

## PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS AND IDENTIFICATION OF ACTINOMYCETE STRAINS ISOLATED FROM SOIL SAMPLES IN HANOI

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### ABSTRACT

Actinomycetes are a rich source of natural compounds with diverse biological activities. In this study, the actinomycete strain *Streptomyces* sp. CH8.1 was identified as *Streptomyces virginiae* through 16S rDNA sequence analysis, alongside the determination of its physiological and biochemical characteristics. Strain CH8.1 demonstrated robust growth on media typical for actinomycetes and could utilize most common nitrogen and carbon sources, including sucrose, maltose, lactose, mannitol, galactose, xylose, cellobiose, glycerin, raffinose, tyrosine, tryptone, peptone, and certain ammonium salts. CH8.1 exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* CCARM 3640 (MIC of 32 µg/mL), *Salmonella enterica* ATCC 14028 (MIC of 16 µg/mL), *Kocuria rhizophila* NBRC 12708 (MIC of 32 µg/mL), *Bacillus subtilis* VTCC 6633 (MIC of 8 µg/mL), *Escherichia coli* ATCC 25922 (MIC of 32 µg/mL), and *Vibrio* sp. (MIC of 16 µg/mL). The R2YE medium was identified as the most suitable for producing antimicrobial compounds. And the CH8.1 strain was demonstrated to contain the type II PKS gene cluster, while lacking the type I PKS gene cluster.

**Keywords:** Actinomycetes, antibacteria, isolation, natural product, *Streptomyces*

### INTRODUCTION

*Actinobacteria* are Gram-positive bacteria and one of the most prevalent groups among the 18 major bacterial lineages. They exhibit diverse morphologies, such as highly differentiated branching filaments, fragmented filaments, rod-shaped coccoids, or spherical coccoids (Ventura *et al.*, 2007). *Streptomyces*, one of over 120 genera within the order *Actinomycetales*, is widely

distributed in soil, characterized by a distinctive earthy odor and branched filamentous cells (Chater, 2016).

*Streptomyces* plays a significant role in ecosystems, including promoting plant growth, enhancing resistance to plant pathogens, and suppressing plant diseases. For instance, *Streptomyces* spp. has shown the ability to inhibit *Fusarium oxysporum*, a fungus causing wilt in mung beans and

bananas. Endophytic *Streptomyces* from citrus plants can resist various fungi, such as *F. oxysporum*, *Phytophthora parasitica*, *Colletotrichum sublineolum*, *Pythium* sp., and *Guignardia citricarpa* (Gopalakrishnan *et al.*, 2020). However, some *Streptomyces* species are plant or vertebrate pathogens, including humans. For example, *S. turgidiscabies*, *S. acidiscabies*, and *S. scabies* cause scab disease in potatoes, leading to significant economic losses (Joshi *et al.*, 2007). *Streptomyces lanatus* has been associated with hypersensitivity pneumonitis in humans (Kofteridis *et al.*, 2007).

*Streptomyces* is renowned for its ability to synthesize a wide range of bioactive natural compounds, making it a primary source of bioactive substances (Seipke *et al.*, 2012). These compounds include antifungals, antibacterials, immunosuppressants, anticancer agents, antidiabetic drugs, herbicides, anthelmintics, cholesterol-lowering drugs, insecticides, and phytohormones (Chater, 2016). Approximately 25% of known antibiotics have been derived from *Streptomyces* (Olanrewaju and Babalola, 2019). These compounds belong to various classes, including non-ribosomal peptides (NRPs), polyketides (PKs), ribosomal peptides (RiPPs), terpenes, siderophores, and melanins.

In our previous research, 58 actinomycete strains were isolated from four agricultural soil samples in Hanoi. These samples were collected from the surface layer at various locations, reflecting ecological diversity. The strains were cultured in different media to determine their physiological and biochemical characteristics, and antibacterial activities against Gram-positive and Gram-negative bacteria. These

strains grew well on carbon sources such as glucose, sucrose, maltose, and glycerol, and nitrogen sources like tryptone, peptone,  $(\text{NH}_4)_2\text{SO}_4$ , and  $\text{NH}_4\text{NO}_3$ . They are currently preserved in the strain collection at the Institute of Biotechnology and Food Technology, Hanoi Open University. This study focuses on analyzing the physiological and biochemical characteristics, and antibacterial activity of the actinomycete strain CH8.1, along with its taxonomic classification.

