

Isolation and Characterization of *Paenibacillus peoriae* JC-3jx: An Endophytic Bacterium from *Dendrobium nobile* with Antibacterial and Growth-Promoting Potential

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Research Article

Keywords: antibacterial effect, *Dendrobium nobile*, endophytic bacteria, growth-promotion effect, *Paenibacillus peoriae*

Posted Date: May 18th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2940079/v1>

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Abstract

Dendrobium is an important source of natural components with high medicinal value. While numerous endophytic fungi associated with these plants have been extensively studied, research on endophytic bacteria remains limited. In this study, an improved tissue separation method was used to isolate endophytic bacteria from *Dendrobium nobile* and enrich the resources of high-quality biocontrol bacteria with both antibacterial and growth-promoting effects. We assessed the inhibitory effect of the isolated bacteria on pathogenic bacteria and fungi using agar diffusion and plate confrontation methods. We further investigated the growth-promoting effect of bacterial suspensions at various concentrations on corn seeds and *Dendrobium nobile* plantlets. The obtained endophytic bacterium, JC-3jx, isolated for the first time from the stem of *Dendrobium nobile*, exhibited significant inhibitory activity against the tested indicator bacteria. Morphologic and phylogenetic analysis confirmed the closest similarity of JC-3jx to *Paenibacillus peoriae*, leading to its final identification as *Paenibacillus peoriae* JC-3jx. This bacteria exhibited varying degrees of inhibition against the six pathogenic plant fungi tested, achieving a significant 56% inhibition rate against *Pyricularia oryzae*. Furthermore, the sterile fermentation supernatant of JC-3jx demonstrated pronounced inhibitory activity against the three tested pathogenic bacteria, particularly the gram-negative *Escherichia coli*. Moreover, the results demonstrate that the bacterial suspension of JC-3jx significantly promoted the rooting of corn seeds and the growth of *Dendrobium nobile* plantlets, indicating its excellent antibacterial and growth-promoting potential.

Introduction

The term “endophytes” refers to a group of microorganisms, including fungi, bacteria, and actinobacteria, that reside within host plant tissues during certain stages of their life cycle without significantly altering the phenotypic characteristics and functions of the plant tissues (Pang et al. 2021; Williams and de Vries 2020). Endophytes establish mutually beneficial relationships with their hosts, promoting host growth and development while also producing similar or identical secondary metabolites as the host (Berrios 2022; de Medeiros Azevedo et al. 2021). Recent research on endophyte metabolites has led to the discovery of numerous structurally novel compounds from traditional medicinal plants, such as *Dendrobium*, *Millettia pachycarpa* Benth, and *Pairs polyphylla*. These newly identified compounds have various pharmacological activities, including antifungal, antibacterial, and antitumor activities, and have emerged as promising drug candidates for treating emerging diseases in humans, plants, and animals (Ngashangva et al. 2021; Sarsaiya et al. 2020; Liu et al. 2021; Zhang et al. 2019). The exploration of medicinal plant endophyte resources and the investigation of secondary metabolites have become focal points for researchers aiming to discover new natural bioactive compounds, thereby facilitating the identification of novel active ingredients with medicinal value.

In traditional Chinese medicine, the fresh and dried stems of the *Dendrobium* species (*D. officinale*, *D. huoshanense*, and *D. nobile*) are collected for their dendrobine components and polysaccharides, which possess significant medicinal value (Su et al.2020; Zhang et al.2021; Li et al.2020). Many of the described endophytes associated with *Dendrobium* have unique biological activities. New polypeptide

compounds with demonstrated antibacterial effects were isolated from an endophytic bacterium, *D. officinale* (Zhao et al.2021). Transcriptome analysis revealed that the mycorrhizal fungus MF23 potentially enhances dendrobine synthesis by regulating the expression of genes involved in the mevalonate pathway (Li et al.2017). Moreover, the mycorrhizal fungus MF23 has shown the ability to promote both the growth of *D. nobile* and the accumulation of active ingredients in laboratory and natural environments (Chen et al.2021). Endophytes from diverse sources have been found to stimulate the germination and growth of *D. nobile* Lindl. seeds (Tsavkelova et al.2016). Eleven novel polyketides were isolated from the fermentation products of the endophytic fungus *Diaporthe* sp. JC-J7 obtained from *D. nobile* (Hu et al.2018). Additionally, a total of 165 endophytic bacteria were isolated from sterilized *Dendrobium* stems, out of which 14 exhibited antibacterial activity against plant pathogens (Wang et al.2019). The long-term co-evolution between endophytes and their hosts plays a pivotal role in the growth and reproduction of *D. nobile*, and recent focus has been directed toward specific metabolites as researchers endeavor to explore novel natural bioactive products.

As an important member of the *Dendrobium* genus, *D. nobile* has been listed in the Chinese Pharmacopoeia, and its endophyte species are providing more strain resources to study the interaction between endophytes and plants and the function of endophyte metabolites. At present, the mining of endophyte resources from *D. nobile* is primarily focused on endophytic fungi, while research on endophytic bacteria is scarce (Chen et al.2021; Hu et al.2018; Li et al.2017; Tsavkelova et al.2016; Wang et al.2019; Yang et al.2018; Yi et al.2022).

In this study, we aimed to isolate and culture endophytic bacteria from various tissues of *D. nobile*. Subsequently, we screened the strains exhibiting the most effective antibacterial performance using the confrontation culture method. Our investigation focused on studying the antibacterial and growth-promoting effects of these selected strains. The outcomes of this study provide a theoretical foundation for understanding the specificity and diverse applications of endophytic bacteria in *D. nobile*. Furthermore, these findings contribute to the comprehensive development and utilization of *D. nobile* resources.

