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Characterization of Plant Pathogenic - Resistant *Streptomyces aureofaciens* CP1.3 Strain

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ABSTRACT

Plant pathogenic bacteria cause severe damage to crop yields and product quality in Vietnam, as well as in many countries around the world. The objective of this study was to isolate and identify new sources of biological materials that exhibit resistance to plant pathogens. In this study, a total of 72 different strains of actinomycetes were isolated from soil samples collected from various locations. Among these strains, CP1.3 demonstrated intense antagonism towards five tested bacteria and the *Fusarium oxysporum* fungus. The CP1.3 strain displayed robust growth and exhibited strong antibacterial activity at a temperature of 30°C, pH range of 7.0-8.0, and salt concentrations up to 1.0%. It also utilized D-glucose, D-sorbitol, and maltose as good carbon sources while producing extracellular enzymes such as chitinase, cellulase, and protease. By analyzing the nucleotide sequence of CP1.3's 16S rRNA and compared to the nucleotide sequence from Genbank, it revealed that this strain has a close relationship with the *Streptomyces aureofaciens* species (99.03% similarity). As a result attained, the strain CP1.3 was named as *Streptomyces aureofaciens* CP1.3.

Keywords: Phytopathogenic bacteria, actinomycetes, antagonism, Streptomyces spp.

Introduction

Plant diseases cause numerous losses of agricultural products in Vietnam as well as in numerous other countries in Southeast Asia and around the world. The outbreak of diseases on valuable plants has a strong impact on farmers' livelihoods, especially in areas where alternative plants are not available. According to the report of Velu *et al*. (2015), there have b approximately 7100 plant-pathogenic microbes, including viruses, bacteria, fungi, nematodes, and insects, with about 150 of them being planted pathogenic bacteria. Bacteria are responsible for most plant diseases, such as bacterial wilt, leaf spot, leaf burn, swelling, and ulceration, etc. Diseases caused by bacteria are common in tropical regions. Some species also cause soft rot in fruits and vegetables before and after harvest. Plant pathogenic bacteria belonging to the genera *Erwinia*, *Pectobacterium*, *Pantoea*, *Agrobacterium*, *Pseudomonas*, *Ralstonia*, *Burkholderia*, *Acid ovorax*, *Xanthomonas*, *Clavibacter*, *Xylella*, *Spiroplasma*, and *Phytoplasma*, which can attack many cultivated plants and typically cause the most significant damage.

Nowadays, farmers in many countries are moving towards sustainable agriculture, which involves minimizing chemical applications and increasing the use of biological preparations in biocontrol and treating plant diseases. Biological preparations help to improve product quality and consumer health as well as environmentally friendly. Actinomycetes are a group of microorganisms that have received much attention from many worldwide microbiologies because they can inhibit pathogens by secreting antibiotics and competing for nutrients (Giang et al., 2020). Actinomycetes can also stimulate disease resistance and help plants tolerate to adverse environmental conditions (Hasegawa *et al*., 2006). Approximately about 23,000 bioactive secondary metabolites from microorganisms have been discovered, with over 10,000 of them being synthesized by actinomycetes. These actinomycetes-derived metabolites account for 45% of all active microbial metabolites (Sudha *et al*., 2019). Additionally, actinomycetes were reported to play a key role in decomposing lignocellulose, solubilizing inorganic phosphate, and nitrogen fixation (Ha *et al*., 2010). Hence, applying actinomycetes caused by bacteria is one of the most feasible ways to manage plant diseases. Therefore, this study attempted to isolate and identify the potential actinomycetes with promising antimicrobial and antibacterial activities.

Materials and Methods

Materials

In this study, the tested microbes used were *Xanthomonas axonopodis*, *Rastonia solanacearum*, *Clavibacter michiganensis*, *Xanthomonas* sp., *Bacillus subtilis*, and *Fusarium oxysporum*. All these microbes were kindly provided by Vietnam Institute of Industrial Chemistry.

Isolation of Actinomycetes

Streptomyces were isolated from soil samples collected in Cuc Phuong National Park according to the method described by Dong *et al*. (2014) and Giang et al. (2022) with some minor modifications. Briefly, one gram of soil sample was taken and serially diluted up to 10^{-2} using distilled water as a diluent. The mixture was vigorously shaken using a vortex; 0.1 ml of each dilution was placed on a Gause I agar disc (g/l): starch 20, K_2HPO_4 0.5, $MgSO_4.7H_2O$ 0.5, NaCl 0.5, KNO_3 1, $FeSO_4$ 0.01, agar 20, pH 7.0-7.2; and the inoculum was spread properly using a sterile glass spreader. The inoculated plates were allowed to stand at room temperature for 5–10 min to allow the liquid to be absorbed and were then incubated at 30°C for 7 days. Identification of the actinomycetes was done based on macroscopic and microscopic examination and

physiological tests as suggested by Bergey's Manual of Systematic Bacteriology, 2nd Edition, Vol 5, The Actinobacteria, Part A.

