Article

Antagonistic potential of bacteria and marine yeasts for the control of phytopathogenic fungi

Luis Guillermo Hernández Montiel[1]
Tomas Rivas García[1]
Mirella Romero Bastidas[2]
César Josué Chiquito Contreras[3]
Francisco Higinio Ruiz Espinoza[2]
Roberto Gregorio Chiquito Contreras[3§]

[1]Center for Biological Research of the Northwest, SC. La Paz, Baja California Sur, Mexico. CP. 23096. Tel 01 (612) 1238484. (lhernandez@cibnor.mx; eltom-r@hotmail.com). [2]Autonomous University of Baja California Sur. La Paz, Baja California Sur, Mexico. CP. 23080. Tel 01(612) 1238800. (mirerome22@hotmail.com; fruiz@uabcs.mx). [3]Faculty of Agricultural Sciences-Campus Xalapa, Universidad Veracruzana. Xalapa, Veracruz, Mexico. CP. 91090. Tel. 01(228) 8421749 (cchiquito@uv.mx).

[§]Corresponding author: rchiquito@uv.mx.

Abstract

The application of synthetic fungicides is a common practice in the control of phytopathogenic fungi. However, its use indiscriminately has brought problems to human, animal and environmental health and has generated resistance in phytopathogens. In the search for alternatives, biological control using microorganisms can be an efficient option to the use of synthetic fungicides. Although bacteria and yeasts isolated from soil and plants have been evaluated as biological control agents, the search for new antagonists continues. The oceanic microflora can be an option for the selection of new antagonistic agents. The objective of this study was to evaluate the antagonistic potential of different bacteria (*Stenotrophomonas rhizophila*, *Bacillus amyloliquefaciens* and *B. subtilis*) and yeasts (*Debaryomyces hansenii*, *Cryptococcus diffluens* and *Rhodotorula minuta*) previously isolated from a hyperhialin lagoon against 13 phytopathogenic fungi of agronomic importance. The results show that different strains of the *S. rhizophila* bacterium were those that exerted greater inhibition on spore germination and mycelial growth of all the phytopathogenic fungi, surpassing the treatments with synthetic fungicides. Among the yeasts, the strains of *D. hansenii* stood out. According to their antagonistic capacity, marine microorganisms can be an option for the management of diseases caused by phytopathogenic fungi.

Keywords: biological control, synthetic fungicides, phytopathogenic fungi, marine microorganisms.

Reception date: December 2017
Acceptance date: January 2018

Introduction

Worldwide, phytopathogenic fungi cause considerable economic losses due to the damage they cause to crops during the different stages of their development (eg. flowering, maturity, harvest) (Pusztahelyi *et al*., 2015; Mumford *et al*., 2016). Traditionally, its control has been based on the application of synthetic fungicides; however, the use of these products can cause harm to human, animal and ecosystem health (Tu *et al*., 2013; Moshi and Matoju, 2017), as well as generating resistance in phytopathogens (Liu *et al*., 2016; Romanazzi *et al*., 2016).

Biological control using antagonistic microorganisms may represent a viable and environmentally safe alternative to synthetic fungicides (Weaver *et al*., 2016; Bach *et al*., 2016). Bacteria and yeasts have been successfully used to control diseases (Eljounaidi *et al*., 2016; Wisniewski *et al*., 2016). Some of its main antagonistic mechanisms are competition for space and nutrients (Droby *et al*., 2016), inhibition by volatile organic compounds (CVO's) (Raza *et al*., 2016a; Arrarte *et al*., 2017), siderophores (Sasha *et al*., 2016; Sasirekha and Srividya, 2016), antibiotics (Sharifazizi *et al*., 2017), hydrolytic enzymes (Ferraz *et al*., 2016; Tokpah *et al*., 2016), induction of resistance (Punja *et al*., 2016) among others.

Bacteria and yeasts are commonly isolated from the surfaces of plants and soil (Sharma *et al*., 2009; Larkin, 2016); however, there are other environments such as the ocean where microorganisms with antagonistic capacity could be isolated as efficient as synthetic fungicides. Studies in recent years on the oceanic microflora have been based mainly on describing its pharmaceutical properties, such as antimicrobial, antituberculous, antiviral, antiparasitic, anthelmintic, among others (Dewapriya and Kim, 2014; Jin *et al*., 2016). However, bacteria and yeasts from marine environments are being considered as new sources of products that can be applied in various areas such as agriculture because they have been shown to be highly efficient microorganisms in the biological control of phytopathogens (Wang *et al*., 2008; Hernández-Montiel *et al*., 2010; Wang *et al*., 2011; Medina-Córdova *et al*., 2016).

The antagonistic potential of marine microorganisms should be studied to select those as promising biological control agents that in the medium term can be a treatment that promote food security, ecological-sustainable production (Usall *et al*., 2016) and the development of new biological products (Vero *et al*., 2013). Therefore, the objective of this work was to evaluate the antagonistic potential of strains of bacteria and marine yeast against various phytopathogenic fungi of agronomic importance.

