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Disease-reducing effects of *Streptomyces albaduncus* ATB 24 on shallot basal rot caused by *Fusarium oxysporum* under postharvest storage conditions

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ABSTRACT

Shallot basal rot caused by Fusarium oxysporum severely affects bulb quality and causes significant economic losses during postharvest storage. This study identified antagonistic soil bacteria isolated from shallot fields capable of reducing the disease under postharvest storage conditions of local farmers. The three strongest antagonistic bacterial isolates against the pathogen in dual cultures on petri plates, i.e., ATA 33, ATB 24, and ATB 32, were tested for their disease-reducing effects. Each isolate was tested using three different cell densities (10^7, 10^8, and 10^9 CFU/mL) in a completely randomized experimental design. The 10^8 CFU/mL suspension of ATB 24 significantly reduced disease incidence (53.33%) as compared to the untreated control (96.67%) after 35 days of storage. ATB 24 was identified as Streptomyces albaduncus using morphological characterization and 16S rRNA gene sequencing. Streptomyces albaduncus ATB 24 could be a biological control agent of shallot basal rot during postharvest storage in practice.

1. INTRODUCTION

Shallots (*Allium ascalonicum* L.), members of the genus *Allium,* are widely used as culinary spices and condiments. Their name is derived from "Ascalon," a town in ancient Syria, suggesting the crop's origin in Asia Minor (Cochran, 1953). In Southeast Asia, shallots are more popular and culturally significant than onions, serving not only to enhance flavor but also as a key ingredient in traditional dishes (Tashiro et al., 1983).

In Viet Nam, shallots (*Allium cepa* var. *aggregatum*) are concentrated in several well-defined production regions. Vinh Chau, formerly belonging to Soc Trang province and currently administered under the expanded Can Tho city following the 2025 administrative reorganization, remains the largest shallot-producing area in the Mekong Delta, where shallot cultivation is a key agricultural livelihood. Ly Son Island, Quang Ngai Province, is another major production center, characterized by its long-established shallot-based farming system under distinctive coastal agroecological conditions (Intellectual Property Office of Viet Nam, 2019). Vinh Chau is particularly renowned for its shallot production, supplying the Mekong Delta and other parts of the country. Shallots are considered specialty crops that contribute significantly to the local economy and agricultural structure. However, in recent years, shallot cultivation in Vinh Chau has faced challenges, including declining productivity, unstable yields, poor quality, and difficulties in postharvest storage. These problems are largely attributed to intensive farming practices, the

expansion of cultivated areas, and the overuse of chemical pesticides (Dang, 2008).

A critical constraint in shallot production is basal rot, primarily caused by the fungal pathogen *F. oxysporum*. This soil-borne pathogen is notoriously difficult to control because it can survive in the soil for extended periods and cause latent infections (Taylor et al., 2013). Latent infections imply that bulbs may appear healthy at harvest but harbor fungal mycelia that reactivate under postharvest storage conditions, leading to rapid decay (Wang et al., 2019; Diabankana et al., 2024). Conventional management relies heavily on synthetic fungicides; however, their efficacy against latent infections is limited. Furthermore, public concern regarding chemical residues and the development of fungicide resistance necessitates the search for safer, sustainable alternatives (Gullino et al., 2000).

Biological control using antagonistic microorganisms has emerged as a promising strategy. Among potential biological control agents, actinobacteria, particularly species of the genus *Streptomyces*, have garnered significant attention. *Streptomyces* species are prolific producers of bioactive secondary metabolites, including antibiotics and lytic enzymes such as chitinases and glucanases, which can degrade fungal cell walls (Palaniyandi et al., 2013; Barka et al., 2016). Moreover, as spore-forming bacteria, they are highly stable and long-lived, making them well-suited for formulation and application in postharvest storage environments where environmental resilience is crucial.