## MATERIALS AND METHODS

### Microorganisms and culture media

The actinomycete strain CH8.1, isolated from agricultural soil in Hanoi, was preserved in 20% glycerol and stored at  $-80^\circ\text{C}$  at the Institute of Biotechnology and Food Technology, Hanoi Open University. After preservation, the strain was activated and cultured on R2YE and ISP3 media.

Six test microorganisms, including *Staphylococcus aureus* CCARM 3640, *Kocuria rhizophila* NBRC 12708, *Salmonella enterica* ATCC 14028, *Bacillus subtilis* VTCC 6633, *Escherichia coli* ATCC 25922, and *Vibrio* sp., were provided by the Institute of Biotechnology and Food Technology, Hanoi Open University. These strains were cultured on LB medium, containing tryptone (1 mg/mL), yeast extract (0.5 mg/mL), sodium chloride (1 mg/mL), and agar (10 mg/mL).

Various culture media were used to study the morphology of actinomycetes. The International *Streptomyces* Project (ISP) media, R2YE, and NDYE were applied with the following compositions: **ISP1**: tryptone (5 g/L), yeast extract (3 g/L), and agar (20 g/L), pH 7.0; **ISP2**: yeast extract (4 g/L),

malt extract (10 g/L), glucose (4 g/L), and agar (20 g/L), pH 7.3; **ISP3**: oat meal (20 g/L), agar (20 g/L), and trace salt solution (1 mL/L), pH 7.0; **ISP4**: starch solution (10 g/L),  $K_2HPO_4$  (1 g/L),  $MgSO_4 \cdot 7H_2O$  (1 g/L), NaCl (1 g/L),  $(NH_4)_2SO_4$  (2 g/L),  $CaCO_3$  (2 g/L), trace salt solution (1.0 mL/L), and agar (20 g/L), pH 7.5; **ISP5**: L-asparagine (1 g/L), glycerin (10 g/L),  $K_2HPO_4$  (1 g/L), trace salt solution (1.0 mL/L), and agar (20 g/L), pH 7.0; **ISP6**: peptone (10 g/L), yeast extract (1 g/L), ferric ammonium citrate (0.5 g/L), and agar (20 g/L), pH 7.0; **ISP7**: glycerin (15 g/L), L-tyrosine (0.5 g/L), L-asparagine (1 g/L),  $K_2HPO_4$  (0.5 g/L),  $MgSO_4 \cdot 7H_2O$  (0.5 g/L), NaCl (0.5 g/L), ferrous sulfate heptahydrate (0.01 g/L), trace salt solution (1.0 mL/L), and agar (20 g/L), pH 7.0; **R2YE**: sucrose (10.3 g/L),  $K_2SO_4$  (0.025 g/L),  $MgSO_4 \cdot 6H_2O$  (1.012 g/L), glucose (1.0 g/L), yeast extract (0.5 g/L), trace salt solution (1.0 mL/L), TES (5.72 g/L),  $CaCl_2 \cdot 2H_2O$  (14.7 g/L), trace salt solution (2 mL/L), L-proline (1.5 g/L), and NaOH (0.2 g/L), pH 7.2; **NDYE**: yeast extract (0.5 g/L),  $NaNO_3$  (0.428 g/L), maltose (1.5 g/L), glucose (2.5 g/L), HEPES (0.477 g/L),  $MgSO_4 \cdot 7H_2O$  (0.012 g/L),  $KH_2PO_4$  (0.023 g/L), trace salt solution (2 mL/L) and NaOH (0.4 g/L).