Materials and Methods

Materials

Strains and plants

Test pathogenic fungi were used, including *Pestalotiopsis* sp., *Phytophthora sojae*, *Phytophthora capsici* LT1534, *Fusarium oxysporum* f. sp. *eleocharidis*, *Alternaria solani*, and *Pyricularia oryzae*, provided by the Institute of Plant Protection, Fujian Academy of Agricultural Sciences.

Test pathogenic bacteria were used, namely *Escherichia coli* CICC 10003, *Staphylococcus aureus* ATCC25923, and *Clostridium perfringens* ATCC13124, preserved and provided by the Engineering

Research Center of Industrial Microbiology, Ministry of Education, Fujian Normal University.

Endophyte isolation materials were utilized in this study, including healthy *D. nobile* collected from Quanzhou Bosenlong Farm *Dendrobium* planting base, Fujian, China.

Main media

Nutrient Agar (NA): 15 g sucrose, 5 g peptone, 3 g beef paste, 1 g yeast extract powder, and 20 g agar powder in 1 L distilled water with a pH of 7.0–7.2.

Nutrient Broth (NB): 15 g sucrose, 5 g peptone, 3 g beef paste, and 1 g yeast extract powder in 1 L distilled water with a pH of 7.0–7.2.

Potato Dextrose Agar (PDA): 200 g potato, 20 g glucose, and 20 g agar powder in 1 L distilled water at natural pH.

Luria-Bertani (LB): 10 g tryptone, 5 g yeast extract powder, and 10 g NaCl in 1 L distilled water at a pH of 7.0–7.2.

Fermentation medium: 20 g corn starch, 15 g sucrose, 15 g yeast paste, 4.5 g $(\text{NH}_4)_2\text{SO}_4$, 2.0 g KH_2PO_4 , 0.5 g MgSO_4 , and 3 g CaCO_3 in 1 L distilled water at a pH of 7.0–7.2.

Primers

Specific primers for PCR amplification of the bacterial 16S rDNA sequence were designed and purchased from Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China:

27F: 5'-AGAGTTTGATCCTGGCTCAG-3'

1492R: 5'-GGTTACCTTGTTACGACTT-3'

Methods

Isolation and Screening of Endophytic Bacteria from *D.nobile*

A modified tissue separation method was used to isolate endophytes from surface-sterilized *D.nobile* tissues (Herrera et al.2020):

(1) The surface of *D.nobile* plants was rinsed with tap water for 2 hours and blot dried with filter paper; 2 g each of roots, stems, and leaves was soaked in 75% ethanol (3 minutes for roots, 2 minutes for stems, and 1 minute for leaves) and 0.1% HgCl_2 solution, then washed three times with sterilized physiological saline solution.

(2) The washed dendrobium tissue was inoculated on NA plates and incubated in a 30°C incubator. The roots were cut into approximately 1 cm sections; both ends were discarded, and 2–3 pieces from the middle section were placed in a dish. The stems were cut into approximately 0.5 cm sections; both ends were discarded, and 3–4 pieces from the middle section were evenly placed in a dish. The leaves were cut into small slices with a side length of approximately 0.5 cm; 3–4 pieces were evenly placed in a dish. Sterilized saline from the last washed dendrobium tissue was applied evenly on the NA plate as a control for surface disinfection effects.

(3) After bacteria growth, further purification was performed by streaking the plate consecutively 3–5 times.

(4) Antibacterial confrontation screening: Using *Pestalotiopsis sp.* as an indicator, single colonies separated by streaking were planted on the confrontation plate and cultured at 30°C for 3–5 days; subsequently, the strains with obvious antibacterial effect were selected for preservation.

Morphological features

According to the results of the initial screening, the strain with the best activity against the indicator bacteria was selected, streaked on a PDA plate, and cultured at 37°C for 24 hours. Colony morphology was observed, and Gram staining was performed.

Molecular identification of JC-3jx

The isolated strain was inoculated into a test tube containing 3 mL of LB medium and cultured overnight at 30°C with shaking at 200 r/min. After the bacterial solution was centrifuged at $10,000 \times g$ for 5 minutes, the supernatant was removed, and bacterial genomic DNA was extracted according to the kit instructions (Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China). Using genomic DNA as a template and 27F and 1492R as primers, the 16S rDNA gene was amplified, the target fragment was detected and recovered using agarose gel electrophoresis, and the recovered product was sent to Sangon Biotech (Shanghai) for sequencing. The obtained sequences were analyzed using BLAST (NCBI database), and a phylogenetic tree was constructed. For the phylogenetic analysis, the 13 homologous sequences with the highest similarity, two *Bacillus* sequences, and *E.coli* sequences with lower similarity were selected. The neighbor-joining method was used to obtain the branch phylogenetic tree, and confidence levels were tested by bootstrap analysis. The bootstrap data set was used 1,000 times to construct the phylogenetic tree.

Antibacterial activity of JC-3jx

The antibacterial activity of JC-3jx was detected using plate confrontation and agar diffusion methods. For the plate confrontation method, the test fungus was inoculated on the sterile PDA plate, and after covering the entire petri dish, a circular fungus cake with a size of 4 mm was cut off at the edge of the colony with a sterile 4 mm diameter puncher, placed on a new PDA plate, and stored at 4°C until use. For the control group, a line was created using a sterile inoculation ring in the center of a new PDA plate, and a point was recorded 15 mm from the line. The pathogenic fungus cake was inoculated at this point. For

the test group, a line was created with the selected endophytic bacteria JC-3jx in the center of a new PDA plate, and a point was recorded 15 mm from the line. The pathogenic fungus cake was inoculated at this point. The plates were incubated at 30°C for 5–7 days, and the growth of pathogenic fungi in the control group and the experimental group was observed. The diameter of the bacterial block and fungus was measured, and the inhibition rate was calculated:

Inhibition rate = (radius of fungi in control group - radius of fungi in experimental group) / (radius of fungi in control group - 4.0 mm) × 100%

For the agar diffusion method, a single colony was picked from the strain JC-3jx plate, streaked into slant medium, and incubated at 30°C for 24 hours. We added 5 mL sterile water to the slant, scraped the bacteria off, mixed thoroughly and placed 0.6 mL into a shaker flask containing 30 mL of fermentation medium. The flask was incubated at 30°C, 220 r/min, for 12, 24, 36, and 48 hours, and antibacterial activity was detected.

