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Effects of Culture Conditions on the Antibacterial Activity of Streptomyces Spp. against Erwinia Spp. Causing Soft Rot Disease on Asparagus Officinalis

Nguyen Thanh Trung[1,2], Nguyen Thi Van Anh[3], Tran Thi Dao[3], Nguyen Thanh Huyen[3], Pham Le Anh Minh[3], Pham Hong Hien[4] & Nguyen Xuan Canh[3]

[1]Institute of Research and Development, Duy Tan University, Da Nang 550000, Vietnam
[2]Faculty of Pharmacy, Duy Tan University, Da Nang 550000, Vietnam
[3]Faculty of Biotechnology, Vietnam University of Agriculture, Hanoi 131000, Vietnam
[4]Science and Technology Office, Vietnam Academy of Agricultural Sciences, Hanoi 12513, Vietnam

Abstract

Erwinia is a genus of Enterobacteriacea containing mostly pathogens, which cause soft rot disease in many ornamental plants and crops, including *Asparagus officinalis*. Chemical treatments to control Erwinia have lost their attractiveness because of the development of resistant strains and the negative impacts on the environment and human health. Therefore, the study of biological controls of soft rot disease has gained great importance. There are several types of microorganisms that show activity against *Erwinia* spp. such as *Pseudomonas fluorescence*, *Bacillus subtilis*, and *Streptomyces* spp. Among them, Streptomyces spp. are found to be the most effective control agents. In this study, 64 isolates of Streptomyces were screened for their antibacterial activity against Erwinia spp. The results indicated that 18 isolates showed an antagonistic reaction against *Erwinia* spp. Among them, isolate D5.1 showed the highest inhibition activity. In addition, the morphological and antibacterial activities of isolate D5.1 grown in different conditions were also characterized.

Keywords

Antibacterial activity, *Asparagus officinalis*, *Erwinia* spp., Soft rot disease, *Streptomyces* spp.

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Correspondence to
nxcanh@vnua.edu.vn

ORCID
Nguyen Xuan Canh
https://orcid.org/0000-0002-7791-6397

Introduction

Asparagus officinalis is one of the healthiest vegetables as it is rich in several types of vitamins, minerals, amino acids, and fiber. In addition, it contains steroid saponins including asparagosides A, B, D, F, G, H, and I, fructans (asparagose and asparagosine), ferulic acid, and flavonoids (quercetin, rutin hyperoside, and isoquercitrin)

(Nature Gate, 2013). Furthermore, *A. officinalis* is also eaten as food with anticancer, antimicrobial, antioxidant, hypolipidemic, and antidiabetic properties.

Today, in Vietnam, the asparagus-growing area is increasing due to increased demand for domestic consumption as well as for exportation. Asparagus has been classified as a drought-tolerant plant, however, it cannot tolerate the increased water amounts during the rainy season. When the moisture content of the soil is too high, asparagus plants are easily infected, especially by *Erwinia* spp., which cause soft rot disease (Smith*et al.*, 1982). Conventional chemical treatments for infected asparagus plants are not optimal as these methods can increase the development of resistant strains of pathogens as well as have undesirable effects on the environment and human health. Therefore, the study of biological agents to control soft rod disease in asparagus plays an important role. A previous study showed that several bacteria such as *Pseudomonas fluorescence*, *Bacillus subtilis*, and *Erwinia herbicola* Eh252 show activity against *Erwinia carotovora* subsp. *carotovora* (Vanneste & Yu, 1996).

Actinomycetes, particularly members of the *Streptomyces* genus, are well known for their ability to produce a broad spectrum of biologically active compounds including antibiotics, hydrolytic enzymes, and enzyme inhibitors (Singh *et al.*, 2006). In addition, they produce about 75% of commercially useful antibiotics (Bhavana *et al.*, 2014). These compounds not only enhance soil fertility but also possess antagonistic activities against a wide range of soil-borne plant pathogens (Aghighi *et al.*, 2004). This allows *Streptomyces* to develop symbiotic interactions with plants by protecting them from various pathogens, and at the same time, plant root exudates promote *Streptomyces* growth (de Lima Procópio *et al.*, 2012). Oskay *et al.* (2004) screened and obtained three strains of *Streptomyces* that inhibited the growth of *Erwinia amylovora.* Abdallah *et al.* (2013) isolated *Streptomyces lavendulae* HHFA1 and *Streptomyces coelicolor* HHFA2 that inhibited the growth of *Erwinia carotovora subsp. carotovora*, which causes soft rot disease on onion. Moreover, the application of *S. coelicolor* HHFA2 on onion in storage reduced disease incidence pronouncedly compared with the untreated control. Salem & El-Shafea (2018) indicated that among three bioagent treatments, *Streptomyces* spp. showed the strongest effects against *Erwinia* Ecc1 and Ecc2, which cause soft rot disease on potato. Nguyen Xuan Canh *et al.* (2017) identified that *Streptomyces* strain L2.5 had the strongest antibacterial activity against *Erwinia carotovora*, which causes soft rot disease on several crops.