Antibacterial activity of isolated actinomyces strains

In this experiment, the Well-diffusion Method was done according to the previously described by Mounyr *et al*. (2016), the test bacterial strains were separately inoculated in the Petri dishes with LB agar media at 30^{o}C for two days in the incubator. A hole was made in this disc using a sterile well cutter with a diameter of 0.5 cm. Each newly isolated streptomyces strain was incubated in liquid Gause I medium on a shaker with a speed of 200 rpm at 30^{o}C. After five days of incubation, taken 100 µl of streptomyces culture and then put in the hole on the agar disk with the test bacteria. The Petri dishes were incubated under 30^{o}C for 6 h. The antimicrobial compounds diffused into the agar and inhibited the germination and growth of the test bacteria, and then the diameters of the inhibition growth zones were measured (Figure. 1). The antibacterial activity of streptomyces strains was measured using the formula D-d (mm), where d was colony diameter; and D was the inhibition zone diameter.

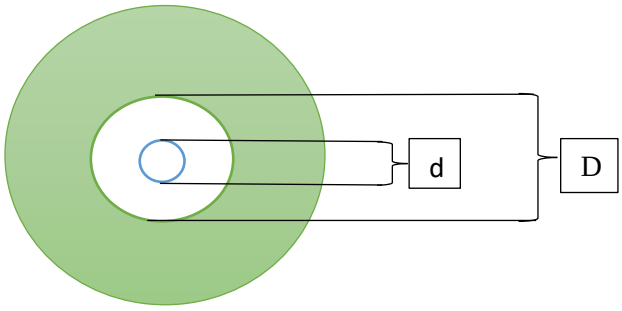

Figure 1. *Measurement of antibacterial activity by the well diffusion method; D implied the inhibition zone diameter, d: was colony diameter.*

Determination of Minimum Inhibitory Concentrations (MICs). Test bacterial strains were separately inoculated into the Petri dish with LB agar media at 30^{o}C for two days in the incubator. Using the sterile-well cutter to make a hole (0.5 cm in diameter), each newly isolated streptomyces strain was incubated in liquid Gause I medium on a shaker (centrifuged 200 rpm at 30^{o}C). After 7 incubation days, the culture was centrifuged at 8000 rpm, 10 min, 4^{o}C, and free cell solution was collected. To measure the MIC value, two-fold serial dilutions of 50, 25, 12.5, 6.25, 3.125, 1.562, and 0.781 µl of the collected solution were prepared and added to the wells in the test discs (These dishes were kept in an incubator under 30; the results were assayed after 12 h. The MIC is the lowest concentration able to inhibit any visible fungal growth (Dhanasekaran *et al.,* 2012).

Antifungal activity of selected Streptomyces strains

The antifungal activity of Streptomyces isolates was tested using a dual culture technique using PDA medium (Dhanasekaran *et al*., 2012). A mycelial disc of the fungal pathogen (5mm dia.) was placed at one end of the Petri plate. The streptomyces antagonists have streaked 1 cm away from the periphery of the Petri plate just opposite the mycelial disc of the pathogen. Visual observation on the inhibition of pathogenic fungal growth was recorded after 96 h of incubation compared to

the PDA plate simultaneously inoculated with fungal pathogen only as the control. The percentage of test pathogen inhibition by the streptomyces isolate was evaluated using the dual culture technique. The radial growth of mycelium in mm was measured, and percent inhibition (PI) was calculated as formula: PI= (C-T)/C×100, where C was the growth of the test pathogen (mm) in the absence of the antagonistic isolate; T was the growth of the test pathogen (mm) in the presence of the antagonistic isolate.

Morphological characteristics

Macroscopic characteristics of newly isolated streptomyces strains were observed, including aerial mycelium, submerged mycelium, color, and diffusible pigments (Alice *et al.*, 2018). In this experiment, the streptomyces strains were cultured on agar media, specifically, Gause I (g/l): starch 20, K_2HPO_4 0.5, $MgSO_4.7H_2O$ 0.5, NaCl 0.5, KNO_3 1, $FeSO_4$ 0.01, agar 20, pH 7.0-7.2; Gause II (g/l): meat extract 30ml, peptone 5, NaCl 5, glucose 10, agar 20, and ISP media: ISP1, ISp2, ISP3, ISP4, ISP5, ISP6, and ISP7. Each medium has different nutrient sources: (ISP1 (g/l): tryptone 5, yeast extract 3, agar 20, pH 7.0; ISP2 (g/l): yeast 4, malt extract 10, glucose 4, agar 20, pH 7.3; ISP3 (g/l): wheat starch 20, agar 20, solution off micromineral salts 1.0 ml, pH 7.0; ISP4 (g/l): starch 10, K_2HPO_4 1, $MgSO_4.7H_2O$ 1, NaCl 1, $(NH_4)_2SO_4$ 2, $CaCO_3$ 2, solution of micromineral salts 1.0 ml, agar 20, pH 7.0; ISP5 (g/l): L-asparagin 1, glycerin 10, K_2HPO_4 1, solution of micromineral salts 1.0 ml, agar 20, pH 7.0; ISP6 (g/l): peptone 10, yeast extract 1, iron citrate ($FeC_6H_5O_7$) 0,5, agar 20, pH 7.0; ISP7 (g/l): glycerin 15, L-tyrosin 0.5, L-asparagin 1, K_2HPO_4 0.5, $MgSO_4.7H_2O$ 0.5, NaCl 0.5, $FeSO_4.7H_2O$ 0.01, solution of micromineral salts 1.0 ml, agar 20, pH 7.0; The compounds of solution of micromineral salts (g/ml) were: $FeSO_4.7H_2O$ 0.01, $MnCl_2.4H_2O$ 0.1g, $ZnSO_4.7H_2O$ 0.1g).

