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Isolation, antimicrobial activity of myxobacterial crude extracts and identification of the most potent strains

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Abstract: Broad spectrum antimicrobial agents are urgently needed to fight frequently occurring multidrug-resistant pathogens. Myxobacteria have been regarded as "microbe factories" for active secondary metabolites, and therefore, this study was performed to isolate two bacteriolytic genera of myxobacteria, *Myxococcus* sp. and *Corallococcus* sp., from 10 soil/sand samples using two conventional methods followed by purification with the aim of determining the antimicrobial activity of methanol extracts against 11 test microorganisms (four Gram-positive, four Gram-negative, two yeasts and one fungus). Out of thirty-nine directly observed strains, 23 were purified and analyzed for antimicrobial activities. Based on the broth microdilution method, a total of 19 crude extracts showed antimicrobial activity. The range of inhibited wells was more important in the case of anti-Gram-positive-bacterial activity in comparison with the anti-Gram-negative-bacterial and antifungal activity. In light of the established degree and range of antimicrobial activity, two of the most active isolates (BNEM1 and SFEC2) were selected for further characterization. Morphological parameters and a sequence similarity search by BLAST revealed that they showed 99% sequence similarity to *Myxococcus xanthus* – BNEM1 (accession no. KX669224) and *Corallococcus coralloides* – SFEC2 (accession no. KX669225). As these isolates had antimicrobial activity, they could be considered for use in the development of antibiotics for pharmaceutical use.

Key words: *Myxococcus* sp., *Corallococcus* sp.; antimicrobial activity; multidrug-resistant organisms

INTRODUCTION

Myxobacteria are flexible rod-shaped Gram-negative bacteria [1], which can perform cooperative feeding, and development into fruiting bodies [2]. They are characterized by an unusual way of life, as they move by gliding or creeping on surfaces and synthesize a large number of biologically active secondary metabolites (more than 80 basic structures with nearly 350 structural variants) [3,4]. The chemical structures of the myxobacteria metabolome are rare both in diversity and biological activities [4,5], because their secondary metabolites are unusual hybrids of polyketides and nonribosomal made peptides which possess high pharmacological importance. Unlike metabolites from other microorganisms, myxobacterial metabolites are not glycosylated [6,7,8]. Members of the Deltaproteobacteria, order *Myxococcales*, are well-known for their ability to produce secondary metabolites with the capability to exert different biological effects [9]. Within the order *Myxococcales*, the genera *Myxococcus* and *Corallococcus* are very interesting. They are typically found in topsoil where they grow as saprophytes by decomposing degradable polymers or as predators by preying on other microorganisms [10]. They are distinguished from other Deltaproteobacteria by three traits: they are strictly aerobic, during starvation they form multicellular fruiting bodies [10] and most members of the *Myxococcales* have large genomes of around 10 Mb [11,12].

Therefore, the present study focused on the isolation and purification of *Myxococcus* sp. and *Corallococcus* sp. from soil/sandy samples, examination of their biological activity against 11 test microorganisms and identification of the most active strains using morphological, physiological and molecular methods.

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MATERIALS AND METHODS

Soil collection

Soil samples were collected about 10-15 cm below the soil or sandy surface from Bahamas, Nassau (25°02'12.92" N; 77°22'27.63" W), Florida, Hollywood Beach (26°00'32.29" N; 80°06'55.89" W), Massachusetts, Sandisfield (42°06'41.90" N; 73°08'36.62" W), Mexico, Mexico city (19°26'09.26" N; 99°08'37.03" W), Argentine, Iguazu (25°41'30.35" S; 54°26'11.45" W), Argentine, Buenos Aires (34°36'33.32"S; 58°21'22.35" W), Bolivia, Santa Cruz (17°49'11.74" S; 64°09P12.45" W), Florida, Miami (25°45'30.87"N; 80°11'45.36" W), British Columbia, Vancouver (49°18'18.80"N; 123°08'41.31" W), East-central Canada, Ontario (53°03'40.73" N; 93°19'15.28" W) in 2014 and 2015. Samples were air-dried for 2-5 days at room temperature, crushed and sieved. The sieved samples were then used for direct isolation of myxobacteria.