The production of active compounds of bacterial hosts depends on both physical and chemical factors such as temperature, pH, fermentation period, and the culture medium compositions (Kiviharju *et al.*, 2004; Gopi *et al.*, 2011*et al.*). Thus, in order to enhance and achieve the maximum production of active compounds by the bacterial host, it is very important to optimize the culture conditions and nutritional factors. Therefore, the aim of this study was to screen *Streptomyces* isolates for inhibition activity against *Erwinia* spp. that cause soft rod disease in *Asparagus officinalis.* Furthermore, we also characterized the most effective isolates by morphological and biochemical properties and identified the optimal culture conditions.

Materials and Methods

Materials

Streptomyces strains used in this study were isolated and stored in the Laboratory of the Department of Microbial Biotechnology, Faculty of Biotechnology, Vietnam National University of Agriculture. *Erwinia* spp. were isolated from infected *A. officinalis* collected at Thuong Tin-Ha Noi and were tested for their pathogenicity on stems of *A. officinalis.*

Antibacterial bioassays

Agar disk method (Dhingra & Sinclair, 1995):

Each Streptomyces isolate was spread individually on a Gause-1 plate (20g soluble

starch, 1g KNO_3, 0.5g NaCl, 0.5g $K_2HPO_3.3H_2O$, 0.01g $FeSO_4.7H_2O$, 20g agar, and distilled water up to 1000mL, pH = 7.2) and was incubated at 30°C. After 5 days, 6mm agar disks containing a Streptomyces colony were prepared using a sterilized cork borer. Then, the agar disks were transferred to a new LB plate (10g yeast extract, 5g NaCl, 10g peptone, 20g agar, and distilled water up to 1000mL, pH = 7.0), which was spread previously with Erwinia spp. The plates were incubated at 30°C for 24h. The antibacterial activities of the Streptomyces isolates were evaluated by measuring the diameter of the inhibitory zones (mm). The negative control test was prepared from fresh Gause-1 medium. The examination was repeated three times. The data were processed using Excel 2007 software.

Characterization of *Streptomyces*

The culture characteristics of the *Streptomyces* spp. were analyzed on six solid medium plates containing either Gause-1, Gause-2 (4g meat extract, 5g peptone, 5g NaCl, 10g glucose, 20g agar, and distilled water up to 1000mL, pH = 7.0), ISP1 (5g tryptone, 3g yeast extract, 20g agar, and distilled water up to 1000mL, pH = 7.0-7.2), ISP2 (4g yeast extract, 10g malt extract, 4g glucose, and distilled water up to 1000mL, pH = 7.3), ISP3 (20g oatmeal, 20g agar, 1mL trace salts solution, and distilled water up to 1000mL, pH = 7.0-7.4), or ISP4 (10g soluble starch, 1g K_2HPO_4, 1g $MgSO_4.7H_2O$, 1g NaCl, 2g $(NH_4)_2SO_4$, 2g $CaCO_3$, 1mL trace salts solution, 20g agar, and distilled water up to 1000mL, pH 7.0-7.4), with the trace salts solution being made with 0.01g $FeSO_4.7H_2O$, 0.1g $MnCl_2.4H_2O$, 0.1g $ZnSO_4.7H_2O$, and distilled water up to 100mL, pH 7.0-7.2. The colors of the mature sporulating aerial mycelium and substrate mycelium were monitored in the 3, 5, 7, 9, and 14-day-old cultures. Microscopic characterization was done by the cover slip culture method (Kawato & Sinobu, 1979). The shapes of the substrate mycelium and spores were observed using a microscope (1000x). The *Streptomyces* isolates were identified by comparing the morphological properties with the actinomycetes morphology provided by *Bergey's Manual of Determinative Bacteriology* (Bergey & Holt, 2000).

Effects of culture conditions

Effects of the stage and time of fermentation

The selected Streptomyces strain was inoculated in ISP2 medium with and without shaking at 30°C, 180 rpm. After 3, 5, 7, 9, and 14 days, the broth culture was centrifuged at 10,000 rpm for 10 minutes to remove the mycelium. The antibacterial activity of the supernatant was analyzed by the agar well diffusion method against *Erwinia* spp.

Effects of pH and temperature

After the optimal stage and the growth duration were defined, the selected Streptomyces strain was inoculated in ISP2 medium with a range of initial pH values from pH 5.0 to pH 10.0 in order to define the optimal pH for the highest antibacterial activity.

The optimal temperature for the maximum antibacterial activity of the selected strain was tested on ISP2 medium at the different temperatures of 20, 25, 30, and 37°C.

Effects of carbon and nitrogen sources

The carbon sources of glucose, fructose, maltose, xylose, D-sucrose, lactose, dextrin, and soluble starch were tested in ISP2 medium at the concentration of 2% to identify the best carbon source for the highest antibacterial activity.

The optimal nitrogen sources for the maximum antibacterial activity were tested in ISP2 medium supplemented with 1% peptone, $(NH_4)_2SO_4$, $NaNO_3$, KNO_3, and NH_4Cl, respectively.

Results and Discussion

Antibacterial activity of the *Streptomyces* spp.