Materials and methods

Microorganisms used

The phytopathogenic fungi used in this study were *Colletotrichum gloeosporioides*, *Penicillium italicum*, *P. digitatum*, *Alternaria solani*, *Fusarium oxysporum*, *Neoscytalidium dimidiatum A. alternata*, *F. solani* y *Curvularia* sp. (Table 1), which belong to the Phytopathology Laboratory of the Center for Biological Research of the Northwest (CIBNOR) and the Autonomous University

of Baja California Sur. The fungi were cultivated in Petri dishes with potato and dextrose agar culture medium (PDA) at 27 °C for 7 days. Their concentrations were adjusted to $1x10^4$ spores mL^{-1}. The collection of bacteria and marine yeasts were provided by the CIBNOR and were originally isolated from the Ojo de Liebre hyperhaline lagoon, located between 27 °35' and 27° 52' north latitude and 113° 58' and 114° 0' of latitude west in the municipality of Mulege, Baja California Sur, Mexico.

Table 1. Origin of different phytopathogenic fungi of agricultural importance.

Key[Ŧ]	Phytopathogen	Hospedero	Disease
CIB-CGP	*Colletotrichum gloeosporioides*	*Carica papaya* L.	Anthracnose
CIB-CGM	*C. gloeosporioides*	*Mangifera indica* L.	Anthracnose
CIB-PIL	*Penicillium italicum*	*Citrus aurantifolia* (Christm.) Swingle	Blue rot
CIB-PDN	*P. digitatum*	*Citrus sinensis* (L.) Osbeck	Green rot
CIB-AST	*Alternaria solani*	*Lycopersicon esculentum* Mill.	Early blight
CIB-FOC	*Fusarium oxysporum*	*Capsicum annuum* L.	Root rot
CIB-FOA	*F. oxysporum*	*Agave tequilana* Weber	Root rot
MR-HF12	*Neoscytalidium dimidiatum*	*Ficus Carica* L.	Descending death
MR-AA16	*A. alternata*	*Ocimum basilicum* L.	Leaf spot
MR-FG16	*F. solani*	*Cicer arietinum* L.	Root rot
MR-FE16	*F. oxysporum*	*Asparagus officinalis* L.	Root rot
MR-FA16	*F. oxysporum*	*Ocimum basilicum* L.	Root rot
MR-CP16	*Curvularia sp.*	*Washingtonia robusta* Wendl.	Leaf spot

[Ŧ]= fungi with the CIB key belong to the collection of the Center for Biological Research of the Northwest and with the key MR belong to the collection of the Autonomous University of Baja California Sur.

The bacteria selected were *Stenotrophomonas rhizophila* (strain KM01 and KM02), *Bacillus amyloliquefaciens* (strain RB01 and RB02) and *B. subtilis* (strain RBM01 and RBM02), which were cultivated in 250 mL Erlenmeyer flask with medium broth trypticasein (CST) at 25 °C for 24 h and 180 rpm. Selected yeasts were *Debaryomyces hansenii* (strain L01, L02 and L03), *Cryptococcus diffluens* (strain N02) and *Rhodotorula minuta* (strain R04 and R06), which were cultivated in 250 mL Erlenmeyer flask with half potato broth and dextrose (CPD) at 25 °C for 24 h and 180 rpm. Bacteria and yeast were used at a concentration of $1x10^6$ cells mL^{-1}.

Inhibition of spore germination of phytopathogenic fungi

In order to determine the antagonistic capacity of the microorganisms on the germination of spores of the different phytopathogens, the methodology proposed by Hernández-Montiel *et al*. (2010). One 1.5 mL Eppendorf tube, 500 µL of each bacterial or yeast suspension (both pre-adjusted to $1x10^6$ cells mL^{-1}) were combined with 500 µL of each fungus suspension (previously adjusted to $1x10^4$ spores mL^{-1}) and incubated 28 °C for 48 h. Another combination was made with 500 µL of

each fungus with a synthetic fungicide, which was selected for each species of phytopathogen (Tecto 60 [ia 2-4-thiazolyl-1H-benzimidazole] at 5 g L^{-1} for *Colletotrichum gloeosporioides*, *Penicillium italicum* and *P. digitatum*, Amistar G. [ia Methyl E-2-2-6-2-cyanophenoxy pyrimidin-4-yloxy-phenyl-3-methoxyacrylate] at 3 g L^{-1} for *Alternaria solani* and *A. alternata*, Derosal 50 [ia Methyl-2-benzimidazol-carbamate] at 2 g L^{-1} for *Fusarium oxysporum* and *F. solani* and Cantus [ia 2-Chloro-N-4'-chlorobiphenyl-2-yl nicotinamide] at 1 g L^{-1} for *Neoscytalidium dimidiatum* and *Curvularia* sp.).

