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Original Article

LC-ESI-QTOF-HRMS-Based Myxobacterial Metabolite Profiling for Potential Anti-Breast Cancer Extracts

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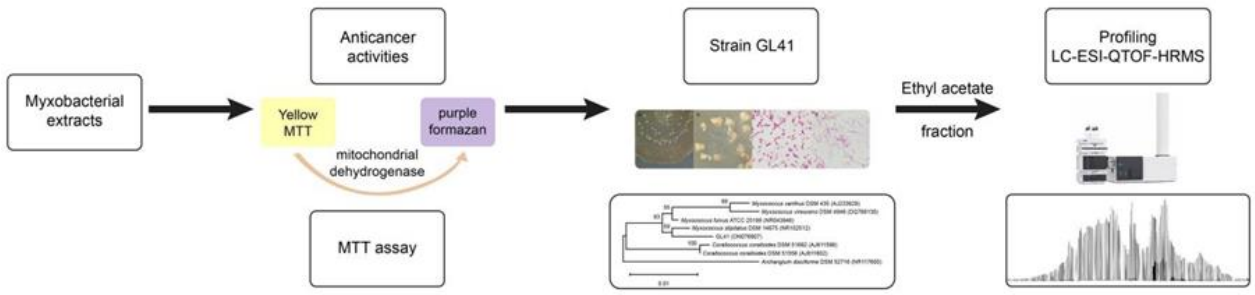
ABSTRACT

Members of Myxococcales have been known as slime bacteria, a unique microbiome in natural habitats with complex multicellular behaviour, sliding movement, and unusual fruiting body morphologies. According to previous reports, myxobacteria typically produced diverse families of secondary metabolites with various biological functions, including antimicrobial, antiviral, and antitumor properties. This study used an MTT cytotoxicity assay to evaluate total extracts from 43 myxobacterial strains on the MDA-MB-231 breast tumour cell line. Among these, one strain was determined to produce the highest anticancer activity with IC_{50} values of 6.25 ± 0.07 µg/mL, 3.9 times more than doxorubicin. Based on the morphological characteristics (colonies, vegetative cells, fruiting bodies, and myxospores) and 16S rDNA gene sequence, the potent strain was classified as belonging to the genus *Myxococcus* (*Myxococcus stipitatus*) named GL41 (Accession number ON076907). Then, profiling the ethyl acetate extract from the GL41 strain was performed to analyze the principle components using liquid chromatography coupled with electrospray ionization mass spectrometry (LC-ESI-QTOF-HRMS). As a result, 5 metabolite peaks were revealed based on the exact pseudomolecular ion $[M+H]^+$ (exactly to 0.0001 m/z) and isotopic distributions. In addition, unknown compound peaks were predicted and exhibited as the putative molecular formulas, contributing to variation among the metabolite profiles under investigation. In conclusion, LC-HRMS-based metabolite screening is an effective and rapid identification approach for discovering potent candidates for subsequent characterization.

GRAPHICAL ABSTRACT



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Introduction

According to International Agency for Research on Cancer, the supplied data exhibited approximately 20 million new cancer cases and 10 million deaths worldwide. Today, with a predicted 2.3 million new cases, female breast cancer (11.7%) has surpassed lung cancer (11.4%) and prostate cancer (7.3%) to be the most prevalent cancer diagnosed (19.3 million cases) [1]. The burden of disease on health services and people's quality of life has led to an increasing trend over the years, regardless of the country's or region's development level. Therefore, enhanced preventive measures are recommended for these countries [2].

Substantial efforts have been made to search for new anticancer medications, and natural compounds are a remarkable source. There have been numerous reports of bioactivities in myxobacteria that belong to a class of slime bacteria known for their complicated multicellular behaviours and coordinated movement [3]. Over the last 40 years, myxobacteria have drawn the attention of many scientists not only for their unique morphological characteristics and complex interactions of cell communication, but also their potential source of novel secondary metabolites responsible for valuable bioactive diversity and applications in drug development [4].