In this study, 64 *Streptomyces* isolates were screened for their antibacterial activities against *Erwinia* spp. by the agar disk method. The results indicated that 18 *Streptomyces* isolates showed high antibacterial activity against *Erwinia* spp. Among them, isolate D5.1 showed the highest antibacterial activity with an inhibition zone diameter of 23mm (**Figure 1**).

Several previous studies have shown similar results. Kovácsová *et al.* (2015) indicated that there were five *Actinomyces* strains that showed antibacterial activity against *Erwinia amylovora*, which causes fire blight in apple plants. Among them, *Streptomyces* isolate 104 K14 showed the highest activity with an inhibition zone diameter of 22mm. The study of Zamanian *et al.* (2005) indicated that *Streptomyces plicatus* strain 101 showed the highest antibacterial activity against *Erwinia carotovora* subsp. *carotovora*. El-Karkouri *et al.* (2010) isolated *Streptomyces cinereoruber* from a Moroccan biotope that inhibited the growth of *Erwinia chrysanthemi* 3937VIII.

Characterization of isolate D5.1

Isolate D5.1 was grown on solid Gause-1 medium at 37°C. The morphological characteristics of the colony were observed at 3, 5, 7, 9, and 14 days. At the early stage of colony formation (3 days-old), the color of the colony was white. After further development, it changed into dark pink (from 5-14 days-old). The shape of the isolate D5.1 colony was radial lines. The dominant type was presented by a round, umbonate colony, completely covered by pale pink aerial mycelium with smooth edges.

Isolate D5.1 grew well on all of the tested media. The abundance and the color of the aerial mycelium depended on the medium composition and the age of the colony. When grown on Gause 1, ISP2, ISP3, and ISP4, the aerial mass and the substrate mycelium color varied from white and pale pink to red. On ISP1 medium, however, the aerial mass and the substrate mycelium color were soil brown. On the other hand, isolate D5.1 produced purple soluble pigment on Gause 1 medium and dark pink on ISP3 medium (**Table 1**).

Effects of culture conditions on the antibacterial activity of isolate D5.1 against *Erwinia* spp.

Effects of the stage of growth and growth duration

The antibacterial ability of each Streptomyces strain was affected by the different growth durations and the stages of growth. In this study, isolate D5.1 was inoculated in 100mL flasks containing 25mL ISP2 medium with and without shaking at 180rpm, 30°C. The optimal growth duration of Streptomyces albidoflavus C247 was 4 days (Islam *et al.*, 2009).

The results in **Figure 2** show that isolate D5.1 started to express antibacterial activity against *Erwinia* spp. after 3 days of growth. The antibacterial activity of this strain increased after 3 days and reached the highest activity at 7 days in both stages of growth. After 7 days, the antibacterial activities decreased slightly, however, they remained at high levels after 14 days of cultivation. The results also indicated that isolate D5.1 grown under vigorous agitation produced much higher antibacterial activity than in growth conditions without shaking (**Figure 2**).

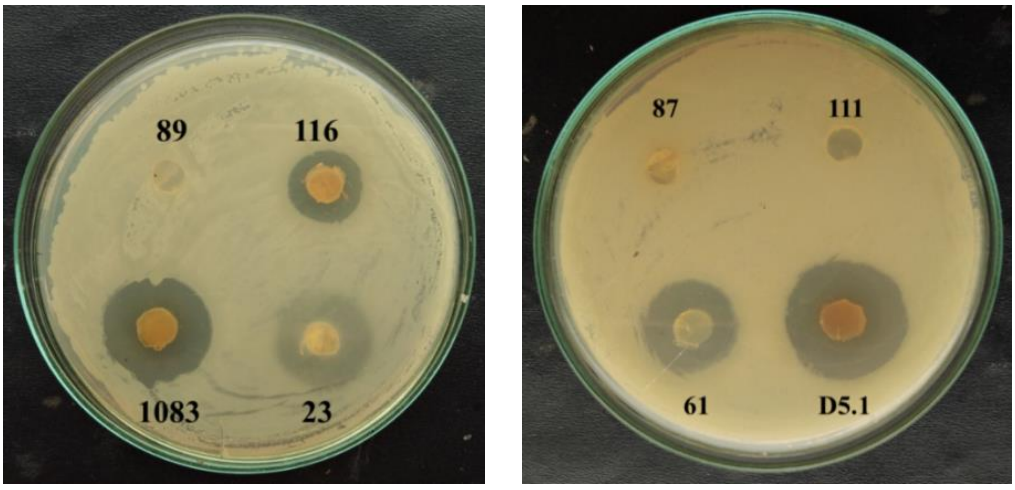


Figure 1. The antibacterial activity of isolate D5.1 against *Erwinia* spp.