To prepare the test plate, the slant medium was heated and then cooled to 45–50 °C, and an appropriate amount of *S. aureus* (G⁺), *E. coli* (G⁻), or *C. perfringens* (G⁺) was added. The liquid culture was well shaken and poured onto a flat plate. After cooling and solidification, holes were punched with a 6 mm hole-punch.

The fermentation broth was centrifuged at 10,000 × *g* for 5 minutes with different incubation times. The supernatant was collected and sterilized through a 0.22 µm filter, and then 50 µl sterile supernatant was placed into agar diffusion wells. The plate was placed at 4°C for approximately 2 hours, until the sample was fully infiltrated. After culturing at 30°C for 16–24 hours, the diameter of the transparent circle was observed and measured as follows:

Inhibition circle diameter (mm) = transparent circle diameter - 6.0 mm

Growth-promoting effect of strains

For the corn seed germination test, corn seeds were soaked in a 75% (volume fraction) ethanol solution for 2 minutes, washed twice with sterile water, and dried under sterile conditions. Fifty sterilized cornseeds were transferred to a square dish containing sterile filter paper, and each dish received 5 mL of bacterial suspension with concentrations of 10⁴, 10⁶, and 10⁸ CFU/ml, respectively; sterile water was set as a blank control (CK). The seeds were cultivated in a 25 °C biochemical incubator for seven days, watered every day (same amount of water per dish), and the rooting and germination of the seeds under different bacterial concentrations were observed after seven days.

A full pod of *D.nobile* species was selected, disinfected, and inoculated. Each bottle was connected to 1 ml of diluted bacterial suspension with concentrations of 10⁴, 10⁶, 10⁸, and 10¹⁰ CFU/ml, respectively; sterile water was set as a blank control (CK), and all bottles were incubated at 25°C with a light intensity of 1800 lux for 12 h/d. After seed germination, on days 30 and 90, the difference between the experimental group and the control group was observed to determine the effect of the strain.

Results

Isolation and screening of endophytic bacteria from *D. nobile*

Using the surface disinfection method, a total of ten endophytic bacteria were isolated by streaking and purifying multiple times (Fig. 1). Using *Pestalotiopsis sp.* as the indicator bacteria, the single colonies isolated by streaking were planted on the opposing plates and cultured at 30°C for 5–7 days. Among them, the strain numbered JC-3jx formed an obvious confrontational arc towards the indicator bacteria (Fig. 2). After slanted pure culture, a 25% bacterial glycerol stock was prepared and stored at – 70°C for later use.

Morphological characteristics of the JC-3jx strain

After being cultured on a PDA medium plate for 24 hours, the JC-3jx colonies were white to light yellow, opaque, round, smooth-surfaced, with no obvious folds, slightly raised, and neat-edged. The bacteria were straight rod-shaped, and the Gram stain was purple-black. These gram-positive bacilli (G⁺) grew individually or in pairs. A small number of spores could be seen in the vision field, and the sporangia were enlarged and oval (Fig. 3).

Molecular identification

The JC-3jx 16S rDNA gene fragment was amplified, and electrophoresis showed a band of the expected target size (Fig. 4). The target gene fragment was extracted and sequenced. The results of the phylogenetic analysis (Fig. 5) showed that the JC-3jx strain had the closest genetic distance to *Paenibacillus* and clustered into one branch. Among them, *P. peoriae* had the highest homology (99.67% similarity). Based on its morphological characteristics and the phylogenetic analysis, the JC-3jx strain was identified as *P. peoriae*. The JC-3jx strain was deposited in the Guangdong Provincial Microbial Culture Collection Center (www.gdmcc.net) with deposit number GDMCC62384, and its corresponding 16S rDNA sequence was submitted to the NCBI database (Genbank accession number OM914713).

Antibacterial activity of the JC-3jx strain

Inhibitory effect of the JC-3jx strain on pathogenic plant fungi

Figure 6 shows that strain JC-3jx had an important inhibitory effect on the six pathogenic fungi tested, among which it had the most obvious inhibitory effect on *Pyricularia oryzae* (56% inhibition rate), while the inhibitory effect on *Alternaria solani* was the weakest, with an inhibition rate of only 31% (Table 1).

Table 1
Inhibitory activity of the JC-3jx strain against different pathogenic fungi.

Pathogenic fungi	Inhibition rate	Antagonistic bandwidth (mm)
<i>Pestalotiopsis sp.</i>	46.6 ± 0.4%	8.0 ± 0.1
<i>Fusarium oxysporum f. sp. eleocharidis</i>	46.2 ± 0.3%	8.0 ± 0.2
<i>Phytophthora capsici</i>	46.3 ± 0.4%	8.0 ± 0.3
<i>Phytophthora sojae</i>	50.2 ± 0.5%	8.0 ± 0.3
<i>Alternaria solani</i>	31.4 ± 0.8%	6.0 ± 0.1
<i>Pyricularia oryzae</i>	56.1 ± 0.6%	6.0 ± 0.4

Inhibitory effect of the JC-3jx strain on pathogenic bacteria

The sterile supernatant of JC-3jx from different culture times showed a relatively clear inhibition zone among the three pathogenic bacteria tested, suggesting reasonably broad-spectrum antibacterial activity. Among them, the inhibitory effect on gram-negative pathogen *E.coli* was significantly better than that on the gram-positive pathogens. Among the fermentation broths from different incubation times, the supernatant from 24 hours had the strongest antibacterial performance, and the diameter of the inhibition zone against *E. coli* reached 22 mm (Fig. 7).