Despite this potential, research on the application of indigenous *Streptomyces* strains to control postharvest diseases in shallots in the Vinh Chau region remains limited. Therefore, this study aimed to evaluate the disease-reducing effect and to conduct taxonomic identification of indigenous antagonistic bacteria for controlling shallot basal rot caused by F. oxysporum under storage conditions, and to identify the potential biological control agent, thereby providing a scientific basis for developing biological products that ensure product quality and comply with Global GAP standards.

2. MATERIALS AND METHODS

2.1. Materials

Pathogen: The fungus *Fusarium oxysporum* was provided by the plant pathology research group at the Institute of Food and Biotechnology, Can Tho University.

Bacteria: Antagonistic bacteria were isolated from shallot-cultivated soils in Vinh Chau and stored at the molecular biology laboratory for antagonism assays (Nguyen et al., 2017).

Shallot bulbs: Planting materials were supplied by the Plant Protection Sub-Department of Soc Trang Province.

2.2. Evaluation of the disease-reducing effects of antagonistic bacteria on shallot basal rot under postharvest storage conditions

2.2.1. Experimental design

The experiment was conducted to evaluate the effects of antagonistic bacterial strains on reducing shallot basal rot caused by *F. oxysporum* under postharvest storage conditions. A completely randomized design (CRD) was applied with three replicates. The study included three bacterial strains (ATA 33, ATB 24, and ATB 32), each tested at cell densities of 10^7, 10^8, and 10^9 CFU/mL. Each treatment replicate consisted of 100 bulbs. For comparative purposes, two control groups were included in the design: a positive control treated with the commercial fungicide Score 250 EC and a negative control treated with sterile distilled water.

2.2.2. Pathogen inoculation

The pathogen was cultured on potato dextrose agar (PDA) to produce spores. A spore suspension was prepared in sterile distilled water and adjusted to 10^7 CFU/mL. Inoculation was performed by spraying 20 mL of the suspension evenly onto the surface of shallot bulbs to ensure uniform wetting.

2.2.3. Bacterial treatment

Antagonistic bacteria were cultured on nutrient agar (NA) for 24–48 h. Bacterial suspensions were prepared in sterile distilled water at concentrations of 10^9, 10^8, and 10^7 CFU/mL. The suspensions were sprayed onto shallot bulbs immediately after pathogen inoculation.

2.2.4. Disease assessment

The disease-reducing effect of antagonistic bacteria was evaluated based on the disease incidence relative to the total number of bulbs per treatment. The treated bulbs were stored at room temperature (approximately 30 ± 2°C) throughout the experiment. Observations were recorded at 7, 14, 21, 28, and 35 days after inoculation (DAI). To evaluate the cumulative disease severity over the entire storage period, the area under the disease progress curve (AUDPC) was calculated using the

trapezoidal integration method described by Shaner and Finney (1977):

$$\text{AUDPC}= \sum_{i=1}^{n-1}\left[\frac{Y_{i+1}+Y_i}{2}\right][X_{i+1}-X_i]$$

where Y_i is the disease incidence at the i^{th} observation, X_i is the time (days) at the i^{th} observation, and n is the total number of observations.

2.3. Identification of antagonistic bacteria

Selected antagonistic bacterial strains that inhibited *F. oxysporum* in storage conditions were identified using 16S rRNA gene sequencing and morphological characterization according to Bergey's classification system.

For molecular identification, DNA was extracted by transferring ten pure colonies into 2 mL tubes containing sterile steel beads and shaking vigorously. One milliliter of lysis buffer was added, mixed, and incubated at room temperature for 10 min. The samples were centrifuged at 13,000 rpm for 5 min, and the supernatant was transferred to a new tube. An equal volume of 95% ethanol was added, the mixture was centrifuged at 13,000 rpm for 5 min, and the supernatant was discarded. The pellet was washed with 500 µL of 70% ethanol, centrifuged, vacuum-dried at 45°C for 10 min, and resuspended in 100 µL TE buffer (0.1×). DNA was stored at –20°C.