Carbon sources utilizing ability: Isolated streptomyces strains were cultured on medium ISP9 (g/l) [$(NH_4)_2SO_4$ 2.64, KH_2PO_4 2.38, $K_2HPO_4.3H_2O$ 5.65, $MgSO_4.7H_2O$ 1, B solution 1.0 ml, agar 20, pH 7.0] supplemented with 1% different carbon sources including: D-glucose, maltose, D-fructose, D-sobitol, L-arabinose, dextrin, D-xylose, D-galactose, and L-rhamnose. B solution compounds (g/ml): $CuSO_4.5H_2O$ 0.64, $FeSO_4.7H_2O$ 0.11, $MnCl_2.4H_2O$ 0.79, $ZnSO_4.7H_2O$ 0.15, distilled water 100ml.

Enzyme activity: Extracellular chitinase, cellulase, amylase, and protease activities were determined using agar disc diffusion. Streptomyces strains were cultivated in liquid Gause I medium at a suitable temperature and pH on the shaker at 200 rpm. After 7 days of incubation, the culture was centrifuged at 8000 rpm for 15 min and the supernatant was collected and used as the crude enzyme. It was then made a well in the prepared agar discs containing substances corresponding to the test enzyme, put 100 µl of the crude enzyme into these wells, and kept these dishes in an incubator at 30^{o}C for 24 h. Enzyme activity was evaluated by the diameter of the clear zone around the well after staining with lugol for amylase, cellulase, chitinase, and black amino for protease. Effects of temperatures, pH, and NaCl concentrations on the growth and antibacterial activity of isolated Streptomyces strains were examined. For this aim, streptomyces strains were cultured at different temperatures (30℃, 37℃, 45℃, and 50℃), pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10, 11, and 12), and NaCl concentrations (0.0%, 0.5%, 1.0%, 3.0%, 5.0%, 7.0%, and 9.0%).

Molecular Identification and Statistical Analyses

Total DNA extraction*:* New selected Streptomyces strain was cultured in liquid Gause I medium. After 7 days of incubation, the total DNA of this strain was extracted using the method described by Farhad *et al*. (2016). The 16S rRNA genes were amplified by primers 27F (5'AGAGTTTGATCCTGG CTCAG3') and 1492R (5′- GGTTACCTTGTTA CG ACTT - 3'). The PCR reaction cycles included: 95℃: 5 min, 29 cycles (94℃: 30 seconds, 53℃: 30 second, 72℃: 1 min), 72℃: 10 min, keep samples at 4℃. The PCR products were electrophoresed on agarose gel 1% and sent to the Molecular Laboratory of The Department of Molecular Biology and Applied Biotechnology, Faculty of Biotechnology, Vietnam National University of Agriculture for nucleotide sequencing. Sequencing of 16S rRNA has been made and compared with bacterial nucleotides in the GenBank database (www.ncbi.nlm.nih.gov). All raw data were statistically analyzed by the Excel version 2016.

Results and Discussion

Isolation of Streptomyces Strains

From the collected soil samples, we isolated 72 Streptomyces strains on the Gause I agar medium. These strains were distinguished from each other by the color of the aerial mycelia, based on the color table of Tresner and Buckus (Hien *et al*., 2014) and divided into 8 different color groups as shown in Table 1.