Table 1. Culture characteristics of isolate D5.1 grown on different media

Medium	Growth	Age (days)	Color of aerial mycelium	Color of substrate mycelium
		3	Pale pink	Pale pink
		5	Pale pink	Pale pink
Gause-1	moderate	7	Pale pink – red	Pale pink – red
		9	Pale pink – red	Pale pink – red
		14	Purple	Purple
		3	Brown - pink	Brown
		5	Soil brown	Soil brown
ISP1	abundant	7	Soil brown	Soil brown
		9	Soil brown	Soil brown
		14	Soil brown	Soil brown
		3	Red-yellow	Pink
		5	Pink	Pink
ISP2	abundant	7	Dark red	Dark red
		9	Dark red	Dark red
		14	Brown red-white	Brown red
		3	Pink	Pink
		5	Red	Red
ISP3	abundant	7	Dark pink	Dark pink
		9	Dark pink	Dark pink
		14	Dark pink	Dark pink
		3	Pale pink	Pale pink
		5	Pale pink – purple	Pink
ISP4	abundant	7	Pale pink – purple	Pink
		9	Pale pink – purple	Pink
		14	Pale pink – purple	Pink

Thus, isolate D5.1 showed the highest antibacterial activity after 7 days of cultivation with shaking at 180rpm.

Reddy *et al.* (2011) reported that the production of antimicrobial metabolites of *S. rochei* were detected in the growth supernatant after 48h of cultivation and reached a maximum level in the late stationary phase (5 days of cultivation).

Effects of pH and temperature

The pH of the growth medium might play an important role in the production of antibacterial compounds by actinomycetes (Pandey *et al.*, 2005). In this study, isolate D5.1 was cultivated in ISP2 medium with an initial pH ranging from pH 5 to pH 10 (**Figure 3**). In the medium with an initial pH ranging from pH 5 to pH 8, the antibacterial activity increased as the pH increased and reached the highest level at pH 8, corresponding to an inhibition zone of 24mm. At higher pH values (from pH 8 to pH 10), the antibacterial activity of isolate D5.1 significantly decreased. Similarly, the highest antibacterial activity of *S. violaceoruber* was observed at pH 8.0 (Palanichamy *et al.*, 2011) while the maximum production of antimicrobial metabolites in *S. rochei* was found at pH 7.5 (Reddy *et al.*, 2011).

In order to analyze the effects of the growth

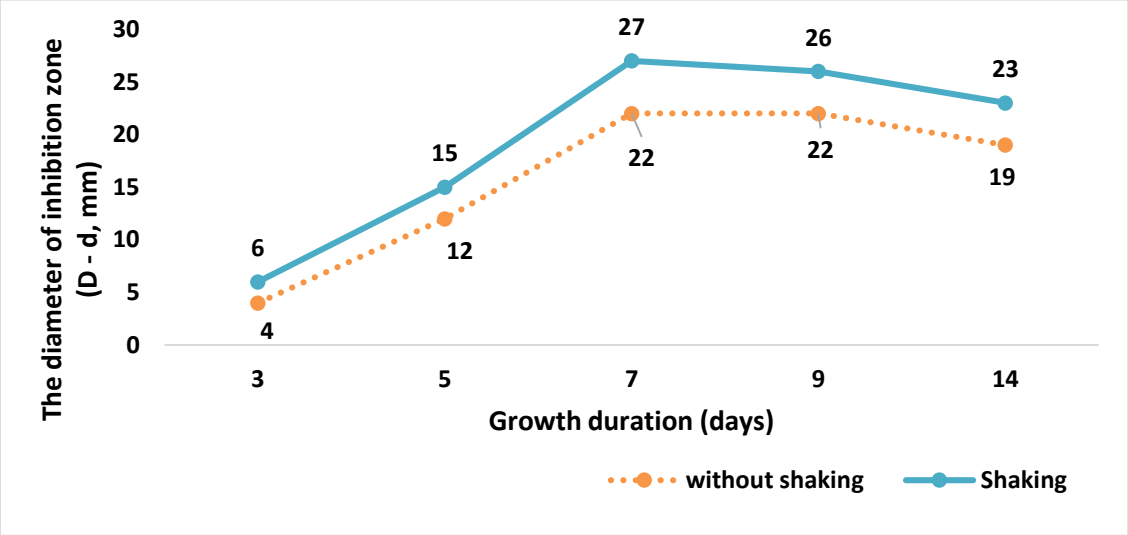
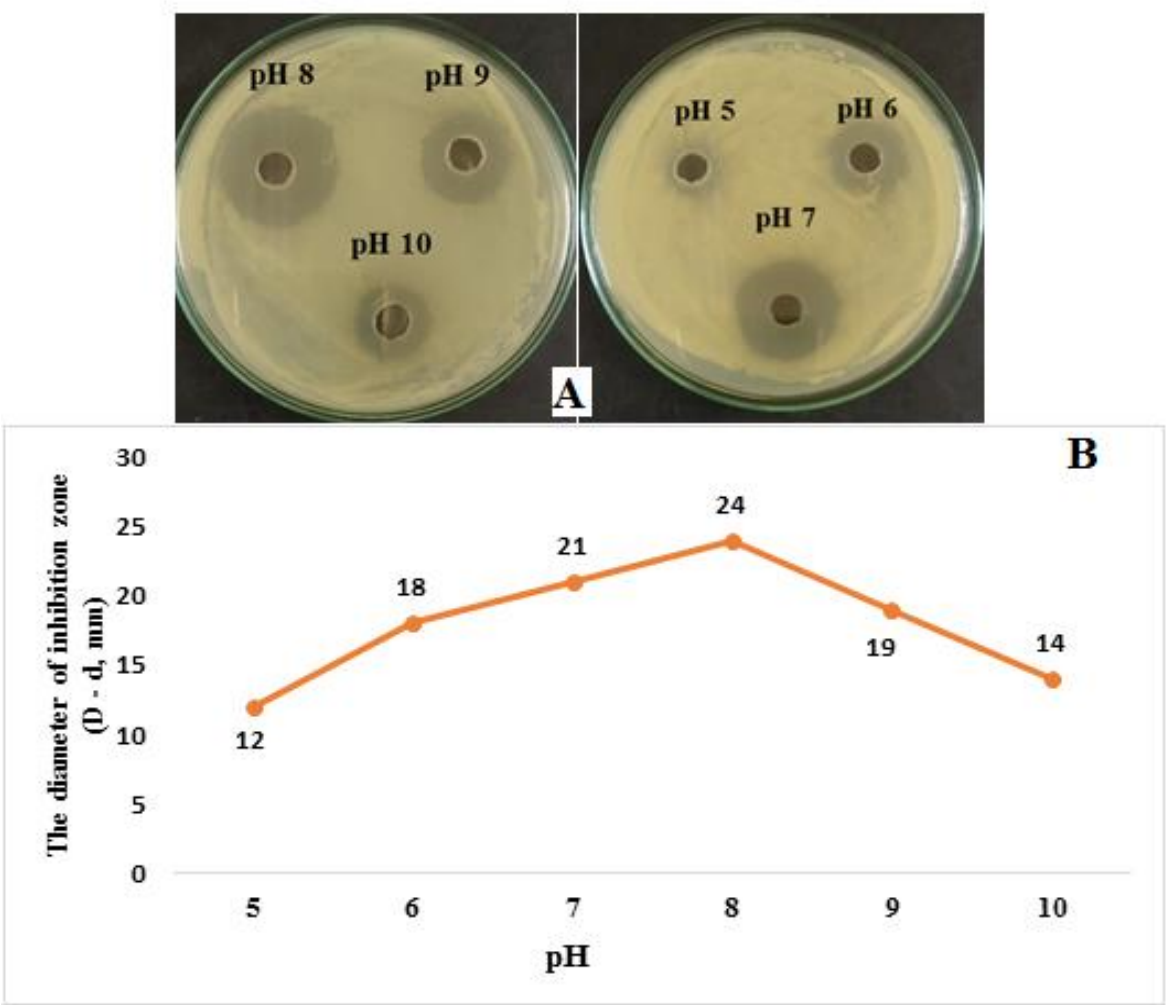