Isolation and purification procedure

For isolation of *Myxococcus* sp. and *Corallococcus* sp. two conventional isolation methods were used: baiting with living *Escherichia coli* streaks [13], and STAN21 agar [10] with slices of filter paper on the top. Living *E. coli* was cross-streaked on the surface of WY (water) medium [10] supplemented with 50 mg/mL of cycloheximide, 50 mg/mL of levamisole and 10 mg/mL of soraphen A for growth inhibition of other organisms. The central parts of the cross were inoculated with a pea-sized aliquot of the dry soils. Pea-sized clumps of soil were inoculated onto Whatman #1 filter paper (Brentford, UK) placed on the surface of STAN21 medium with the addition of 1 mL of 500-fold diluted vitamin solution [14], cycloheximide (50 mg/mL) and levamisole (50 mg/mL). Plates were incubated at 30°C for 4-12 days. Recognition of myxobacteria by the morphology of fruiting bodies was performed using an Olympus stereomicroscope (SZX-10, Japan). Using a sterile needle, colonies looking like *Myxococcus* sp. and *Corallococcus* sp. were transferred to new WY plates with *E. coli* and purified several times. Finally, pure isolates were transferred to CY (casitone-yeast) medium [13] and to 20 mL of liquid CY/H medium [15], which were used for the preparation of glycerol stocks (0.5 mL of culture + 1.0 mL of glycerol, 50% v/v) conserved at -80°C in the Helmholtz Center for Infection Research, Microbial Strain Collection Group, Braunschweig, Germany.

Morphological identification of pure strains

Growth and morphology of strains were observed by stereomicroscope. According to criteria given in "The Prokaryotes" [13] and "Bergey's Manual of Systematic Bacteriology" [16], the taxonomy of all pure isolates was determined by observation of vegetative cells, fruiting bodies, myxospores and swarms.

Test strains

The organisms used in this study included four Gram-positive (*Bacillus subtilis* (DSM 10), *Micrococcus luteus* (DSM1790), *Staphylococcus aureus* (Newman), *Mycobacterium smegmatis* (ATCC 700084)), four Gram-negative (*Escherichia coli* (DSM 1116), *Escherichia coli* (TolC), *Pseudomonas aeruginosa* (PA14), *Chromobacterium violaceum* (DSM 30191)), yeasts *Candida albicans* (DSM 1665) and *Pichia anomala* (DSM 6766) and the filamentous fungus *Mucor hiemalis* (DSM 2656). Test microorganisms were obtained from DSM (German Collection of Microorganisms and Cell Cultures), Braunschweig, Germany and ATCC (American Type Culture Collection), Manassas, VA 20110, USA.

Crude extract preparation

The purified isolates were cultivated in 100 mL of P (peptone) medium [10], with 1-2 % Amberlite XAD (XAD-16 adsorbent resin) (Sigma-Aldrich, USA) and incubated for 5-7 days at 30°C. At the end of the fermentation, the XAD-16 absorber resin was separated by sieving, extracted with acetone for 1 h under the flue and separated with filter paper to bottom flasks. At 40°C the acetone was evaporated in a rotary evaporator (Heidolph, Germany). Finally, the dried precipitate was dissolved in 1 mL of methanol (Sigma-Aldrich, USA), resulting in raw extracts of 1:100 concentration.