As a control, 500 µL of each fungus suspension was placed in a 1.5 mL Eppendorf tube. Aliquots of each treatment were taken to determine the number of whole and germinated spores, considering an entire spore as that which showed no change of color or rupture in its cell wall and a germinated spore when the size of hypha was equal to or greater than the diameter of the spore (Yao *et al*., 2004). Ten repetitions were made per treatment, observing 200 spores per repetition.

Inhibition of radial growth of phytopathogenic fungi

A 0.5 cm diameter PDA tampon containing the culture of each 7-day fungus was placed in the center of Petri dishes with PDA medium. Subsequently, 10 µL of each bacterial or yeast concentration was inoculated and striated at the two ends of the Petri dish. As a control, Petri dishes were inoculated only with the fungus. All Petri dishes were incubated at 25 °C for 7 days. At the end, the area of mycelial growth of each fungus was quantified using the ImajeJ® program and the (%) inhibition was determined by the formula I%= DC-DT/DCX100, where I%= inhibition of the fungus in percentage, DC= diameter of the mycelium of the control treatment and DT= diameter of the mycelium in the presence of the antagonist. Five repetitions were made per treatment.

Statistical analysis

A completely randomized design was used in all the experiments and the data were processed by a one-way analysis of variance (Anova). The statistical package Statistica® v. 10.0 for Windows (StatSoft) and for comparison of means the Fisher LSD post-hoc test was used ($p< 0.05$).

Results and discussion

The germination of spores in all the phytopathogenic fungi was inhibited between 90 to 94% by strain KM01 of *S. rhizophila*, significantly surpassing ($p< 0.05$) the inhibition exerted by other marine microorganisms and synthetic fungicides (Table 2). Strains of *B. amyloliquefaciens* and *B. subtilis* inhibited between 81 to 93% and 51 to 69%, respectively. In relation to the yeasts, strains L01 and L02 of *D. hansenii* with a range of inhibition between 50 to 91% stood out. The lowest values of inhibition were observed with the strains of *R. minuta* and *C. diffluens*. The various fungicides used in this study inhibited spore germination by 80 to 90%. On the other hand, mycelial growth was inhibited in all fungi by strains KM01 and KM02 of *S. rhizophila* by 90 to 98% and 88 to 97%, respectively, significantly exceeding ($p< 0.05$) the inhibition exerted by the other marine microorganisms and synthetic fungicides (Table 3).

Table 2. *In vitro* effect of marine microorganisms on the inhibition of spore germination of phytopathogenic fungi.

Cepa	Inhibition of spore germination (%)												
	CIB-CGP	CIB-CGM	CIB-PIL	CIB-PDN	CIB-AST	CIB-FOC	CIB-FOA	MR-HF12	MR-AA16	MR-FG16	MR-FE16	MR-FA16	MR-CP16
KM01	91.6a[¥]	93.5a	94.2a	94.7a	90.2a	91.4a	91.8a	91.8a	92.3a	90.5 a	90.1 a	91.4 a	91.8a
KM02	90.8a	83.6e	90.6c	94.6a	74.6d	75.6e	86.7e	81.6e	80.2d	84.7c	81.9c	90.7b	90.1b
RB01	88.3b	93.4a	87.3e	90.4c	81.7b	81.9d	90.1b	85.8d	86.5b	82.7d	87.3b	91.2a	91.7a
RB02	83.9d	85.6d	91.8b	88.9d	81.9b	87.4c	87.7d	89.7c	85.6c	81.1e	87.5b	84.9c	89.1c
RBM01	60.4e	69.4f	57.6g	60.2f	58.9f	58.1h	54.6h	66.1h	62.5f	63.7f	66.5d	55.7f	59.5f
RBM02	60.2e	61.7g	54.7h	57.4h	58.6f	54.9i	58.4f	57.3i	51.9h	51.8i	59.1f	60.1e	61.7e
L01	89.1b	90.1b	91.7b	91.7b	69.5e	64.7f	52.6i	65.8f	66.2e	57.7h	54.1g	62.7d	58.1g
L02	89.3b	87.9c	87.1e	86.3e	49.7g	54.7i	53.7g	53.8j	53.1g	50.9 j	50.4h	51.4g	51.2h
L03	58.3f	60.3h	63.9f	58.7g	41.9h	61.8g	30.3j	63.7g	43.7i	60.7	60.7e	50.1h	61.7e
N02	13.4h	13.5k	15.7i	17.2i	13.8i	15.3j	14.8k	16.2k	12.9k	17.3k	16.9i	17.3i	10.7k
R04	18.5g	18.7j	15.6i	16.6j	13.4i	11k	13.6l	15.9k	17.4 j	13.2l	15.3j	13.4j	15.8i
R06	12.9i	19.9i	13.2j	10.7k	13.6i	6.9l	9.8m	10.9l	11.7l	11.7m	9.7k	12.7k	11.8j
Fungicida	85.4c[§]	90.2b[§]	89.6 d[§]	90.6c[§]	80.2c[Ŧ]	90.1b[₡]	87.8c[₡]	90.2b [ℓ]	85.8c[Ŧ]	88.1b[₡]	87.7b[₡]	90.3b[₡]	87.7d [ℓ]

[§]= Tecto 60 (ia. 2-(4-tiazolil)-1H-bencimidazol) a 5 g L^{-1}. [Ŧ]= Amistar G. (ia. Metil (E)-2-2-6-(2-cianofenoxi) pirimidin-4-iloxi-fenil-3-metoxiacrilato) a 3 g L^{-1}; [₡]= Derosal 50 (ia. Metil-2-bencimidazol-carbamato) a 2 g L^{-1}. [ℓ]= Cantus (ia. 2-Cloro-N-(4'-clorobifenil-2-il) nicotinamida) a 1 g L^{-1}. [¥]= different letters in the columns indicate significant differences according to the Fisher LSD post-hoc test (p <0.05).