Table 1. *Color groups of isolated Streptomyces strains*

Color Group	Quantity of isolated strains	Ratio (%)
White	37	51.39
Grey	16	22.22
Yellow	6.0	8.33
Red	4.0	5.56
Blue	1.0	1.39
Green	1.0	1.39
Violet	2.0	2.78
Brown	5.0	6.94

Antibacterial Activity of the Isolated Strains

Actinobacteria can presently synthesize different types of secondary metabolites; many of these have exhibited pharmaceutically important biological activities with various promising biological activities. In fact, over 70% of anti-infection agents in nature were derived from actinomycetes. Streptomyces is the most prominent genus of actinomycetes known for the production of numerous bioactive metabolites (Sudha *et al*., 2019). In this study, after isolation and purification, 72 *Streptomyces* isolates were first evaluated against the 5 tested bacterial strains by the agar diffusion method as previously described by Mounyr *et al*. (2015). As the results shown

in Figure 2, it documented only one strain CP1.3 was able to antagonize all 5 tested bacterial strains. In a previous report, Keser *et al*. (2009) isolated 15 Streptomyces strains against *X. campestris* pv. *vesicatoria* with a diameter of antagonistic holo ranges from 8 mm to 21 mm. Some actinobacterial strains in the study of Mustafa *et al*. (2004) reported that *C. michiganensis* displayed the inhibition zone in diameter of these strains ranging from 9-13 mm, equivalent antagonistic zone diameter of strain CP1.3 in our study. In the same investigation with the bacterial strain, Mustafa *et al*. (2004) found that the greatest antagonistic diameter was 13 mm and the lowest was 9 mm, both smaller than the diameter of the antagonistic clear halo diameter of CP1.3. The author identified 16 actinomycete strains that were antagonistic to 10 bacterial strains (Mustafa *et al*., 2004). On the other hand, only two actinomycete strains were able to combat at least 5 or more bacterial strains. The *Bacillus subtilis* strain was hostile to both actinomycete strains, while only one was antagonistic to the *Clavibacter michiganensis*. In another study, Zhao *et al*. (2019) showed antibacterial efficacy against two strains of bacteria, *Ralstonia solanacearum* and *Micrococcus luteus*, with an antagonist diameter of 23 mm for *Ralstonia solanacearum* bacteria. However, there was no inhibition of *Micrococcus luteus* activity. In this study, the identified CP1.3 strain was shown to be slightly feeble in antibacterial activity compared to the previous report but it can antagonize a wide range of bacterial species.

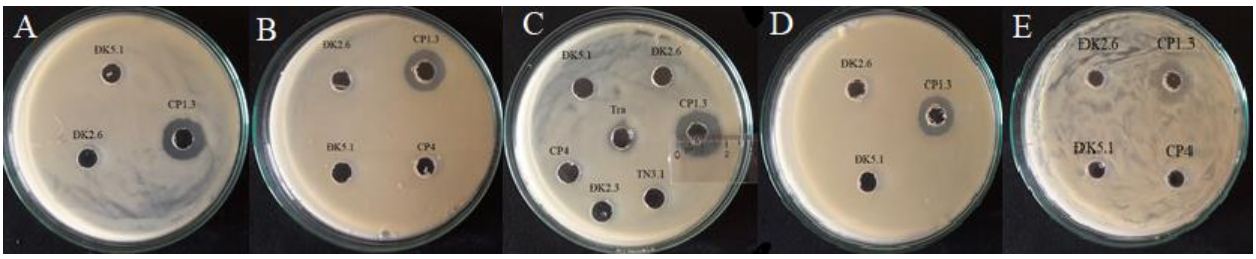

Figure 2. *The antagonistic clear halo of CP1.3*
(A) *Xanthomonas axonopodis;* (B) *Rastonia solanacearum;* (C) *Clavibacter michiganensis;* (D) *Xanthomonas* sp.*;* (E) *Bacillus subtilis*

Table 2. *CP1.3 strain antimicrobial activity against 5 bacterial strains*

Strains	Clear halo (mm)
Xanthomonas axonopodis	5.0±0.1
Rastonia solanacearum	4.0±0.1
Clavibacter michiganensis	11.0±0.1
Xanthomonas sp.	2.0±0.1
Bacillus subtilis	5.0±0.1

Minimum Inhibitory Concentrations (MICs) of CP1.3 Strain

Antibiotics have been routinely utilized to prevent and cure bacterial infections in recent years. On the other hand, drug resistance is caused by the misuse or abuse of antibiotics. As a result, the minimum inhibitory concentration (MIC) must be determined to suggest and manage antibiotic

concentrations. The antagonistic ability of CP1.3 was determined based on the diameter of the clear halo formed when the actinomycete extract was added. The amount of extract for all tested bacterial strains was 100 µl, followed by 50 µl, 25 µl, 12.5 µl, and 6.25 µl. The studied bacterial strains were not inhibited when the volume of extract employed was 3.125 µl. The least inhibitory concentration of the CP1.3 strain to inhibit *Xanthomonas axonopodis* was 6.25 µl, while the remaining *Rastonia solanacearum*, *Clavibacter michiganensis*, *Xanthomonas* sp., and *Bacillus subtilis* strains both had a minimum inhibitory concentration of 12.5 µl (Figure 2).

