# Identification and morphological characterization of marine actinomycetes as biocontrol agents of *Fusarium solani* in tomato

## Identificación y caracterización morfológica de actinomicetos marinos como agentes de biocontrol de *Fusarium solani* en tomate

## Identificação e caracterização morfológica de actinomicetos marinhos como agentes de biocontrole de *Fusarium solani* em tomate

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## Abstract

*Fusarium* spp. damages the roots of crops, its control is with synthetic fungicides, however, marine actinomycetes can be an option to the use of agrochemicals. The objective of this work was the identification and morphological characterization of marine actinomycetes as antagonists to *Fusarium solani* (Mart.) Sacc. *Fusarium* spp. was isolated from diseased tomato plants and mangrove sediment actinomycetes, both identified through taxonomic keys and molecular techniques. Eight isolates of *Fusarium* spp. were obtained, H8 being the most virulent and it was identified as *F. solani*. Thirty actinomycetes were isolated, of which only four inhibited the phytopathogen, being A19 the one that inhibited the fungus by 70% and was identified as *Streptomyces* sp. Marine actinomycetes may be an option for disease management in plants of agricultural interest.

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## Resumen

*Fusarium* spp. daña a las raíces de los cultivos, su control es con fungicidas sintéticos, sin embargo, los actinomicetos marinos puede ser una opción al uso de agroquímicos. El objetivo de este trabajo fue la identificación y caracterización morfológica de actinomicetos marinos como antagonistas a *Fusarium solani* (Mart.) Sacc. *Fusarium* spp. fue aislado de plantas enfermas de tomate y los actinomicetos de sedimento de manglares, ambos se identificaron a través de claves taxonómicas y por técnicas moleculares. Se obtuvieron ocho aislamientos de *Fusarium* spp., siendo H8 el más virulento y fue identificado como *F. solani*. Se aislaron 30 actinomicetos, de los cuales solo cuatro inhibieron al fitopatógeno, siendo A19 el que inhibió en un 70% al hongo y fue identificado como *Streptomyces* sp. Los actinomicetos marinos pueden ser una opción para el manejo de enfermedades en plantas de interés agrícola.

**Palabras clave**: *Streptomyces*, *Nocardiopsis*, antagonismo, *Solanum lycopersicum.*

## Resumo

*Fusarium* spp. danifica as raízes das lavouras, seu controle é feito com fungicidas sintéticos, no entanto, os actinomicetos marinhos podem ser uma alternativa ao uso de agroquímicos. O objetivo deste trabalho foi a identificação e caracterização morfológica de actinomicetos marinhos como antagonistas a *Fusarium solani* (Mart.) Sacc. *Fusarium* spp. foi isolado a partir de tomateiros doentes e actinomicetos de sedimento de manguezais, ambos foram identificados por meio de chaves taxonômicas e técnicas moleculares. Foram obtidos oito isolados de *Fusarium* spp., sendo H8 o mais virulento e identificado como *F. solani*. Foram isolados 30 actinomicetos, dos quais apenas quatro inibiram o fitopatógeno, sendo A19 o que inibiu o fungo em 70% e foi identificado como *Streptomyces* sp. Os actinomicetos marinhos podem ser uma alternativa efetiva para o manejo de doenças em plantas de interesse agrícola.

**Palavras-chave**: *Streptomyces, Nocardiopsis*, antagonismo, *Solanum lycopersicum*.

## Introduction

Tomato (*Solanum lycopersicum* L.), is an important crop in many regions of the world (Reyes-Pérez *et al*., 2018). This crop is susceptible to *Fusarium* spp. (Li *et al*., 2018), which, causes losses close to 80% of production (Akbar *et al*., 2016). The symptoms of plants with *Fusarium* spp. are; defolation, wilting and yellowing of leaves, and stem and root necrosis (Summerell *et al*., 2003). *Fusarium* spp. is controlled by synthetic fungicides such as: Mancozeb, Benomil, Carbendazim, among others (Gadhave *et al*., 2020), However, its application causes resistance to fungi and negative effects on the environment, human and animal health (Torres-Rodriguez *et al*., 2021). The search for alternatives to decrease the use of synthetic fungicides in agriculture is a priority worldwide (Maluin and Hussein, 2020).