Study on the growth-promoting effect of the JC-3jx strain

Corn seed germination test

Compared with the control group, the corn seeds inoculated with various concentrations of bacterial suspension had a better rooting effect and more root branches. Among them, the inoculated suspension concentrations of 1.6×10^6 and 1.6×10^4 CFU/mL were significantly higher, and the inoculated suspension concentration of 1.6×10^8 CFU/mL was relatively low. The most obvious comparison was between the control group and the test group with an inoculated suspension concentration of 1.6×10^4 CFU/mL (Fig. 8). Figure 8 shows that the inoculation of various concentrations of bacterial suspensions offered a certain growth-promoting effect on corn, but the bacterial concentration was not positively correlated with rooting and budding, as the lower bacterial concentration showed a better growth-promoting effect.

Growth promotion test of *D.nobile*

After 90 days of observation, the test group with a concentration of 1.6×10^{10} CFU/mL suspension stopped growing on day 30 (Fig. 9), and the test group with 1.6×10^8 CFU/mL suspension grew significantly slower in the later period, possibly due to the competitive consumption of nutrients from the culture medium. The plant growth rate of the test group with added concentrations of 1.6×10^6 and

1.6×10^4 CFU/mL bacterial suspension was significantly better than that of the control group (Fig. 10). The bacterial concentration was not positively correlated with the growth of *D.nobile* tissue seedlings, but the lower bacterial concentration showed a better growth-promoting effect.

Discussion

D.nobile, a valuable medicinal plant, contains a variety of biologically active substances, such as alkaloids, bibenzyls, phenanthrenes, phenylpropanoids, and polysaccharides (Lei et al.2022; Wang, 2021; Zhang et al.2021). Dendrobine, an important medicinal component extracted from *Dendrobium*, exhibits various pharmacological activities, including anti-tumor, anti-inflammatory, anti-fatigue, hypoglycemic, and hypolipidemic properties, as well as nervous system protection effects (Zhang.et al. 2022; Mou et al.2021). Studies have demonstrated that *Dendrobium* plants are inseparable from the role of endophytes during the growth process (Chen et al.2021; Hu et al.2018; Li et al.2017; Tsavkelova et al.2016; Wang et al.2019; Yang et al.2018; Yi et al.2022). Beneficial endophytes have been found in *D.nobile*, *D.officinale*, and *D.moniliforme*. However, there are few reports on endophytic bacteria associated with *D.nobile*.

Paenibacillus is a class of rod-shaped bacteria that can thrive aerobically or as facultative anaerobes. These bacteria are widely distributed in nature and have the ability to produce endospores. Many *Paenibacillus* strains have been identified for their beneficial effects on plant growth, including nitrogen fixation, phosphorus solubilization, iron acquisition, and the production of plant growth regulators. They can also induce systemic resistance in plants and generate insecticidal or bactericidal compounds such as antimicrobial peptides, bacteriocins, and lipopeptides. These properties have found applications in biomedicine, biocontrol, and bioremediation (Grady et al.2016; Langendries and Goormachtig 2021). In this study, we isolated an endophytic bacterium named JC-3jx from the *Paenibacillus* genus, which was found for the first time in the stem tissue of *D.nobile*. Through comprehensive morphological and phylogenetic analysis, we identified this bacterium as *P.peoriae*. Notably, JC-3jx exhibited significant antagonistic activity against plant pathogenic fungi and also displayed inhibitory effects against various common pathogenic bacteria. The observed inhibitory activity may be attributed to the synthesis and secretion of metabolites by JC-3jx. Further investigation is required to elucidate the composition, synthesis mechanisms, and antibacterial mechanisms of the active substances present in the sterile supernatant.

Pseudomonas fluorescens and *Klebsiella oxytoca* have demonstrated varying degrees of growth-promoting effects on the germination of *D.nobile* seeds (Pavlova et al.2017). The *P. peoriae* strain NRRL BD-62 possesses nitrogen-fixing capabilities and can produce proteases and chitinases, which are hypothesized to contribute to its growth-promoting effects (von der Weid et al.2003). Another strain, *P.peoriae* SP9, has been shown to stabilize the root microbial community and enhance wheat growth in polluted soils (Araujo et al.2020). In this study, we conducted rooting and sprouting experiments on corn seeds and mung beans using different concentrations of JC-3jx strain suspension. The findings revealed a pronounced growth-promoting effect of JC-3jx. Furthermore, the extent of this effect was found to be dependent on the concentration of the bacterial suspension, with the lowest concentration exhibiting the

most potent growth-promoting activity. Future research will delve into the mechanisms underlying the growth-promoting effects of this strain on *D.nobile* and explore its relationship with metabolite synthesis, such as dendrobine. These efforts aim to provide a more comprehensive theoretical basis for the multi-scale development of the JC-3jx strain.

Conclusion

In this study, we successfully isolated the endophytic bacterium JC-3jx from the stem tissue of *D. nobile* using tissue separation and confrontational culture techniques with indicator bacteria. Through comprehensive analysis, JC-3jx was identified as belonging to the species *P. peoriae*. This discovery represents the first isolation of *P. peoriae* from *D. nobile*.

Our investigation revealed that JC-3jx exhibited significant antagonistic activity against a range of pathogenic fungi, indicating its potential as a biocontrol agent. Moreover, the sterile fermentation supernatant of JC-3jx demonstrated inhibitory effects against various pathogenic bacteria, highlighting its broad-spectrum antibacterial properties. These findings suggest that JC-3jx possesses valuable antibacterial and antifungal properties that can contribute to the development of effective control strategies.