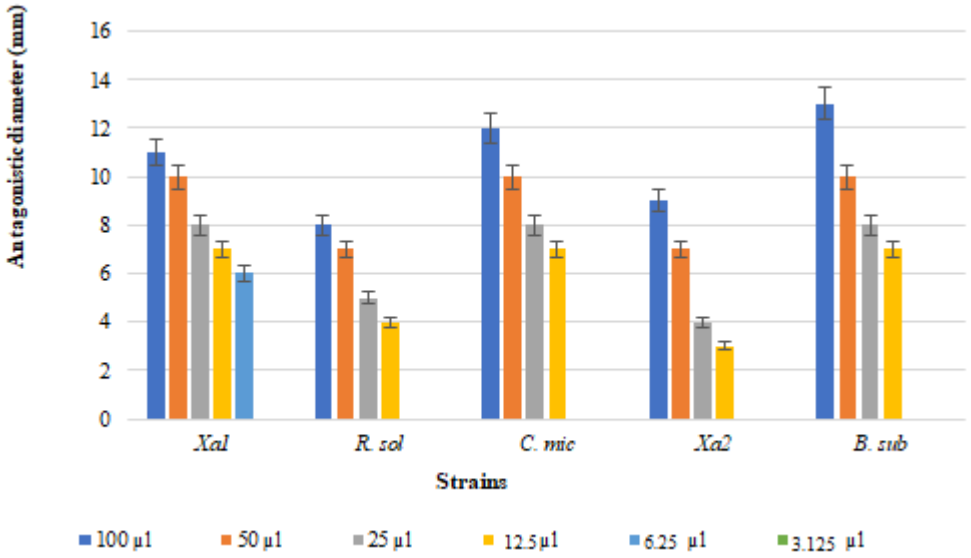

Figure 2. *The minimum inhibitory concentrations (MICs) of CP1.3 strain*

Investigation of Antifungal Activity of Actinomycetes CP1.3

Fusarium oxysporum is a fascinating fungus that extensively spreads in the environment and causes significant crop damage. In this experiment, the dual culture method approach was used to investigate the antagonistic activity of the selected CP1.3 strain against *F. oxysporum*. The results revealed that antagonism began after 4 days, and after 7 days, there was a definite antagonism with a percentage inhibition of 32.5%. The fungus developed over 42 days but did not reach the actinomycete surface (Figure 3). Previously, Hien *et al* (2014) reported that the strain HN1 could antagonize *F. oxysporum* and retain resistance for 19 to 22 days, which was shorter than the strain CP1.3 in our study.

In this study, the CP1.3 strain was selected to continue the research as it could biosynthesize the powerful antifungal agent *F. oxysporum*, retain great antifungal resistance, and have the ability to protect plants from *F.oxysporum* infection.

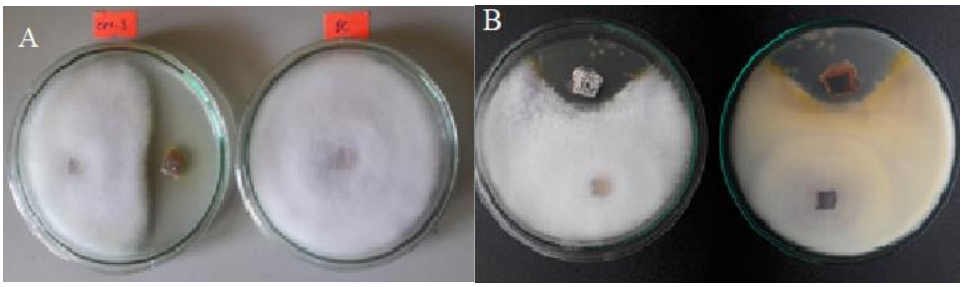

Figure 3*. Antagonist activity of CP1.3 strain against Fusarium oxysporum*

(A) 7 days; (B) 42 days

Biological characteristics of the selected actinomycete strain

Morphological characteristics

Actinomycetes are a group of Gram-positive bacteria with morphological characteristics similar to fungi. However, when cultivated on different mediums, actinomycetes have a wide variety of colors (Alice *et al*., 2018). One of the main criteria for distinguishing actinomycetes strains is the composition of the growth medium, the substrate mitochondria, the gaseous mycelium's capacity to create soluble pigments, and the development of melanin (Table 3). Because melanin production causes the color of the culture medium to change from yellow to dark brown or black, the color of the CP1.3 strain's culture media did not change from 24 h of culture to the 21st day of culture. As a result, strain CP1.3 did not produce melanin pigment. Some previous studies reported the same our findings (Hien et al., 2014; Lan et al., 2015; Loi et al., 2013). However, Thao *et al.* (2016) and Tuong *et al* (2015) have shown that there are actinomycete strains capable of generating melanin pigment. The morphology of the filaments on the slide was observed under the optical microscope after 24 h of incubation, and spores developed after 36 h. The sporophyte chain of the CP1.3 strain is branching, segmented, and helical (Figure 4).

