



Antifungal and antitumor activities of cellulose-degrading myxobacteria isolated from soil in Vietnam

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Abstract

Introduction: Myxobacteria are known to produce epothilone, a natural macrolide compound with significant antitumor activity achieved through the inhibition of microtubules formation during cell division, which is a mechanism critical to chemotherapy. This study investigated the antifungal and antitumor activities of myxobacteria isolated from soils enriched with decomposed plant material.

Methods: Myxobacterial strains were isolated and screened by examining macroscopic characteristics, assessing cellulose degradation using filter paper as the sole carbon source, and observing the presence of fruiting bodies and motility on slides. Selected strains were cultured in CK6 medium containing Amberlite XAD-16 resin, and active compounds were eluted using methanol. Antifungal activity was evaluated using disk diffusion and thin-layer chromatography autoradiography, and cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

Results: Among the 41 soil samples, three strains (QN01, QN02, and QN04) demonstrated cellulose degradation capability and exhibited morphological characteristics consistent with the *Sorangium* sp. The QN02 strain exhibited antifungal activity against pathogens, including *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum gypseum*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium* sp., and *Mucor* sp. Furthermore, extracts from QN02 inhibited MCF-7 breast cancer cells ($IC_{50}=159.1$ ng/mL) and human embryonic kidney cells (HEK-293) human kidney cells ($IC_{50}=528.1$ ng/mL).

Conclusions: The QN02 strain is a promising source of antifungal and antitumor compounds.

Keywords: antifungal agents; antineoplastic agents; cellulose; cytotoxins; myxococcales

1. INTRODUCTION

Cancer remains one of the most significant global health challenges and the second leading cause of death, with over

19 million new cases and 9.7 million cancer-related deaths reported annually [1]. In Vietnam, cancer is the second leading cause of mortality, following cardiovascular diseases [2]. Despite advancements in treatment, cytotoxic drugs remain cen-

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tral to chemotherapy; however, their non-specific mechanisms often lead to adverse effects in healthy cells and tissues [3].

Recent advances in cancer biology, particularly in understanding oncogenes and their mechanisms, have driven the development of targeted therapies. Among them, cytotoxic drugs that disrupt microtubule function, such as taxanes and vinca alkaloids, have demonstrated notable efficacy. However, their clinical application is often restricted because of resistance and toxicity issues [4]. In the 1990s, a new class of antitumor agents named epothilone was discovered in the myxobacterium *Sorangium cellulosum* [5]. Epothilones, with their macrolide structure, inhibit cancer cell division by targeting functional microtubules [6]. Notably, they exhibit 1,000 to 5,000 times the potency of Taxol, with significantly reduced side effects. Additionally, analogs of epothilone B have been reported to be up to 35,000 times more potent than Taxol against multidrug-resistant cell lines, highlighting their promise in cancer treatment [7].

The first epothilone was isolated from the *S. cellulosum* strain So ce90 in 1985 at the Gesellschaft für Biotechnologische Forschung in Braunschweig, Germany, and it exhibited selective antifungal activity against *Zygomycete* and *Mucor hiemalis* when cultured in liquid medium [8]. Myxobacteria, typically found in soils rich in decaying vegetation and herbivorous feces, can degrade crystalline cellulose and are cultured using filter paper as the sole carbon source in mineral-salt agar with an inorganic nitrogen source, such as KNO_3 or $(\text{NH}_4)_2\text{SO}_4$. These bacteria are prolific producers of biologically active secondary metabolites, including epothilone, which has been isolated from diverse *S. cellulosum* strains globally [9,10].

Despite this potential, limited research in Vietnam has examined myxobacteria for their antifungal and antitumor properties, particularly those related to epothilone. This study identified myxobacterial strains from Vietnam capable of producing novel bioactive compounds with antifungal and antitumor activities.