It has been reported that over 600 bioactive secondary metabolites categorized into 100 core structures have been produced from over 7000 myxobacterial isolates [5]. Mainly these metabolites specialize in incredibly unusual action mechanisms that they could not find in other microbial producers [6]. The ability to synthesize particular compounds in myxobacteria is often strain-specific than species-specific. Although there have been certain advances and achievements in genome mining, the characterization of gene clusters encoding biosynthetic enzymes, and recombinant engineering [7], current research strategies mainly depend on the continued discovery and characterization of new strains based on bioactivities as the primary source of promising metabolites. With a traditional approach, bioactive screening is still widely applied and is the most successful way to date [8]. By the 2000s, only about 40 species had been described, but a large number of strains produced hundreds of different biomolecules. Reichenbach (2001) mentioned that his research group screened 6000 strains for 20 years, discovering 4-8 new structures yearly. Therefore, searching for the secondary compounds through the isolation process and preliminary bioactive screening is necessary.

In vitro studies towards tumour cell lines are considered primary screening tests to search for strains producing high cytotoxicity that might be utilized for cancer therapeutic agents [7] and reduce a minimal number of experiments to be carried out. Some structures have been discovered from novel strains, such as disorazol, tubulysin, epothilone, chivosazol, chondramide, and rhizopodin, showed impressive antitumor effects. However, regardless of shared molecular targets and biological properties, every secondary metabolite belongs to a distinct class, such as macrolacton (archazolid, chivosazol, and epothilone), peptide (chronramide and tubulysin), peptolide (argyrine and vioprolide), benzolactone enamide (apcularen and cruentaren) [5]. Epothilone, a *Sorangium cellulosum*-derived paclitaxel mimic, has been approved for breast cancer treatment. In addition, the FDA has authorized the use of ixabepilone, an analogue of epothilone B, for treating metastatic taxane-resistant breast cancer [7].

Currently, liquid chromatography coupled with mass spectrometry is known as a technique for providing information on bacterial secondary metabolites. It promises to detect the most extensive range of practical chemical diversity in a particular sample [9]. Therefore, a combination of liquid chromatography (LC), electrospray ionization (ESI), and high-resolution spectra signals produced from the Quadrupole time of flight analyzer (QTOF) is considered as the screening platform [10]. The data were manually checked for primary component analysis in describing secondary metabolite profiles. The LC-MS technique has yielded promising applications, such as the elucidation of natural components in

extracellular extracts of *Myxococcus* strains (Daniel Krug et al., 2008) [11], identification of corallopyronin A and B, and myxalamid B and C from *Corallococcus coralloides* and *Myxococcus xanthus* (Ivana Charousová, 2017) [12] as well as the discovery of new product rowithocin from *Sorangium* (Thomas Hoffmann, 2018) [13].

In this study, total extracts of soil myxobacteria from different regions in Vietnam were objects to evaluate anti-breast cancer activities from metabolic extracts, followed by practical mining of metabolite profiles by LC-ESI-QTOF-HRMS technique.

Materials and Methods

Myxobacterial strains

Forty-three myxobacterial strains were isolated and stored at Microbiology Department, Nguyen Tat Thanh University.

Preparation of myxobacterial crude extracts

The strain was stored at -80 °C and reactivated on VY/2 medium (Baker's yeast 0.5%, $CaCl_2$ 0.1%, vitamin B12 0.5 µg/mL, agar 1.5%, pH 7.2) [14]. The purified isolates were inoculated in 100-mL P media (soluble starch 0.8%, Baker's yeast 0.4%, peptone 0.2%, yeast extract 0.2%, $CaCl_2$ 0.1%, $MgSO_4.7H_2O$ 0.1%, ferric EDTA 8 µg/mL, Hepes 23.8, pH 7.5) and shaken at 180 rpm, 30 °C for ten days [15]. On the 4th-day of cultivation, 1-2% Amberlite XAD-16N resin was added. After fermentation, the resin was harvested by sieving and desorbed for 2 h with 80 mL mixture of acetone and methanol (1:1, v/v). Finally, the organic solvents were evaporated in a vacuum to obtain crude extracts.

The crude extracts were dissolved in a suitable solvent to give 10 and 20 mg/mL stock solutions. The stock solutions were stored at -20 °C, thawed and diluted in a medium to reach the investigated concentrations.

