

Biological control of *Alternaria alternata*-induced black-spot disease in *Dioscorea opposita* using endophytic *Bacillus* sp. E-Do8

Controle biológico da doença da mancha negra induzida por *Alternaria alternata* em *Dioscorea opposita* utilizando *Bacillus* sp. E-Do8 endofítico

Control biológico de la enfermedad de la mancha negra inducida por *Alternaria alternata* en *Dioscorea opposita* mediante el uso de *Bacillus* sp. E-Do8 endófito

Received: 02/21/2026 | Revised: 03/05/2026 | Accepted: 03/06/2026 | Published: 03/07/2026

Louis Antoniel Joseph

ORCID: <https://orcid.org/0000-0002-7747-0743>
Federal University of Tocantins, Brazil
E-mail: anthoniello@gmail.com

Manoucheca Jean

ORCID: <https://orcid.org/0009-0004-4638-377X>
Federal University of the Jequitinhonha and Mucuri Valleys, Brazil
E-mail: manoucheca.jean@ufvjm.edu.br

Bento Gil Uane

ORCID: <https://orcid.org/0009-0008-6143-9654>
Federal University of the Jequitinhonha and Mucuri Valleys, Brazil
E-mail: bentogilwane@gmail.com

Frantzdy Luc

ORCID: <https://orcid.org/0009-0004-0027-4033>
Federal University of Mato Grosso do Sul, Brazil
E-mail: f.luc@ufms.br

Meque Samuel Tivane

ORCID: <https://orcid.org/0009-0002-1142-731X>
Federal University of the Jequitinhonha and Mucuri Valleys, Brazil
E-mail: meque.tivane@ufvjm.edu.br

Kerley-Vivaldi Jean

ORCID: <https://orcid.org/0009-0000-8786-1556>
State University of Santa Catarina, Brazil
E-mail: kerley.jean@ufvjm.edu.br

Inocêncio Oliveira Mulaveia

ORCID: <https://orcid.org/0009-0006-0326-0969>
Federal University of the Jequitinhonha and Mucuri Valleys, Brazil
E-mail: inocencio.mulaveia@ufvjm.edu.br

Abstract

Plant endophytic bacteria play crucial roles in the prevention and control of crop diseases. Yam (*Dioscorea opposita*), an important tuber and root crop, is renowned for its rich vegetable and medicinal values. However, the black-spot disease is relatively prevalent during its cultivation process, and there are no reports on using endophytes to control this disease. In this study, we aimed to investigate the biological control of black spot disease caused by *Alternaria alternata* in *Dioscorea opposita* using the endophytic bacterium *Bacillus* sp. E-Do8. An endophytic bacterium that strongly antagonized *Alternaria alternata*, the pathogen causing yam black-spot disease, was isolated from Tiegun yam leaves, and it was identified as a strain of *Bacillus* and named E-Do8. Further investigations revealed that the E-Do8 fermentation broth could significantly inhibit the spore germination and mycelial growth of *A. alternata*, and the mycelia showed uneven thickness, swelling, and irregular entanglement. Moreover, the ability of *A. alternata* to penetrate cellophane was weakened when cultured with the E-Do8 fermentation broth, which could also significantly inhibit the pathogenicity of *A. alternata*. In addition, the UHPLC-Orbitrap Exploris 240 system was employed to analyze the active ingredients in the E-Do8 fermentation broth that inhibit *A. alternata*, and it was found that E-Do8 only produced surfactin-type compounds, C14-surfactin and surfactin.

Keywords: *Dioscorea opposita*; Endophytic bacterium; *Bacillus*; Black-spot disease; *Alternaria alternata*; Surfactin.

Resumo

As bactérias endofíticas de plantas desempenham papéis cruciais na prevenção e no controle de doenças em culturas agrícolas. O inhame (*Dioscorea opposita*), um importante tubérculo e raiz cultivada, é conhecido por seus ricos valores hortícolas e medicinais. No entanto, a mancha-preta é relativamente prevalente durante seu cultivo, e não há relatos sobre

o uso de endófitos para controlar essa doença. Neste contexto, este artigo teve como objetivo apresentar um estudo sobre o controle biológico da mancha negra causada por *Alternaria alternata* em *Dioscorea opposita* utilizando a bactéria endofítica *Bacillus* sp. E-Do8. Uma bactéria endofítica que antagonizou fortemente *Alternaria alternata*, o patógeno causador da mancha-preta do inhame, foi isolada de folhas de inhame Tiegun e identificada como uma cepa de *Bacillus*, denominada E-Do8. Investigações posteriores revelaram que o caldo de fermentação de E-Do8 inibiu significativamente a germinação de esporos e o crescimento micelial de *A. alternata*, e o micélio apresentou espessura irregular, inchaço e emaranhamento irregular. Além disso, a capacidade de *A. alternata* de penetrar celofane foi reduzida quando cultivada com o caldo de fermentação de E-Do8, o que também inibiu significativamente a patogenicidade de *A. alternata*. Além disso, o sistema UHPLC-Orbitrap Exploris 240 foi empregado para analisar os ingredientes ativos no caldo de fermentação E-Do8 que inibem *A. alternata*, e constatou-se que E-Do8 produziu apenas compostos do tipo surfactina, surfactina C14 e surfactina.

Palavras-chave: *Dioscorea alternata*; Bactéria endofítica; *Bacillus*; Doença da mancha negra; *Alternaria alternata*; Surfactina.

Resumen

Las bacterias endófitas asociadas a las plantas desempeñan un papel fundamental en la prevención y el control de enfermedades en los cultivos. El ñame (*Dioscorea opposita*), un importante tubérculo de uso alimentario y medicinal, es ampliamente reconocido por su valor nutricional y fitoterapéutico. No obstante, durante su cultivo es relativamente frecuente la aparición de la enfermedad de la mancha negra, y hasta el momento no existen informes sobre el empleo de endófitos para su control. En este contexto, este artículo tuvo como objetivo presentar un estudio sobre el control biológico de la enfermedad de la mancha negra causada por *Alternaria alternata* en *Dioscorea opposita* utilizando el endófito *Bacillus* sp. E-Do8. A partir de hojas de ñame Tiegun una bacteria endófito con una fuerte actividad antagonista frente a *Alternaria alternata*, el patógeno causante de la mancha negra del ñame. Dicha bacteria fue identificada como una cepa del género *Bacillus* y denominada E-Do8. Estudios posteriores demostraron que el caldo de fermentación de E-Do8 inhibió significativamente la germinación de esporas y el crecimiento micelial de *A. alternata*, observándose alteraciones morfológicas en el micelio, tales como grosor desigual, hinchazón y enredos irregulares. Asimismo, la capacidad de *A. alternata* para penetrar el celofán se redujo al cultivarse en presencia del caldo de fermentación de E-Do8, lo que indica una posible disminución significativa de su patogenicidad. Además, mediante el sistema UHPLC-Orbitrap Exploris 240 se analizaron los compuestos activos presentes en el caldo de fermentación de E-Do8 responsables de la inhibición de *A. alternata*, identificándose exclusivamente compuestos del tipo surfactina, específicamente C14-surfactina y surfactina.