2. MATERIALS AND METHODS

2.1. Soil sample collection and strain isolation

Following established protocols for isolating cellulose-de-

grading microorganisms from humid, high-temperature environments with high organic carbon contents (e.g., decaying wood and haystacks), 41 soil samples were collected from various provinces and cities in Vietnam (Fig. 1). Each sample was labeled with a unique code consisting of the first two letters of the sampling site and a two-digit ordinal number. Samples were stored in sterile 50 mL Falcon tubes (Thermo Fisher Scientific, Waltham, MA, USA; catalog number: 339653), with each tube containing 25% to 50% soil.

Strains of dermatophytes, molds, and cell lines were obtained from the Department of Microbiology - Parasitology, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam. These included *Trichophyton mentagrophytes*, *Microsporium gypseum*, *Trichophyton rubrum*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* sp., *Fusarium* sp., and *Mucor* sp. The cell lines used were breast cancer cells (MCF-7), human embryonic kidney cells (HEK-293), and human liver carcinoma cells (HepG2).

2.2. Isolation of cellulose-degrading myxobacterial species

Myxobacterial strains were isolated using the following four-step process:

Step 1: Macroscopic examination

Bacteria were initially cultured on CY agar (containing 0.3% casitone, 0.1% yeast extract, 0.1% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.5% agar, and pH adjusted to 7.2) and VY/2 agar (containing 0.5% bakers' yeast, 0.1% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.5 mg/ μL cyanocobalamin, 1.5% agar, and pH adjusted to 7.2) to observe colony characteristics. Cultures were prepared in triplicate to ensure consistency.

Step 2: Microscopic observation

Gram staining was conducted using a Gram staining kit (Sigma-Aldrich, St. Louis, MO, USA; catalog number: 77730), followed by microscopic analysis of bacterial morphology. Each observation was repeated three times independently to confirm reproducibility.

Step 3: Cellulose degradation assessment

Strains were screened for cellulose degradation in carboxy methyl cellulose (CMC) medium (containing 1 g K_2HPO_4 , 1

slide and solidified. A fruiting body was inoculated onto the slide, sealed with a 1:1 mixture of vaseline and paraffin, and incubated at 30°C for 3–10 days. Fruiting bodies were collected using a sterile toothpick and were transferred to CMC medium. *Bacillus subtilis* PY79 served as the positive control for cellulose degradation, whereas *Escherichia coli* served as the negative control. After 4–5 days of incubation at 30°C, cellulose degradation was detected using Congo red staining (0.1% w/v; Sigma-Aldrich; catalog number: C6767) and NaCl (1M; Sigma-Aldrich; catalog number: S9888). A clear zone around the colony indicates positive cellulose degradation. All degradation tests were conducted in triplicate to ensure consistent results.

2.3. Crude extracts from myxobacterial culture

Primary bacterial cultures were initially grown in M26 medium (containing 0.8% potato starch, 0.2% glucose, 0.2% tryptone, 0.2% yeast extract powder, 0.1% CaCl₂·2H₂O, 1 mL/L trace elements solution, 1% agar and pH adjusted to 7.0) at 200×g for 3 days. The strains were then transferred to 100 mL of CK6 medium (containing 0.15% MgSO₄·7H₂O, 0.002% Fe³⁺ citrate, 0.2% KNO₃, 0.025 K₂HPO₄, 0.5% glucose, and 0.15% CaCl₂·2H₂O) supplemented with 0.72 g of Amberlite XAD-16 resin (MilliporeSigma Supelco, Burlington, USA; Cat. No. XAD16500G), cultured at 200×g, and incubated at 30°C for 10 days. After incubation, the resin was separated, rinsed with water, and dried in a desiccator.

The dried resin was eluted with 50 mL of methanol (Merck KGaA; catalog number: 106009) and then evaporated under vacuum at 40°C. The residue was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich; catalog number: D8418) for use in antifungal and cytotoxicity assays and in 1 mL of methanol for thin-layer chromatography (TLC) analysis. All cultivation and extraction procedures were performed in triplicate to ensure consistency.

2.4. Thin-layer chromatography

Myxobacterial extracts were analyzed by TLC on silica gel Si 60 F254 plates (Merck KGaA; catalog number: 105554). The mobile phase was a mixture of dichloromethane (Merck KGaA; catalog number: 106050) to methanol (Merck

KGaA; catalog number: 106009) in a 90:10 ratio. Potential bioactive compounds were detected by UV irradiation at 254 nm. Each TLC analysis was performed in triplicate to ensure consistent detection of bioactive compounds.