Evaluation of anticancer activities

Cell lines

The epithelial, human breast cancer cell line MDA-MB-231 was supplied from ATCC Company (American type culture collection, USA) and stored at Pasteur Institute in Ho Chi Minh City, Vietnam.

Cell culture and treatment

Cells were activated and cultured in DMEM (Dulbecco's Modified Eagle Medium), supplemented with 10% fetal calf serum (FBS), 2 mM L-glutamine, 100 IU/mL penicillin, 100 µM streptomycin and incubated at a humidified 5% CO_2 atmosphere at 37 °C in the incubator. Until the cell growth reached about 70-80% confluence, cells were collected and delivered into 96-well culture plates with appropriate density. Cells were incubated at 37 °C, 5% CO_2, for 18-24 h to adhere to the bottom and grow stably. After 72 h treatment of MDA-MB-231 cells with samples, the percentage of viable cells was assessed by MTT assay. Negative control (diluent solvent) was carried out simultaneously [16].

In vitro cancer cell viability assays

Cell viability was determined by the activity of enzyme succinate dehydrogenase in the mitochondria of living cells. This enzyme converts MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] to purple formazan crystals, which are soluble in organic solvents such as isopropanol. The percentage of viable cells was calculated based on the absorbance of samples at the wavelength of 570 nm [17].

After treatment with the test samples, the cells were removed culture medium and supplemented with a serum-free medium containing 0.5 mg/mL of MTT reagent. Cells were incubated at 37 °C, 5% CO_2, to formazan crystals formed. After 3 hours, the medium with MTT was removed and the formed formazan crystals were solubilized in acidified isopropanol (0.1% HCl); the mixture was shaken at ambient temperature for 10 min. The optical density was measured at 570 nm using a microplate reader [16]. The antitumor activity was assessed by the growth inhibition percentage (GI%) using the formula [18]:

$$GI\% = \left(1 - \frac{A_{sample}}{A_{control}}\right) \times 100$$

Where, A_{sample} is the absorbance of myxobacterial extract and $A_{control}$ is the absorbance of negative control (without samples) based on 100% viable cells. Each measurement was performed in triplicates. All data are shown as the mean ± standard deviation (SD). The IC_{50} values of crude extracts were calculated from the regression graph. Doxorubicin was included as a positive control.

Identification of the most potent strain

Morphological characteristics of colonies, fruiting bodies, vegetative cells, and myxospores on VY/2 medium were observed and described after being cultured at 30 °C for 5-10 days. Data were compared with previous publications [14, 19, 20]. 16S rDNA gene sequencing: DNA was extracted using the Genomic DNA Extraction Kit (ABT, Vietnam) according to the manufacturer's instructions. Purified PCR products were sent for sequencing at Axil Scientific, Singapore. Gene sequences were assembled using the Lasergene program. The nucleotide BLAST (NCBI) was used to align and determine the similarity between query sequences and references published on NCBI Genbank. Phylogenetic relationships were analysed using MEGA version 11.0.0 software [21, 22].

Analysis of natural products from the potent extract by high-performance liquid chromatography coupled with high-resolution mass spectrometry

Myxobacterial strain exhibiting high cytotoxic activity was cultured on P-medium to obtain total extracts [15]. The extract was distributed liquid-liquid with solvents of increasing polarity (n-hexane, chloroform, and ethyl acetate) to separate into four fractions. The cytotoxic activity against cancer cell line MDA-MB-231 on extracts was evaluated at a 100 µg/mL concentration by MTT method [16]. Potentially active fractions were injected into the LC-MS system to predict data mining for metabolites [11]. The fraction was analysed by an LC-HRMS system consisting of an ultra-high-pressure liquid chromatography system combined with a QTOF mass spectrometry system.

Chromatographic conditions: C18 XTerra column (4.6×50 mm, 2.5 µm, Waters, USA); flow rate was 0.35 mL/min; column chamber temperature was set at 30 °C; sample injection volume was 5 µL. The mobile phase mixture consisted of methanol (phase A) and water with 0.1% formic acid (phase B). Elution according to the gradient program varied by the portion phase A to phase B (0-5 min isocratic step: 10% A; 5-10 min gradient step: 10 → 100% A; 10-15 min isocratic step: 100% A).