Palabras clave: *Dioscorea alternata*; Bacteria endófito; *Bacillus*; Enfermedad de la mancha negra; *Alternaria alternata*; Surfactina.

1. Introduction

Globally, the reduction in food crop yields caused by diseases accounts for approximately 20% of the total production each year (Fisher et al., 2018; Eneas et al., 2022). In the past few decades, the prevention and control of crop diseases in agricultural production have predominantly dependent on chemical fungicides. Although chemical fungicides have undeniably played a vital role in safeguarding food supply, their long-term utilization has engendered a multitude of adverse impacts on the soil, water, and the broader ecological environment. Concurrently, it also presents a latent threat to human health and food security (Savary et al., 2012; Avgoustaki & Xydis, 2020; Joseph et al., 2022). Biogenic pesticide preparations, distinguished by their low toxicity and facile degradability, are acknowledged as environmentally friendly substitutes for chemical pesticides. At present, a variety of microbial preparations, such as *Bacillus*, *Pseudomonas* and *Trichoderma*, have been applied to combat the plant diseases. These microorganisms are capable of influencing or inhibiting the growth of pathogens via mechanisms such as competition, antagonism, or by inducing systemic resistance in plants, and are thus regarded as significant potential biocontrol strains (Pieterse et al., 2014; Joseph et al., 2025).

Plant endophytic bacteria are a group of microorganisms that live in healthy plants and spend the whole or part of their life cycle without causing any damage to plant growth. An integrated entity is established between these endophytes and their host plants, the plants furnish a habitat and essential nutrients to the endophytes, while, reciprocally, the endophytes bestow advantages upon the plants by virtue of their distinctive functions (Afzal et al., 2019; Joseph et al., 2025). Since 1993, when the endophyte with the capacity to produce taxol was initially isolated from Pacific yew (Stierle et al., 1993), plant endophytes have

come to the forefront as one of the prime focuses in the realm of microbial research. Endophytes have been detected in nearly all presently known plant species, and they are adept at mitigating a diverse array of stresses that plants endure. On the one hand, endophytes assume a pivotal role in responses to biotic and abiotic stresses by augmenting plant nutrient absorption and modulating plant hormones implicated in growth and stress responses (Zhang et al., 2019; Joseph et al., 2022). On the other hand, they confer indirect benefits to the host plants by manufacturing antibiotics, enzymes, and activating plant defense mechanisms (Joo et al., 2021; Joseph et al., 2025a).

Bacillus sp. are widely distributed in soil and plants. They possess antagonistic and antimicrobial effects, and their antimicrobial substances exhibit a wider bactericidal spectrum and higher stability, such as *B. subtilis* and *B. amyloliquefaciens* that can inhibit the growth of *Aspergillus parasiticus* and the production of aflatoxins (Jin et al. 2018; Siahmoshteh et al., 2018). Therefore, they are important biological control microorganisms that are currently widely developed and applied. The inhibitory effect of *Bacillus* sp. on pathogen is usually the result of a combination of mechanisms (Van Wees et al., 2000). They can occupy the dominant ecological niche and compete with pathogens for the limited nutrients available in the environment (Eljounaidi et al., 2016; Joseph et al., 2023), induce systemic resistance in plants (Tahir et al., 2017), and synthesize lipopeptide compounds that inhibit the growth of pathogen (Gond et al., 2015; Eneas et al., 2022). Furthermore, *Bacillus* sp. can secrete a variety of enzymes, including cellulase, lipase, protease, and chitinase, as well as various antagonistic substances such as antimicrobial peptides and proteins, and these active substances have a wide antimicrobial range and exert a significant inhibitory effect on a variety of pathogens, such as bacteria, fungi and viruses (Sun et al., 2006; Joseph et al., 2025b).

Yam (*Dioscorea opposita*), a perennial twining plant, is a prime example of a plant with both medicinal and edible properties. It ranks fourth among root/stem crops in terms of output, trailing only potatoes, cassava, and sweet potatoes. In 2022, its annual production surpassed 88 million tons (FAO, 2019), providing sustenance for hundreds of millions of people worldwide (Wang et al., 2022). In Brazil, yam has been cultivated for more than 2000 years and is widely consumed as a vegetable. *Dioscorea opposita* Thunb., including the Tiegum yam cultivated in the regions of Alfredo Chaves and São Bento de Urânia, is recognized for its exceptional quality and notable medicinal properties, establishing it as a highly valued medicinal resource. Currently, although numerous reports exist regarding the application of endophytic bacteria in disease control, research specifically focused on yams remains relatively limited. In this study, we aimed to investigate the biological control of black spot disease caused by *Alternaria alternata* in *Dioscorea opposita* using the endophytic bacterium *Bacillus* sp. E-Do8.

2. Methodology

2.1 Isolation and purification of endophytic bacteria

An experimental, laboratory-based study was conducted using a qualitative and quantitative approach (Pereira et al., 2018; Risemberg et al., 2026) employing simple descriptive statistics with column graphs, mean and standard deviation values, and statistical analysis (Vieira, 2021). The experiments were conducted at the Phytopathology Laboratory at UFT in Gurupi, Tocantins, Brazil (11°43'36.86" S, 49°02'57.18" W). The region has a tropical climate (Aw) according to the Köppen classification system, with an average annual precipitation of 1617 mm and monthly temperatures ranging from 19 °C to 36 °C (Joseph et al., 2025). The fresh leaves of Tiegum yam were first rinsed under running water for a duration of 2-3 h. Subsequently, they were immersed in 70% ethanol for 30 s, and then transferred in a 0.1% mercuric chloride solution for 5 min. The leaves were then washed six times with sterile water and blotted on sterile absorbent paper to air dry on the clean bench. To validate the adequacy of surface sterilization, the final wash water was spread evenly onto an LB (Luria-Bertani) medium (0.5% yeast extract, 1% tryptone and 1% NaCl and 2% agar, w/v) and incubated at 28 °C. Once it was ascertained that the sterilization process was complete, a sterile scalpel blade was employed to cut the sterilized leaves into small pieces (1 cm × 1 cm). Which were then inoculated onto an LB medium and cultured at 28 °C for two days. Upon the emergence of colonies, the purification procedure was

initiated and continued until the colony morphology on the Petri dish became uniform. Through microscopic observation, strains exhibiting pronounced differences in morphology, color, and size were carefully selected and then stored for utilization in subsequent experimental procedures. For example, Gram staining procedure was performed as previously described (Hanapi et al., 2023).