2.5. Investigation of antifungal activity

Dermatophytes and molds were cultured on SDA agar slants (HiMedia Laboratories; catalog number: MH063) at 30°C for 3–7 days, depending on the species. Fungal suspensions were adjusted to an optical density (OD) of 0.1 at 530 nm, corresponding to 1–5×10⁶ CFU/mL. The suspension was spread onto agar plates, and 6-mm-thick wells were drilled under sterile conditions. Crude extracts, dissolved in DMSO to a final concentration of 20.48 mg/mL, were added at 60 µL per well. The plates were then incubated at 30°C for 3–7 days, with antifungal activity indicated by an inhibition zone around each well. Ketoconazole (HiMedia Laboratories; catalog number: CMS4322) was used as a positive control to validate the antifungal activity of the myxobacterial extracts. Each antifungal assay was conducted in triplicate to ensure reproducibility.

Additionally, antifungal activity was assessed using TLC bioautography. TLC plates were placed in contact with agar plates inoculated with fungal suspensions and incubated at 30°C for 3–7 days. Inhibition zones on the agar, corresponding to spots on the TLC plate, indicated antifungal activity. All tests were performed in triplicate to confirm the consistent results.

2.6. Cytotoxicity assays of cancer cell lines

Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma-Aldrich; catalog number: M2128). MCF-7, HEK-293, and HepG2 cells were cultured in MEM medium (Sigma-Aldrich; catalog number: M2279) with 10% fetal calf serum (FCS; Sigma-Aldrich; catalog number: F4135) at 37°C for 24 h in 96-well microtiter plates (Thermo Fisher Scientific; catalog number: 167008). The MTT reagent was prepared at concentrations ranging from 50 ng/mL to 6400 ng/mL in MEM with 10% FCS. After 24 h of incubation, 10 µL of 5 mg/mL MTT solution was added to each well, followed by an additional 4 h of incubation. The culture me-

dium was then removed, and 0.2 mL of DMSO was added to dissolve the formazan crystals. Absorbance was measured at 570 nm using a microplate reader (Bio-Rad, Hercules, CA, USA; model: iMark). The assay was conducted in triplicate, and IC_{50} values were calculated using GraphPad Prism v5.04 (GraphPad Software, San Diego, CA, USA). The percentage of cell inhibition was calculated as $\text{inhibition} = [(OD_{\text{control}} - OD_{\text{test}}) / OD_{\text{control}}] \times 100$.

3. RESULTS

3.1. Isolation and screening of cellulose-degrading myxobacteria

From 41 soil samples collected across various provinces in Vietnam, 33 strains were isolated, exhibiting colonies with yellow, orange-yellow, brown, or black-brown pigmentation on CY and VY/2 media. These strains included AG01, AG02, AG03, BD01, BD02, BD03, BD04, BD05, BT01, DL01, DL02, DL03, DL04, DL05, DN01, H02, H03, HCM01, HCM03, KH03, KT01, LD02, LD03, PR01, PR02, QB01, QN01, QN02, QN03, QN04, QN05, TN01, and TN02. Of these, six strains (AG01, BD04, H02, QN01, QN02, and QN04) demonstrated cellulose degradation activity CMC medium. Additionally, 17 strains were identified as Gram-negative bacilli, and three strains (QN01, QN02, and QN04)

formed fruiting bodies. The detailed results are presented in Table 1 and Figs. 2–4.

Macroscopic and microscopic examinations revealed that QN01, QN02, and QN04 exhibited characteristics consistent with *S. cellulosum*, according to *Bergey's Manual of Systematic Bacteriology*. These strains, isolated from soil from Quang Nam province, demonstrated cellulose degradation, as confirmed by comparing the clear zones around the colonies with those of the positive control strain *B. subtilis* PY79 and the negative control strain *E. coli*. Their degradation capabilities confirmed their identification as *S. cellulosum*.