Broth microdilution detection of antimicrobial activity

The antimicrobial activity was screened by the broth microdilution method according to [17] in 96-well microplates (BRAND, Germany). Indicator microorganisms were prepared by dilution with Mueller-Hinton broth (Merck, Germany) of 4-6-h-old cultures of bacteria to obtain 0.05 McFarland turbidity, and with Mycosel broth [18] of 4-6-h-old cultures of yeasts and a 48-h-old culture of fungus to obtain 0.01 McFarland turbidity. The dilution stages of crude extracts were determined by inhibited wells (A-H). As more wells with inoculated test microorganisms were inhibited with increasing activity of the tested raw extract. For example, inhibition till well G is twofold higher than till well E.

Characterization of the most active isolates

DNA isolation, PCR reaction, sequencing and phylogenetic analysis

The genomic DNA was extracted using a Spin Plant Mini Kit (Invisorb, Germany) and amplified by PCR using primers F27 and R1525 [19]. Reaction mixtures were made in a total volume of 50 μL (25.0 μL of JumpStar Ready Mix (SigmaAldrich, Germany), 1 μL of each primer, 22 μL of PCR water and 1μL of template DNA). The PCR reaction ran in a Mastercycler Gradient (Eppendorf, Germany) under the following conditions: initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and elongation at 72°C for 120 s, with a final extension at 72°C for 10 min. The purified PCR products (NucleoSpin® Gel and PCR Clean-up-Kit (Macherey-Nagel, Germany) were sequenced in the research group Genomic Analytics of the Helmholtz Center for Infection Research (HZI), Braunschweig (Germany) using primers F27 and R518. The obtained 16Sr RNA gene sequences were checked for quality using BioEdit alignment. The similarity of the 16S rRNA gene sequences was compared with available sequences deposited in the NCBI database using BLAST search (http://www.ncbi.nlm.nih.gov/BLAST). A phylogenetic tree was constructed with the maximum likelihood method [20] using PhyML [21], with bootstrap values based on 100 replications.

Physiological and biochemical characterization

Three approaches were used for physiological testing. Determination of optimal temperature (20, 30, 37 and 42°C), pH tolerance (levels of 5-8) and the spectrum of resistance (ampicillin 100 μg/mL, gentamycin 50 μg/mL, hygromycin 150 μg/mL, chloramphenicol 30 μg/mL, polymyxin 50 μg/mL, kanamycin 50 μg/mL, spectinomycin 50 μg/mL, cephalosporin 100 μg/mL, bacitracin 50 μg/mL, fusidic acid 50 μg/mL, oxytetracycline 10 μg/mL, thiostrepton 50 μg/mL and trimethoprim 50 μg/mL) was observed on VY/2 (baker´s yeast) medium [10]. From the outer fringes of the swarm, agar pieces were cut off and put on the agar plates for 5-7 days at 30°C.

Biochemical characteristics were established using the commercially available test kit ApiZym® (bioMérieux, France). The API ZYM system consists of a plastic gallery of cupules; at the bottom of each is a fabric support carrying the substrates, which detect 19 enzymes. The isolates to be tested were grown in liquid CY/H medium. API strips were inoculated following the manufacturer's instructions. The strips were incubated for 5 h at 30°C. After incubation, reagents were added to the cupules. After 5-10 min, we evaluated the strips according to manual criteria.

RESULTS AND DISCUSSION

Myxobacteria are one of the pivotal antibiotic producers that contribute to the production of bioactive compounds with an unusual mode of action. Despite the importance of myxobacteria, the screening of myxobacteria is not high, due to a shortage in trained microbiologists and difficulties in the process of isolation, purification and maintenance. The microorganisms studied in this research corresponded to the general description of the order *Myxococcales* according to [22]. They produced long, thin motile rods on agar plates, moving by creeping motion. The use of two isolation methods followed by the 3-4 purification steps on the 10 soil samples provided a total of 24 pure strains of 40 directly observed on the isolation agar plates. Both used methods were suitable for the isolation of the bacteriolytic genera *Myxococcus* and *Corallococcus*, but in case of STAN21 medium with

filtrate paper on the top, we recorded more myxobacterial strains (Table 1). *E. coli* baiting were a more effective method for the isolation of *Corallococcus* sp. as opposed to STAN21 medium, where we observed similar amounts of both studied genera. Direct picking of fruiting bodies or agar pieces made from the fringes of swarms transferred to new agar plates with inoculated *E. coli* worked very well as a purification procedure. Similar results were reported in [23], where the majority of pure cultures from soils was obtained by the baiting procedure with a dung pellet and with *E. coli* streaks.