The use ofactinomycetes as biocontrol agents towards phytopathogens is an alternative to the application of agrochemicals (Wang *et al*., 2018). Among the antagonistic mechanisms of actinomycetes are, antibiotics, siderophores, induction of host resistance, hydrolytic enzymes, among others (Gopalakrishnan *et al*., 2021; Igarashi *et al*., 2021; Shen *et al*., 2021). Actinomycetes from terrestrial environments have been extensively studied, however, actinomycetes from marine environments are an understudied resource, which may be more efficient in controlling plant phytopathogens (Gong *et al*., 2018). Mangroves are ecosystems that are found in tropical and subtropical intertidal regions worldwide (Sangkanu *et al*., 2017). In these ecosystems, salinity and nutrient availability are highly variable, resulting in unique characteristics for the isolation of marine actinomycetes (Soldan *et al.,* 2019). The objective of this work was the identification and morphological characterization of marine actinomycetes as biocontrol agents against *Fusarium solani.*

## Materials and methods

**Isolation and morphological identification of *Fusarium* spp.**

Roots of tomato plants with *Fusarium* symptoms were collected from a commercial orchard located in El Carrizal, Baja California Sur, Mexico at 23°47'00" north latitude and 110°17'40" west latitude. Root pieces of 5 mm were disinfected with 2% sodium hypochlorite for 30 sec. They were washed with sterile distilled water and dried on absorbent paper. After, they were sown in Petri dishes with potato-dextrose-agar (PDA, Difco 39 $g.L^{-1}$) plus streptomycin (0.1 $g.L^{-1}$) and ampicillin (0.1 $g.L^{-1}$) and were incubated at 28 °C for 6 days in complete darkness. Colonies were purified in Petri dishes with PDA and stored in slant tubes at 4 °C. Fungal sporulation was determined (+++ abundant, ++ good, + moderate) (Sivakumar *et al*., 2018) and for macroscopic and microscopic identification the taxonomic keys of Summerell *et al*. (2003) were used.

**Pathogenicity test**

The concentration of each fungus was adjusted to $1.10^6$ conidia.$mL^{-1}$ using a Neubaüer chamber**.** Tomato seedlings var. Saladet of 25 days, were immersed in conidia suspension of each isolation for 15 min, prior to transplantation. As a control, plants were immersed in sterile distilled water. The plants were kept at 28 °C, 80% relative humidity (HR) and 12 h light in a growth chamber (Convairon®) for 26 days. The severity of the disease was determined using the Marlatt scale *et al*. (1996)'; 1=plants without symptoms; 2=slight chlorosis and wilting; 3=moderate chlorosis and wilting; 4=severe chlorosis and wilting; 5=dead plant. In addition, we quantified; height, stem diameter, root fresh weight and disease incidence (%DI) (Saravanakumar *et al*., 2016) using the formula: %DI= (PI/TP)×100%, where Pi=number of infected plants and TP=total plants. Koch's postulates were tested. Five replicates (seedlings) per treatment were performed and the experiment was repeated twice.

**Molecular identification a nd p hylogenetic a nalysis of *Fusarium* spp.**

The extraction of DNA was carried out according to the methodology of Ochoa *et al*. (2007). The ITS1-5.8s-ITS2 region of rDNA was amplified using primers ITS1 (5' TCCGTAGGTGAACCCTGCGG 3') and ITS4 (5" TCCTCCGCTTATTGATATGC 3") (White *et al*., 1990). A thermal cycler (Applied Biosystems®) was used with a denaturation period of 3 min at 95 °C, followed by 30 cycles (denaturation at 95 °C for 1 min, alignment for 30 s at 50 °C and an extension of 1 min at 72 °C), with a final extension at 72 °C for 10 min. PCR products were sequenced in Genewiz®. Phylogenetic analysis was performed with the MEGA 7 program (Kumar *et al*., 2018) with the maximum parsimony (MP)

method, using the Kimura-2 parameter model (Tamura, 1992) and Gama distribution and 10,000 replicates (bootstrap). The MP tree was obtained using NNI (Nearest-Neighbor-Interchange).