Morphological analysis of VY/2 yeast agar revealed distinct features for each strain. QN01 displayed yellow-brown colonies with undulating edges, dark veins, and scattered spherical fruiting bodies near the colony boundary. QN02 initially formed milky-white, round colonies with serrated edges and concentric rings. After 5–7 days, the colonies developed branched vein-like structures and brown spots with thin, brittle patches. Under 150 \times magnification, brown sporocarps with oval or polygonal shapes were observed. QN04 showed yellow-brown colonies with rounded margins and radial pocket-shaped structures, with visible oval-shaped fruiting bodies at 150 \times magnification. All three strains exhibited large fruiting bodies (30–50 μm), Gram-negative bacilli with slightly round heads, and spores with gliding motility,

Table 1. Criteria for macroscopic characteristics used to screen for cellulose-degrading myxobacteria

Medium	Criteria
CY	Yellow, yellow-orange, orange, brown, and slimy colonies.
CMC	Viscous colonies with cellulose degradation.
VY/2	Yellow, orange, brown, and viscous colonies with their edges, which were branched or wavy, and fruiting bodies would appear 10 days later on.

CMC, carboxymethyl cellulose.

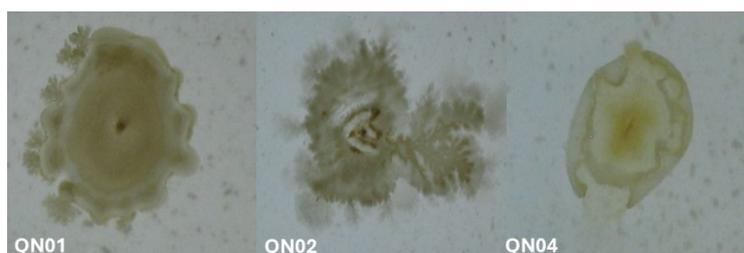


Fig. 2. Colony morphology of QN01, QN02, and QN04 on VY/2 agar. $\times 40$ magnification, direct observation of bacterial colonies (unstained method).

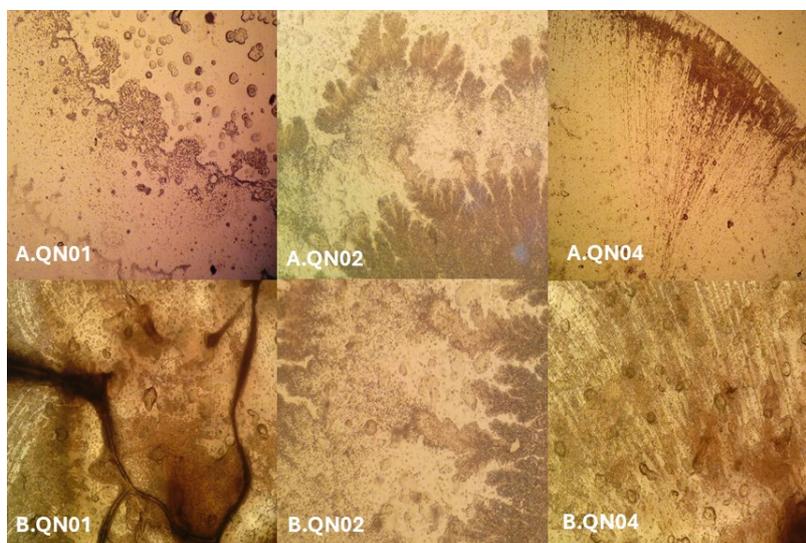


Fig. 3. Fruiting bodies of QN01, QN02, and QN04. A. $\times 150$ magnification, B. $\times 600$ magnification.