2.2 Detection of antifungal activity of endophytic bacteria

After the pathogenic fungus *A. alternata* HB7 responsible for black-spot disease had been cultured on PDA (potato dextrose agar) medium (20% potato, 2% glucose and 2% agar, w/v) for 15 d, a punch with a diameter of 5 mm was utilized to obtain fungal plugs from the periphery of the colony. These plugs were then inverted and affixed to the central region of fresh PDA plates, and the purified endophytic bacteria were inoculated at a separation distance of 2-3 cm from the fungal plugs. Subsequently, the plates were cultured at 28 °C for eight days to monitor the effects of endophytic bacteria on the growth of *A. alternata* (Nunes et al., 2023; Joseph et al., 2025).

2.3 Identification of endophytic bacteria and construction of phylogenetic tree

The universal primers 27F (5'-AGAGTTTGATC-MTGGCTCAG-3') and 1492R (5'-GGTTACCTT GTTACGACTT-3') were utilized to amplify the 16S rDNA fragment of the endophytic bacterium E-Do8, which demonstrated antagonistic effects against *A. alternata* (Lane, 1991). PCR was performed under the following conditions: a preheating step at 95 °C for 3 min, 35 cycles consisting of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 40 s, and a final extension step at 72 °C for 5 min. Subsequently, and the PCR product was performed for Sanger sequencing. After obtaining the sequence, it was submitted to GenBank to acquire the accession number PV067162. Then, the NCBI database was used to perform a BLAST comparative analysis between PV067162 and the type strains. To determine the taxonomic status of the E-Do8 strain, the MEGA6 software and the Neighbor-Joining method were used to construct a phylogenetic tree based on the homologous sequences of the type strains employing the Kimura 2-parameter model with 1000 bootstrap replicates (Tamura et al., 2013).

2.4 Endospore staining

The E-Do8 colonies that were cultured for 16 h were transferred into an empty test tube using an inoculating loop. Subsequently, 500 µl ddH₂O was added to the tube and stirred thoroughly to prepare a concentrated bacterial suspension. Two drops of malachite green dye were then added into the test tube, and incubated in a water bath for 10 min at 100 °C. Afterward, an inoculating loop was used to pick up several loopfuls of the bacterial suspension from the test tube and spread onto a clean glass slide to form a thin film. The smear was fixed by passing it through a gentle flame, and then rinsed with ddH₂O until the outflowing water was colorless. Next, the smear was stained with safranin solution for 1 min and gently rinsed with ddH₂O. The residual liquid was absorbed with filter paper, dried, and observed under an optical microscope (Milky Way counter; Taichung, Taiwan) (Joseph et al., 2025b, 2025c).

2.5 Detection of the antagonistic activity of endophyte fermentation broth

To investigate the effect of the fermentation broth on the growth of pathogenic fungus, E-Do8 was subjected to shaken and cultured in 50 ml LB broth for 12 h, 24 h, and 36 h. Subsequently, the supernatant was obtained by centrifuging at 8000 × g for 20 min using the SL1 Plus centrifuge (Thermo Fisher Scientific, MA, USA), and then uniformly mixed with 2 × PDA solid medium in equal volumes before being poured into Petri dishes. In contrast, for the control group, sterile LB liquid medium was mixed with 2 × PDA solid medium in equal volumes. After that, the pathogenic fungus *A. alternata* was inoculated at the center of these plates and incubated at 28 °C for several days (Nunes et al., 2023; Joseph et al., 2025).

To assess the antagonistic activity of the crude extract from the fermentation broth, the 24 h fermentation broth of E-Do8 was extracted using ethyl acetate. The extract was then concentrated using a RE52CS-type rotary evaporator at 40 °C and dissolved in methanol. An *A. alternata* disc with a diameter of 5 mm was inoculated at the center of a PDA plate. On one side (approximately 2-3 cm away) of the disc, 20 µl of the fermentation broth was inoculated, while on the other side, 20 µl of methanol was inoculated as a control. The plate was then incubated at 28 °C for several days to observe the colony growth. The spore germination rate was measured according to Joseph et al. (2025). Briefly, the *A. alternata* conidia suspension (10^7 conidia ml⁻¹) was evenly spread on the solid PDA plates either with or without the 24 h E-Do8 fermentation broth. After culturing the plates at 28 °C for 24 h, the spore germination rate was measured under an optical microscope.

2.6 Pathogenicity analysis

To examine whether the mycelium could penetrate cellophane, conidia of *A. alternata*, which had been cultured for 15 days under conditions both with and without the 24 h E-Do8 fermentation broth, were harvested and used to prepare the conidial suspensions (10^7 conidia ml⁻¹). Subsequently, 3 µl suspension was carefully pipetted and placed at the center of a PDA plate covered with sterile cellophane. After a two-day incubation period, the cellophane was removed, and the plate was returned to the incubator for an additional seven days to monitor the colony growth. To evaluate the effect of the fermentation broth on the pathogenicity of the pathogen, the 36 h E-Do8 fermentation broth was uniformly sprayed onto the leaves of Tiegun yam plants (cultivated under controlled conditions: 25 °C, a L:D 12:12 photo-period, 60% RH, and Hoagland nutrient solution applied every seven days), and the LB liquid medium was used as the control group. Following this, the *A. alternata* conidial suspensions (10^7 conidia ml⁻¹) were sprayed onto the leaves. Subsequently, these plants were cultivated in an artificial climate chamber under a L:D 16:8 photoperiod at 25 °C and a 50–60% RH for 15 days to observe the development of pathogenic symptoms.

2.7 Detection of fermentation broth components using MS

The 24 h crude extract of the E-Do8 fermentation broth was analyzed via the UHPLC-Orbitrap Exploris 240 high resolution mass spectrometry instrument (ThermoFisher Scientific Inc. USA). The liquid chromatography system employed was the Vanquish horizon, and the chromatographic column used was a Hypersil gold C18 (2.1 mm × 150 mm, 1.9 µm). The column temperature was set at 30 °C, the injection volume was set to 10 µl, and the detection wavelength was fixed at 214 nm. For the mobile phase, its composition and gradient were as follows: component A was ultrapure water, and component B was acetonitrile. The flow rate of the mobile phase was kept constant at 0.3 ml min⁻¹. The elution gradient was specified as follows: from 0 to 5 min, 20% B; from 5 to 10 min, 40% B; from 10 to 12 min, 95% B; and from 12 to 15 min, 20% B. The mass spectrometry system utilized was the Orbitrap Exploris 240, operating in both ESI⁺ and ESI⁻ detection modes. The drying temperature was set at 350 °C, the sheath temperature at 350 °C, the nebulizer pressure at 60 psig, the capillary voltage at 3500 V, the nozzle voltage at 1000 V, the fragmentor voltage at 175 V, and the skimmer voltage at 65.0 V. The mass range was set from 150 to 3000 m/z. The data were analyzed using Thermo Xcalibur 4.6 software (ThermoFisher Technologies, USA).