Strains of *B. amyloliquefaciens* and *B. subtilis* inhibited between 80 to 92% and 50 to 75%, respectively. In relation to the yeasts, the strains of *D. hansenii* presented an inhibition of 10 to 20%, the lowest values were observed with the strains of *R. minuta*. The various fungicides inhibited between 86 to 91%. This ability to inhibit spore germination and the mycelial growth of phytopathogenic fungi by bacteria and yeasts has already been studied in isolated plant or soil strains (Mnif and Ghribi, 2015; Kröber *et al*., 2016; Palazzini *et al*., 2016; Reiss and Jørgensen, 2017).

Table 3. *In vitro* effect of marine microorganisms on the inhibition of radial growth of phytopathogenic fungi.

Cepa	Inhibition of radial growth (%)												
	CIB-CGP	CIB-CGM	CIB-PIL	CIB-PDN	CIB-AST	CIB-FOC	CIB-FOA	MR-HF12	MR-AA16	MR-FG16	MR-FE16	MR-FA16	MR-CP16
KM01	97.2a[¥]	98.4	97.1a	96.7a	93.6a	95.4a	90.7a	93.1a	93.2a	90.6a	91.1a	93.2a	95.9a
KM02	97.4a	94.7b	94.5b	96.3a	87.5b	93.2b	90.5a	90.5b	88.1b	90.5a	90.9a	91.8b	93.7b
RB01	91.5b	83.1d	89.3d	88.2d	84.1c	85.1e	82.1c	84.3d	86.7c	83.1c	86.5c	86.3d	85.1d
RB02	91.1b	83.4d	89.7d	92.5b	87.3b	86.9d	82.6c	84.4d	83.2d	82.7c	83.2d	80.1e	85.4d
RBM01	64.3d	57.4f	73.4e	61.4e	75.4e	50.7g	56.7e	58.2f	71.3e	55.6e	54.7f	61.7g	71.3e
RBM02	64.7d	61.7e	59.6f	58.3f	74.1f	60.6f	68.3d	68.7e	64.1f	60.7d	63.3e	68.1f	62.8f
L01	15.5f	19.3g	13.4h	15.2i	12.7i	13.1i	18.7f	14.3g	12.8h	15.1g	17.4g	18.3h	15.3h
L02	15.2f	14.2i	13.2h	18.7h	20.2g	18.1h	10.8h	14.4g	19.6g	15.4g	16.2h	14.5i	17.4g
L03	18.1e	18.4h	20.7g	19.2g	15.6h	18.5h	15.1g	10.1h	11.1i	17.3f	16.5h	18.3h	17.7g
N02	10.7g	11.8j	12.1i	8.1j	9.1j	9.4j	14.9g	8.5i	11.3i	11.3h	10.1i	11.7j	10.8i
R04	6.1h	5.2k	4.3k	7.8k	5.1l	6.8k	5.6i	5.1k	6.2j	5.8i	7.8j	7.6k	6.6j
R06	5.7i	4.7l	7.1j	7.6k	6.2k	9.7j	5.7i	6.2j	4.9k	6.1i	6.3k	7.4k	5.1k
Fungicida	86.1c[§]	91.1c[§]	90.3c[§]	89.7c[§]	81.5d[Ŧ]	90.7c[₡]	89.3b[₡]	86.6c[ℓ]	86.3c[Ŧ]	88.9b[₡]	88.1b[₡]	90.1c[₡]	90.2c[ℓ]

[§]= Tecto 60 (ia. 2-(4-tiazolil)-1H-bencimidazol) a 5 g L^{-1}; [Ŧ]= Amistar G. (ia. Metil (E)-2-2-6-(2-cianofenoxi) pirimidin-4-iloxi-fenil-3-metoxiacrilato) a 3 g L^{-1}. [₡] = Derosal 50 (ia. Metil-2-bencimidazol-carbamato) a 2 g L^{-1}. [ℓ] = Cantus (ia. 2-Cloro-N-(4'-clorobifenil-2-il) nicotinamida) a 1 g L^{-1}; [¥] = different letters in the columns indicate significant differences according to Fisher's LSD post-hoc test (p <0.05).