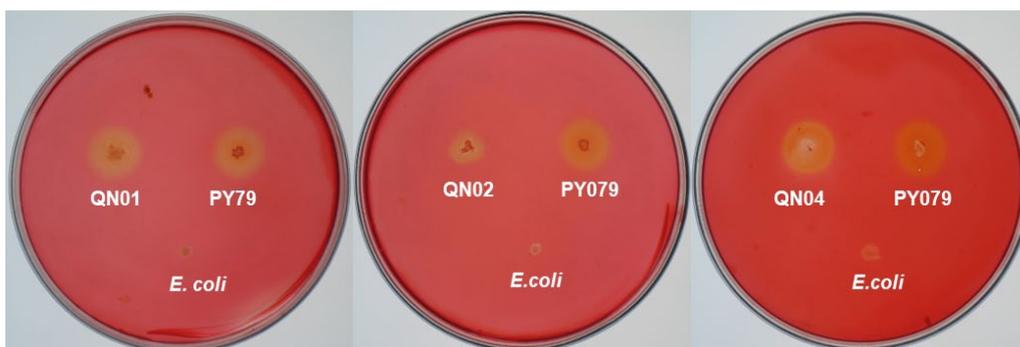


Fig. 4. Cellulose degradation ability of strains QN01, QN02, and QN04 compared to positive control (*Bacillus subtilis* PY079) and negative control (*Escherichia coli* ATCC 25922).

consistent with myxobacterial features.

3.2. Extraction and qualitative analysis of the crude extracts

TLC analysis of the crude extracts indicated that only QN02 exhibited a UV absorption spot at 254 nm with an R_f value of 0.746 (Fig. 5). Visualization with vanillin or 1% concentrated H_2SO_4 revealed three distinct spots on the chromatogram, with R_f values of 0.5, 0.7, and 0.746.

3.3. Antifungal activity of culture extracts

The antifungal and antibacterial activities of the crude extracts of QN01, QN02, and QN04 were evaluated using the disk diffusion method, and the results are summarized in Ta-

bles 2 and 3, and Figs. 6 and 7. The QN02 strain showed significant antifungal activity against all tested fungi, except for *Candida albicans* and *Fusarium* sp., with inhibition zones greater than 15 mm. TLC bioautography further confirmed the antifungal activity of the QN02 extract, particularly at $R_f=0.746$, against several fungi, including *T. mentagrophytes*, *M. gypseum*, *Mucor* sp., *Penicillium* sp., *A. flavus*, and *A. niger*. The consistent antifungal activity at $R_f=0.746$ indicates that QN02 contains a potent bioactive compounds, making it a strong candidate for further cytotoxicity testing.

3.4. Cytotoxicity of QN02 crude extract

The cytotoxicity of the QN02 crude extract was evaluated using the MTT assay. Results indicated a dose-dependent