### **Biological characteristics of strain CH8.1**

The morphological characteristics of actinomycetes were assessed based on the color of aerial and substrate mycelia, the formation of soluble pigments, and melanin production, following the method of Tresner and Backus (1963). According to the International Streptomyces Project (ISP, 1970), aerial mycelium colors were classified into eight groups: White, Grey, Red, Yellow, Green, Blue, Violet, and Undefined (X). Substrate mycelia could be

grey or colorless. These observations are crucial for the classification and identification of actinomycete strains and provide insights into their pigment production under specific culture conditions.

### **Morphological characteristics**

After 7 and 14 days of cultivation, the spore chains and spore surfaces of strain CH8.1 were analyzed using Gram staining and observed under an electron microscope, following the procedures of Shirling and Stanley (Shirling and Gottlieb, 1966; Wood and Krieg, 1989; Lambert and Loria, 1989). Spore chain morphologies were classified as straight (R), slightly curved (RF), hooked (RA), or spiral (S). Spore surfaces were categorized as smooth (SM), warty (WA), spiny (SP), or hairy (HA).

### **Biochemical characteristics**

The ability of the strain to assimilate carbon and nitrogen sources was evaluated by culturing on ISP9 medium, containing ammonium sulfate (2.64 g/L), potassium dihydrogen phosphate (2.38 g/L), potassium hydrogen phosphate trihydrate (5.65 g/L), magnesium sulfate heptahydrate (1 g/L), solution B (1.0 mL), and agar (20 g/L), adjusted to pH 7.0. Assimilation experiments were conducted by adding 1% carbon sources and 0.1% nitrogen sources, following Shirling and Stanley (Shirling and Gottlieb, 1966; Wood and Krieg, 1989; Lambert and Loria, 1989).

The growth of strain CH8.1 was influenced by environmental factors, including temperature, initial pH, and sodium chloride concentration. ISP2 medium was used to evaluate growth under varying conditions, following Stanley and Holt (Wood and Krieg, 1989).

### **Temperature effects**

Strain CH8.1 was cultured at temperatures of 20°C, 24°C, 28°C, 32°C, 36°C, and 40°C to determine the optimal growth range.

#### **Initial pH effects**

The pH of the culture medium was adjusted from 5.0 to 10.0 (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0) to assess its impact on growth.

#### **NaCl concentration effects**

Experiments were conducted with NaCl concentrations ranging from 1% to 10% (including 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, and 10%) to determine the strain's salt tolerance.

These results helped identify the optimal environmental conditions for the growth of strain CH8.1, providing a foundation for further studies on its biological applications.

#### **Antibacterial activity of strain CH8.1**

The antibacterial activity of strain CH8.1 was evaluated using the agar well diffusion method, following Shirling and Gottlieb (1966) (Shirling and Gottlieb, 1966; Trinh *et al.*, 2016). The strain was cultured in liquid ISP1, ISP2, ISP4, NDYE, and R2YE media at 150 rpm and 28°C. After 5 days, the culture broth was centrifuged at 4000 rpm to remove cells. The supernatant was loaded into wells on LB agar plates, containing tryptone (10 g/L), yeast extract (5 g/L), NaCl (10 g/L), and agar (10 g/L) pre-inoculated with test microorganisms. Antibacterial efficacy was determined by the formation of inhibition zones around the wells after 18–24 hours of incubation. The diameter of the inhibition zones was used to assess the antibacterial activity of the culture supernatant.

The minimum inhibitory concentration (MIC) values for the active compound against six bacterial strains were determined using a microdilution technique, as outlined in a prior study (Al-Dhabi *et al.*, 2020; Minh *et al.*, 2016). The CH8.1 extract was solubilized in 1% DMSO (v/v) and subjected to serial dilutions in Mueller-Hinton broth (HiMedia, India) to prepare varying stock concentrations. Approximately 180 µL of freshly prepared bacteria ( $2 \times 10^4$  CFU/mL) was dispensed into 96-well plates, followed by the addition of 20 µL of the extract. The plates were incubated at 37°C over 24–48 hours. The assay was conducted in triplicate, and the MIC was defined as the lowest concentration that completely prevented microbial growth.