Figure 2. Effects of the stage of growth and growth duration on the antibacterial activity of isolate D5.1



Note: A: Antagonistic activity of strain D5.1 on agar plates with different pH-levels of culture medium (5, 6, 7, 8, 9, and 10); B: Inhibition zone diameter chart

Figure 3. Effects of pH on the antibacterial activity of isolate D5.1

temperature, strain D5.1 was cultivated in a range of temperatures from 20 to 37°C. It was shown that strain D5.1 had good antagonistic activity against Erwinia spp. when grown at 25 to 37°C. The optimal temperature for the maximum antibacterial activity was 30°C with an inhibition zone of 26mm (**Figure 4**).

In previous studies, the optimum temperature for the growth of *Streptomyces spp.* was close to 30°C. The optimum temperature for the production of antimicrobial metabolites in *S. rochei* was 32°C (Reddy *et al.*, 2011). The highest growth and antimicrobial activity of *Streptomyces violaceoruber* were observed at 30°C (Palanichamy *et al.*, 2011). The study of Islam *et al.* (2009) indicated that the optimal temperature for the antifungal activity of *Streptomyces albidoflavus* C247 was 30°C. Pudi *et al.* (2016) also showed that the maximum growth and alkaloid production of actinomycetes isolated from marine sediments collected on the Kakinada coast were obtained at the temperature 30°C.