Furthermore, we evaluated the impact of bacterial suspensions at different concentrations on the rooting of corn seeds and the germination of mung beans. The results clearly demonstrated the substantial growth-promoting effects of JC-3jx, indicating its potential as a valuable resource for promoting plant growth. These observations highlight the potential of JC-3jx as a high-quality biocontrol and growth-promoting bacterial resource.

Overall, our study provides valuable insights into the isolation, identification, and characterization of the endophytic bacterium JC-3jx from *D. nobile*. The observed antibacterial and antifungal activities, as well as the significant growth-promoting effects, underscore the potential applications of JC-3jx in the field of biocontrol and plant growth promotion.

Declarations

Funding statement

This project was financially supported by Young and middle-aged project of Education Department of Fujian Province (No. JAT201224).

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

All authors contributed to the study conception and design. ^{1#}Li ZhiPing and Lin Yuan contributed equally to this work. Material preparation, data collection and analysis were performed by Li ZhiPing and Lin Yuan. The first draft of the manuscript was written by Li ZhiPing and Huang QinGeng . All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Acknowledgements

We thank Fujian Academy of Agricultural Sciences for providing the test pathogenic fungi.

We would like to thank Editage (www.editage.cn) for English language editing.

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Figures

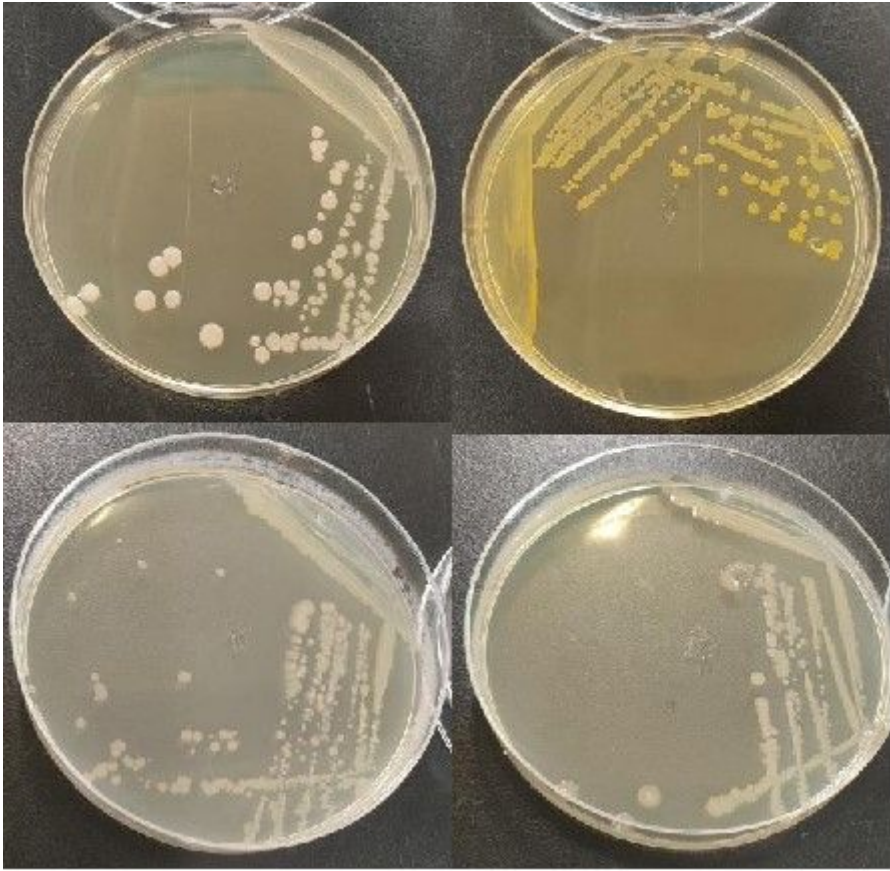


Figure 1

Part of the single-colony isolation from *D.nobile*



Figure 2

Part of the confrontation culture of different colonies and indicator bacteria from *D.nobile*

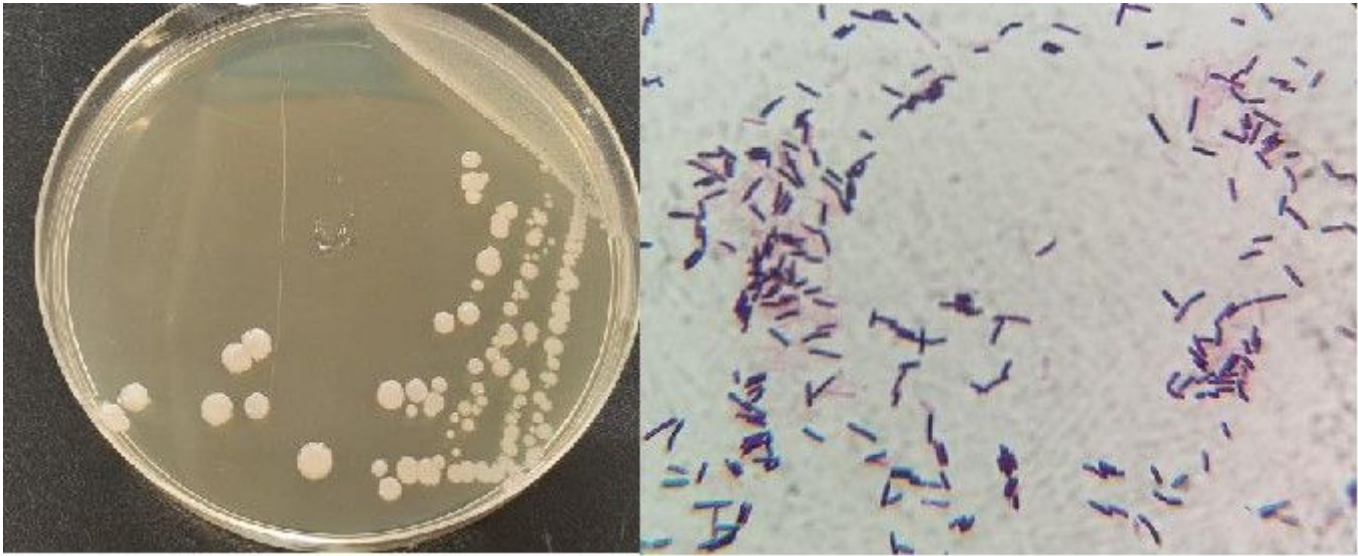


Figure 3

Colonies (left) and cell morphology (right) of strain JC-3jx (1000×)