#### **Taxonomic classification based on 16S rDNA sequence analysis and PKS gene detection by PCR**

Total DNA of strain CH8.1 was extracted after 72 hours of cultivation in liquid R2YE medium, following the method of Sambrook and Russell (2001) (Sambrook, 1989). The extracted DNA was used as a template for PCR amplification of the 16S rDNA region, using the primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') (Genset). Additionally, genes related to PKS I and PKS II systems were amplified using primers K1F (5'-TSAAGTCSAACATCGGBCA-3') and M6R (5'-CGCAGGTTSCSGTACCAGTA-3') for PKS I, and KSaF (5'-TSGCSTGCTTGGAYGCSATC-3') and KSaR (5'-TGGAANCCGCCGAABCCGCT-3') for PKS II (Metsä-Ketelä *et al.*, 1999; Ayuso-Sacido *et al.*, 2005). The PCR thermal cycle

consisted of initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C (for 27F/1492R) or 58°C (for KSaF/KSaR) for 30 seconds and extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. PCR products were stored at 4°C.

PCR products were analyzed by electrophoresis on 1.0% agarose gel, with a 1Kb Plus DNA Ladder Marker as the reference. The products were purified and sent for sequencing at Apical Scientific Sequencing, Malaysia. The obtained nucleotide sequences were compared with the GenBank database using BLAST ([www.ncbi.nih.gov](http://www.ncbi.nih.gov)) to determine the taxonomic relationship of strain CH8.1.

## RESULTS AND DISCUSSION

### Morphological characteristics of strain CH8.1

After 7 days of cultivation on ISP1, ISP2, ISP3, ISP4, ISP5, ISP6, R2YE, and NDYE media, the morphological characteristics of

strain CH8.1, including the color of aerial and substrate mycelia and pigment formation, were observed and recorded. The mycelium colors were compared with the standard color chart proposed by Tresner and Backus to identify characteristic pigments (Tresner and Backus, 1963).

Table 1 summarizes the morphological characteristics of strain CH8.1 on different culture media. The color of aerial mycelia (AM) and substrate mycelia (SM) varied depending on the nutrient composition of each medium. Specifically, on ISP1, the aerial mycelia were white, while on ISP2–ISP6, R2YE, and NDYE, they ranged from light grey to dark grey. The substrate mycelia were white on ISP1 and ISP3, light yellow on ISP2, ISP4, ISP5 and ISP6, and dark yellow on R2YE and NDYE.

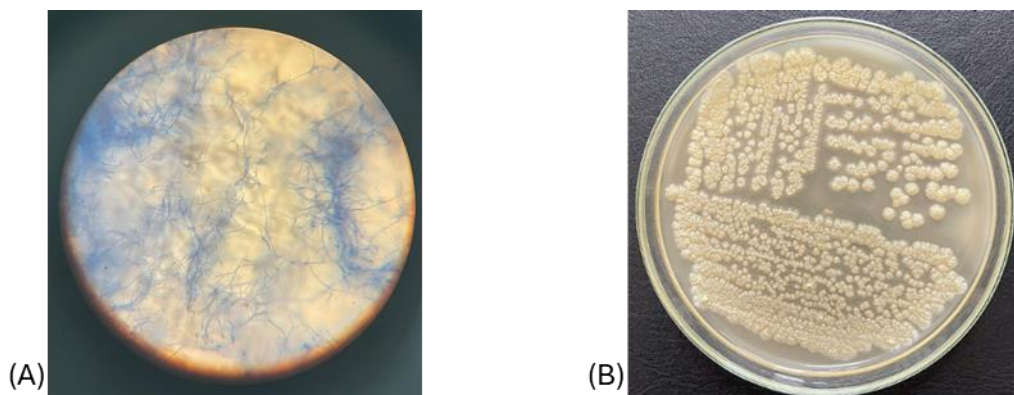
Strain CH8.1 did not produce melanin pigments on any of the tested media. However, soluble pigments were observed on ISP2, ISP4, R2YE, and NDYE, with colors ranging from light yellow to dark yellow.