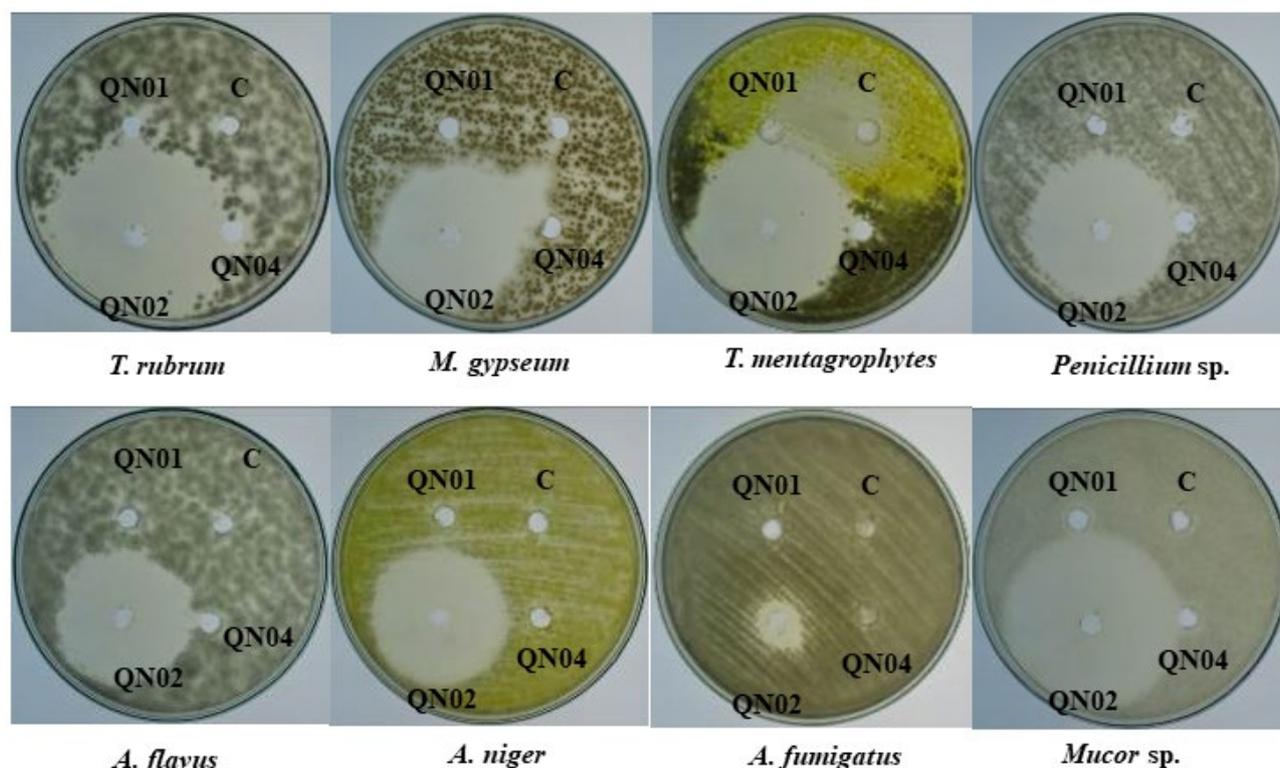


Fig. 5. Chromatography of methanol extracts: C (MeOH), 1 (QN01), 2 (QN02), 3 (QN04).

Table 2. Antifungal activity of extracts from strains QN01, QN02, and QN04 (inhibition zone diameter in mm)

Strain	QN01	QN02	QN04
Fungi/Bacteria			
<i>Trichophyton rubrum</i>	-	60	-
<i>Trichophyton mentagrophytes</i>	-	60	-
<i>Microsporium gypseum</i>	-	40	-
<i>Candida albicans</i>	-	-	-
<i>Mucor sp.</i>	-	50	-
<i>Penicillium sp.</i>	-	50	-
<i>Fusarium sp.</i>	-	-	-
<i>Aspergillus flavus</i>	-	40	-
<i>Aspergillus niger</i>	-	40	-
<i>Aspergillus fumigatus</i>	-	15	-

Table 3. Diameter of antifungal zone at Rf=0.746 (mm)

Fungal strains	Diameter (mm)
<i>Ticorporum gypseum</i>	35
<i>Trichophyton rubrum</i>	30
<i>Trichophyton mentagrophytes</i>	25
<i>Penicillium sp.</i>	10
<i>Aspergillus flavus</i>	8
<i>Aspergillus fumigatus</i>	8
<i>Aspergillus niger</i>	10
<i>Mucor sp.</i>	30

4. DISCUSSION

decrease in absorbance at 570 nm, correlating with reduced cell viability as the extract concentration increased (Table 4, Fig. 8). The IC₅₀ values of the QN02 extract were 0.16 µg/mL in MCF-7 and 0.53 µg/mL in human kidney cells (HEK-293). However, the extract did not inhibit the proliferation of HepG2 cells at concentrations up to 6.4 µg/mL.

The isolation and characterization of myxobacteria from 41 soil samples collected across 15 provinces in Vietnam led to the identification of three strains, designated QN01, QN02, and QN04, which meet the taxonomic criteria for *S. cellulorum* as outlined in *Bergey's Manual of Systematic Bacteriology*. All three strains were derived from soil samples collected in Quang Nam province. The geographic dis-