2.8 Data analysis

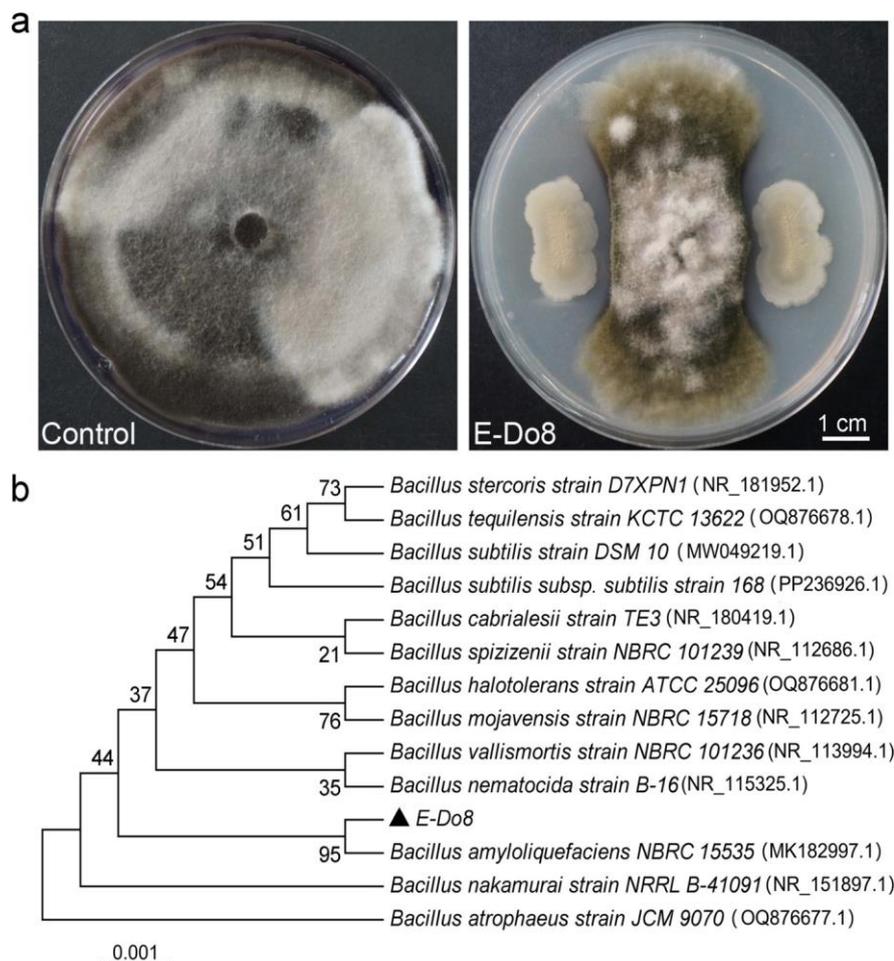
The normality of the dataset was evaluated using the Shapiro-Wilk test. In addition, a Levene's test was also performed to verify the homogeneity of variance. Once the normal distribution had been checked, a one-way analysis of variance (ANOVA) was performed, and Tukey's test at a 5% significance level. All experiments were repeated three times, and the data were presented as the mean ± SE. Data analysis was performed using SPSS 24.0 software.

3. Results

3.1 Isolation and identification of endophyte E-Do8

In this study, a total of thirty-two endophytic bacteria were isolated from yam leaves. Using the plate confrontation assay, it was found that the endophyte E-Do8 exerted a significant inhibitory effect on *A. alternata*, the pathogen causing yam black-spot disease. Compared with the control group, the mycelial growth of the pathogen in the vicinity of E-Do8 was inhibited (Figure 1a). After acquiring the 16S rDNA sequence of E-Do8 strain (PV067162), the NCBI BLAST and comparative analysis was performed against the gene sequences of related species in the GenBank database. The phylogenetic tree determined that E-Do8 is a strain of *Bacillus* bacteria and it clustered into the same clade as the type strain *B. amyloliquefaciens* NBRC 15535 (Figure 1b). Moreover, after 18 h of cultivation, the surface of the single colony of the E-Do8 strain was wrinkled, opaque, and featured an irregularly raised middle part (Figure S1a). Gram staining reaction indicated that strain E-Do8 is G⁺ bacterium (Figure S1b). Endospore staining showed that the spores were green and the sporangia were light red (Figure S1c), which was consistent with the characteristics of the *Bacillus* genus. In summary, E-Do8 was identified as a member of the *Bacillus* sp.

Figure 1- Identification of strain E-Do8. **(a)** Antagonistic activity of E-Do8 against *A. alternata*. **(b)** Phylogenetic tree based on 16S rDNA sequence of E-Do8 and related strains. The phylogenetic tree was constructed using the Neighbor-Joining method with 1000 bootstrap replicates and a p-distance correction, and the bootstrap values (%) are indicated at nodes. The scale bar indicates 0.001 substitutions per base position. The black triangle symbol indicates the position of E-Do8.

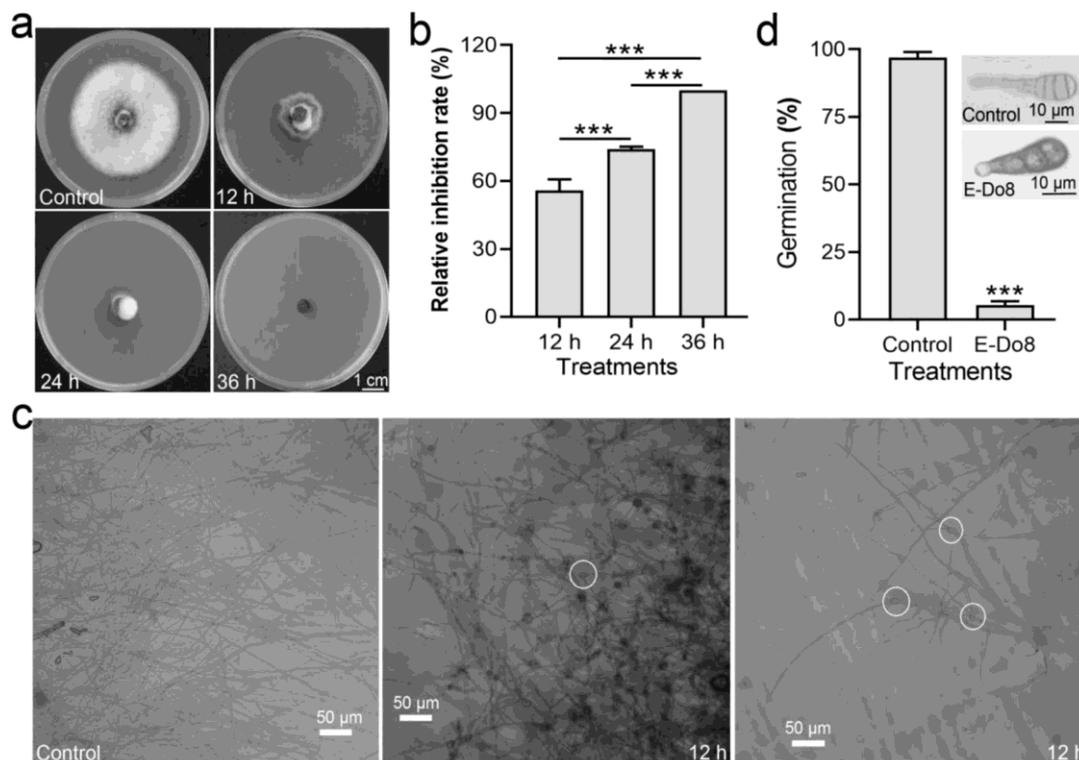


Source: Research data (2025).