Taxonomic identification of the *Myxococcus* and *Corallococcus* genera was performed by the keys provided by [13] and [24]. The *Myxococcus* genus was characterized by short thin or fat rods clustered in separated large and globular fruiting bodies on a stalk. The color of swarms varied from light yellowish orange when young to bright orange when mature. The cell shape of *Corallococcus* species were thin long rods with cigar shape that built fruiting bodies with a coralloid shape and orange, brown or pale brown color. In general, the observed older cultures of myxobacteria tended to become shorter and thicker.

Biological screening against test microorganisms

All pure strains dispersed easily in liquid P-medium with the addition of XAD. A quick switch to dispersed growth was obtained from all the strains by transferring 1 mL of myxobacterial culture in liquid CY/H medium before fermentation. Twenty-four crude extracts prepared from the fermentation were tested for growth inhibition against the test microorganisms, which included four Gram-positive, four Gram-negative, two yeasts and one fungus. Table 2 gives the antimicrobial potential of myxobacteria against each target microorganism. No growth of the test organisms in wells after 24-48 h indicated an antimicrobial activity of the isolates.

In the present study, the antimicrobial activity of myxobacterial isolates was varied. Nineteen myxobacteria of 24 pure strains were active: 19 produced inhibitory secondary metabolites against all Gram-positives, 14 against Gram-negative bacteria and 13 showed antifungal activities. Very high activities were detected against the Gram-positive bacteria *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus*, and middle activity against *Mycobacterium smegmatis*, *Chromobacterium violaceum*, *Escherichia coli* (TolC)

Table 1. Distribution of *Myxococcus* sp. and *Corallococcus* sp. according to isolation method and origin.

Geographical origin	Isolation source	Method of isolation	Number of isolates/ genera	Labelling of pure strains in internal database
Bahamas, Nassau	soil with sandy	*E. coli* baiting Filter paper	1M, 1C -M, 2C	BNEM1, BNEC1 BNFC1
Florida, Hollywood beach	sandy	*E. coli* baiting Filter paper	-M, -C -M, -C	- -
Massachusetts, Sandisfield	soil	*E. coli* baiting Filter paper	2 M, 4C 3M, 2C	SFEM1,2; SFEC1,2 SFFM1, SFFC1
Mexico, Mexico City	soil	*E. coli* baiting Filter paper	1M, 1C 4M, 4C	MXEC1 MXFM1,2;MXFC1
Argentina, Iguazu	soil	*E. coli* baiting Filter paper	-M, -C -M, -C	- -
Argentina, Buenos Aires	soil	*E. coli* baiting Filter paper	-M, 1C -M, 2C	BAEC1 BAFC1,2
Bolivia, Santa Cruz	soil	*E. coli* baiting Filter paper	1M, 2C 3M, -C	SCEM1, SCEC1 SCFM1,2
Florida, Miami	soil with sandy	*E. coli* baiting Filter paper	-M, 1C 1M, -C	- MIFM1
British Columbia, Vancouver	soil	*E. coli* baiting Filter paper	-M, 2C 1M, -C	VREC1 VEFM1
East-central Canada, Ontario	soil	*E. coli* baiting Filter paper	-M, -C -M, 1C	- ORFC1

M – *Myxococcus*; C – *Corallococcus*

Table 2. Distribution of antimicrobial activity of *Myxococcus* and *Corallococcus* taxonomic groups.