ESI-HRMS conditions: Nozzle voltage, capillary, and fragmentor voltage were 400 V, 3.5 kV, and 10 eV, respectively; Nebulizer pressure was 35 psi; the dry gas temperature and gas flow were 300 °C and 8 L/min. The ions formed by electron injection ionization (ESI) method were loaded into the QTOF mass analyser of spectrometry system. Scans were recorded in positive ion mode in the 50-1000 m/z at a rate of 4 spectra/sec.

Data analysis: High-resolution mass data were analysed using Masshunter Qualitative Analysis software B.07.00 (Agilent). The monoisotopic mass accuracy from the isotope pattern was used to calculate the molecular formulas by Chemcalc.org, where recommendations are ranked based on mass deviation [23]. Moreover, the reported metabolite database from the genus *Myxococcus* sp. helps predict molecular formulas suitable for logical chemical structures. The m/z values (accurate to 0.0001 m/z) for assumed ions are limited by an absolute detection tolerance of ± 5 ppm or ± 3 mDa compared with recorded ions from chromatogram [10].

Table 1: Inhibition effect of crude extract from 43 myxobacterial strains on MDA-MB-231 cell line

Dose (µg/mL)	Number of strains with different inhibition rates		
	> 30%	> 60%	> 90%
100	12	7	0
200	32	10	6

Table 2: *In vitro* growth inhibition percentage of 43 crude extracts to MDA-MB-231 cell lines

No.	Strains	GI% (Mean ± SD)		No.	Strains	GI% (Mean ± SD)	
		100 µg/mL	200 µg/mL			100 µg/mL	200 µg/mL
1	AG11	18.47 ± 1.33	58.68 ± 1.02	23	LA41	15.21 ± 0.67	20.19 ± 1.55
2	BD2	79.61 ± 1.15	91.61 ± 0.38	24	LA43	13.13 ± 2.11	33.95 ± 0.28
3	BDi23	22.42 ± 1.13	43.31 ± 0.70	25	LA44	9.27 ± 0.57	49.19 ± 0.58
4	BDi22	80.60 ± 0.60	93.40 ± 0.48	26	NB71	26.50 ± 1.16	34.40 ± 0.39
5	BP161	15.85 ± 1.55	21.98 ± 2.34	27	NB8x1	21.29 ± 1.23	36.03 ± 0.83
6	BP181	9.41 ± 1.93	35.84 ± 0.46	28	NB83	49.82 ± 2.06	60.15 ± 1.62
7	BP182	19.63 ± 2.26	41.99 ± 0.29	29	PY1	9.89 ± 0.94	34.84 ± 0.38
8	BP213	36.04 ± 0.58	63.90 ± 0.44	30	QT15	13.01 ± 1.60	38.13 ± 1.56
9	BRVT1	14.70 ± 1.26	25.82 ± 1.78	31	QT72	8.93 ± 1.14	24.31 ± 1.40
10	BT101	14.32 ± 0.68	20.86 ± 2.26	32	ST11	22.17 ± 1.27	49.86 ± 0.80
11	BT43	8.71 ± 3.91	25.13 ± 0.46	33	TG131	24.51 ± 0.72	40.79 ± 0.71
12	BT92	34.03 ± 3.68	53.96 ± 0.28	34	TG23	26.46 ± 0.47	55.88 ± 0.68
13	CT21	78.25 ± 1.90	86.64 ± 0.19	35	TH41	11.59 ± 1.97	21.49 ± 2.20
14	DN11	27.62 ± 1.70	32.53 ± 1.45	36	TH48	11.40 ± 1.03	18.80 ± 1.06
15	DN21	7.86 ± 2.42	29.04 ± 2.09	37	TH52	19.33 ± 1.12	55.91 ± 0.61
16	DN23	13.07 ± 2.58	19.50 ± 0.70	38	TV11	22.67 ± 0.87	33.40 ± 2.41
17	GL41	80.85 ± 1.43	90.03 ± 0.33	39	TV22	17.83 ± 1.23	49.01 ± 0.18
18	GL43	15.82 ± 0.41	48.53 ± 0.21	40	TV51	35.25 ± 0.47	59.58 ± 1.08
19	HCM21	8.08 ± 2.71	12.04 ± 1.35	41	VL25	79.75 ± 1.08	90.79 ± 0.34
20	HCM81	77.22 ± 1.99	93.37 ± 1.11	42	VL21	16.50 ± 0.85	41.51 ± 0.78
21	HG223	29.05 ± 1.05	62.10 ± 0.72	43	VL32	32.90 ± 1.01	57.36 ± 1.31
22	HG225	80.40 ± 1.84	92.60 ± 0.66	*Control: Doxorubicin (5 µg/mL) 74.44 ± 0.68			