**Table 1.** Morphological characteristics of strain CH8.1 on different culture media

Culture medium	Color		Pigment	
	Aerial mycelia	Substrate mycelia	Soluble pigment	Melanin pigment
ISP1	White	White	-	-
ISP2	Light grey	Light yellow	Light yellow	-
ISP3	Light grey	White	-	-
ISP4	Light grey	Light yellow	Light yellow	-
ISP5	Light grey	Light yellow	-	-
ISP6	Light grey	Light yellow	-	-
R2YE	Grey	Dark yellow	Light yellow	-
NDYE	Grey	Dark yellow	Light yellow	-

Under electron microscopy at 1000X magnification, strain CH8.1, after 7 days of cultivation in liquid ISP4 medium, exhibited long, branched filamentous mycelia with secondary filaments branching from the primary ones. The spore-bearing filaments were slightly curved. Additionally, after

dilution and plating on solid ISP4 medium, followed by incubation at 28°C for 7 days, the strain formed colonies with light yellow color, convex surfaces, distinct wrinkles, and radial grooves. Colony diameters ranged from 3–5 mm (Figure 1).



**Figure 1** Morphological characteristics of strain CH8.1 on ISP4 medium (A) Mycelia observed under a microscope at 1000X magnification. (B) Colony morphology after 7 days of cultivation.

### Physiological and biochemical characteristics

According to Nomomura (1974), the ability to utilize carbon and nitrogen sources is a critical physiological characteristic of actinomycetes. In this study, strain CH8.1 was cultured on ISP9 medium supplemented with various carbon and nitrogen sources to evaluate its assimilation capabilities.

Strain CH8.1 could not grow in media lacking carbon or nitrogen sources (Table 2), confirming the essential role of these compounds in its development. Regarding carbon assimilation, CH8.1 effectively utilized sucrose, maltose, D-galactose, D-cellobiose, glycerol, and raffinose, as evidenced by strong growth on media containing these compounds. In contrast, it exhibited weak or no assimilation of lactose, D-mannitol, and D-xylose, indicating limitations in its enzyme systems for

degrading these sugars. For nitrogen sources, CH8.1 grew well on all tested organic and inorganic nitrogen sources, including L-tyrosine, tryptone, peptone,  $(\text{NH}_4)_2\text{SO}_4$ , and  $\text{NH}_4\text{NO}_3$ , demonstrating its ability to metabolize both organic and inorganic nitrogen for growth.

Environmental conditions significantly influenced the growth and development of strain CH8.1. The strain was cultured on ISP4 medium under varying temperature, pH, and NaCl concentrations to determine optimal growth conditions. The results showed that CH8.1 grew within a temperature range of 25–35°C, with an optimum at 28°C, indicating a preference for moderate temperatures typical of many actinomycetes. Regarding pH, CH8.1 grew within a pH range of 6–10, with an optimum at pH 7.0, suggesting adaptability to neutral to slightly alkaline environments, a trait common among antibiotic-producing

actinomycetes. For NaCl concentration, CH8.1 grew at 1–10%, with an optimum at 3% (Table 2).

**Table 2.** Carbon and nitrogen source utilization and culture conditions for strain CH8.1

Carbon and nitrogen source (1.0%, w/v) and culture conditions	Growth ability
No carbon	-
Sucrose	+
Mantose	+
Lactose	+/-
D-mannitol	+/-
D-galactose	+
D-xylose	+/-
D-cellobiose	+
Glycerin	+
Raffinose	+
No nitrogen	-
L-tyrosin	+
Tryptone	+
Peptone	+
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	+
NH <sub>4</sub> NO <sub>3</sub>	+
Optimal temperature	28°C
Optimal pH	7.0
NaCl concentration	3%