Strain	Panel of tested microorganisms /range of inhibited wells										
	B.s.	Ch.v.	E.c.1	E.c.2	M.l.	Ps.a.	M.s.	S.a.	M.h.	P.a.	C.a.
Myxococcus sp.											
BNEM1	D	C	-	C	F	A	C	H	A	A	A
SFEM1	F	A	-	B	E	A	D	H	A	-	-
SFEM2	B	C	A	B	-	A	-	-	-	-	-
SFFM1	C	B	A	B	C	A	C	D	-	-	-
MXFM1	H	-	-	-	G	-	C	H	C	-	-
MXFM2	H	-	-	-	G	-	C	H	D	D	B
SCEM1	-	-	-	-	-	-	-	-	-	-	-
SCFM1	G	B	A	D	E	-	B	C	D	E	C
SCFM2	F	A	A	C	E	A	D	H	A	C	B
MIFM1	G	-	-	-	E	-	C	H	B	C	A
VEFM1	-	-	-	-	-	-	-	-	-	-	-
Corallococcus sp.											
BNEC1	H	-	-	D	H	-	C	H	C	A	A
BNFC1	H	-	-	C	H	-	D	H	B	A	A
SFEC1	G	-	-	C	F	-	B	F	-	-	-
SFEC2	H	C	A	D	F	A	D	G	A	C	C
SFFC1	-	-	-	-	-	-	-	-	-	-	-
MXEC1	-	-	-	-	-	-	-	-	-	-	-
MXFC1	-	-	-	-	-	-	-	-	-	-	-
BAEC1	D	C	A	C	C	-	C	C	A	A	A
BAFC1	E	B	-	A	F	-	D	H	-	B	B
BAFC2	D	A	-	A	D	-	C	D	-	A	-
SCEC1	C	D	-	C	-	B	-	-	-	-	-
VREC1	G	-	-	-	F	-	G	-	-	-	-
ORFC1	C	-	-	-	D	-	C	-	-	-	-

B.s. – *Bacillus subtilis*; Ch.v. – *Chromobacterium violaceum*; E.c. 1 – *Escherichia coli* DSM1665; E.c.2 – *Escherichia coli* TolC; M.l. – *Micrococcus luteus*; Ps.a. – *Pseudomonas aeruginosa*; M.s. – *Mycobacterium smegmatis*; S.a. – *Staphylococcus aureus*; M.h. – *Mucor hiemalis*; P.a. – *Pichia anomala*; C.a. – *Candida albicans*

and fungal pathogens. Very low activities were recorded in the case of the Gram-negatives *Escherichia coli* (DSM1665) and *Pseudomonas aeruginosa*.

There has been little mention of the antimicrobial activity of myxobacteria to date, but study of [23] reported that 62 tested myxobacterial isolates showed antifungal activities and inhibition potential against *Escherichia coli* and *Bacillus subtilis*. The majority of active strains belonged to the *Myxococcus* genus, which was indeed the most frequently isolated. Foster et al. [25] reported that out of 64 strains, 77% inhibited growth of *Micrococcus luteus* and 23% shown antifungal activities. Norén and Raper [26] found that the tested myxobacteria were able to produce antibacterial agents that, however, were active only in Gram-positive eubacteria, the Gram-negative species being unaffected by the agents. *Micrococcus* sp. and *Staphylococcus* sp. were the most susceptible. The different inhibition pattern between Gram-positive and Gram-negative bacteria is caused by the component differences of the cell wall morphology [27]. Among the underexplored bacterial taxa, myxobacteria certainly deserve the attention [9]. In recent years, myxobacteria have matched fungi, actinomycetes as well as some species of the genus *Bacillus* as top producers of microbial metabolites [28,29].

Considering these results, it could be seen that two of the investigated strains, *Myxococcus* sp. BNEM1 and *Corallococcus* sp. SFEC2, exhibited higher activity against pathogenic bacteria and fungi. The antimicro-

Table 3. Enzymatic activity and resistance to various antibiotics of isolates BNEM1 and SFEC2.