**Isolation and identification of marine actinomycetes**

Sixteen samples of marine mangrove sediment were collected from four sampling sites in the Zacatecas estuary located in La Paz, Baja California Sur, Mexico at 24°9'30" north latitude and 110°25'37" west latitude. The collection was performed at a distance of 0-1, 1-3 and 3-5 m from the shoreline, at a depth of 25 cm. Sediment samples were collected with a 10 cm diameter soil auger. The central portion of the samples was extracted with the help of a sterile spoon. The samples were dried at room temperature for 7 days. One g of sample was weighed and suspended in 9 mL of sterile seawater, it was incubated for 20 min in water bath at 60 °C and the solution was serially diluted to $10^{-6}$ (Palla *et al*., 2018). It was added 1 mL of each dilution to 15 mL of malt extract yeast agar medium (ISP2; malt extract 10 g, yeast extract 4 g, dextrose 4 g, agar 20 g, seawater 1 L, pH 7.2). Plates were incubated at 28 °C for 15 days. Each colony was purified in ISP2 and maintained in 20% glycerol at -80 °C. Actinomycetes were identified with the keys of Shirling and Gottlieb (1966) and Gram staining (Duraipandiyan *et al*., 2010).

***In vitro* antagonism of marine actinomycetes vs. *F. solani***

A 0.5 cm disc of each actinomycete grown in ISP2 was placed in Petri dishes with PDA 1 cm from the edge of the plate and in the center a 0.5 cm disc of *F*. *solani* from a 7-day culturein PDA. A group of Petri dishes were inoculated with a reference strain of terrestrial origin cataloged as ED48 of *Streptomyces* sp. provided by the Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, Mexico. Another group was inoculated with the phytopathogen plus the fungicide Carbendazim (6 $mg.mL^{-1}$) and another group was inoculated only with *F. solani*. The plates were incubated for 10 days at 28 °C. The percentage of radial growth inhibition (RGPI, %) of the fungus was determined with the formula: [(R1-R2)/R1]×100% where R1=radial growth of *F. solani* on the control plate and R2=growth of *F. solani* in the direction towards the actinomycete colony (Azadeh *et al*., 2010). Five plates per treatment were used and the experiment was repeated twice.

**Antimicrobial activity in actinomycete supernatants**

Each actinomycete was cultivated in ISP2 at 30 °C for 7 days. After, 5 mL of sterile water were added and the spores were scraped and transferred to a 250 mL Erlenmeyer flask containing 50 mL of starch casein broth and incubated at 150 rpm and 28 °C for 2 days. The cells were harvested, washed and re-suspended in 25 mL of sterile saline solution, subsequently, 10 mL of each inoculum was deposited in a 250 mL flask containing 100 mL of nutrient broth (millet 10 $g.L^{-1}$, glucose 10 $g.L^{-1}$, $CaCO_3$ 2 $g.L^{-1}$, NaCl 2.5 $g.L^{-1}$, peptone 3 $g.L^{-1}$, pH 7.2-7.4) and incubated at 150 rpm and 28 °C for 12 days. The medium was centrifuged at 8000 x g. for 20 min at 4 °C, the supernatant was passed through a 0.22 µm membrane filter and stored at 4 °C. It was placed 1 mL of the supernatant from each actinomycete on a sensidisc 1 cm from the edge of the Petri dishes with PDA and a disk of *F. solani* was placed in the center. One group of Petri dishes was inoculated with strain ED48, another group was inoculated with the phytopathogen plus the fungicide Carbendazim (6 mg.mL-1) and another group was inoculated only with *F. solani*. The percentage of radial growth inhibition (RGPI, %) of the fungus was determined with the formula: [(R1-R2)/R1]×100% where R1=radial growth of *F. solani* on the control plate and R2=growth of *F. solani* in the direction towards the actinomycete colony (Azadeh *et al*., 2010). Five plates per treatment were used and the experiment was repeated twice.

**Molecular identification and phylogenetic analysis of marine actinomycetes**

DNA was extracted using the modified Ochoa method *et al*. (2007). PCR amplification of the 16S rRNA gene sequence was performed using primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5′-GGTTACCTTGTTACGACTT-3') (Wang *et al*., 2018). A thermal cycler (Applied Biosystems®) was used with a denaturation period of 3 min at 98 °C, followed by 30 cycles (denaturation at 94 °C for 1 min, alignment for 1 min at 52 °C and an extension of 1 min at 72 °C), with a final extension at 72 °C for 10 min. PCR products were sequenced in Genewiz®. Phylogenetic analyses were performed with the MEGA 7 program (Kumar *et al*., 2018) by the neighbor-joining method using the Tamura-3 parameter model (Tamura, 1992) and Gama distribution and 10,000 repeats (bootstrap).