3.2 E-Do8 fermented broth inhibits the growth of *A. alternata*

To further explore the inhibitory effect of E-Do8 on *A. alternata*, the E-Do8 fermentation broth obtained at different time points was incorporated into the culture medium. After six days of incubation, it was found that the growth of *A. alternata* was significantly inhibited ($F_{2,27} = 175.20$, $p < 0.001$) by the 12 and 24 h fermentation broths, with inhibition rates of $55.84 \pm 2.83\%$ and $74.03 \pm 0.65\%$, respectively. The 36-h fermented broth, however, completely arrested the growth of *A. alternata* (Figure 2a and b). This finding indicated that the inhibitory effect became more prominent as the fermentation time increased. Taking the 12 h fermented broth as a representative, once it was introduced into the medium, the *A. alternata* hyphae displayed uneven thickness, some parts were obviously thinner or thicker than others, along with obvious swelling and chaotic, irregular entanglement. In contrast, the mycelia in the control group remained plump and smooth, without any of the abnormalities observed in the experimental group (Figure 2c). Furthermore, the addition of the E-Do8 fermentation broth into the PDA medium significantly inhibited ($F_{1,18} = 2908.65$, $p < 0.001$) the germination of *A. alternata* conidia, where the germination rate in the fermentation broth-treated group ($5.33 \pm 0.88\%$) was notably lower than that in the control group ($97.00 \pm 1.15\%$) (Figure 2d).

Figure 2- E-Do8 fermented broth affected the growth of *A. alternata*. (a) Influence of the fermentation broth over different time periods on the colony growth of *A. alternata*. (b) Relative inhibition rate of *A. alternata* growth by the fermentation broth at varying time points. The calculation for relative inhibition rate used the following equation: $(D_{\text{Control}} - D_{\text{Treatment}}) / D_{\text{Control}} \times 100\%$, where D_{Control} is the colony diameter of the control group and $D_{\text{Treatment}}$ is the colony diameter of the E-Do8 fermented broth treatment group, $***$, $p < 0.001$. (c) Effect of the fermentation broth on the mycelium growth of *A. alternata*. The white circle indicates the swelling of the hypha. (d) Effect of the 24 h E-Do8 fermentation broth on the conidial germination of *A. alternata* (cultured on the PDA plates at 28 °C for 24 h). The image in the upper-right corner depicts spore germination in the control and E-Do8 treatment groups. $***$, $p < 0.001$. The error bars shown in the figure correspond to SE.

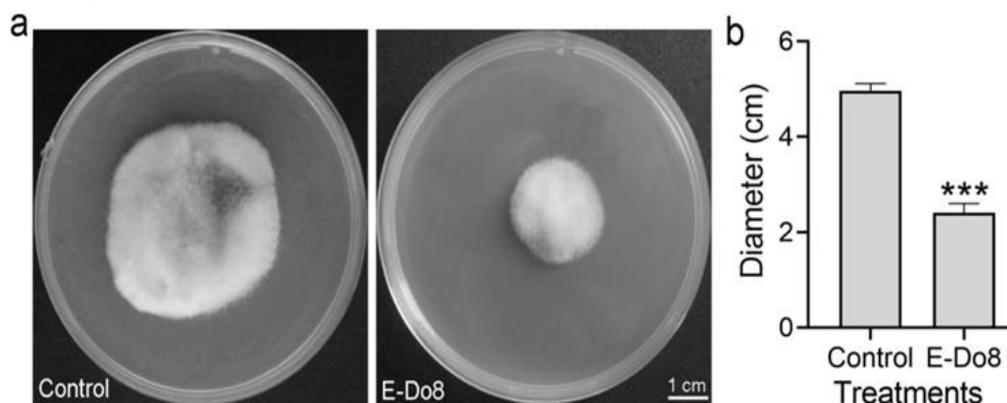


Source: Research data (2025).

3.3 The fermentation broth of E-Do8 can inhibit the infection of *A. alternata*

To further explore the effect of the fermentation broth on pathogen infection, we initially investigated the ability of *A. alternata* conidia, subjected to different treatments, to penetrate cellophane, which mimics plant cell wall tissue penetration (López-Berges et al., 2010). Our findings revealed that the cellophane-penetrating ability of conidia, grown for 15 days in PDA medium supplemented with 24 h E-Do8 fermentation broth, was decreased (Figure 3a). After removing the cellophane, the colony size (2.40 ± 0.12 cm) was significantly smaller compared to that of the control group (4.97 ± 0.88 cm) ($F_{1,18} = 191.26$, $p < 0.001$) (Figure 3b). Given that the 36 h fermentation broth of E-Do8 was proven to completely inhibit the growth of *A. alternata* (Figure 2a), we selected the 36 h fermentation broth as a representative sample to study its effect on pathogenicity. After spraying it onto the healthy yam leaves, it was found that the fermentation broth could effectively prevent *A. alternata* infection, and no disease spots appeared on the leaves (Figure S2).

Figure 3- E-Do8 fermented broth exerted an impact on the infection and pathogenesis of *A. alternata*. (a) Colonies that form on the plate after cellophane spores' penetration assay. (b) Statistics of the colony growth diameter. ***, $p < 0.001$. The error bars shown in the figure correspond to SE.

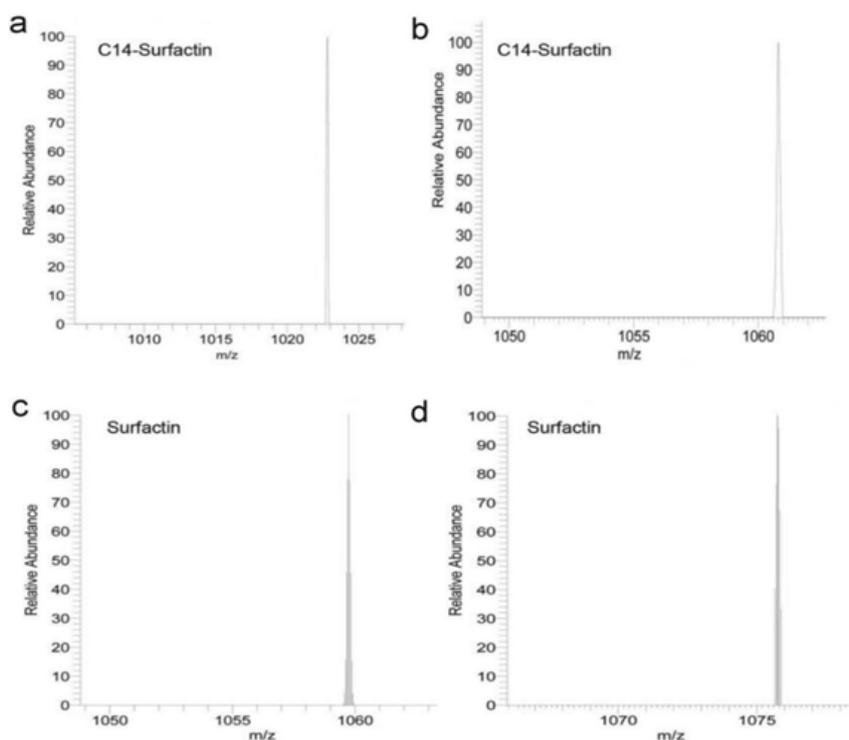


Source: Research data (2025).