### Antibacterial activity of strain CH8.1

The antibacterial activity of crude extracts from strain CH8.1 cultured in different media is presented in Table 3 and Figure 2. The results demonstrate that CH8.1 produced antimicrobial compounds with a broad spectrum of activity against both Gram-positive (*S. aureus*, *B. subtilis*, and *K. rhizophila*) and Gram-negative (*S. enterica*, *E. coli*, and *Vibrio* sp.) bacteria. Notably, the culture medium significantly influenced the

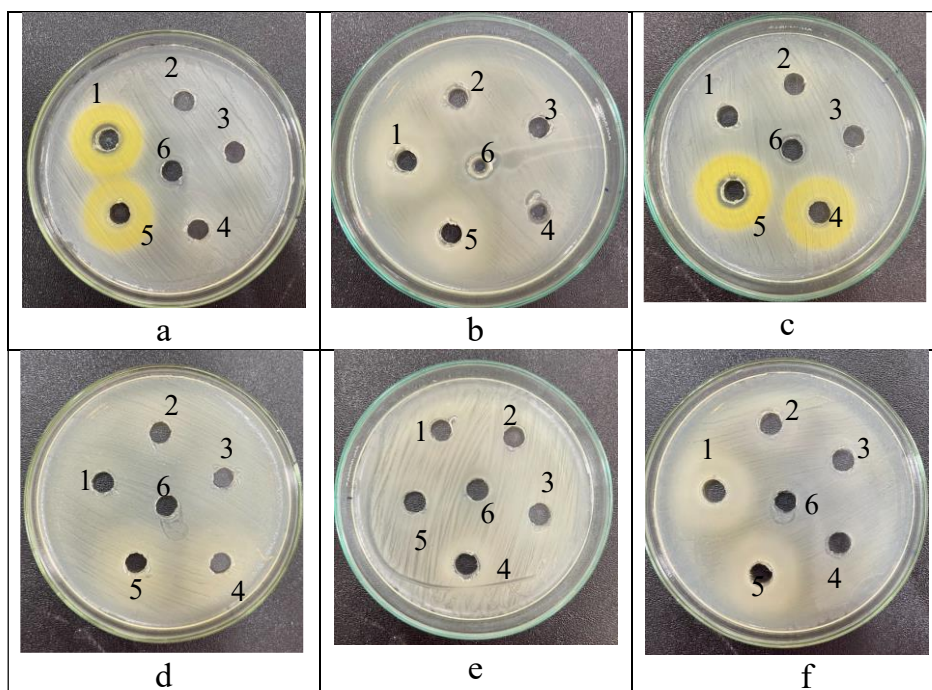
antibacterial efficacy of the extracts. ISP1 and R2YE media yielded the highest inhibition, with the largest inhibition zone diameters of 26 mm against *S. aureus* and *S. enterica* on ISP1, and 26 mm against *Vibrio* sp. on R2YE. ISP2, ISP4, and NDYE showed lower inhibition, with zone diameters ranging from 11–16 mm. The methanol control produced no inhibition zones, confirming that the observed activity was due to bioactive compounds produced by CH8.1. These findings highlight the

critical role of culture media in modulating antibiotic biosynthesis in strain CH8.1, with ISP1 and R2YE being optimal conditions.

Identifying suitable media enhances the production of antibacterial compounds and opens avenues for further research into the bioactive compounds synthesized by CH8.1.

**Table 3.** Antibacterial activity of crude antibiotics from strain CH8.1

Test microorganism	Inhibition Zone Diameter (D - d, mm)					
	ISP1 (1)	ISP2 (2)	ISP4 (3)	NDYE (4)	R2YE (5)	MeOH (6)
<i>Staphylococcus aureus</i> CCARM 3640	26	14	12	11	24	0
<i>Salmonella enterica</i> ATCC 14028	26	12	14	20	24	0
<i>Kocuria rhizophila</i> NBRC 12708	12	16	8	24	24	0
<i>Bacillus subtilis</i> VTCC 6633	14	14	14	24	26	0
<i>Escherichia coli</i> ATCC 25922	12	14	16	12	18	0
<i>Vibrio</i> sp.	24	14	14	14	26	0