Table 3: *In vitro* IC_{50} (µg/mL) of extracts from seven potent strains on the MDA-MB-231 cell line

No.	Strains	IC_{50} (µg/mL)	Identification	Accession number
1	BD2	16.78 ± 0.19	*Myxococcus stipitatus*	ON076901
2	BDi22	8.32 ± 0.05	*Melittangium boletus*	ON055893
3	CT21	7.91 ± 0.08	*Myxococcus* sp.	ON076507
4	GL41	6.25 ± 0.07	*Myxococcus stipitatus*	ON076907
5	HCM81	22.08 ± 0.15	*Myxococcus virescens*	ON076908
6	HG225	20.28 ± 0.51	*Myxococcus* sp.	ON076554
7	VL25	17.72 ± 0.11	*Myxococcus fulvus*	ON055737
8	Doxorubicin	1.60 ± 0.01		

Results and Discussion

Effect of extracts with different concentrations on the viability of MDA-MB-231 cells

Myxobacteria have been more deeply studied in several unexplored parts of the world over the most recent decades. However, there have not been any reports relating to myxobacteria in Vietnam focusing on isolation and primary investigation of bioactive properties to find novel species. In this study, forty-three myxobacterial strains isolated and stored by Pharmacy Faculty at Nguyen Tat Thanh University were fermented and extracted by organic solvents. Myxobacterial extracts were screened for their potential as anticancer agents. MTT method using a 96-well plate, with MDA-MB-231 cell line as screening models, was used to evaluate the growth inhibitory activity. The results are presented in Tables 1 and 2.

The crude extracts inhibited breast-cancer cell proliferation in a dose-dependent mode. The anticancer activity from most strains was negligible at 100 µg/mL, and higher effects were observed at 200 µg/mL. Thirty-two and twelve strains inhibited more than 30% of the tested cells at 200 and 100 µg/mL doses, respectively. Seven strains at 100 µg/mL showed toxicity on 60% of the cancer cells, whereas this value was ten at 200 µg/mL. Similarly, six isolates had an inhibition threshold of over 90% at a concentration of 200 µg/mL and none were found at a low dose. Therefore, MDA-MB-231 cell lines showed different susceptibilities dependent on surveyed concentrations (Table 1).

The results demonstrated that the extracts of the 43 myxobacterial strains decreased the viability percentage towards cancer cell lines but to various extents. The percentages of growth inhibition fluctuated from 7.86 ± 2.42 to 80.85 ± 1.43% (at the test concentration of 100 µg/mL) and 12.04 ± 1.35 to 93.40 ± 0.48% (200 µg/mL) are listed in Table 2. Among these, the breast cancer cells were found to be the most vulnerable to the treatment of GL41 extracts with the most substantial growth inhibition at 100 µg/mL concentration tested (80.85 ± 1.43%), seconded by the treatment activity displayed by BDi22 extract (80.60 ± 0.60%) (Table 2). Generally, differences in the chemical components of crude extracts are likely to have an impact on the effect strengths.

Evaluation of IC_{50} values of potential strains

The IC_{50} values of potent strains that amounts to the high anticancer activity of MDA-MB-231 cell lines are presented in Table 3.