Table 3. *Morphological characteristics of strain CP1.3 on different media*

Medium	**7 days**		**14 days**		**21 days**	
	AM	**SM**	**AM**	**SM**	**AM**	**SM**
Gause I	White	Yellow	White, violet	Brown	Grey, violet	Dark brown
Gause II	Brown	Brown	Brown	Brown	Brown	Brown
ISP1	White	Brown	White	Brown	White	Dark brown
ISP2	Brown	Brown	White, brown	Brown	Grey	Dark brown
ISP3	White, grey	Brown	Gray, white	Dark brown	Grey	Dark brown
ISP4	White	Brown	Grey	Dark brown	Grey	Dark brown
ISP5	White, dark brown	Black	White, dark brown	Black	Grey	Black
ISP6	White	Brown	White	Brown	White	Brown
ISP7	White, black	Black	White, black	Black	Grey, black	Black

Note: AM: Aerial mycelium, SM: Substrate mycelium

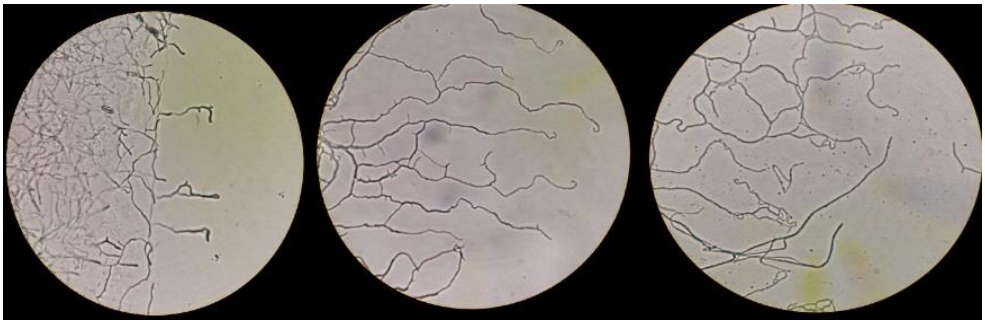

Figure 4*. Spore production sequence of CP1.3 strain*

Physiological and biochemical properties

In this study, our survey on the growth ability of strain CP1.3 on ISP9 medium supplemented with different carbon sources showed that CP1.3 strain could grow well in carbon sources such as D-glucose, D-sorbitol, and maltose; medium growth in carbon sources such as L-arabinose, D-galactose, L-rhamnose, dextrin and weak growth in D-fructose and D-xylose carbon sources. We further examined for the presence of chitinase, cellulase, and protease enzymes, it showed that the CP1.3 strain could synthesize all 3 enzymes, most noticeably chitinase (16mm) activity, as shown in Figure 5. As a consequence, actinomycete extract would be digested by animal corpses such as crabs, shrimp, and crab shells, reducing smell pollution in the environment. On the other hand, chitin is a crucial component of the fungal cell wall, and actinomycetes can make chitinase, thus when utilizing them to prevent fungal illnesses, the chitinase enzyme will come into play and boost the antagonistic impacts.

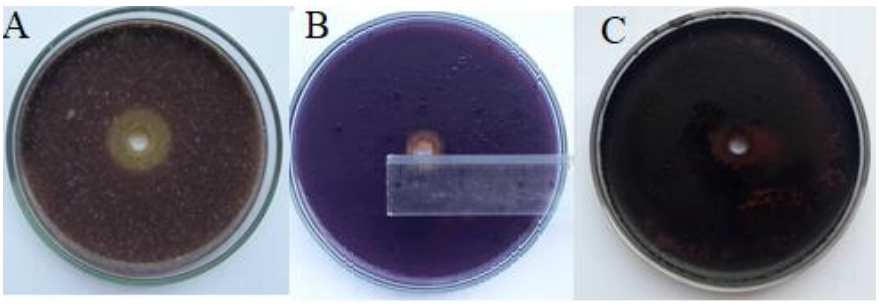

Figure 5. *The ability to produce extracellular enzymes of actinomycetes CP1.3*
(A) *Chitinase; (B) Cellulase; (C) Protease*

Environmental conditions have a significant impact on microorganisms' life and metabolic activities. As a result, strain CP1.3 was grown on Gause I medium at various temperatures, pH levels, and salt concentrations. The results revealed that the CP1.3 strain developed and had high antibacterial activity at 30°C, pH 7.0-8.0, and was tolerant to salt concentrations up to 1.0% and was ranked as a low salt tolerance group (Larsen *et al*., 1986).

Molecular Identification of Actinomycetes CP1.3

In this experiment, we employed a molecular biology approach based on the similarity of this strain's 16S rRNA gene fragment with actinomycetes strains in the GeneBank in order to identify the actinomycetes in this study. Actinomycetes' DNA was isolated using the method of Farhad *et al* (2016). To amplify the 16S rRNA gene segment of CP1, the primers 27F and 1492R were applied. Three strains were utilized, and electrophoresis yielded a single DNA band of roughly 1500 bp, which was within the theoretically possible size, while using this primer for multiplexing (Figure 6).