The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCCTGGCTC-3') and 1492R (5'-TACGGTTACCTTGTTACGACT-3') (Weisburg et al., 1991). PCR reactions (25 µL) contained 2.5 µL 10× buffer, 4 µL dNTPs, 3 µL $MgCl_2$, 10 pmol of each primer, 0.25 µL Taq polymerase, and sterile distilled water (Tran, 2010). The thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 2 min; final extension at 72°C for 10 min; and hold at 4°C. PCR products were visualized by agarose gel electrophoresis and sequenced using an automated sequencer. Sequence homology was analyzed against the GenBank database using BLASTn. Phylogenetic and molecular evolutionary analyses were conducted using MEGA X software (Kumar et al., 2018). A phylogenetic tree was constructed using the neighbor-joining method. The robustness of the tree topology was evaluated using bootstrap analysis with 1,000 replicates. Evolutionary distances were computed using the Kimura 2-parameter method

Morphological characteristics of antagonistic bacterial isolates capable of inhibiting shallot basal rot were examined microscopically and by observing colony growth on Petri dish. Isolates were cultured on ISP-3 medium for 7 days, and morphological traits such as aerial mycelium color and spore mass were recorded according to Bergey's classification key (Whitman, 2012). Observations were performed following the methods of Tresner and Buckus (1963), including spore chain morphology under light microscopy and spore surface structure under scanning electron microscopy.

For micro-morphological observations, the humid-chamber method of Krug (2004) was used. Sterile filter paper moistened with distilled water was placed in a Petri dish, overlaid with a sterile glass slide, and topped with a 5 × 5 mm agar block (ISP-3 medium). Actinomycetes were inoculated onto the four vertical edges of the agar block, covered with a coverslip, and incubated at room temperature. Microscopic observations were performed after 2–3 days. Spore surface morphology was further examined using scanning electron microscopy at the Institute of Food and Biotechnology, Can Tho University.

2.4. Data analysis

Data on disease reduction by antagonistic bacterial strains under storage conditions were analyzed using SPSS version 16 at a significance level of 5%. The experiments were arranged in a completely randomized design with three replicates. Mean comparisons were performed using Duncan's multiple range test, and non-significant differences were indicated by identical letters.

3. RESULTS AND DISCUSSION

3.1. Effects of antagonistic bacterial strains in reducing disease incidence under storage conditions

The data presented in Table 1 indicate that the disease incidence (%) of shallot bulbs caused by *F. oxysporum* progressively increased over the storage period across all treatments, including both the antagonistic bacterial applications and the control group. This trend reflects the biological characteristics of *F. oxysporum*, a latent pathogen that thrives under post-harvest storage conditions (Wesoly et al., 2024). Initial infection typically

occurs in the field without manifesting overt symptoms, subsequently spreading and causing the most significant economic losses during storage (Khalifa et al., 2016).

At **7** DAI, the majority of treatments involving antagonistic bacteria exhibited very low disease incidence (0.00–16.67%), which was notably lower than that of the negative control (11.67%). In particular, treatments ATB 24 and ATB 32 at a concentration of 10^9 CFU/mL showed no diseased bulbs (0.00%), demonstrating a potent inhibitory effect during the early stages of storage.

By 14, 21, 28, and 35 DAI, although disease incidence increased across all treatments, the antagonistic bacterial treatments consistently maintained significantly lower disease incidence than the negative control.

Table 1. Disease incidence (%) and area under the disease progress curve (AUDPC) of shallot bulbs treated with antagonistic bacterial suspensions at concentrations of 10^9, 10^8, and 10^7 CFU/mL at 7, 14, 21, 28, and 35 DAI