Among the main antagonistic mechanisms of bacteria and yeasts, there is the production of hydrolytic enzymes, competition for space and nutrients and siderophores (Droby *et al*., 1989; Kai *et al*., 2007; Ryan *et al*., 2009; Herzog *et al*., 2016; Medina-Córdova *et al*., 2016; Grzegorczyk *et al*., 2017). In relation to hydrolytic enzymes (chitinases, glucanases and proteases), these present an activity directly on the cell wall of the fungus, which, is composed mainly of chitin, β-glucan and proteins, which are hydrolyzed to produce oligosaccharides from smaller size that are harnessed as carbon by bacteria and yeast (Sharma *et al*., 2009).

The competition for nutrients and space is another antagonistic mechanism presented by microorganisms (Jamalizadeh *et al*., 2011) and is directly related to carbon competition in the environment, which is rapidly diminished by bacteria and yeast, limiting the fungus in its host germination and infection processes (Janisiewicz and Korsten, 2002; Liu *et al*., 2013).

On the other hand, the siderophores produced by bacteria or yeasts are molecules of low molecular weight related to the Fe^{3+} ion, which is trapped and transported by the microorganisms in an active transport process, using a multitude of membrane receptors. Once inside the cell, the iron is released through a redox process. Without iron in the environment, microorganisms cannot continue with their vital biological processes such as the synthesis and repair of nucleic acids, respiration, photosynthetic transport, nitrate reduction, free radical detoxification, among others.

This strategy of siderophore production by bacteria and yeast has been involved in the control of phytopathogens and has been recognized as an important antagonist trait found in many of the biological control agents (Yu *et al*., 2011; Sasha *et al*., 2016; Liu *et al*., 2017).

In relation to the in vitro effect of COV's, the inhibition exerted by the marine strains KM01 and KM02, both of *S. rhizophila*, towards all the phytopathogenic fungi was from 92 to 95%, significantly exceeding ($p< 0.05$) the inhibition exerted by other marine microorganisms (Table 4). The strains of *B. amyloliquefaciens* and *B. subtilis* inhibited between 81 to 89% and 60 to 69%, respectively. In relation to the yeasts, the strains of *D. hansenii* presented a range of inhibition between 44 to 59%. The lowest inhibition values were observed with *R. minuta* strains. The production of COV's has already been identified as a way to inhibit spore germination and mycelial growth of fungi (Raza *et al*., 2016a; Arrarte *et al*., 2017).

Table 4. *In vitro* effect of marine microorganisms on the inhibition of radial growth of phytopathogenic fungi by COV's.

Cepa	Inhibition of growth (%)												
	CIB-CGP	CIB-CGM	CIB-PIL	CIB-PDN	CIB-AST	CIB-FOC	CIB-FOA	MR-HF12	MR-AA16	MR-FG16	MR-FE16	MR-FA16	MR-CP16
KM01	94.1a¥	92.2a	95.4a	93.6a	96.1a	93.1a	94.9a	94.4a	94.8a	91.5a	95.5a	95.2a	93.2a
KM02	93.8a	91.8b	93.9b	92.9b	94.7b	92.7b	94.4a	93.8b	92.6b	91.5a	95.9a	94.8a	93.1a
RB01	83.4b	85.4c	87.8c	85.6d	84.1c	89.1c	83.5c	84.8d	86.1c	80.2c	82.4c	83.4c	89.4b
RB02	81.1c	81.6d	85.6d	87.8c	84.2c	83.9d	85.6b	87.1c	83.8d	84.5b	88.1b	87.6b	87.5c
RBM01	61.9d	68.7e	69.4f	63.4f	68.4d	69.7e	66.4d	69.1e	63.5e	67.1d	64.7d	63.8e	66.3d
RBM02	60.8e	60.1f	70.1e	68.7e	65.5e	65.8f	62.9e	67.5f	61.5f	63.4e	64.1d	67.4d	63.1e
L01	52.9g	48.5h	51.7h	57.2g	54.4f	52.7g	51.2g	58.2g	54.4h	56.8f	55.7f	54.9g	56.1f
L02	56.6f	49.1g	54.3g	53.6h	52 g	52.9g	59.5f	55.3h	57.5g	54.1g	58.9e	57.2f	54.7g
L03	50.1h	49.3g	47.5i	51.2i	50.3h	49.8h	48.3h	48.7i	45.6i	48.5h	44.1g	49.7 h	45.7h
N02	20.4i	22.6i	21.5j	23.6j	20.8i	20.7i	26.9i	22.4j	21.5j	19.5i	17.4h	20.5i	22.8i
R04	10.1j	10.6j	9.1l	9.5k	8.7k	10.1j	9.9j	9.3k	8.5k	9.8j	7.9i	9.5j	8.4k
R06	9.8j	10.4j	10.7k	9.7k	9.9j	9.8j	10.4j	10.1l	8.9k	9.7k	8.2i	9.6j	10.4j
Testigo	0 k	0 k	0 m	0 l	0 l	0 k	0 k	0 m	0 l	0 l	0 j	0 k	0 l

¥ = different letters in the columns indicate significant differences according to Fisher's LSD post-hoc test ($p< 0.05$).