**Statistical analysis**

Data were analyzed by one-way analysis of variance method (ANOVA) using STATISTICA 10.0 software (software StatSoft, Tulsa, OK) and Fisher's LSD test was used ($P<0.05$) for separation of means.

## Results and discussion

**Isolation and identification of *Fusarium* species**

Eight isolates were obtained: H1, H2, H3, H4, H5, H6, H7 and H8 of *Fusarium* spp. that presented different morphological characteristics, related to color, sporulation and mycelium (table 1).

According to Summerell *et al*. (2003) variability in morphology is common among *Fusarium* species. Choi *et al*. (2018) report *Fusarium* spp. colonies with diverse shapes, textures and colors. Duarte Leal *et al*. (2016) observed red, violet, salmon and yellow *Fusarium* spp. isolates. Macroconidia showed semi-curved, straight and slender shapes, with 3 to 5 septa, with a blunt or papillate apical cell type and foot-shaped basal cell or light notch. Microconidia were reniform and fusiform in shape (table 2). Differences were found among macroconidia and chlamydospores formed by the eight isolates, according to Murugan *et al*. (2020) and Sivakumar *et al*. (2018), there is variability among reproductive structures of *Fusarium* species.

**Pathogenicity of *Fusarium* spp.**

Of the eight *Fusarium* spp. isolates, only H3, H6, H7 and H8 were pathogenic, observing 100% incidence in plants (table 3). Control plants showed the greatest height, stem diameter and root fresh weight because there is a relationship between *Fusarium* spp. pathogenicity with decreased plant growth (Chang *et al*., 2018). It has been reported that there is difference in virulence among isolates of *Fusarium* spp. (Murugan *et al*., 2020). In this regard, Nirmaladevi *et al*. (2016) observed variation in virulence of *Fusarium* spp. isolates, 45% were highly virulent and 30% were moderately virulent. The pathogenicity of *Fusarium* spp. is mediated by the action of lytic enzymes (endopolygalacturonase, exopolygalacturonase, endoxylanase and endopectate lyase) that depolymerize all cell wall components, such as cellulose, pectins and proteins (extensins). In addition, these enzymes serve to inactivate the plant's defense protein components, such as chitinase and β-1,3-glucanase (Roncero *et al*., 2000).

**Table 1. Morphological characteristics of *Fusarium* spp. isolated from roots of *Solanum lycopersicum* L., in Baja California Sur, Mexico.**

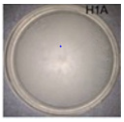
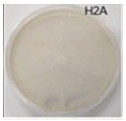
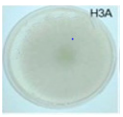
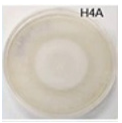
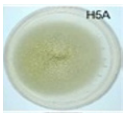
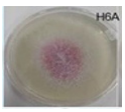
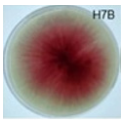
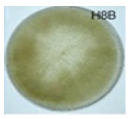
| Isolation | | Color | Sporulation | Mycelium |
|---|---|---|---|---|
| H1 |  | Gray | + | Sparse,white, aerial mycelium |
| H2 |  | Light yellow | + | Abundant, white, cottony aerial mycelium |
| H3 |  | White to cream | ++ | Sparse, white, cottony, hyaline, aerial mycelium |
| H4 |  | White to cream | +++ | Dense, white, cottony, hyaline, aerial mycelium |
| H5 |  | Light yellow | +++ | Abundant, white, cottony aerial mycelium |
| H6 |  | Pale violet | +++ | Abundant, white pinkish, floccus, aerial mycelium |
| H7 |  | Dark violet | +++ | Abundant, white pinkish, floccus, aerial mycelium |
| H8 |  | Brown yellow | +++ | Abundant, white, cottony, hyaline, aerial mycelium |