Bacterial strains	Concentration (CFU/mL)	Disease incidence (%)					AUDPC
		7 DAI	14 DAI	21 DAI	28 DAI	35 DAI	
ATA 33	10^9	10.00 ^{ab}	13.33 $^{a-d}$	35.00 ^{bcd}	56.67 ^{bc}	86.67 ^{bc}	705.83 ^{c}
	10^8	15.00 ^{a}	16.67 ^{ab}	41.67 ^{ab}	63.33 ^{ab}	86.67 ^{bc}	781.67 ^{b}
	10^7	15.00 ^{a}	20.00 ^{a}	43.33 ^{ab}	65.00 ^{a}	91.67 ^{ab}	822.50 ^{ab}
ATB 24	10^9	0.00 ^{c}	6.67 ^{de}	18.33 ^{e}	25.00 ^{f}	51.67 ^{f}	355.83 ^{f}
	10^8	3.33 ^{bc}	8.33 ^{cde}	21.67 ^{e}	30.00 ^{ef}	53.33 ^{f}	408.33 ^{f}
	10^7	16.67 ^{a}	13.33 $^{a-d}$	31.67 ^{d}	46.67 ^{d}	63.33 ^{e}	600.83 ^{d}
ATB 32	10^9	0.00 ^{c}	10.00 $^{b-e}$	26.67 ^{de}	36.67 ^{e}	76.67 ^{d}	525.00 ^{e}
	10^8	10.00 ^{ab}	15.00 ^{abc}	33.33 ^{cd}	53.33 ^{cd}	78.33 ^{cd}	665.00^{c}
	10^7	13.33 ^{a}	18.00 ^{a}	45.00 ^{a}	61.67 ^{ab}	83.33 ^{bcd}	775.83^{b}
Positive control		0.00 ^{c}	3.33 ^{e}	6.67 ^{f}	13.33 ^{g}	30.00 ^{g}	186.67 ^{g}
Negative control		11.67 ^{a}	20.00 ^{a}	46.67 ^{a}	68.33 ^{a}	96.67 ^{a}	851.67^{a}
F-ratio		7.78	6.42	18.96	51.89	53.82	98.78
%CV		47.27	28.78	15.71	9.39	6.68	6.18

Values within each column followed by a common letter do not differ significantly at P = 0.05 as determined by Duncan's post-hoc test.

Among the three bacterial strains, ATB 24 demonstrated the highest effect in disease control, followed by ATB 32, while ATA 33 exhibited lower inhibitory effects. At 35 DAI, the disease incidence in the ATB 24 treatments ranged from 51.67% to 63.33%, depending on the applied concentration, which was significantly lower than that of ATA 33 (86.67–91.67%) and the negative control (96.67%). To evaluate this suppressive effect over time, the Area Under the Disease Progress Curve (AUDPC) was analyzed. The AUDPC values were consistent with disease incidence trends, confirming that the antagonistic bacteria slowed the overall progression of the epidemic. Strain ATB 24 at 10^9 CFU/mL exhibited the lowest AUDPC (355.83), reducing the cumulative disease burden compared to ATA 33 (up to 822.50) and the negative control (851.67). This indicates that ATB 24 not only reduced the final infection rate but also delayed and slowed the pathogen's spread throughout the storage period.

Strain ATB 32 showed intermediate effect, with disease incidence at 35 DAI ranging from 76.67% to 83.33%. Although this was significantly lower than the negative control, it remained higher than the incidence recorded for ATB 24. This variation suggests that the antagonistic effect is highly dependent on the intrinsic biological characteristics of each strain, including its competitive ability, antifungal compound biosynthesis, and adaptability to storage conditions.

Within the same bacterial strain, treatments with a higher concentration (10^9 CFU/mL) consistently resulted in a lower disease incidence than 10^8 and 10^7 CFU/mL across all observation points. For instance, at 35 DAI, ATB 24 at 10^9 CFU/mL recorded only 51.67% disease incidence, whereas at 10^7 CFU/mL, it reached 63.33%. These results indicate that initial bacterial density plays a crucial role in establishing antagonistic populations on the bulb surface, thereby enhancing biological competition and inhibiting fungal invasion. When

the bacterial concentration is low, the disease-reducing effect declines markedly during storage.

The positive control maintained very low disease incidence in the early stages (0.00–6.67%) and increased to only 30.00% at 35 DAI, representing the highest level of control. However, the antagonistic bacterial treatments, particularly ATB 24 at 10^9 CFU/mL and 10^8 CFU/mL, still provided significant protection compared to the negative control, demonstrating potential as an alternative or supplement to chemical measures. The negative control recorded the highest disease incidence throughout the study, reaching 96.67% at 35 DAI, confirming the high pathogen pressure of *F. oxysporum* during storage when left untreated.