The COV's produced by bacteria such as dimethyl disulfide, dimethylhexadecylamine, phenylethyl alcohol, furan 2-methyl-5-methyl, among others, and those produced by yeasts such as 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, among others, have already been reported in the inhibition of phytopathogens (Hernández-León *et al*., 2015; Raza *et al*., 2016b). In general, the antimicrobial activity of these compounds is attributed to their interaction with the cell membrane of the phytopathogen, which breaks down the acceleration of the diffusion of their ions and essential metabolites of their membrane (Heipieper *et al*., 1994).

Finally, the management of diseases caused by phytopathogenic fungi in plants through antagonistic microorganisms is a priority worldwide (Bardin *et al*., 2015; Stenberg *et al*., 2015; Van Bruggen and Finckh, 2016). This is the first study to demonstrate the antagonistic potential of marine bacteria of the species *Stenotrophomonas rhizophila*, *Bacillus amyloliquefaciens* and *B. subtilis* and of marine yeasts of the species *Debaryomyces hansenii*, *Cryptococcus diffluens* and *Rhodotorula minuta* towards different phytopathogenic fungi of soil and plants, surpassing the inhibition of the different synthetic fungicides used in this work.

Subsequent studies will study the antagonistic mechanisms (e.j competition space and nutrients, hydrolytic enzymes, siderophores, among others) of the best marine strains of bacteria and yeast, in addition to determine their ability to control diseases caused by fungi *in vivo*. The selection of the best marine microorganisms as antagonistic agents can be an alternative in the production of food in a sustainable way, reducing the dependence on synthetic fungicides and lowering the production costs of crops.

Conclusions

The greater antagonistic capacity of the different marine strains of bacteria and yeasts towards the different phytopathogenic fungi was observed with the KM01 strain of the *S*. rhizo*ph*ila bacterium, which inhibited spore germination between 90 and 94% and between 90 and 94%. 98% mycelial growth of the fungi *Colletotrichum gloeosporioides*, *Penicillium italicum*, *P. digitatum*, *Alternaria solani*, *Fusarium oxysporum*, *Neoscytalidium dimidiatum*, *A. alternata*, *F. solani* y *Curvularia* sp., surpassing the effect of synthetic fungicides. Among the marine yeasts, strains L01, L02 and L03 of *D. hansenii* stood out. The antagonistic efficiency of marine microorganisms suggests that they may be a medium-term option in the management of diseases caused by phytopathogenic fungi.

Gratefulness

The authors thank the financial support of the National Council of Science and Technology (CONACYT) through project SEP-2012-181972 and Geol. Ernesto Díaz Rivera for his excellent technical assistance.

Cited literature

Arrarte, E.; Garmendia, G.; Rossini, C.; Wisniewski, M. and Vero, S. 2017. Volatile organic compounds produced by Antarctic strains of *Candida sake* play a role in the control of postharvest pathogens of apples. Biol. Control. 109:14-20.

Bach, E.; dos Santos, S. G. D.; de Carvalho, F. G.; Lisboa, B. B. and Passaglia, L. M. P. 2016. Evaluation of biological control and rhizosphere competence of plant growth promoting bacteria. Appl. Soil Ecol. 99:141-149.

Bardin, M.; Ajouz, S.; Comby, M.; Lopez, F. M.; Graillot, B.; Siegwart, M. and Nicot, P. C. 2015. Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? Front. Plant Sci. 6(566):1-14.

Dewapriya, P. and Kim, S. 2014. Marine microorganisms: an emerging avenue in modern nutraceuticals and functional foods. Food Res. Int. 56:115-125.

Droby, S.; Chalutz, E.; Wilson, C. L. and Wisniewski, M. 1989. Characterization of the biocontrol activity of *Debaryomyces hansenii* in the control of *Penicillium digitatum* on grapefruit. Can. J. Microbiol. 35(8):794-800.

Droby, S.; Wisniewski, M.; Teixidó, N.; Spadaro, D. and Jijakli, M. H. 2016. The science, development, and commercialization of postharvest biocontrol products. Postharvest Biol. Technol. 122:22-29.

Ferraz, L. P.; da Cunha, T.; da Silva, A. C. and Kupper, K. C. 2016. Biocontrol ability and putative mode of action of yeasts against *Geotrichum citri-aurantii* in citrus fruit. Microbiol. Res. 188-189:72-79.

Eljounaidi, K.; Lee, S. K. and Bae, H. 2016. Bacterial endophytes as potential biocontrol agents of vascular wilt diseases-Review and future prospects. Biol. Control. 103:62-68.

Grzegorczyk, M.; Żarowska, B.; Restuccia, C. and Cirvilleri, G. 2017. Postharvest biocontrol ability of killer yeasts against *Monilinia fructigena* and *Monilinia fructicola* on stone fruit. Food Microbiol. 61:93-101.

Heipieper, H.J.; Weber, F. J.; Sikkema, J.; Keweloh, H. and de Bont, J. A. M. 1994. Mechanisms of resistance of whole cells to toxic organic solvents. Trends Biotechnol. 12(10):409-415.