**Table 2. Characteristics of the reproductive structures of *Fusarium* spp. isolation.**

| Isolation | Macroconidia | | | | Microconidia | | chlamydospore |
|---|---|---|---|---|---|---|---|
| | Shape | Septum | Apical cell | Basal cell | Shape | Septum | |
| H1 | Semicurve | 3 | Blunt | Foot shape | Reniform | 0 | Paired, smooth wall |
| H2 | Straight, slender | 3-4 | Blunt | Light notch | Reniform | 0-1 | ---------- |
| H3 | Semicurve | 3 | Blunt | Foot shape | Reniform | 0 | ---------- |
| H4 | Dorsiventral curvature | 4-5 | Narrow | Foot shape | Reniform | 0 | Paired, smooth wall |
| H5 | Semicurve | 3 | Blunt | Light notch | Reniform | 0-1 | Simple warty |
| H6 | Dorsiventralcurvature | 3-5 | Papillate | Foot shape | Reniform, fusiform | 0 | Paired, smooth wall |
| H7 | Dorsiventral curvature | 3-5 | Papillate | Foot shape | Reniform, Fusiform | 0 | Paired, smooth wall |
| H8 | Semicurve | 3-5 | Blunt | Foot shape | Reniform, fusiform | 0-1 | Paired, smooth wall |

**Table 3. Severity of *Fusarium* spp. isolation and their effect on growth variables in tomato.**

| Treatment | Severity* | Height (mm) | Stem diameter (mm) | Root fresh weight (mg) |
|---|---|---|---|---|
| Control | 0 | 233.6±11.3[a] | 4.1±0.3[a] | 55.0±4.3[a] |
| H3 | 3 | 166.4±11.1[b] | 2.2±0.4[b] | 32.4±3.7[b] |
| H6 | 4 | 125.2±,12.4[c] | 1.6±0.3[c] | 28.4±3.9[c] |
| H7 | 4 | 129.1±11.7[c] | 1.6±0.3[c] | 27.8±4.1[c] |
| H8 | 5 | 75.4±12.8[d] | 1.2±0.4[d] | 18.4±3.8[d] |

[a, b, c, d] Different letters between columns differ significantly according to Fisher ($P<0.05$). Severity*: 1=plants without symptoms; 2=slight chlorosis and wilting; 3=moderate chlorosis and wilting; 4=severe chlorosis and wilting; 5=dead plant.

**Molecular identification and phylogenetic analysis of *Fusarium* sp.**

The size of the PCR product of the ITS1-5.8s-ITS2 region of isolate H8 was 550 bp. Its sequence was 100% identical to *F. solani*. In the phylogenetic analysis, the ITS1-5.8s-ITS2 sequences were grouped with *F. solani* as a single group (figure 1). Morphology of the fungi allows their identification to the genus (Summerell *et al*., 2003), however, the similarities in the reproductive structures make their identification at the species level complex (Al-Fadhal *et al*., 2019). Singha *et al*. (2016) observed differences in morphological and molecular identification (ITS region) of *Fusarium* species. The ITS region is studied for fungal identification due to the species specificity of this region and provides better resolution at the subspecies level and therefore, sequence analysis is a superior option for phylogenetic studies (Okubara *et al*., 2005). The dendogram indicated that the sequence obtained in the present study clustered with other *F. solani* sequences. The colony morphology and sequence of the ITS region of isolate H8, identified it as *F. solani.*

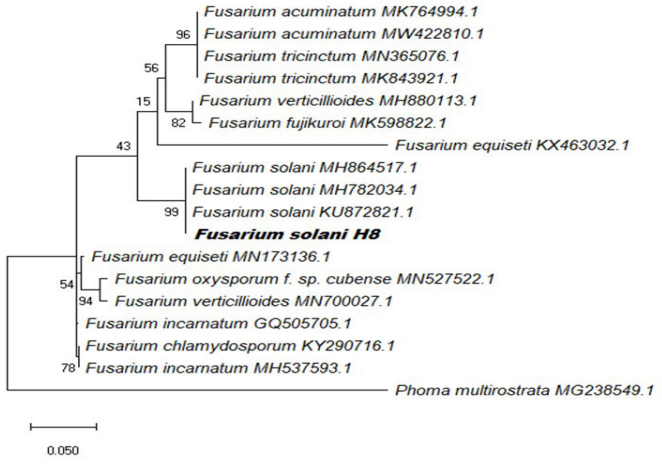



**Figure 1. Phylogenetic tree based on the ITS1-5.8s-ITS2 region of the H8 fungus isolated from tomato plants diseased by *Fusarium* spp.** Bootstrap values are indicated as percentages over the nodes in this analysis (10,000 bootstrap).