Strain ATB 24 showed a high effect at both 10^9 CFU/mL and 10^8 CFU/mL, with no statistically significant difference between these two concentrations. This conclusion is supported by the AUDPC analysis, where the values for ATB 24 at 10^9 CFU/mL (355.83) and 10^8 CFU/mL (408.33) showed no significant difference. Therefore, to achieve the best economic efficiency, ATB 24 (10^8 CFU/mL) treatment is recommended.

According to Janisiewicz and Korsten (2002), the effectiveness of antagonistic microorganisms in post-harvest disease control depends on their ability to establish an initial population and maintain a sufficiently high density on the host surface. This is consistent with the current findings, in which treatments at 10^9 CFU/mL consistently yielded lower disease incidence than lower concentrations across all monitoring intervals.

The distinct variation in effect among strains also aligns with the observations of Compant et al. (2005), who noted that biocontrol success depends not only on density but also on unique biological traits, including nutrient competition, the biosynthesis of antifungal metabolites, and adaptation to adverse environments. Competition for nutrients and space is a fundamental mechanism by which bacteria protect crops, particularly by producing siderophores to sequester iron. These results are further supported by Raaijmakers et al. (2002), who reported that different bacterial strains exhibit varying levels of *Fusarium* inhibition due to the diversity and potency of their antibiotic activities.

An upward trend in disease incidence over time, even with antagonistic treatments, has been documented in previous literature. Ahmed et al. (2021), in their study on onion basal rot caused by *F. oxysporum*, suggested that the effect of antagonistic microbes often wanes during prolonged storage. This phenomenon aligns with the results of this study, in which disease incidence rose significantly from 21 DAI onward but remained substantially lower than in the negative control.

Overall, the results of this study are consistent with previous reports on the biological control of *F. oxysporum* under post-harvest conditions. The observed differences between strains and concentrations are biologically plausible and strengthen the scientific basis for utilizing antagonistic bacteria in managing shallot basal rot during storage.

3.2. Identification of antagonistic bacterial strains

3.2.1. 16S rRNA Gene sequence analysis

Genomic DNA of the antagonistic strain ATB 24 was extracted and amplified via Polymerase Chain Reaction (PCR) using the universal primer pair 27F and 1492R. The resulting PCR products were subjected to electrophoresis on a 1.5% agarose gel.

The analysis revealed a single, distinct band of approximately 1,500 bp (Figure 1), consistent with the expected size of the 16S rRNA gene fragment. Subsequent sequencing of the PCR product yielded a high-quality 1,035-nucleotide sequence. This substantial sequence length provides a robust foundation for enhancing the reliability of comparative analyses of global genomic databases.

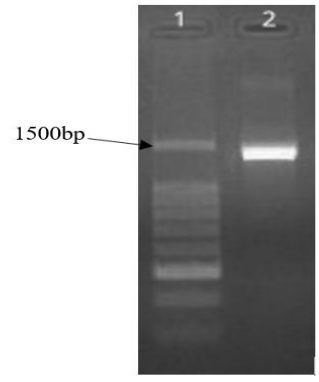

Figure 1. Agarose gel electrophoresis of the 16S rRNA gene PCR product of strain ATB 24

1: DNA ladder, 2: ATB 24.

3.2.2. Sequence homology analysis

The partial 16S rRNA gene sequence of strain ATB 24 (1,031 bp, covering 97% of the query sequence) was analyzed using BLASTn in the NCBI GenBank database. BLAST results indicated that strain ATB 24 belongs to the genus *Streptomyces*. The highest sequence similarity was observed with *S. fimbriatus*, which showed a maximum identity of 97.3%. Other closely related *Streptomyces* species included *S. griseoluteus*, *S. alboflavus*, *S. griseoflavus*, and *S. albaduncus*, with sequence similarity values ranging from approximately 96.8% to 97.2%.