3.4 Analysis of active substances in the E-Do8 fermentation broth

To analyze the effective constituents in the E-Do8 fermentation broth with inhibitory effects against *A. alternata*, the 36 h E-Do8 fermentation broth was extracted using ethyl acetate, and then the crude extract was dissolved in methanol. The plate confrontation assay demonstrated that the crude extract of the E-Do8 fermentation broth could effectively inhibit the growth of the pathogen (Figure S3), indicating that an antifungal crude extract was successfully obtained through this method. To further investigate the antifungal mechanism of E-Do8 and elucidate the components of the antifungal substances, the chemical components of the crude extract of the E-Do8 fermentation broth were analyzed using the UHPLC- Orbitrap Exploris 240 system. The results showed that the major antifungal components were surfactin type compounds, specifically C14-surfactin [M + H]⁺, C14-surfactin [M + K]⁺, surfactin [M + Na]⁺, and surfactin [M + K]⁺, with retention times of 13.12 min, 11.28 min, 13.08 min, and 12.78 min (Figure 4).

Figure 4- Analysis of the antifungal chemical components in the crude extract of E-Do8 fermentation broth. (a) C14-surfactin [M + H]⁺. (b) C14-surfactin [M + K]⁺. (c) surfactin [M + Na]⁺. (d) surfactin [M + K]⁺.



Source: Research data (2025).

4. Discussion

Crop diseases are a crucial factor impeding the sustainable development of agriculture, posing a severe threat to food security and ecological stability. In the field of biological control, plant endophytes have become a valuable resource. For example, the fermentation broth of an endophyte strain *B. vallismortis* ZZ185, isolated from the *Ilex latifolia* Thunb., exhibited remarkable antimicrobial activity against multiple pathogens, including *Cryphonectria parasitica*, *A. alternata*, *Fusarium graminearum*, *Phytophthora capsici*, and *Rhizoctonia solani* (Zhao et al., 2010). Similarly, Liu et al. (2007) isolated an endophytic bacteria *Acinetobacter baumannii* LCH001 from *Cinnamomum camphora*, and the inhibition rate of its fermentation broth against *Botrytis cinerea* achieved a remarkable 78.6% when the concentration was 100 $\mu\text{l ml}^{-1}$. Root and tuber crops are of vital importance in the global food supply chain. Here, we focused on the yam black-spot disease as the entry point to explore the control situation of endophytic bacteria against pathogens.

Bacillus sp. can not only promote plant growth but also enhance plant resistance against diseases, such bifunctional characteristics make them highly promising for application in sustainable agricultural development (Sun et al. 2022). *B. amyloliquefaciens* strain Ba168, as a case in point, has been proven to effectively control apple blue mold caused by *Penicillium expansum* (Joseph et al., 2025c). Also, strain DHA55 exhibited a staggering 74.90% inhibition rate against watermelon fusarium wilt triggered by *F. oxysporum* (Al-Mutar et al., 2023). In this study, a bacterium with outstanding antifungal characteristics was isolated from the yam leaves, and it was identified as a number of the *Bacillus* sp. and named as E-Do8. Our experiments unambiguously demonstrated that E-Do8 could substantially impede the growth of pathogen. Notably, its fermentation broth exhibited remarkable efficacy in multiple facets. Firstly, it proved extremely effective in suppressing the germination of pathogen conidia, thereby thwarting the initial dissemination of the disease. Secondly, it significantly retarded the growth of hyphae, disrupting the normal development and proliferation of the pathogen. Thirdly, it attenuated the pathogen's infectivity and disease-causing ability, reducing the likelihood of successful infection and subsequent damage to the host plant. Collectively, these

findings strongly imply that E-Do8 holds substantial promise as a biocontrol agent, meriting further exploration and application in agricultural settings.

Lipopeptides secreted by *Bacillus* exhibit antifungal activity against plant pathogenic fungi. These lipopeptides mainly include surfactin, iturin and fengycin (Chen et al., 2018; Kim et al., 2021), exist in diverse isomeric forms and possess favorable antimicrobial activity (Zerouh et al., 2011; Carolin et al., 2021). Among them, surfactin is a powerful biosurfactant with both antiviral and antimicrobial activities. Surfactin can effectively inhibit the growth of *Propionibacterium acnes* by disrupting its cell wall and cell membrane (Shan et al., 2021). In this study, the crude extract of E-Do8 fermented broth obtained by ethyl acetate extraction exhibited significant antifungal activity. To further analyze the chemical composition of the crude extract, the UHPLC-Orbitrap Exploris 240 system was employed, and it was found that it contained surfactin, an antifungal peptide, while iturin and fengycin were conspicuously absent. Although a large number of studies have confirmed that *Bacillus* has excellent biological activity against various pathogens, it should be emphasized that different endophyte species, or even different strains within the same species, can exhibit substantial differences in their antimicrobial efficacies, such as *B. amyloliquefaciens* Cas02 being capable of producing iturin A (Chu et al., 2021).

5. Conclusion

The results show that the strain effectively suppressed the pathogens growth, reduced conidial germination, and caused pronounced morphological abnormalities in fungal hyphae. Additionally, the fermentation broth significantly impaired the pathogens' ability to penetrate host-mimicking substrates and completely prevented disease development on yam leaves, confirming its capacity to inhibit both infection and pathogenicity. Chemical analyses identified surfactin and C14-surfactin as the major antifungal compounds produced by E-Do8, suggesting that lipopeptide-mediated antagonism plays a central role in its biocontrol activity. Overall, these findings establish *Bacillus* sp. E-Do8 as a promising, environmentally safe agent for the management of yam black-spot disease, and providing a scientific basis for integrating endophytic *Bacillus* strains into sustainable disease-control strategies for *Dioscorea* crops.