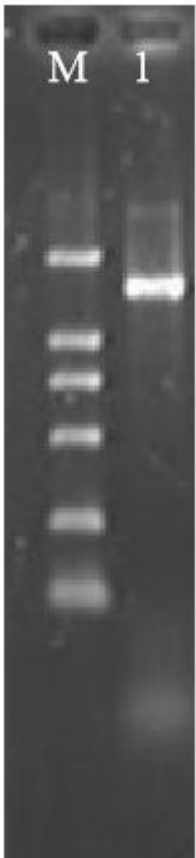


Figure 4

PCR amplification of the JC-3jx16S rDNA gene (M: DL 2000 bp DNA Marker; I: lane 1, 16S rDNA PCR-product)

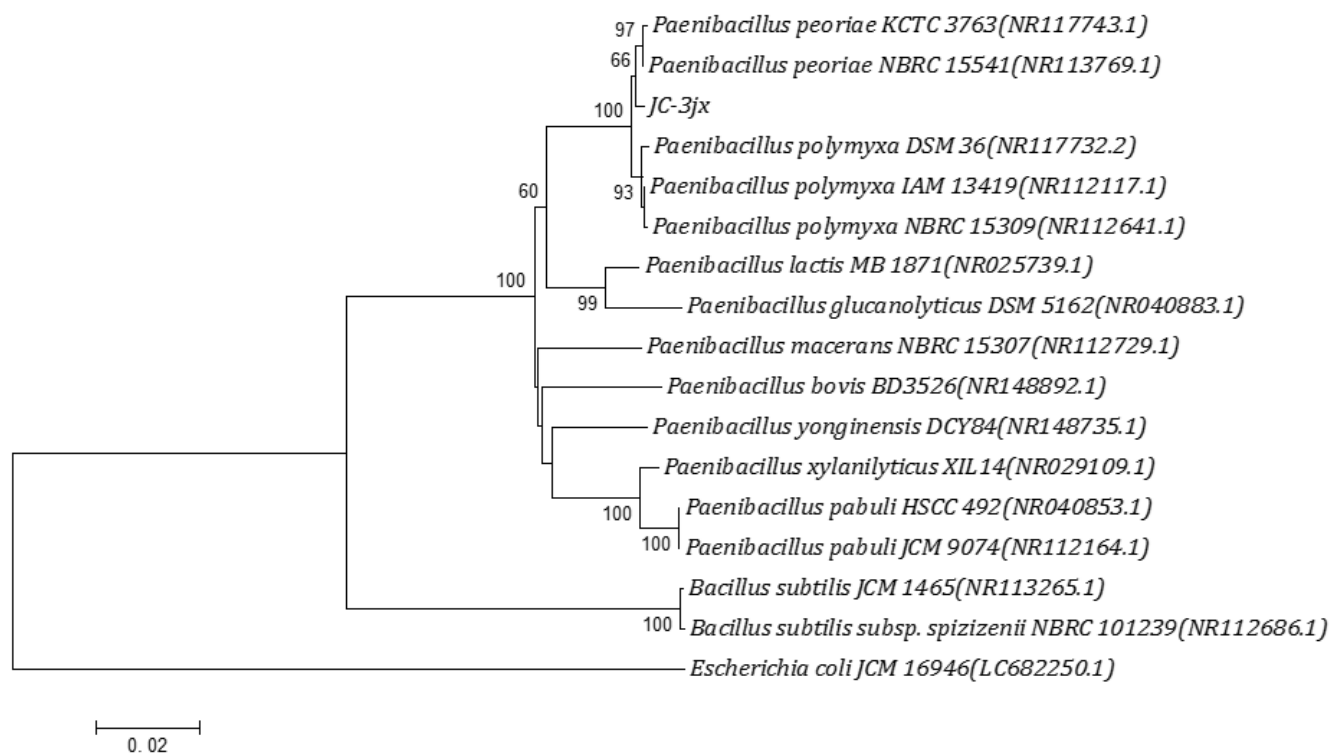


Figure 5

Phylogenetic tree of the JC-3jx strain based on its 16S rDNA gene sequence

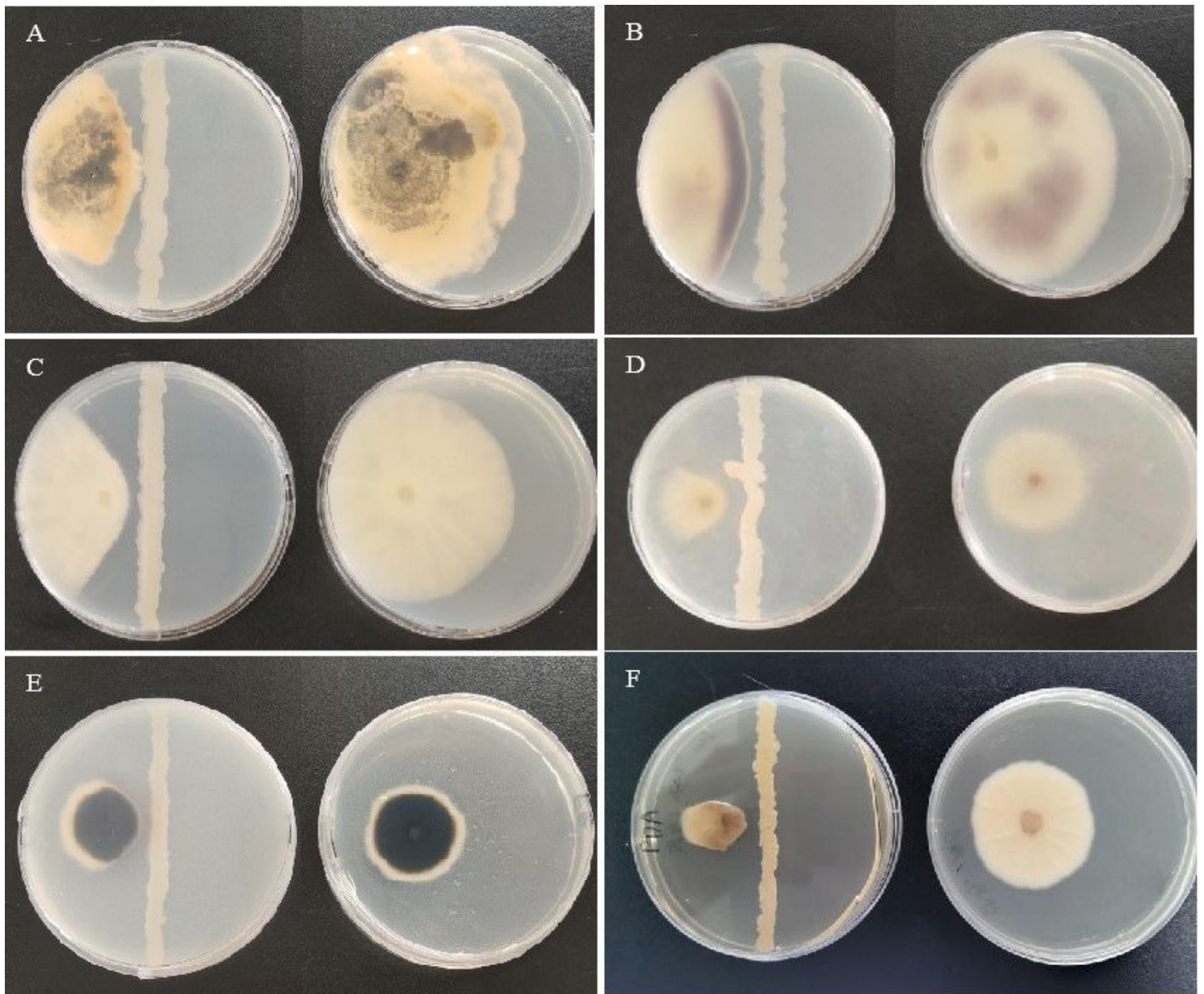


Figure 6

Inhibitory effect of strain JC-3jx against pathogenic fungi: **A)** *Pestalotiopsis* sp.; **B)** *Fusarium oxysporum* f. sp. *eleocharidis*; **C)** *Phytophthora capsici*; **D)** *Phytophthora sojae*; **E)** *Alternaria solani*; **F)** *Pyricularia oryzae*

