in Saudi Arabia, which synthesizes EPSR3, a polysaccharide consisting of glucose, galacturonic acid, and arabinose (Selim *et al*., 2022).

In contrast to *B. cereus*, the polysaccharide extracts of *L. albus* (strain D) and *Alcaligenes* sp. (strain F) showed a concentration-dependent inhibitory effect. *Leucobacter albus* is a recently identified species within the genus (Lin *et al*., 2004), a taxonomic group from which isolates have been obtained from various environments, including the phyllosphere of potato plants (Nouioui *et al*., 2018; Xu *et al*., 2021). However, no reports were found on the use of *L. albus* in agriculture or studies on the chemical composition of its exopolysaccharides. Members of the genus *Alcaligenes* are also common inhabitants, apparently saprophytes of the intestinal tract of vertebrates, and have been found in freshwater, marine, and terrestrial environments (Shoda, 2020). Some analyses of the composition of exopolysaccharides reported the presence of curdlan (β-(1,3) glucose residues) and succinoglycan (a compound of glucose, galactose, pyruvic acid, and succinic acid) in *Alcaligenes faecalis* var mixogenes, and levan (fructose polymers) in *Alcaligenes viscosus* (Mishra and Jha, 2013). Although there are studies showing that isolates of this genus increased root growth and chlorophyll content in alfalfa plants subjected to salt stress (Tirry *et al*., 2021) and that the application of siderophore-rich supernatant showed significant antifungal and antibacterial potential (Sayyed and Patel, 2011), the results were not related to the participation of the exopolysaccharides constituting this bacterial species.

Although the use of polysaccharides for plant disease control has been evaluated as an alternative to synthetic products, they have largely been of algal origin (Righini *et al*., 2019; Li *et al*., 2022; Krishnan *et al*., 2024). To a lesser extent, polysaccharides of microbial origin have been explored, with efforts focusing on β-glucans, chitins, and chitosans from fungi and yeasts (Riseh *et al*., 2024), and on rare occasions on exopolysaccharides secreted by bacteria (Kumari *et al*., 2025), among which the study by Guzzo *et al*. (1993) stands out. They evaluated the induction of resistance in coffee plants diseased with rust by applying xanthan gum extracted from *Xanthomonas* spp., achieving a reduction in disease severity of up to 92%. Therefore, coupled with the favorable results obtained in our study, the search for bacterial polysaccharides for phytosanitary purposes is perceived as a promising field to explore, especially since, as natural compounds, polysaccharides in general have been called eco-compatible compounds due to their affinity with the environment (Mukarram *et al*., 2021).

CONCLUSION

The inhibition or stimulation of *Hemileia vastatrix* spore germination by crude polysaccharide extracts varied according to the bacterial species of origin and extract concentration. Polysaccharides produced by *Bacillus cereus* (strain E) showed consistent inhibitory activity across all tested dilutions, suggesting that extract composition may play a key role in this effect. In contrast, extracts from *L. albus* (strain D) and *Alcaligenes* sp. (strain F) exhibited concentration-dependent activity. These findings indicate that crude polysaccharide extracts from *B. cereus*, *L. albus*, and *Alcaligenes* sp. may have potential as alternative strategies for controlling coffee leaf rust, considering regional variability in fungal sporulation and germination periods. Further studies are required to chemically characterize these extracts, elucidate the mechanisms of action, and evaluate their efficacy under field conditions.

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Data availability. The data can be requested from the corresponding author.

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