To further clarify the phylogenetic position of strain ATB 24, a phylogenetic tree was constructed based on partial 16S rRNA gene sequences using the neighbor-joining method (Figure 2). In the phylogenetic tree, strain ATB 24 clustered within a well-defined group comprising *S. albaduncus* and *S. fimbriatus*, forming a distinct clade that is clearly separated from other *Streptomyces* lineages. Although this clustering was supported by a bootstrap value of 63% at the main node, the internal node support between ATB 24 and *S. albaduncus* indicated a very close evolutionary relationship. This high level of sequence conservation suggests that while the 16S rRNA gene data confirms the placement of ATB 24 within this specific cluster, it provides insufficient resolution for a definitive species-level assignment when used as a standalone molecular marker. According to the currently accepted taxonomic criteria, a 16S rRNA gene sequence similarity threshold of approximately 98.65–99.0% is generally required for reliable species delineation within the genus *Streptomyces*. Given that strain ATB 24 exhibited a maximum sequence similarity of only 97.3% and was analyzed using a partial 16S rRNA gene sequence, it cannot be conclusively assigned to any recognized species of the genus *Streptomyces*. Therefore, strain ATB 24 was provisionally designated as *Streptomyces* sp. ATB 24. To further characterize its taxonomic position, subsequent analyses focused on detailed morphological and spore ultrastructural features using scanning electron microscopy as part of a polyphasic taxonomic approach.

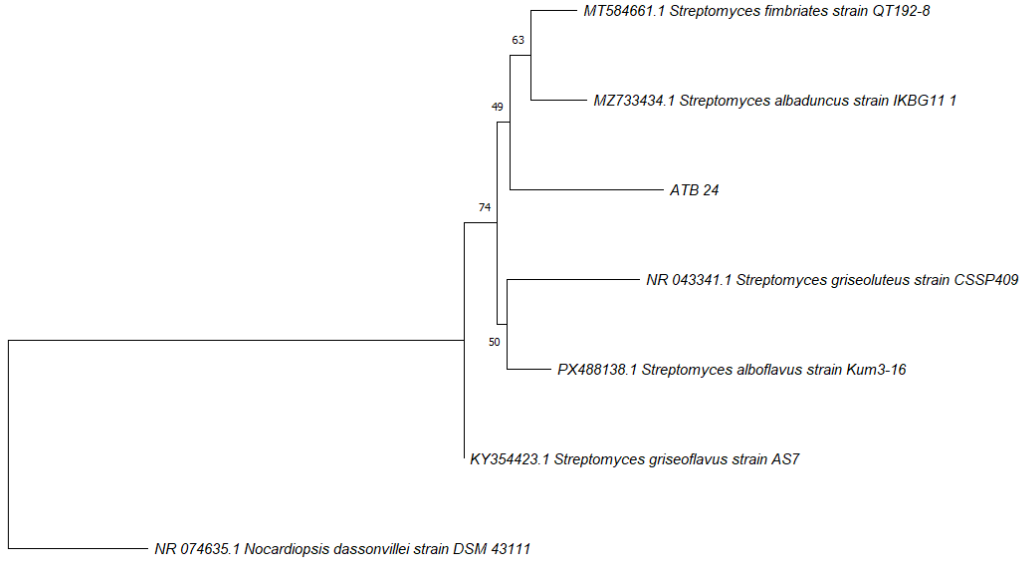

Figure 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the evolutionary relationship between strain ATB 24 and related species of the genus *Streptomyces*. Bootstrap values (1,000 replicates) are indicated at the nodes.

3.2.3. Morphological identification

To resolve the taxonomic position of strain ATB 24 within its phylogenetic cluster, a comparative phenotypic analysis was performed. Given the high conservation of the 16S rRNA gene among the 19 closely related species, morphological and ultrastructural traits on Oatmeal Agar (ISP-3) served as the primary criteria for species-level differentiation.