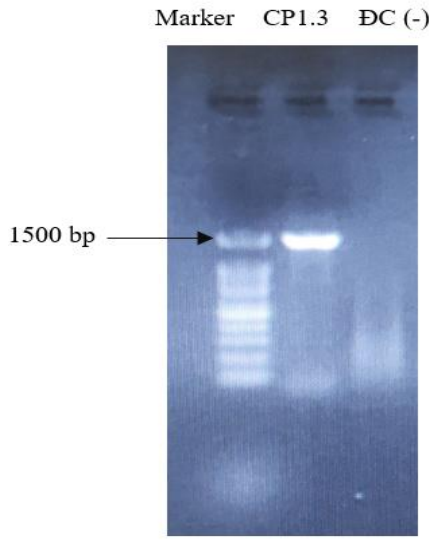

Figure 6. *PCR results were generated with primers from the actinomycete CP1.3 16S rRNA region*

As the results attained, the nucleotides in PCR products were sequenced. After getting the sequence, use the BLAST tool to compare it to other sequences in the gene bank and use Mega X software to create a phylogenetic tree for the CP1.3 strain. The results are shown in Figure 7.

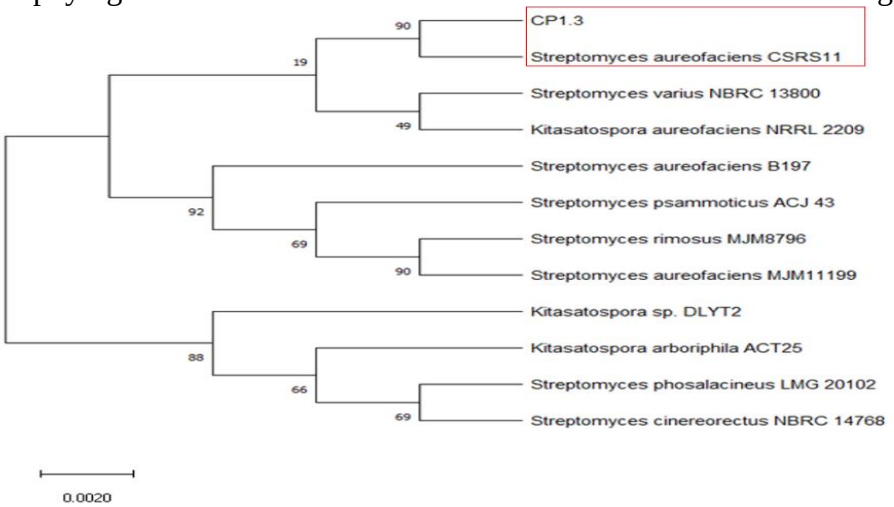

Figure 7. *Phylogenetic tree based on 16S rRNA sequence of actinomycete strain CP1.3*

With a bootstrap value of 90, this phylogenetic tree showed that the CP1.3 strain belongs to the same value as *Streptomyces aureofaciens* CSRS11. Furthermore, nucleotide sequencing data revealed a 99.03 % similarity of 16S rRNA between actinomycete strains CP1.3 and *Streptomyces aureofaciens* CSRS11. These two strains are comparable in terms of dependability and resemblance. As a result, we concluded that the CP1.3 strain is closely related to *Streptomyces aureofaciens* CSRS11, and I called it *Streptomyces aureofaciens* CP1.3.

Conclusions

In conclusion, 72 actinomycetes strains were isolated and purified, and CP1.3 actinomycetes strains were chosen for their significant antimicrobial activity against 5 *Xanthomonas axonopodis*, *Rastonia solanacearum*, *Clavibacter michiganensis*, *Xanthomonas* sp., and *Bacillus subtilis* strains. Strain CP1.3 is not only has significant antibacterial properties, but it can also inhibit the growth of the fungus *Fusarium oxysporum*. At 30°C, pH 7-8, salt tolerance up to 1.0%, and good utilization of carbon sources (D-glucose, D-sorbitol, and maltose), the CP1.3 strain develops and has high antibacterial potential. This strain can produce extracellular enzymes: chitinase, cellulase, and protease. The comparison and analysis of 16S rRNA nucleotide sequences from actinomycete strain CP1.3 with nucleotide sequences from *Streptomyces aureofaciens* on Genbank discovered that this actinomycete strain is closely related to *Streptomyces aureofaciens*.

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References

Alice, M.B.F., Aruna, S.S., Anbumalarmathi, J. (2018). Isolation and characterization of actinomycetes from marine soil. MOJ Biology and Medicine. 3(6):221-225.

Dhanasekaran D., N. Thajuddin and A. Panneerselvam (2012). Applications of Actinobacterial Fungicides in Agriculture and Medicine. In book: *Fungicides for Plant and Animal Diseases*. (Dharumadurai et al eds), 2012, 29-54.