References

- Afzal, I., Shinwari, Z.K., Sikandar, S. & Shahzad, S. (2019). Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. *Microbiology Research*, 221(2), 36–49. <https://doi.org/10.1016/j.micres.2019.02.001>
- Al-Mutar, D.M.K., Alzawar, N.S.A., Noman, M., Azizullah, L.I.D.Y. & Song, F.M. (2023). Suppression of *Fusarium* wilt in water-melon by *Bacillus amyloliquefaciens* DHA55 through extracellular production of antifungal lipopeptides. *Journal of Fungi*, 9(3), 1–22. <https://doi.org/10.3390/jof9030336>
- Avgoustaki, D.D. & Xydis, G. (2020). Plant factories in the water-food-energy nexus era: a systematic bibliographical review. *Food Security*, 12(1), 253–268. <https://doi.org/10.1007/s12571-019-01003-z>
- Carolin, C.F., Kumar, P.S. & Ngueagni, P.T. (2021). A review on new aspects of lipopeptide biosurfactant: types, production, properties and its application in the bioremediation process. *Journal of Hazardous Materials*, 407(14), 1–7. <https://doi.org/10.1016/j.jhazmat.2020.124827>
- Chen, L., Heng, J., Qin, S. & Bian, K. (2018). A comprehensive understanding of the biocontrol potential of *Bacillus velezensis* LM2303 against *Fusarium* head blight. *PLoS One*, 13(6), 1–22. <https://doi.org/10.1371/journal.pone.0198560>
- Chu, D.P., Ilyas, N., Peng, L.J., Wang, X.Q., Wang, D.K., Xu, Z.C., Gao, Q., Tan, X.L., Zhang, C.S., Li, Y.Q. & Yuan, Y. (2021). Genomic insights on fighting bacterial wilt by a novel *Bacillus amyloliquefaciens* strain Cas02. *Microbial Biotechnology*, 15(4), 1152–1167. <https://doi.org/10.1111/1751-7915.13925>
- Eljounaidi, K., Lee, S.K. & Bae, H. (2016). Bacterial endophytes as potential biocontrol agents of vascular wilt diseases-review and future prospects. *Biological Control*, 103(1), 62–68. <https://doi.org/10.1016/j.biocontrol.2016.07.013>
- Eneas, J.S.M., Joseph, L.A., Rodrigues, R.C.M., Santos, E.A., Casais, L.C.N., Reis, K.H.B., Cardoso, J.N. & Santos, A.C. (2022). Variabilidade espacial das propriedades dendrométricas do *Eucalyptus urophylla* no Bioma Cerrado. *Research, Society and Development*, 11(11), 1–9. <https://doi.org/10.33448/rsd-v11i11.33638>
- FAO (Food and Agriculture Organization) (2022). Online statistical database: Crops and livestock products. *FAOSTAT (Food and Agriculture Organization Statistics)*.
- Fisher, M.C., Hawkins, N.J., Sanglard, D. & Gurr, S.J. (2018). Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science*, 360(6390), 739–742. <https://doi.org/10.1126/science.aap7999>

- Gond, S.K., Bergen, M.S., Torres, M.S. & White, J.F. (2015). Endophytic *Bacillus* spp. produce antifungal lipopeptides and induce host defense gene expression in maize. *Microbiological Research*, 172, 79–87. <https://doi.org/10.1016/j.micres.2014.11.004>
- Hanapi, N.H.M., Monajemi, H., Ismail, A., Suhaili, Z. & Juahir, H. (2023). Identification of microbes from textile dye waste- water and its antibiotic resistance from local textile factory. *Scientific Reports*, 27, 44–54. <https://doi.org/10.1038/s41598-025-95359-2>
- Jin, P., Wang, B.L., Liu, W., Fan, Y. & Miao, W. (2018). A new cyclic lipopeptide isolated from *Bacillus amyloliquefaciens* HAB-2 and safety evaluation. *Pesticide Biochemistry and Physiology*, 147, 40–45. <https://doi.org/10.1016/j.pestbp.2017.08.015>
- Joo, H.S., Deyrup, S.T. & Shim, S.H. (2021). Endophyte-produced antimicrobials: a review of potential lead compounds with a focus on quorum-sensing disruptors. *Phytochemistry Reviews*, 20, 543–568. <https://doi.org/10.1007/s11101-020-09711-7>
- Joseph, L.A., Jean, M., Appolon, I., Pierre, J., Jean, K.V., Fils-aimé, F. & Uane, B.G. (2025b). *Trichoderma harzianum* UFT-25 and its relationship with the promotion of *Eucalyptus* plant growth. *Research, Society and Development*, 14(2), 1–10. <https://doi.org/10.33448/rsd-v14i2.48253>
- Joseph, L.A. (2025a). Recent advances in the applications of endophytic *Trichoderma* spp. for biocontrol and plant growth promotion. *Mycological Progress*, 24(51), 1–12. <https://doi.org/10.1007/s11557-025-02071-6>
- Joseph, L.A., Jean, M., Luc, F., Jean, K.V., Uane, B.G., Matsinhe, M.A.D., Tivane, M.S. & Mulaveia, I.O. (2025c). Potential of *Trichoderma asperellum* against root-rot caused by *Fusarium equiseti* in tomato plants. *Research, Society and Development*, 14(12), 1–12. <https://doi.org/10.33448/rsd-v14i12.50223>
- Joseph, L.A., Jean, M., Mial, F., Fragéus, K., Jean, K.V. & Fils-aimé, F. (2023). Avaliação do efeito de pesticidas sobre o crescimento do *Beauveria bassiana*. *Research, Society and Development*, 12(4), 1–9. <https://doi.org/10.33448/rsd-v12i4.44676>
- Joseph, L.A., Lima, N.M.P., Rocha, P.A.L., Chagas Júnior, A.F., Rocha, J.P.L., Pereira, J.S., Martins, A.O., Moraes, C.B., Oliveira, L.M.R., Araújo, W.L., Sarmiento, M.I. & Sarmiento, R.A. (2025). Morphological responses of *Eucalyptus* demonstrate the potential of *Trichoderma harzianum* to promote resistance against *Leptocybe invasa*. *Brazilian Journal of Microbiology*, 56(1), 1–12. <https://doi.org/10.1007/s42770-025-01704-y>
- Joseph, L.A., Sousa, K.A.O., Chagas Junior, A.F. & Luc, F. (2022). Compatibility of fungicides with *Trichoderma asperelloides* and *Azospirillum brasilense*. *Revista Scientia Agraria Paranaensis*, 21(1), 30–35. <https://doi.org/10.18188/sap.v21i1.29155>
- Kim, Y.S., Lee, Y., Cheon, W., Park, J., Kwon, H.T., Balaraju, K., Kim, J., Yoon, Y.J. & Jeon, Y. (2021). Characterization of *Bacillus velezensis* AK-0 as a biocontrol agent against apple bitter rot caused by *Colletotrichum gloeosporioides*. *Scientific Reports*, 11, 1–14. <https://doi.org/10.1038/s41598-020-80231-2>
- Lane, D.J. (1991). 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) *Nucleic acid techniques in bacterial systematics*. Wiley, New York, 115–175.
- Liu, C.H., Chen, X., Liu, T.T., Lian, B., Gu, Y.C., Caer, V., Xue, Y.R. & Wang, B.T. (2007). Study of the antifungal activity of *Acinetobacter baumannii* LCH001 *in vitro* and identification of its antifungal components. *Applied Microbiology and Biotechnology*, 76, 459–466. <https://doi.org/10.1007/s00253-007-1010-0>
- López-Berges, M.S., Rispail, N., Prados-Rosales, R.C. & Di Pietro, A. (2010). A nitrogen response pathway regulates virulence functions in *Fusarium oxysporum* via the protein kinase TOR and the bZIP protein MeaB. *Plant Cell*, 22(7), 2459–2475. <https://doi.org/10.1105/tpc.110.075937>
- Lu, M.H., Chen, Y.H., Li, L.J., Ma, Y.H., Tong, Z.F., Guo, D.S., Sun, P.P. & An, D.R. (2022). Analysis and evaluation of the flagellin activity of *Bacillus amyloliquefaciens* Ba168 antimicrobial proteins against *Penicillium expansum*. *Molecules*, 27(13), 1–13. <https://doi.org/10.3390/molecules27134259>
- Nunes, T.V., Rodrigues, J.N., Pinto, I.O., Pimenta, R.S., Sarmiento, M.I., Silva, R.S., Souza, P.G.C., De Souza, D.J., Joseph, L.A., Souza, M.L.O. & Sarmiento, R.A. (2023). Endophytic development of the entomopathogenic fungus *Beauveria bassiana* reduced the development of galls and adult emergence of *Leptocybe invasa* in susceptible *Eucalyptus*. *Sustainability*, 15(8), 1–13. <https://doi.org/10.3390/su152316411>
- Pereira, A.S. et al. (2018). *Metodologia da pesquisa científica*. [free ebook]. Santa Maria: Editora da UFSM.
- Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C.M. & Bakker, P.A.H.M. (2014). Induced systemic resistance by beneficial microbes. *Induced Systemic Resistance by Beneficial Microbes*, 52, 347–375. <https://doi.org/10.1146/annurev-phyto-082712-102340>
- Risemberg, R.I.C., Wakin, M. & Shitsuka, R. (2026). A importância da metodologia científica no desenvolvimento de artigos científicos. *E-Acadêmica*, 7(1), 1–5, e0171675. <https://eacademica.org/eacademica/article/view/675>.
- Savary, S., Ficke, A., Auberto, J.N. & Hollier, C. (2012). Crop losses due to diseases and their implications for global food production losses and food security. *Food Security*, 4, 519–537. <https://doi.org/10.1007/s12571-012-0200-5>
- Shan, M.Y., Meng, F.Q., Zhou, L.B., Lu, F.X., Bie, X.M., Zhao, H.Z. & Lu, Z.X. (2021). Surfactin inhibits the growth of *Propionibacterium acnes* by destroying the cell wall and membrane. *Letter in Applied Microbiology*, 73(6), 684–693. <https://doi.org/10.1111/lam.13576>
- Shitsuka, R. et al. (2014). *Matemática fundamental para tecnologia*. (2ed). Editora Érica.
- Stahmoshteh, F., Hamidi-Esfahani, Z., Spadaro, D., Shams-Ghah-farokhi, M. & Razzaghi-Abyaneh, M. (2018). Unraveling the mode of antifungal action of *Bacillus subtilis* and *Bacillus amyloliquefaciens* as potential biocontrol agents against aflatoxigenic *Aspergillus parasiticus*. *Food Control*, 89, 300–307. <https://doi.org/10.1016/j.foodcont.2017.11.010>
- Stierle, A., Strobel, G. & Stierle, D. (1993). Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. *Science*, 260, 214–216. <https://doi.org/10.1126/science.8097061>