**Isolation and morphological identification of marine actinomycetes**

Thirty isolates of actinomycetes that showed differences in mycelium, color, texture, shapewere obtained and were determined as Gram-positive bacteria (table 4). The determination of the characteristics of actinomycetes is important in the evaluation of the diversity of the microbial community, these can be differentiated based on texture, color, shape and elevation of colonies, among others (Rathore *et al*., 2019). The morphological results are similar to those reported by Goudjal *et al*. (2014), who obtained isolates with coloration ranging from yellowish white to brownish gray. Mangrove ecosystems are one of the habitats with a large number of organisms (Barka *et al*., 2016; Sangkanu *et al*., 2017). Only 1% of the species in these ecosystems have been studied and there is a lack of knowledge about their ecological role and potential application, especially in agriculture (Palla *et al*., 2018; Ameen *et al.,* 2021).

**Table 4. Morphological characteristics of marine actinomycetes isolation.**

| Isolation | Mycelium | Mycelium color | Texture | Colony |
|---|---|---|---|---|
| A1 | Substrate | Yellowish gray | Wrinkled/coarse | Irregular |
| A2 | Substrate | Pale yellow | Folded | Irregular |
| A3 | Substrate | Yellowish gray | Wrinkled | Circular |
| A4 | Aerial | Dark yellow | Creamy | Irregular |
| A5 | Substrate | Yellowish gray | Wrinkled | Irregular |
| A6 | Substrate | Light yellow | Wrinkled | Circular |
| A7 | Substrate | Pale yellow | Folded | Circular |
| A8 | Aerial | Pale yellow | Creamy | Punctiform |
| A9 | Substrate | Light yellow | Wrinkled | Irregular |
| A10 | Substrate | Gray | Wrinkled | Irregular |
| A11 | Substrate | Red | Creamy | Irregular |
| A12 | Substrate | Yellow | Creamy | Irregular |
| A 13 | Substrate | Light yellow | Creamy | Circular |
| A 14 | Substrate | Dark brown | Wrinkled | Irregular |
| A 15 | Substrate | Light pink | Creamy | Circular |
| A 16 | Substrate | Light gray | Creamy | circular |
| A 17 | Aerial | Dark gray | Creamy | Irregular |
| A 18 | Aerial | Yellow | Creamy | Circular |
| A 19 | Substrate | Yellow | Folded | Rhizoid |
| A 20 | Aerial | Dark gray | Creamy | Irregular |
| A 21 | Substrate | Light yellow | Creamy | Punctiform |
| A 22 | Substrate | White | Creamy | Circular |
| A 23 | Aerial | Yellow | Creamy | Punctiform |
| A 24 | Substrate | Light pink | Creamy | Circular |
| A 25 | Aerial | Dark yellow | Creamy | Irregular |
| A 26 | Aerial | Dark gray | Creamy | Irregular |
| A 27 | Substrate | Light yellow | Wrinkled | Circular |
| A 28 | Aerial | Yellow | Creamy | Circular |
| A 29 | Substrate | Yellow | Creamy | Circular |
| A 30 | Substrate | Yellow | Folded | Circular |

***In vitro* antagonism tests of marine actinomycetes against *F. solani***

Only four isolates showed antifungal activity (A20, A19, A18 and A15) against *F. solani*. A19 showed the highest antagonistic activity with a RGPI (Percentage of radial growth inhibition) of

72%, which did not show difference with the synthetic fungicide. A15 showed the lowest RGPI with 39.53% (figure 2a). Only A19 and A18 showed an effect on supernatant. A19 showed a RGPI of 44.92% and it did not show difference with ED48 (figure 2b).

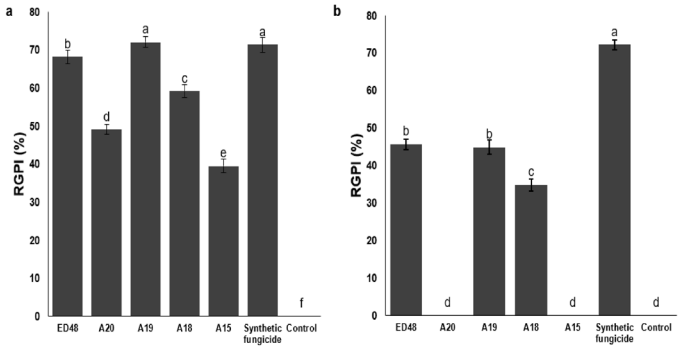



**Figure 2. *In vitro* antagonism of marine actinomycetes against *F. solani*.** (a) Percentage of radial growth inhibition (RGPI) of actinomycetes against F. solani. (b) RGPI of actinomycetes supernatants against F. solani. Synthetic fungicide= Carbendazim (6 mg.mL$^{-1}$). n= 5. ± Standard Desviation. Equal letters in the columns do not differ significantly according to Fisher (P<0.05).