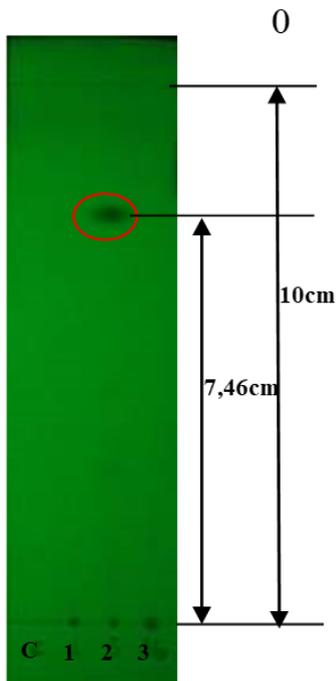


Fig. 6. Antifungal activity of strains QN01, QN02, and QN04 (C: DMSO control). DMSO, dimethyl sulfoxide.

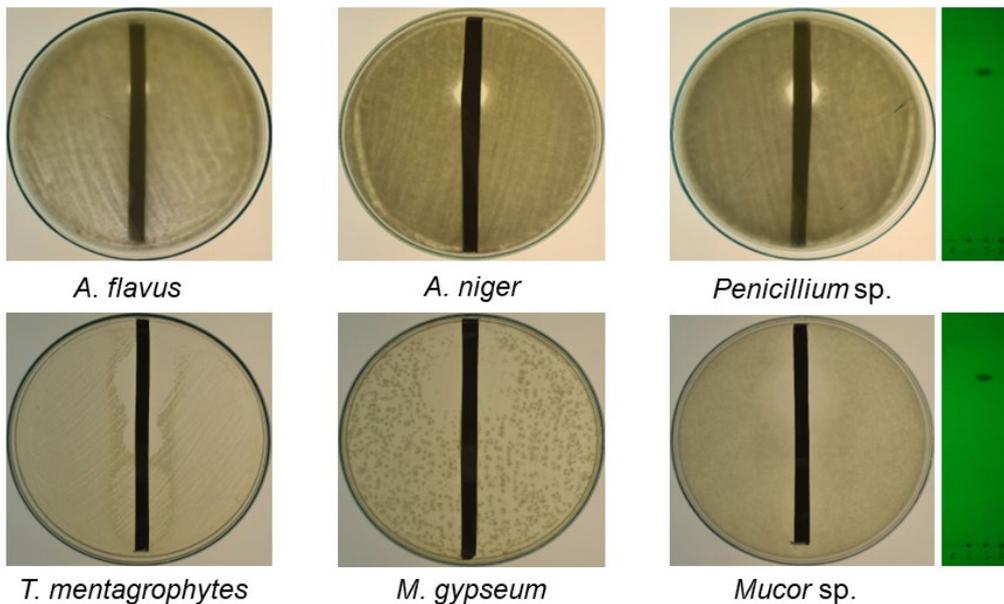


Fig. 7. Bioautography of extracts from QN02 against fungi.

tribution of myxobacteria detected across multiple provinces highlights their widespread presence in diverse environments. This information is crucial not only for understanding environmental preferences and potential regional adaptations but also for guiding future researchers in exploring myxo-

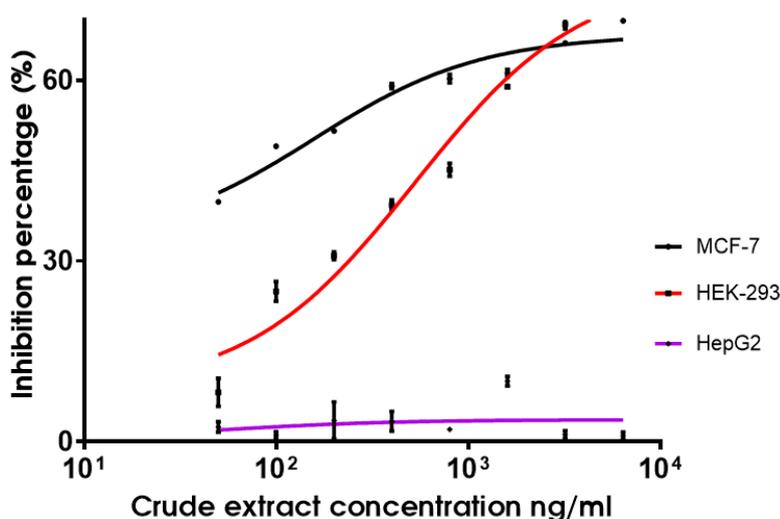
bacteria in similar or neighboring areas.