Seven myxobacterial isolates exhibited inhibitory activity against the growth of MDA-MB-231 cells with an IC_{50} threshold of less than 25 µg/mL. The microscopic morphology and 16S rRNA sequencing are typical criteria for classifying myxobacteria. Most of these strains belong to the genus *Myxococcus* with 6/7 strains; one strain was categorized as the species *Melittangium boletus*. The IC_{50} value of MDA-MB-231 breast cancer cells exposed to the metabolites from the strain GL41 extract was 6.25 ± 0.07 µg/mL and was comparable to that of cells treated with DMSO (100 ± 1.3%), followed by strain CT21 inhibited 50% of cancer cells at a concentration of 7.91 ± 0.08 µg/mL. These values were 3.9-5.2 times higher than the positive control (doxorubicin, IC_{50} = 1.60 ± 0.01 µg/mL). Members of the genus *Melittangium* are generally not as prominent in the production of bioactive secondary substances as *Myxococcus* and *Sorangium*. However, this finding showed impressive cytotoxic activity from *Melittangium boletus* BDi22 with an IC_{50} value of 8.32 ± 0.05 µg/mL.

Identification of GL41 strain

The morphology characteristics of the most remarkable strains (strain GL41) were described in detail. After five days of culture, the colonies were thin with flame-shape edges, reached 4-5 cm in diameter, and produced diffuse light orange/pink pigment (Figure 1a). The VY/2 medium induced the formation of globular or oval fruiting bodies with pink to pale orange, solitary, about 100-600 µm in size, tapering at the short stalks, and can be seen with the naked eye (Figure 1b). The spherical myxospores gather in clusters, 0.8-1.5 µm in diameter (Figure 1c). The vegetative cells are Gram-negative, rod-shaped, 0.4-1.0×3-8 µm, and tapered at both ends (Figure 1d).

GL41 cells were observed sliding movement and cytolytic activity against *E. coli* on WCX agar (w/v, agar 1.5%, $CaCl_2$ 0.07%, cycloheximide 0.01%). Catalase and urease tests were positive, but the cellulose hydrolysis test was negative. Positive results were for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, phosphatase acid, and naphthol-AS-BI phosphohydrolase.

The 16S rDNA sequence data were deposited into Genbank with accession number ON076907 and showed 99.3% similarity with *Myxococcus stipitatus* species. Phylogenetic analysis between the query GL41 strain and the reference strains is expressed in Figure 2.

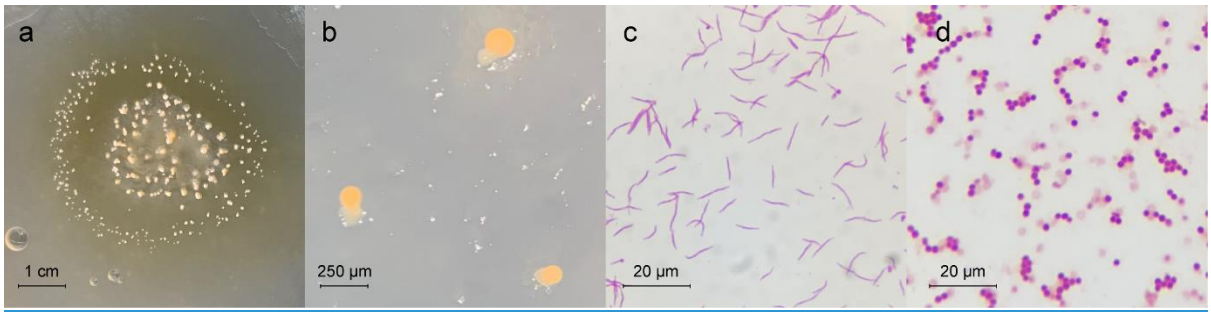

Figure 1: Typical morphological characteristics of GL41 strain. (a) Swam on VY/2 medium, (b) Fruiting bodies on VY/2 medium, (c) Myxospores, and (d) Vegetative cells with Gram-negative stain under microscope 1000X