On ISP-3 medium, strain ATB 24 exhibited vigorous growth characterized by pale yellow aerial mycelia and yellowish-brown substrate

mycelia (Figure 3A). This yellow-series phenotype served as a robust primary filter, allowing the exclusion of the majority of phylogenetic neighbors. Specifically, the gray-series taxa, including *S. griseofuscus, S. griseoflavus, S. werraensis, S. fimbriatus, S. umbrinus, S. fumanus, S. laceus, S. diastatochromogenes, S. novaecaesareae,* and *S. sclerogranulatus*, were eliminated based on their distinct pigmentation. Furthermore, members of the white-series (*S. albogriseolus, S. griseoloalbus,* and *S. pseudogriseolus*) and those belonging to specialized color groups—such as *S. caelestis* (blue-gray), *S. coeruleorubidus* (blue-red), and *S. violaceorectus* (violet)—were also excluded due to these significant cultural discrepancies.

Definitive diagnostic evidence was provided by spore surface ornamentation. SEM analysis revealed that the spores of ATB 24 had a distinctly spiny surface (Figure 3B). This ultrastructural marker is highly specific. While *S. alboflavus*, *S. griseoluteus* and *S. aureoversilis* share similar yellow aerial mycelium, they are characterized by smooth spore surfaces. In contrast, the combination of pale yellow mycelia and spiny spores is a hallmark characteristic of *Streptomyces albaduncus* as described in Bergey's Manual of Systematic Bacteriology.

The morphological profile of strain ATB 24 defined by its pale yellow color series on ISP-3, spiral spore chains, and spiny spore surfaces, shows complete congruence with the type strain of *S. albaduncus*. Although the 16S rRNA gene sequences suggested close affinity to multiple taxa, the integration of these diagnostic morphological markers provides a definitive basis for identification. Consequently, strain ATB 24 was identified as *S. albaduncus.*

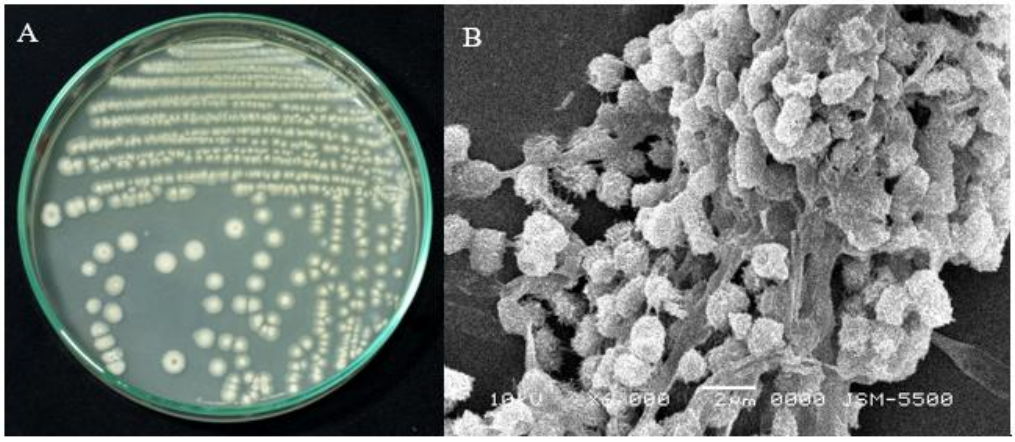

Figure 3. (A) Colonies of strain ATB 24 grown on ISP 3 medium (B) Spore surface morphology of strain ATB 24 observed using scanning electron microscopy (SEM).

4. CONCLUSION

This study demonstrated that antagonistic bacteria isolated from shallot-cultivated soils effectively suppressed shallot basal rot caused by *F. oxysporum* during postharvest storage. Among the evaluated strains, ATB 24 showed the most consistent disease-reducing activity throughout the storage period. Disease suppression was strongly influenced by bacterial cell density, with a concentration of 10^8 CFU/mL providing the most effective and economically feasible level of control**.** Molecular and morphological characterization identified strain ATB 24 as *S. albaduncus*. The strain exhibited good adaptability to postharvest storage environments and maintained antagonistic activity during prolonged storage, confirming its potential as a biological agent for postharvest disease management in shallots.

CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

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