**Figure 2.** Antibacterial activity of crude antibiotics against test microorganisms. a. *S. aureus* CCARM 3640, b. *S. enterica* ATCC 14028, c. *K. rhizophila* NBRC 12708, d. *B. subtilis* VTCC 6633, e. *E. coli* ATCC 25922, f. *Vibrio* sp. 1: CH8.1 culture broth on ISP1, 2: CH8.1 culture broth on ISP2, 3: CH8.1 culture broth on ISP4, 4: CH8.1 culture broth on NDYE, 5: CH8.1 culture broth on R2YE, 6: Methanol



To verify antimicrobial activity, the MIC of the crude CH8.1 extract against various indicator microorganisms were represented in Table 4. The lowest MIC value (8 µg/mL) was observed with *B. subtilis* VTCC 6633

followed by *S. enterica* ATCC 14028 (16 µg/mL) and *Vibrio* sp. (16 µg/mL). The highest MIC of 32 µg/mL was seen with the CH8.1 extract against *S. aureus* CCARM 3640 and *E. coli* ATCC 25922.

**Table 4.** MIC of CH8.1 crude extract against tested bacteria.

Tested pathogens	MIC (µg/mL)
<i>Staphylococcus aureus</i> CCARM 3640	32
<i>Salmonella enterica</i> ATCC 14028	16
<i>Kocuria rhizophila</i> NBRC 12708	32
<i>Bacillus subtilis</i> VTCC 6633	8
<i>Escherichia coli</i> ATCC 25922	32
<i>Vibrio</i> sp.	16

### Identification of strain CH8.1 and PKS gene detection

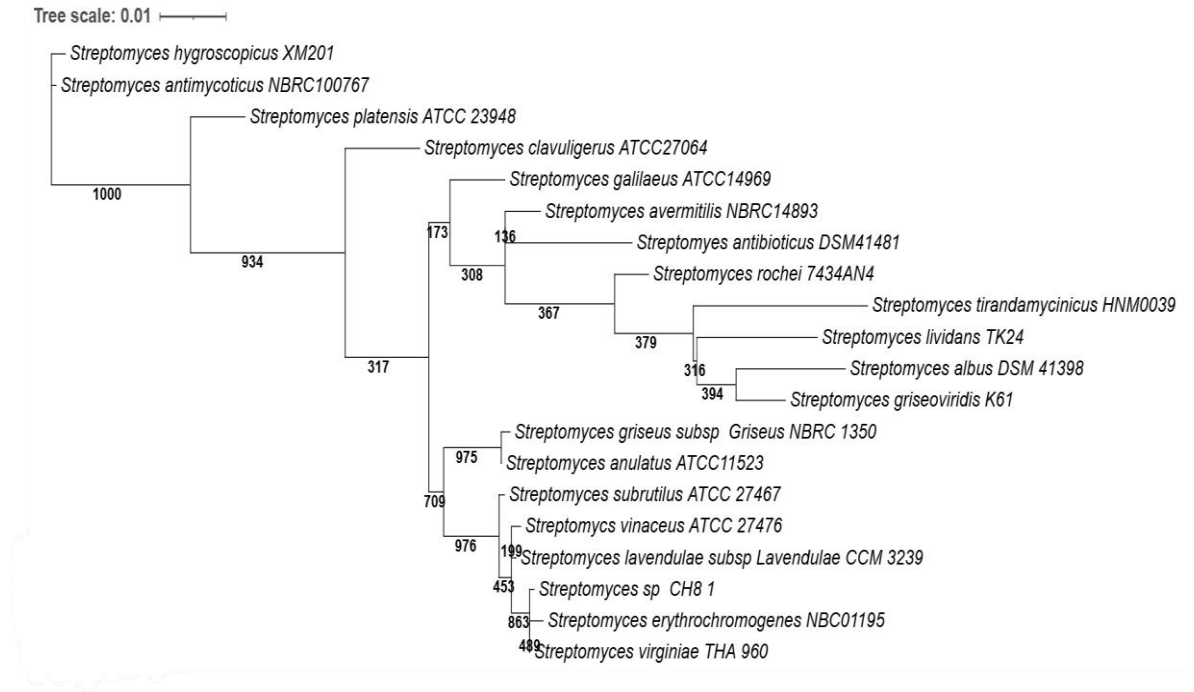
BLAST analysis of the 16S rDNA sequence of strain CH8.1 revealed high similarity with sequences in the GenBank database (NCBI). Specifically, CH8.1 showed 99.93% similarity with *Streptomyces virginiae* THA960, 99.74% with *Streptomyces erythrochromogenes* NBC01195, and 95.27%–99.40% with other strains (Figure 4). To determine evolutionary relationships, the 16S rDNA sequence of CH8.1 was aligned with highly similar strains using BioEdit, and a phylogenetic tree was constructed using MegaX. The analysis (Figure 4) indicated that CH8.1 is closely related to the *S. virginiae* group. Based on morphological, physiological, and biochemical characteristics according to the ISP classification system (Nomomura, 1974) and Bergey (Wood and Krieg, 1989), the strain was identified as *S. virginiae* CH8.1.