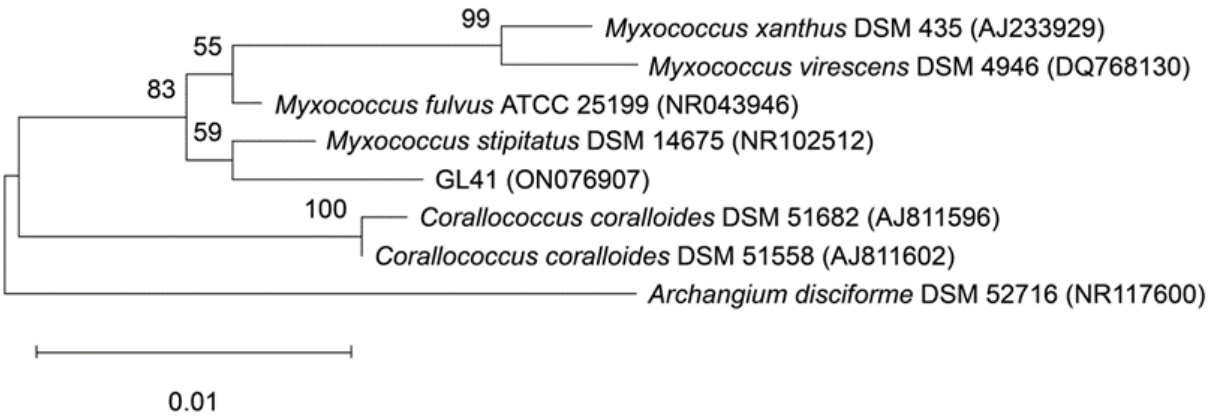

Figure 2: The 16S rRNA gene sequences of the GL41 were aligned to their closest strains and utilized to build a phylogenetic tree using the maximum likelihood method. 1000 bootstrap replications were used to test the phylogeny (accession numbers are mentioned in parenthesis)

Data analysis of secondary compounds in ethyl acetate extract by liquid chromatography coupled with mass spectrometry

Evaluation of anti-MDA-MB-231 cell activity of the fractions at the concentration of 100 µg/mL are shown in Table 4.

The results from Table 4 indicated that the ethyl acetate fraction exhibited the highest inhibitory activity on the MDA-MB-231 cell line with an inhibition percentage of 75.4 ± 0.54% compared with 74.8 ± 1.56% of the total extract.

By ultra-high-pressure liquid chromatography in combination with an HRMS probe, ethyl acetate fraction of strain *M. stipitatus* GL41 was screened for the possible presence of known secondary compounds in the extract, followed by $[M+H]^+$ ion values. The results showed that five substances are similar in characterized molecules and nine unknown compounds (Tables 5 and 6).

Analytical results in Table 5 demonstrated that the mass (m/z) data of the $[M+H]^+$ ions identified five structurally determined secondary metabolite peaks, including althiomycin, DKxanthene-518, myxalamide C, myxochelin A, and myxothiazol, and not significantly different from the published documents (Δ is from 0.20-4.78 ppm and 0.10-2.10 mDa). The high signals in chromatography-mass spectrometry are manually synthesized into a list of 9 precise m/z values with the corresponding proposed molecular formulas (Table 6).

Table 4: MDA-MB-231 inhibition percent of fractions

Fractions	Total extract	*n*-Hexane	Chloroform	Ethyl acetate	Residue
%GI	74.8 ± 1.56	53.2 ± 0.80	41.9 ± 2.31	75.4 ± 0.54	17.8 ± 0.22

Table 5: Published compounds of ethyl acetate fraction and proposed molecular formulas based on the results of LC-ESI-HRMS analysis

Compound	Formula (M)	$[M+H]^+$ (m/z)		Δ (ppm)	Δ (mDa)
		Measured	Calculated		
Myxalamid C	$C_{24}H_{37}NO_3$	388.2845	388.2846	0.31	0.12
Myxochelin A	$C_{20}H_{24}N_2O_7$	405.1662	405.1656	1.41	0.57
Althiomycin	$C_{16}H_{17}N_5O_6S_2$	440.0672	440.0693	4.78	2.10
Myxothiazol A	$C_{25}H_{33}N_3S_2O_3$	488.2027	488.2036	1.87	0.91
DKxanthene-518	$C_{29}H_{34}N_4O_5$	519.2603	519.2602	0.20	0.10