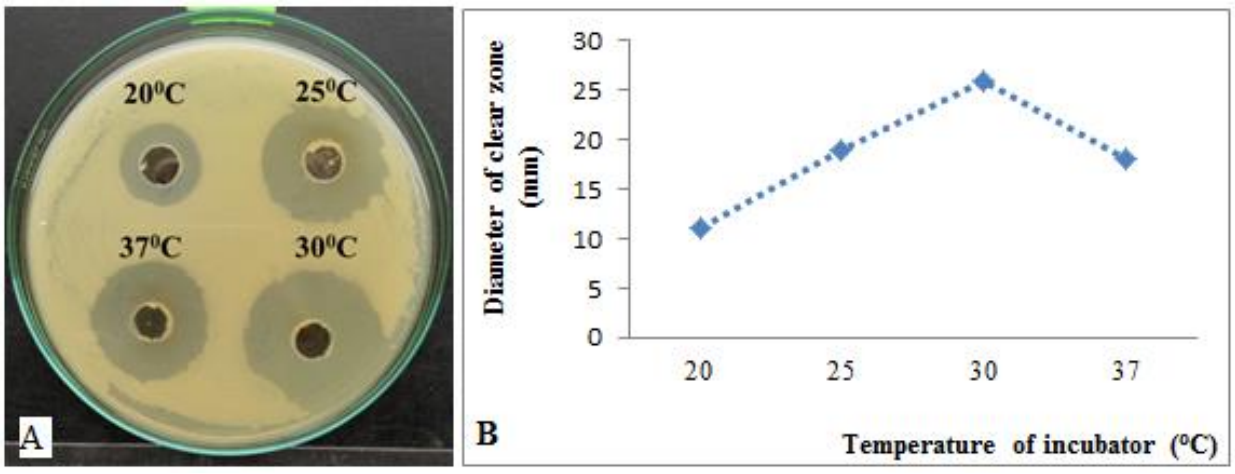
Effects of carbon and nitrogen sources

In this experiment, various carbon and nitrogen sources were supplemented independently in ISP2 medium (**Figures 5 and 6**). The results showed that almost all the carbon sources, except sucrose, enhanced the antibacterial activity in isolate D5.1. Isolate D5.1 produced the highest antibacterial activity (inhibition zone of 28mm) when growing in ISP2 medium with lactose as the only carbon source (**Figure 5**).

The results in **Figure 6** show that peptone, potassium nitrate, and ammonium sulphate seemed to be the most suitable nitrogen sources for the production of high antibacterial activity in isolate D5.1. In addition, the organic nitrogen sources seemed to induce relatively higher antibacterial activity than the inorganic nitrogen sources did. Among them, peptone showed the highest antibacterial activity. These results are in accordance with reports that organic nitrogen sources are superior for antibiotic production in *Streptomyces rimosus* (Yu *et al.*, 2008; Reddy *et al.*, 2011) and *Streptomyces spectabilis* (Holkar *et al.*, 2017).