- Sun, L.J., Lu, Z.X., Bie, X.M., Lu, F.X. & Yang, S.Y. (2006). Isolation and characterization of a co-producer of fengycins and surfactins, endophytic *Bacillus amyloquelaciens* ES-2, from *Scutellaria baicalensis* Georgi. *World Journal of Microbiology and Biotechnology*, 22, 1259–1266. <https://doi.org/10.1007/s11274-006-9170-0>
- Sun, M., Meng, X.G., Peng, T.L. & Hu, X.H. (2022). Effect of *Bacillus methylotrophicus* on tomato plug seedling. *Horticulturae*, 8(10), 1–22. <https://doi.org/10.3390/horticulturae8100947>
- Tahir, H.A.S., Gu, Q., Wu, H.J., Niu, Y.D., Huo, R. & Gao, X.W. (2017). *Bacillus volatiles* adversely affect the physiology and ultra-structure of *Ralstonia solanacearum* and induce systemic resistance in tobacco against bacterial wilt. *Science Reports*, 7, 1–15. <https://doi.org/10.1038/srep40481>
- Tamura, K., Stecher, G., Peterson, D., Filipiński, A. & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Van Wees, S.C.M., de Swart, E.A., Van Pelt, J.A., Van Loon, L.C. & Pieterse, C.M.J. (2000). Enhancement of induced disease resistance by simultaneous activation of salicylate and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences*, 97(15), 8711–8716. <https://doi.org/10.1073/pnas.130425197>
- Vieira, S. (2021). *Introdução à bioestatística*. Editora GEN/Guanabara Koogan.
- Wang, P.T., Shan, N., Ali, A., Sun, J.Y., Luo, S., Xiao, Y., Wang, S.L., Hu, R., Huang, Y.J. & Zhou, Q.H. (2022). Comprehensive evaluation of functional components, biological activities, and minerals of yam species (*Dioscorea polystachya* and *D. alata*) from China. *LWT*, 168, 1–10. <https://doi.org/10.1016/j.lwt.2022.113964>
- Zerriouh, H., Romero, D., García-Gutiérrez, L., Cazorla, F.M., de Vicente, A. & Pérez-García, A. (2011). The iturin-like lipopeptides are essential components in the biological control arsenal of *Bacillus subtilis* against bacterial diseases of cucurbits. *Molecular Plant-Microbe Interactions*, 24(12), 1540–1552. <https://doi.org/10.1094/mpmi-06-11-0162>
- Zhang, Y., Yu, X.X., Zhang, W.J., Lang, D.Y., Zhang, X.J., Cui, G.C. & Zhang, X.H. (2019). Interactions between endophytes and plants: Beneficial effect of endophytes to ameliorate biotic and abiotic stresses in plants. *Journal of Plant Biology*, 62, 1–13. <https://doi.org/10.1007/s12374-018-0274-5>
- Zhao, Z., Wang, Q., Wang, K., Brian, K., Liu, C.H. & Gu, Y.C. (2010). Study of the antifungal activity of *Bacillus vallismortis* ZZ185 *in vitro* and identification of its antifungal components. *Bioresource Technology*, 101(1), 292–297. <https://doi.org/10.1016/j.biortech.2009.07.071>