Hernández, L. R.; Rojas, S. D.; Contreras, P. M.; del Carmen O., M. M.; Macías, R. L. I.; Reyes-de la C. H.; Valencia, C. E. and Santoyo, G. 2015. Characterization of the antifungal and plant growth-promoting effects of diffusible and volatile organic compounds produced by *Pseudomonas fluorescens* strains. Biol. Control. 81:83-92.

Hernández, M. L. G.; Ochoa, J. L.; Troyo, D. E. and Larralde, C. C. P. 2010. Biocontrol of postharvest blue mold (*Penicillium italicum* Wehmer) on Mexican lime by marine and citrus *Debaryomyces hansenii* isolates. Postharvest Biol. Tec. 56(2):181-187.

Herzog, B.; Overy, D. P.; Haltli, B. and Kerr, R. G. 2016. Discovery of keratinases using bacteria isolated from marine environments. Syst. Appl. Microbiol. 39(1):49-57.

Jamalizadeh, M.; Etebarian, H. R.; Aminian, H. and Alizadeh, A. 2011. A review of mechanisms of action of biological control organisms against post-harvest fruit spoilage. EPPO Bull. 41(1):65-71.

Janisiewicz, W. J. and Korsten, L. 2002. Biological control of postharvest diseases of fruits. Annu. Rev. Phytopathol. 40(1):411-441.

Jin, L.; Quan, C.; Hou, X. and Fan, S. 2016. Potential pharmacological resources: natural bioactive compounds from marine-derived fungi. Mar. Drugs. 14(4):76-87.

Kai, M.; Effmert, U.; Berg, G. and Piechulla, B. 2007. Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen *Rhizoctonia solani*. Arch. Microbiol. 187(5):351-360.

Kröber, M.; Verwaaijen, B.; Wibberg, D.; Winkler, A.; Pühler, A. and Schlüter, A. 2016. Comparative transcriptome analysis of the biocontrol strain *Bacillus amyloliquefaciens* FZB42 as response to biofilm formation analyzed by RNA sequencing. J. Biotechnol. 231:212-223.

Larkin, R. P. 2016. Impacts of biocontrol products on *Rhizoctonia* disease of potato and soil microbial communities, and their persistence in soil. Crop Prot. 90:96-105.

Liu, P.; Luo, L. and Long, C. A. 2013. Characterization of competition for nutrients in the biocontrol of *Penicillium italicum* by *Kloeckera apiculata*. Biol. Control 67(2):157-162.

Liu, S.; Che, Z. and Chen, G. 2016. Multiple-fungicide resistance to carbendazim, diethofencarb, procymidone, and pyrimethanil in field isolates of *Botrytis cinerea* from tomato in Henan Province, China. Crop Prot. 84:56-61.

Liu, Y.; Wang, W.; Zhou, Y.; Yao, S.; Deng, L. and Zeng, K. 2017. Isolation, identification and *in vitro* screening of Chongqing orangery yeasts for the biocontrol of *Penicillium digitatum* on citrus fruit. Biol. Control. 110:18-24.

Medina, C. N.; López, A. R.; Ascencio, F.; Castellanos, T.; Campa, C., A. I. and Angulo, C. 2016. Biocontrol activity of the marine yeast *Debaryomyces hansenii* against phytopathogenic fungi and its ability to inhibit mycotoxins production in maize grain (*Zea mays* L.). Biol. Control. 97:70-79.

Mnif, I. and Ghribi, D. 2015. Potential of bacterial derived biopesticides in pest management. Crop Prot. 77:52-64.

Moshi, A. P. and Matoju, I. 2017. The status of research on and application of biopesticides in Tanzania. Crop Prot. 92:16-28.

Mumford, R. A.; Macarthur, R. and Boonham, N. 2016. The role and challenges of new diagnostic technology in plant biosecurity. Food Sec. 8(1):103-109.

Palazzini, J.; Dunlap, C.; Bowman, M. and Chulze, S. 2016. *Bacillus velezensis* RC 218 as a biocontrol agent to reduce *Fusarium* head blight and deoxynivalenol accumulation in wheat. Microbiol. Res. 9:30-36.

Parafati, L.; Vitale, A.; Restuccia, C. and Cirvilleri, G. 2015. Biocontrol ability and action mechanism of food-isolated yeast strains against *Botrytis cinerea* causing post-harvest bunch rot of table grape. Food Microbiol. 47:85-92.

Punja, Z. K.; Rodríguez, G. and Tirajoh, A. 2016. Effects of *Bacillus subtilis* strain QST 713 and storage temperatures on post-harvest disease development on greenhouse tomatoes. Crop Prot. 84:98-104.

Pusztahelyi, T.; Holb, I. J. and Pócsi, I. 2015. Secondary metabolites in fungus-plant interaction. Front Plant Sci. 6(573):1-23.