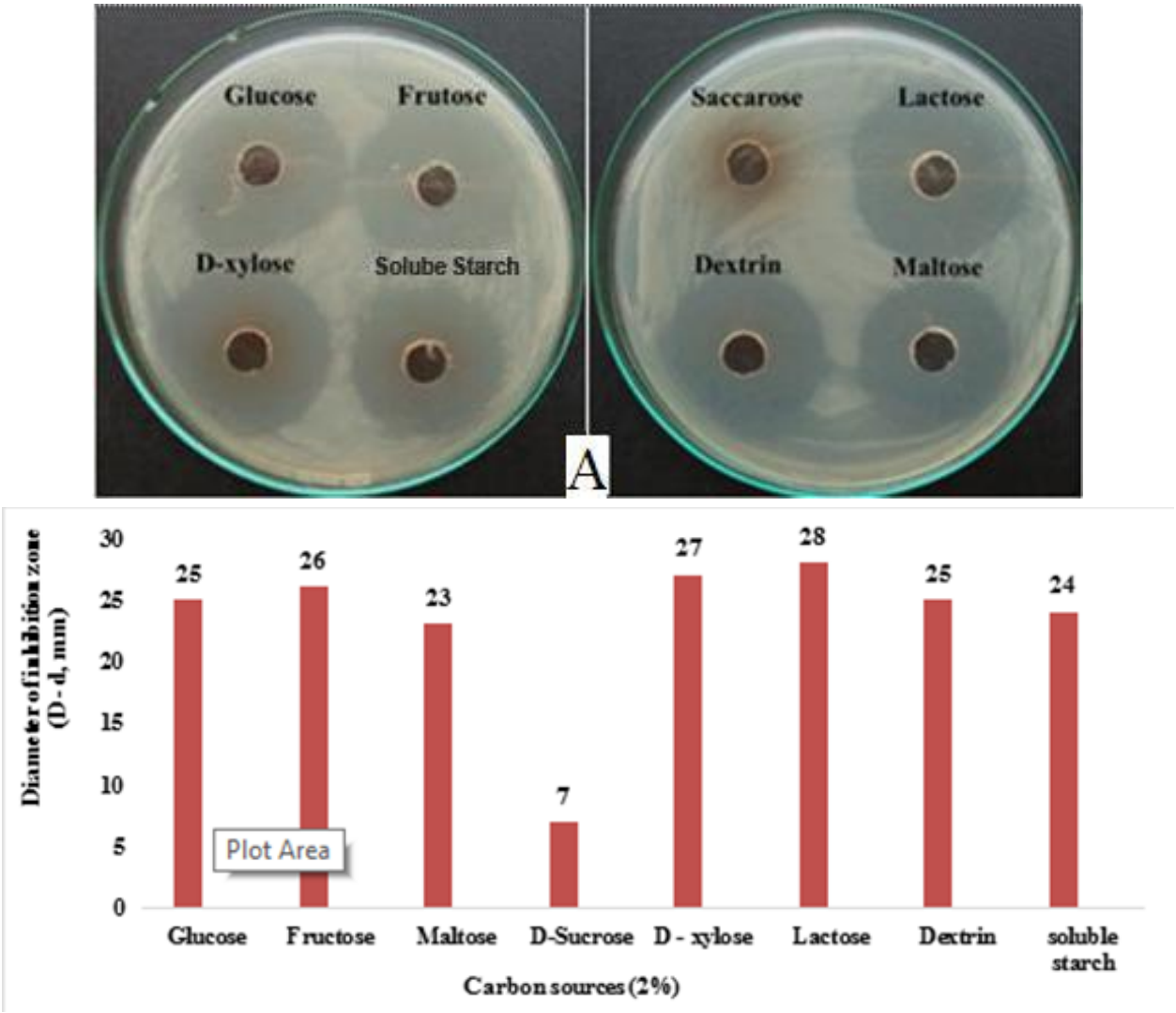
Conclusions

This overview showed that the course of In this study, isolate D5.1 was found to be the most effective strain for the inhibition of *Erwinia* spp. among 64 *Streptomyces* isolates stored in our laboratory. In addition, isolate D5.1 reached the highest antibacterial activity when grown in ISP2 medium (pH 8) supplemented with lactose and peptone as the only carbon and nitrogen sources at 30°C, 180rpm.



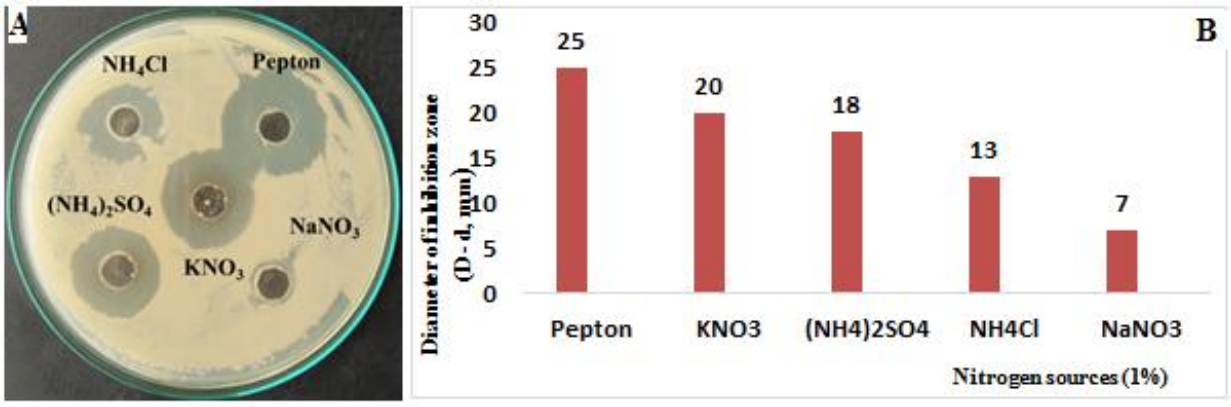
Note: A: Antagonistic activity of isolate D5.1 on agar plates with different culture temperatures (20, 25, 30, and 37°C); B: Inhibition zone diameter chart

Figure 4. Effect of temperature on the antibacterial activity of isolate D5.1



Note: A: Antagonistic activity of isolate D5.1 on agar plates with different carbon sources;
B: Inhibition zone diameter chart

Figure 5. Effect of carbon sources on the antibacterial activity of isolate D5.1



Note: A: Antagonistic activity of isolate D5.1 on agar plates with different carbon sources;
B: Inhibition zone diameter chart

Figure 6. Effect of nitrogen sources on the antibacterial activity of isolate D5.1

References

Abdallah M. E., Haroun S. A., Gomah A. A., El-Naggar N. E. & Badr H. H. (2013). Application of actinomycetes as biocontrol agents in the management of onion bacterial rot diseases. Journal Archives of Phytopathology and Plant Protection. 46(15): 1797-1808.

Aghighi S., Shahidi Bonjar G. H., Rawashdeh R., Batayneh S. & Saadoun I. (2004). First report of antifungal spectra of activity of Iranian actinomycetes strains against Alternaria solani, Alternaria alternate, Fusarium solani, Phytophthora megasperma, Verticillium dahliae and Saccharomyces cerevisiae. Asian Journal of Plant Sciences. 3(4): 463-471.

Bergey D. H. & Holt J. G. (2000). Bergey's manual of determinative bacteriology (9th ed.). Philadelphia: Lippincott Williams and Wilkins.

Bhavana M., Talluri V. P. & Kumar K. S. (2014). Optimization of culture conditions of Streptomyces carpaticus (MTCC-11062) for the production of antimicrobial compound. Interational Journal of Pharmacy and Pharmaceutical Sciences. 6: 281-285.

de Lima Procópio R. E., da Silva. I. R., Martins M. K., de Azevedo R. L. & de Araújo J. M.(2012). Antibiotics produced byStreptomyces. The Brazilian Journal of Infectious Diseases. 16(5): 466-471.