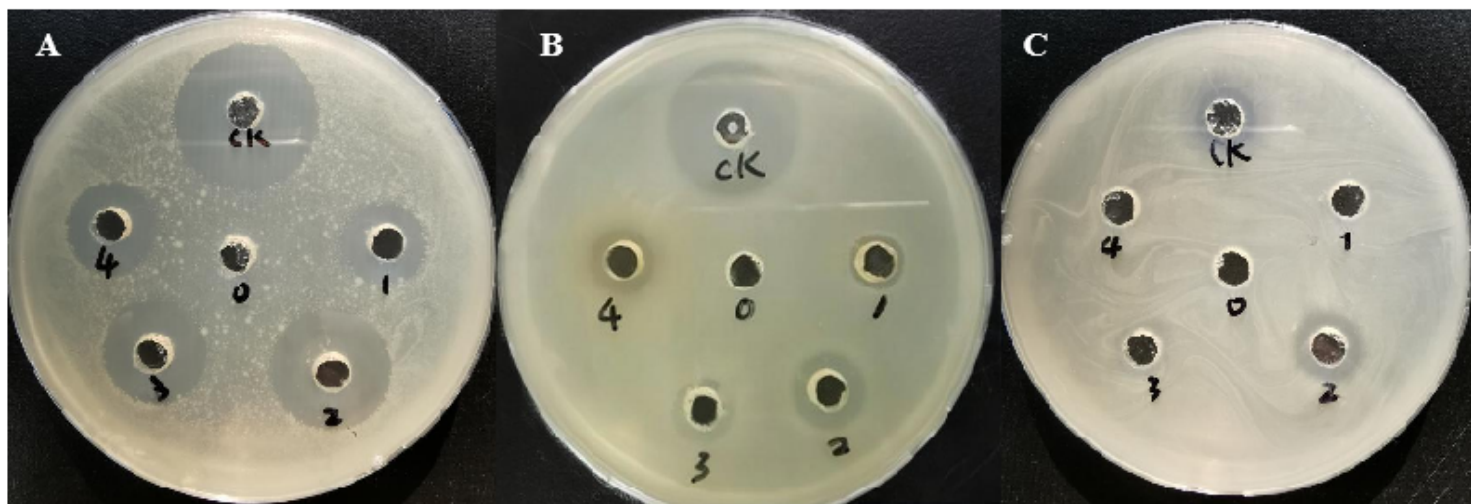


Figure 7

Inhibitory effect of strain JC-3jx against pathogenic bacteria. **A)** Inhibition zone of JC-3jx fermentation broth against *E. coli* CMCC44103 (CK = colistin sulfate, 2 g/L). **B)** Inhibition zone of JC-3jx fermentation broth against *C. perfringens* (CK = nisin, 2.5 g/L). **C)** Inhibition zone of JC-3jx fermentation broth to *S. aureus* ATCC25923 (CK = nisin, 2.5 g/L). 0: sterile water; 1, 2, 3, and 4: JC-3jx fermentation broths fermented for 12, 24, 36, and 48 hours, respectively