Table 6: Potential compounds and proposed molecular formulas

Retention time (min)	$[M+H]^+$ (m/z)	Proposed molecular formula
11.409	332.1930	$C_{13}H_{25}N_5O_5$
10.456	441.2164	$C_{20}H_{32}N_4O_5S$
10.377	523.2567	$C_{28}H_{34}N_4O_6$
9.055	525.2904	$C_{21}H_{36}N_{10}O_6$
10.095	543.1924	$C_{23}H_{34}N_4O_7S_2$
10.725	544.1946	$C_{19}H_{29}N_9O_8S$
9.593	545.2618	$C_{27}H_{36}N_4O_8$
11.334	555.3183	$C_{30}H_{42}N_4O_6$
9.965	815.5858	$C_{46}H_{78}N_4O_8$

Thus, the analysis results show that strain *M. stipitatus* GL41 is capable of producing five metabolites based on the acceptable deviation within acceptable limit between the calculated and recorded molecular weights from the chromatogram (Δ ppm less than 5 ppm and Δ mDa less than 3 mDa). Among these compounds, althiomycin was rarely reported from the genus *Myxococcus*, while the group of myxalamides and DKxanthenes were frequently present in most screened strains, as reported by Daniel Krug *et al.* [11]. Besides the elucidation of molecular structure, the biological activities of these compounds have also been reported in previous publications. In detail, the antibacterial activity of althiomycin was demonstrated by Fujimoto, H. *et al.* [24], whereas myxothiazol A strongly inhibits the growth of gram-positive bacteria and filamentous fungi by acting on the cytochrome bc1 complex [25, 26]. Similarly, DKxanthene-518 was revealed to possess antioxidant activity (although the mechanism is unknown) [27] and inhibit the growth of fungi *A. niger*, *C. albicans* from the extract of strain *M. stipitatus* DSM 14675 [28], while the electron inhibitor myxalamide has inhibitory properties against bacteria, yeast, and fungi. Besides, myxochelin A was demonstrated with the antioxidant, antibacterial, and antiproliferative effects on K-562 white blood cells at the IC_{50} concentration of 1.9 μM and inhibited the invasion of mouse colon carcinoma 26-L5 cells at a non-cytotoxic concentration [29, 30]. The prediction of presence of these compounds explained for antioxidant, antimicrobial, and anticancer activities of the strain GL41 extract. In addition, it is not excluded the hypothesis that 9 compounds with unidentified chemical formulas are biologically active components of the extract from strain GL41. The targeted screening procedure exhibited that strain GL41 is the source of specific myxobacterial secondary metabolites. This finding is not entirely surprising. In fact, it is completely consistent with previous publications when a high percentage of *Myxococcus* sp. has been reported to be a proficient factory of novel bioactive products [31, 32].

Several cytotoxic compounds have been successfully discovered from *Myxococcus stipitatus*, such as rhizopodin (the inhibitor of actin polymerization) with ID_{50} value (measured by MTT assay) was 12-30 ng/mL on BHK, CHO, L929, and K-562 167 cell lines [33] or phenalamides A1-3 were able to inhibit sensitive cancer cells and CL02 and CP70 resistant cells with IC_{50} values in the range of 0.23-0.57 μg/mL

[34]. These molecules opened up new prospects in cancer chemotherapy research.

The soil microorganisms are the promising reservoir of biomedically valuable therapeutic agents [35]. Despite the enormous difficulties of isolation and culture, Myxococcales are now known to be proficient in producing unusual active molecules that cannot be detected in other groups. Even though a series of unique structures and related activities have been published, the potential for secondary substances is still coming up to be discovered. These strains may significantly affect human well-being with unusual antitumor activities [36].

Conclusion

A bioactivity-oriented method was applied to screen the antitumor activity of 43 myxobacterial extracts. The combination of morphological characteristics and 16S rDNA gene sequences supplied the identification of the highest active strain against the MDA-MB-231 cell line as *Myxococcus stipitatus* (GL41). This is the first report describing the antitumor activity of myxobacteria isolated and cultured from Vietnam habitats. This finding is an initial guide for further studies on potent strains to determine the chemical constituents and elucidate the mechanism of antitumor action.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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