Giang, N.V., Hoai, T.T., Trang, V.M., Trung, K.H., Xuan, T.D., Duong, V.X., Huyen, P.K., Diep, V.T.N., Trung, N.T., Khanh, T.D., Thu, P.T.L. (2020). Isolation and characterization of potassium solubilizing bacteria in some Vietnamese soil samples. International Journal on Emerging Technologies, 11(3): 639-643.

Giang, N.V., Quan, N.T., Nhung, N.T., Linh, T.T.D., Anh, T.H., Trung, D.T., Thuy, T.T.D., Tran, N.P.B., Trung, K.H., Khanh, T.D. (2022). Evaluation of the IAA-producing ability of some endophytic bacteria isolated from Panax pseudoginseng roots. Vietnam Journal of Agriculture and Rural Development, 3(2):2-10.

Ha,T.D., Thu, H.C., Dung, P.T.N., Suong, D.T.N. (2010). Research on the distribution and dynamics of soil microflora in Dien Thang Nam commune - Dien Ban - Quang Nam. The University of Danang - Journal of Science and Technology (JST-UD), 4:50. (*in Vietnamese with English abstract*).

Hien, L.T., Loi, D.V., Van, V.T., Giang, N.V. (2014). Isolation and screening Streptomyces spp. Against plant pathogenic fungi. Journal of Science & Development, 12(5): 656-664 (*in Vietnamese with English abstract*).

Dong, S.W., Quan-hong Xue, Yun-yan Ma, Xiao-li Wei, Jie Chen and Fei He (2014). Oligotrophy is Helpful for the Isolation of Bioactive Actinomycetes. Indian J Microbiol. Doi 10.1007/s12088-014-0444-1.

Farhad, M.A., Leila, J., Reza, K.N. and Ali. A. (2016). A Simple and Rapid System for DNA and RNA Isolation from Diverse Plants Using Handmade Kit. Doi:10.1038/protex.2016.015.

Hasegawa S., A. Meguro, M. Shimizu, T. Nishimura and H. Kunoh (2006). Endophytic Actinomycetes and Their Interactions with Host Plants. Actinomycetologica, 20:72-81.

Keser S., Mutlu M.B. and Güven K. (2009). Antimicrobial Effect of Halophilic Actinomycetes on *Xanthomonas* and *Pseudomonas* Tomato Disease Agents. In: *ISHS Acta Horticulture 808: II International Symposium on Tomato Diseases*. pp.401-404. https://doi.org/10.17660/ActaHortic.2009.808.67 .

Larsen, H. (1986). Halophilic and halotolerant microorganism: an overview historical perspective. FEMS Microbiology Reviews, 24: 2235-2241.

Loi, V.V., Hieu, N.V., Lien, N.T.H., Hop, P.T.B., Thao, P.T.H. (2013). *Streptomyces chartreusis* cp23x9 producing xylanase: Biological characteristics and classification. Journal of Biology, 35(3se):45-50 (*in Vietnamese with English abstract*).

Mounyr, B., Moulay, S. and Saad, K.I. (2016). Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2):71-79.

Mustafa, O., A. Üsame, T., and Cem. A. (2004). Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. African Journal of Biotechnology, 3 (9):441-446.

Lan, N.T.P., Ngan, V.T.T., Loc, T.P., Anh, T.H. (2015). election of Streptomyces spp. Isolates with Antifungal Activity against Rice Blast Fungus, *Pyricularia grisea*. Journal of Science and Development, 13(8):1442-1451.

Thao, P.T.H., Linh, N.V.N., Lien, N.T.H., Tam, N.K.B., Hieu, N.V. (2016). Endophytic Actinomyces *Streptomyces parvilus* HNR3X4 in Ha Noi -Dien Grapfruit Trees and the Potential to Biosynthetize Antimicrobial Substances. VNU Journal of Science, 32(1s):327-333 (*in Vietnamese with English abstract*).

Sudha, K.B., Krishna, P.S. and Sreenivasuluc, K. (2019). Screening and identification of novel isolate *Streptomyces* sp., NLKPB45 from Nellore coastal region for its biomedical applications. Saudi Journal of Biological Sciences, 26(7):1655–1660.

Tuong, L.M., Vu, P.T., Giang, V.L. (2015). Identification of actinomycetes as potential antagonistic ability against bacterial leaf blight disease of rice. Can Tho University Journal of Science, 41: 46-52 (*in Vietnamese with English abstract*).

Velu Rajesh Kannan, Kubilay Kurtulus Bastas, and Robert Antony (2015). Plant Pathogenic Bacteria: An Overview. pp. 1-15.

Zhao, J., Han, L., Yu, M., Cao, P., Li, D., Guo, X., Liu, Y., Wang, X., Xiang, W. (2019). Characterization of *Streptomyces sporangiiformans* sp. nov., a Novel Soil Actinomycete with Antibacterial Activity against *Ralstonia solanacearum.* Microorganisms, 7(9): 360; https://doi.org/10.3390/microorganisms7090360 .