Figure 8

Growth-promoting effect of a JC-3jx strain suspension at various concentrations on corn seeds: **A)** Control group; **B)** 1.6×10^8 CFU/mL; **C)** 1.6×10^6 CFU/mL; **D)** 1.6×10^4 CFU/mL

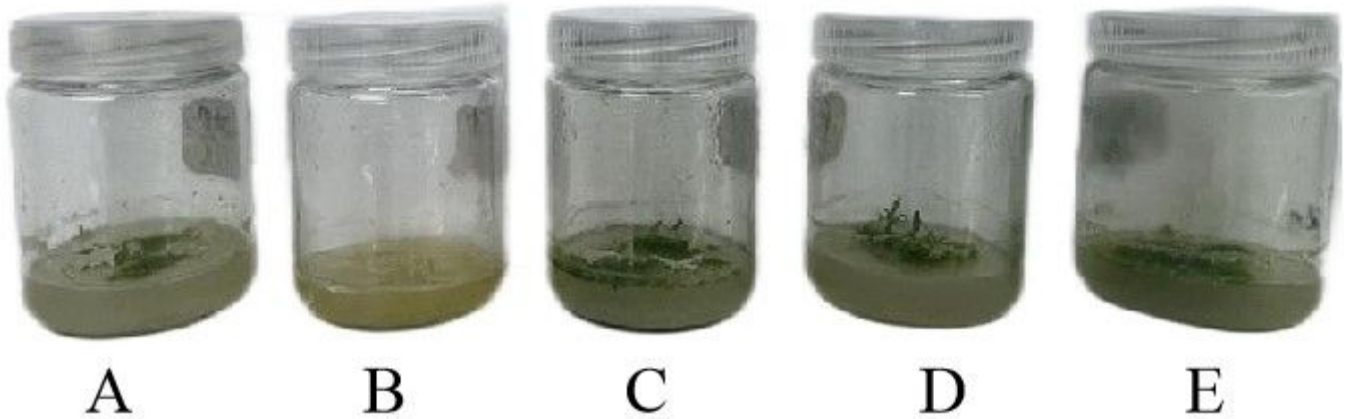


Figure 9

Growth-promoting effect of a JC-3jx strain suspension on *D.nobile* plantlets after 30 days: **A)** Control group; **B)** 1.6×10^{10} CFU/mL; **C)** 1.6×10^8 CFU/mL; **D)** 1.6×10^6 CFU/mL; **E)** 1.6×10^4 CFU/mL

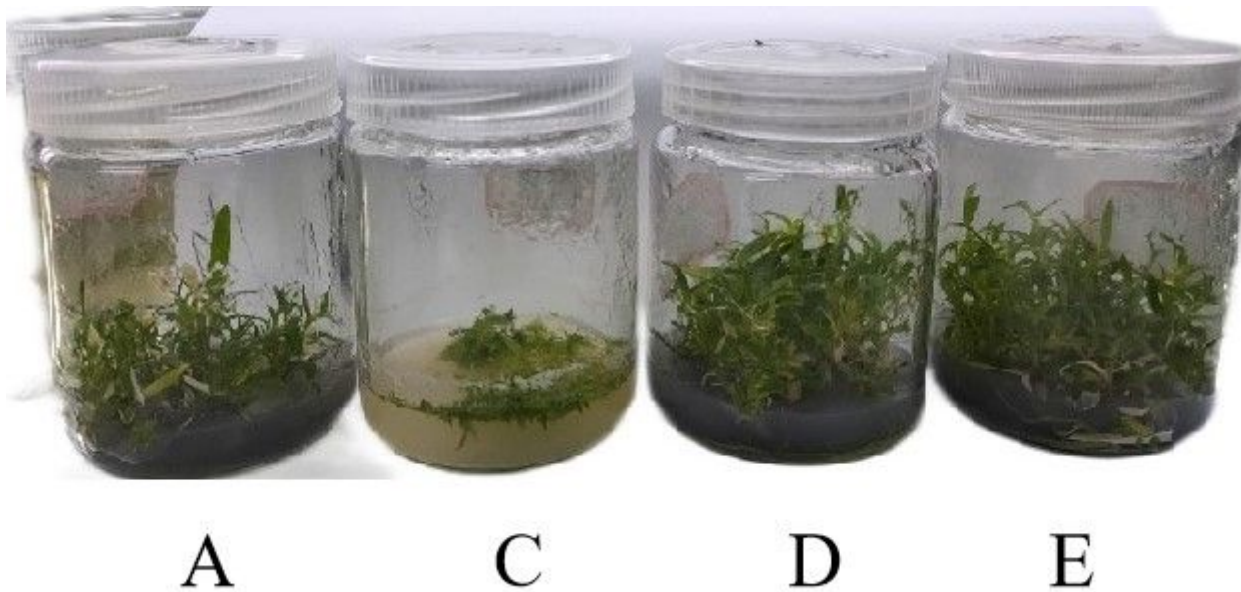


Figure 10

Growth-promoting effect of a JC-3jx strain suspension on *D.nobile* plantlets after 90 days: **A)** Control group; **C)** 1.6×10^8 CFU/mL; **D)** 1.6×10^6 CFU/mL; **E)** 1.6×10^4 CFU/mL