Actinomycetes from terrestial environments have been widely studied as biocontrol agents against phytopathogens (Benhadj *et al*., 2019). Therefore, isolation of actinomycetes from under-researched environments such as marine, glacial, saline, among others, will provide new biocontrol agents that can be applied in agriculture (Gong *et al*., 2018). Several species of *Streptomyces* have been used for the control of soil phytopathogens (Ling *et al*., 2020). Among the bioactive compounds they produce are: hydrolytic enzymes and actinomycins, which are antibiotics belonging to the chromopeptide lactone family that function as growth inhibitors (Chen *et al*., 2020). *Nocardiopsis* species exhibit antifungal activity against *Fusarium* species and produce various antimicrobial metabolites (Intra *et al*., 2011). The antagonistic activity of actinomycetes against plant pathogens depends on their ability to produce hydrolytic enzymes, antifungal metabolites, competition for nutrients, siderophores, among others (Igarashi *et al*., 2021; Shen *et al*., 2021; Vurukonda *et al*., 2018).

**Molecular identification and phylogenetic analysis of marine actinomycetes**

PCR products of the 16S region were 1500 bp in size. A19 and A20 were 99.56% similar to *Streptomyces* sp. and A15 and A18 to *Nocardiopsis lucentensis* (99.50%). Phylogenetic analysis of the 16S gene of A20 grouped it together with *Streptomyces griseoflavus* and A19 was grouped in the *Streptomyces* sp. clade. Phylogenetic analysis of A18 and A15 grouped them with *Nocardiopsis lucentensis* (figure 3).

The 16s rRNA gene is found in prokaryotic organisms and archaeobacteria and its eukaryotic counterpart exists, its size is 1500 bp and it presents a high degree of conservation to distinguish between taxa, even at deep taxonomic levels (Clarridge, 2004). Based on colony morphology and 16s rRNA gene sequence, A20 was identified as *Streptomyces griseoflavus*, isolate A19 as *Streptomyces* sp. and isolates A15 and A18 as *Nocardiopsis lucentensis*.

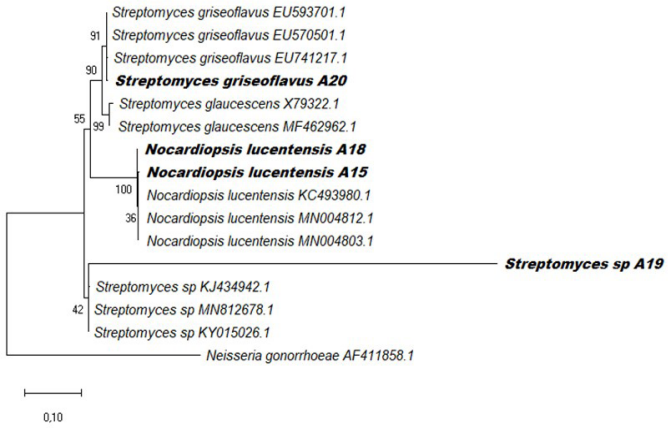



**Figure 3. Phylogenetic tree based on the 16S rRNA gene of actinomycetes A20, A19, A18 and A15 isolated from marine environments.** Bootstrap values are given as percentages over the nodes in this analysis (10,000 bootstrap).

## Conclusions

Thirty isolates of marine actinomycetes were obtained, of which *Streptomyces* sp., S. *griseoflavus* and *Nocardiopsis lucentensis* showed *in vitro* antifungal activity against *F. solani*. *Streptomyces* sp. showed the highest antifungal activity against the phytopathogenic fungus with a (RGPI) of 72%. Marine environments are a new source for isolation of microorganisms that can be used as biocontrol agents against phytopathogens.

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