*Streptomyces virginiae* is a species known for synthesizing antibiotics, particularly virginiamycin, a streptogramin with potent activity against Gram-positive bacteria. It is used in medicine and agriculture to control pathogenic bacteria. Thus, the bioactivity of CH8.1 is likely attributed to virginiamycin.

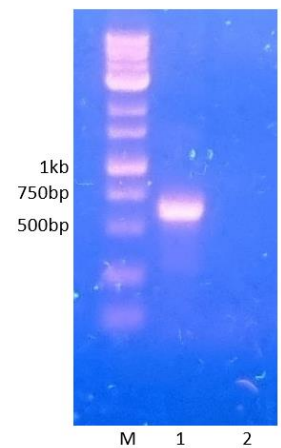
To identify the PKS gene cluster in strain CH8.1, two primer pairs, KSaF/KSaR and K1F/M6R, were used. These primers were designed by Metsa-Ketela *et al.* and Ayuso-Sacido *et al.*. The KSaF/KSaR primer pair was designed to amplify a segment of the KS $\alpha$  gene of the type II PKS gene cluster, while the K1F/M6R pair was used to amplify a part of genes encoding the type I PKS gene cluster. The results, shown in Figure 5, indicate that with the KSaF/KSaR primers, a band ranging from 500 bp to 750 bp was obtained, consistent with the expected amplified gene segment size of 600 bp for this primer pair. This suggests that the CH8.1 strain contains a cluster encoding

type II PKS. In contrast, no PCR product was obtained with the K1F/M6R primers, indicating that CH8.1 may lack a type I PKS gene cluster or that a type I PKS may be present in Ch8.1 but the primers failed to

specifically bind and amplify the target gene segment. To definitively conclude whether CH8.1 lacks the type I PKS-encoding gene, whole-genome sequencing of Ch8.1 is required.



**Figure 4.** Phylogenetic tree of *Streptomyces* sp. CH8.1 based on 16S rDNA sequences.



**Figure 5.** PKS gene detection on CH8.1 strain by PCR. M: FastGene® 1 kb DNA Marker Plus (NIPPON Genetics EUROPE) 1: PCR product using KSaF/KsaR primers 2: PCR product using K1F/M6R primers.

## CONCLUSION

The actinomycete strain CH8.1, isolated from agricultural soil in Hanoi, forms light yellow, convex, wrinkled colonies with radial grooves, ranging from 3–5 mm in diameter. It exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria, including six test microorganisms. Analysis of morphological, physiological, and biochemical characteristics, combined with 16S rDNA sequencing, confirmed high similarity with *S. virginiae*, identifying the strain as *S. virginiae* CH8.1. The strain utilized a wide range of carbon and nitrogen sources and grew optimally at 28°C, pH 7.0, and 3% NaCl. These biological properties suggest that *S. virginiae* CH8.1 has potential applications in the research and development of antibacterial compounds.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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