Dhingra O. D. & Sinclair J. B. (1995). Basic plant pathology methods. CRC press: USA., pp 287-296, 390-391.

El-Karkouri A., El-Hassani F. Z., El-Mzibri M., Benlemlih M. & El-Hassouni M. (2010). Isolation and identification of an actinomycete strain with a biocontrol effect on the phytopathogenic Erwinia chrysanthemi 3937VIII responsible for soft rot disease. Annals of Microbiology. 60: 263-268.

Gopi R., Ramakrishna R. & Rajagopal A. (2011). Optimization of culture conditions of Streptomyces rochei (MTCC 10109) for the production of antimicrobial metabolites. Egyptian Journal of Biology. 13: 21-29.

Holkar S. K., Begde D. N., Nashikkar N. A., Kadam T. A. & Upadhyay A. A. (2017). Optimization of some culture conditions for improved biomas and antibiotic production by Streptomyces spectabilis isolated from soil. International Journal of Pharmaceutical Sciences and Research. 4(8): 2980-2987.

Islam Md. R., Jeong Y. T., Ryu Y. J., Song C. H. & Lee Y. S. (2009). Isolation, identification and optimal culture conditions of Streptomyces albidoflavus C247 producing antifungal agents against rhizoctonia solani AG2-2. Mycobiology. 37(2): 114-120.

Kawato M. & Shinobu R. (1959). A simple technique for the microscopical observation. Memoirs of the Osaka University Liberal Arts and Education: 114.

Kiviharju K., Leisola M. & Eerikäinen T. (2004). Optimization of Streptomyces peucetius var. Caesius N47 cultivation and epsilon-rhodomycinone production using experimental designs and response surface methods. Journal of Industrial Microbiology & Biotechnology. 31(10): 475-481.

Kovácsová S., Javoreková S., Medo J., Charousová I., Elbl J. & Plošek L. (2015). Characteristic of Streptomyces species with antimicrobial activity against selected phytopathogenic bacteria and fungi. Journal of Microbiology Biotechnology Food Sciences. 5(1): 55-59.

Oskay M., Tamer A. U. & Azeri C. (2004). Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. African Journal of Biotechnology. 3(9): 441-446.

Pudi N., Varikuti. G. D., Badana A. K., Gavara M. M., Kumari S. & Malla R. (2016).. Studies on Optimization of Growth Parameters for Enhanced Production of Antibiotic Alkaloids by Isolated Marine actinomycetes. Journal of Applied Pharmaceutical Science. 6(10): 181-188.

Nature Gate (2013). Asparagus officinalis. Retrieved from www.luontoportti.com/suomi/en/kukkakasvit /asparagus on December 9, 2020.

Nguyen Xuan Canh, Nguyen Thi Khanh & Pham Hong Hien (2017). Characterization of Actinomyces strain with bioactivity against Erwinia carotovora causing soft rot disease on some crops. Vietnam Journal of Agricultural Science and Technology. 7(80): 41-46 (in Vietnamese).

Pandey A., Shukla A. & Majumdar S. K. (2005). Utilization of carbon and nitrogen sources by Streptomyces kanamyceticus M27 for the production of an antibacterial antibiotic. African Journal of Biotechnology. 4: 909-910.

Palanichamy V., Hundet A., Mitra B. & Reddy N. (2011). Optimization of cultivation parameters for growth and pigment production by Streptomyces spp. isolated from marine sediment and rhizosphere soil. International Journal of Plant, Animal and Environmental Sciences. 1: 158-170.

Reddy N. G., Ramakrishna D. P. N. & Rajagopal S. V. (2011). Optimization of culture conditions of Streptomyces rochei (MTCC 10109) for the production of antimicrobial metabolites. Egyptian Journal of Biology. 13: 21-29.

Salem E. A & El-Shafea Y. M. A. (2018). Biological control of potato soft rot caused by Erwinia carotovora subsp. Carotovora. Egyptian Journal of Biological Pes Control. 28: 94.

Singh L. S., Baruah I. & Bora T. C. (2006). Actinomycetes of Loktak habitat: isolation and screening for antimicrobial activities. Biotechnology. 5(2): 217-221.

Smith M. A., Mccolloch L. P. & Friedman B. A. (1982). Market Diseases of Asparagus, Onions, Beans, Peas, Carrots, Celery, and Related Vegetables. Agriculture handbook number 303, United States Department of Agriculture.

Vanneste J. L. & Yu J. (1996). Biological control of fire

blight using Erwinia herbicola Eh252 and Pseudomonas fluorescens A506 separately or in combination. Acta Horticulturae Journal. 411: 351-353.

Yu J., Liu Q. & Liu Q. (2008). Effect of liquid culture requirements on antifungal antibiotic production by Streptomyces rimosus MY02. Bioresource Technology. 99: 2087-2091.

Zamanian S., Bonjar G. H. S. & Saadoun I. (2005). First report of antibacterial properties of a new strain of Streptomyces plicatus (strain 101) against Erwinia carotovora subsp. carotovora from Iran. Biotechnology. 4(2): 114-120.