Raza, W.; Wang, J.; Wu, Y.; Ling, N.; Wei, Z.; Huang, Q. and Shen, Q. 2016a. Effects of volatile organic compounds produced by *Bacillus amyloliquefaciens* on the growth and virulence traits of tomato bacterial wilt pathogen *Ralstonia solanacearum*. Appl. Microbiol. Biotechnol. 100(17):7639-7650.

Raza, W.; Ling, N.; Yang, L.; Huang, Q. and Shen, Q. 2016b. Response of tomato wilt pathogen *Ralstonia solanacearum* to the volatile organic compounds produced by a biocontrol strain *Bacillus amyloliquefaciens* SQR-9. Sci. Rep. 6:1-13.

Reiss, A. and Jørgensen, L. N. 2017. Biological control of yellow rust of wheat (*Puccinia striiformis*) with Serenade® ASO (*Bacillus subtilis* strain QST713). Crop Prot. 93:1-8.

Romanazzi, G.; Smilanick, J. L.; Feliziani, E. and Droby, S. 2016. Integrated management of postharvest gray mold on fruit crops. Postharvest Biol. Tec. 113:69-76.

Ryan, R. P.; Monchy, S.; Cardinale, M.; Taghavi, S.; Crossman, L.; Avison, M. B.; Berg, G.; Lelie, V. D. and Dow, J. M. 2009. The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. Nature Rev. Microbiol. 7(7):514-525.

Sasha, M.; Sarkar, S.; Sarkar, B.; Sharma, B. K.; Bhattacharjee, S. and Tribedi, P. 2016. Microbial siderophores and their potential applications: a review. Environ. Sci. Pollut. Res. 23(5):3984-3999.

Sasirekha, B. and Srividya, S. 2016. Siderophore production by *Pseudomonas aeruginosa* FP6, a biocontrol strain for *Rhizoctonia solani* and *Colletotrichum gloeosporioides* causing diseases in chilli. Agric. Nat. Res. 50(4):250-256.

Sharifazizi, M.; Harighi, B. and Sadeghi, A. 2017. Evaluation of biological control of *Erwinia amylovora*, causal agent of fire blight disease of pear by antagonistic bacteria. Biol. Control. 104:28-34.

Stenberg, J. A.; Heil, M.; Åhman, I. and Björkman, C. 2015. Optimizing crops for biocontrol of pests and disease. Trends Plant Sci. 20(11):698-712.

Sharma, R. R.; Singh, D. and Singh, R. 2009. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. Biol. Control. 50(3):205-221.

Tokpah, D. P.; Li, H.; Wang, L.; Liu, X.; Mulbah, Q. S. and Liu, H. 2016. An assessment system for screening effective bacteria as biological control agents against *Magnaporthe grisea* on rice. Biol. Control. 103:21-29.

Tu, Q.; Chen, J. and Guo, J. 2013. Screening and identification of antagonistic bacteria with potential for biological control of *Penicillium italicum* of citrus fruits.Sci. Hort. 150:125-129.

Usall, J.; Torres, R. and Teixidó, N. 2016. Biological control of postharvest diseases on fruit: a suitable alternative? Curr. Opin. Food Sci. 11:51-55.

Van Bruggen, A. H. C. and Finckh, M. R. 2016. Plant diseases and management approaches in organic farming systems. Annu. Rev. Phytopathol. 54(1):25-54.

Vero, S.; Garmendia, G.; González, M. B.; Bentancur, O. and Wisniewski, M. 2013. Evaluation of yeasts obtained from Antarctic soil samples as biocontrol agents for the management of postharvest diseases of apple (*Malus domestica*). FEMS Yeast Res. 13(2):189-199.

Wang, Y.; Bao, Y.; Shen, D. and Feng, W. 2008. Biocontrol of *Alternaria alternata* on cherry tomato fruit by use of marine yeast *Rhodosporidium paludigenum* Fell and Tallman. Int. J. Food Microbiol. 123(3):234-239.

Wang, Y.; Tang, F.; Xia, J. and Yu, T. 2011 A combination of marine yeast and food additive enhances preventive effects on postharvest decay of jujubes (*Zizyphus jujuba*). Food Chem. 125(3):835-840.

Wisniewski, M.; Droby, S.; Norelli, J.; Liu, J. and Schena, L. 2016. Alternative management technologies for postharvest disease control: the journey from simplicity to complexity. Postharvest Biol. Tec. 122:3-10.

Weaver, M. A.; Abbas, H. K.; Jin, X. and Elliott, B. 2016. Efficacy of water-dispersible formulations of biological control strains of *Aspergillus flavus* for aflatoxin management in corn. Food Addit. Contam. 33(2):346-351.

Yao, H.; Tian, S. and Wang, Y. 2004. Sodium bicarbonate enhances biocontrol efficacy of yeasts on fungal spoilage of pears. Int. J. Food Microbiol. 93(3):297-304.

Yu, X.; Ai, C.; Xin, L. and Zhou, G. 2011. The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on *Fusarium* wilt and promotes the growth of pepper. Eur. J. Soil Biol. 47(2):138-145.