This mapping of sample sites (Fig. 1) provide a foundation for identifying regions with high myxobacterial diversity, which could lead to novel bioactive compounds. The identification of these strains aligns with previous studies by Gerth

Table 4. The mean of OD_{570nm} value of MTT test (n=3)

Concentration of extract (ng/mL)	0	50	100	200	400	800	1,600	3,200	6,400
OD ₅₇₀ (MCF-7 cell lines)	1.40	0.85	0.72	0.69	0.58	0.56	0.55	0.48	0.43
The percentage of cell inhibition (%)	0.00	39.29	48.57	50.71	58.57	60.00	60.71	65.71	69.29
OD ₅₇₀ (HEK-293 cell lines)	1.40	1.25	1.05	0.98	0.85	0.78	0.58	0.42	0.37
The percentage of cell inhibition (%)	0.00	10.71	25.00	30.00	39.29	42.29	58.57	70.00	73.57
OD ₅₇₀ (HepG2 cell lines)	1.45	1.43	1.42	1.47	1.50	1.39	1.48	1.49	1.52
The percentage of cell inhibition (%)	0,00	1,38	2,07	<0	<0	<0	<0	<0	<0

OD, optical density; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; MCF-7, breast cancer cells; HEK-293, human embryonic kidney cells; HepG2, human liver carcinoma cells.

**Fig. 8.** Cytotoxicity of crude extract in MTT assay. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

et al. and Hyun et al., who isolated *S. cellulosum* using filter paper or CMC as the sole carbon source [7,10,11].

Our findings suggest that the QN02 strain is a promising producer of epothilone, based on its antifungal and cytotoxic activities. TLC analysis of the QN02 extracts revealed three distinct spots with R_f values of 0.5, 0.7, and 0.746, which align with the separation of epothilone A and B as observed in studies by Gerth et al. [7]. The solvent system (dichloromethane-methanol, 90:10) effectively separated these compounds, supporting the presence of epothilone in the QN02 extract.

In our cytotoxicity assays, the QN02 extract had an IC₅₀ of 0.16 µg/mL against the MCF-7 breast cancer cell line, indicating potent antiproliferative activity. Although Kamath et al. reported an IC₅₀ of 3.5 nM for purified epothilone, this result is consistent with the IC₅₀ values observed in other

studies on crude myxobacterial extracts [12]. For instance, Wang et al. reported an IC₅₀ of 1.32 µg/mL and Guo et al. found an IC₅₀ of 1.07 µg/mL for similar methanol extracts, suggesting that the crude form retains significant cytotoxic activity [13,14].

Comparative studies have demonstrated that epothilone exhibits superior cytotoxicity compared with Taxol across various cancer cell lines, with IC₅₀ values ranging from 0.3 to 2.0 nM, whereas Taxol IC₅₀ values vary significantly from 2 to 9,000 nM, depending on the cell type [8]. These differences in cytotoxicity emphasize the influence of extraction methods and the varying sensitivities of cancer cell lines to epothilone.

Despite these promising results, this study has several limitations. The sample size limited to 41 soil samples from various specific regions in Vietnam may not represent the

full diversity of cellulose-degrading myxobacteria. Additionally, further molecular characterization, including DNA sequencing, is required to confirm the taxonomy of the isolated strains and to elucidate the biosynthetic pathways responsible for producing bioactive compounds.

5. CONCLUSION

This study confirmed the presence of cellulose-degrading myxobacteria in soil, reinforcing their abundance in specific environments, which aligns with previous research findings. Among the isolated strains, QN02 showed significant potential for epothilone production, highlighting its promise as a source of bioactive compounds.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Writing - original draft: MT Quang.

Writing - review & editing: MT Quang, KNT Duong, TBN Tran, DXM Do, TM Nguyen, DAH Nguyen, TM Nguyen.

Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval

Not applicable.

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