No.	Enzyme	BNEM1	SFEC2	Antibiotic	BNEM1	SFEC2
2	Phosphatase alkaline	5	4	Ampicillin	-	+
3	Esterase (C4)	1	1	Gentamycin	+	+
4	Esterase lipase (C8)	3	4	Hygromycin	+	+
5	Lipase (C14)	5	4	Chloramphenicol	-	-
6	Leucine arylamidase	5	5	Polymyxin	+	+
7	Valine arylamidase	4	5	Kanamycin	-	+
8	Cystine arylamidase	1	0	Spectinomycin	+	-
9	Trypsin	1	2	Cephalosporine	+	+
10	Chymotrypsin	2	0	Bacitracin	+	+
11	Phosphatase acid	5	5	Fusidic acid	-	+
12	Naphthol-AS-BI-phosphohydrolase	2	2	Oxytetracycline	-	-
	Gal, Gal, Gluc, Glu, Glu, N-ac-glu, man, fuco			Thiostrepton	-	-
13-20		0	0	Trimethoprim	-	-

For API ZYM: Gal – galactosidase, Gluc – glucuronidase, Glu – glucosidase, N-ac-glu – N-acetyl-glucose amidase, Man – mannosidase, Fuco – fucosidase; 5 – >40 nmol, 4 – 20 nmol, 3 – 10 nmol, 2 – 5 nmol, 1 – 2.5 nmol, 0 – no enzymatic activity. For reactions to antibiotics: + (positive) – visible growth indicates resistance, - (negative) – inhibition of growth indicates sensitivity

Table 4. The secondary metabolites from *Myxococcus xanthus* and *Corallococcus coralloides*, which have already been identified and characterized.

Antibiotic	Class	Effect	Reference
Myxococcus xanthus			
myxoprincomide	linear peptide	antifungal	[33]
myxalamids	polyketide-peptide hybrid	antibacterial	[34]
myxochromid	cyclic peptide	antibacterial	[35]
myxovirescin	macrocyclic antibiotic	antibacterial	[36]
myxochelin	catechol siderophore	antibacterial	[37]
cittalin	macrocyclic peptide	no detected	[38]
saframycin	tetrahydroiso-quinoline	antitumor drug	[39]
althiomycin	peptide	antibacterial	[40]
Corallococcus coralloides			
corallorazine	dipeptide	antibacterial	[41]
corallopyronin	polyketide	antibacterial	[42]

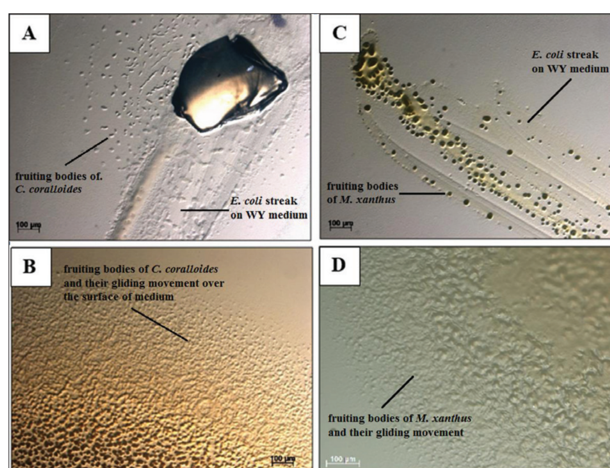

Fig. 1. Fruiting bodies and swarms of *Corallococcus coralloides* SFEC2 on (**A**) WY medium with *E. coli* streaks and on (**B**) CY medium, and *Myxococcus xanthus* BNEM1 on (**C**) WY medium and (**D**) CY medium.

bial activity of the strains is probably due to the presence of an antimicrobial complex of antibiotics. These strains have been found to produce several secondary metabolites. The molecular basis of synthesis for these compounds has been studied in some details (Table 4).

Characterization of the most potent *Myxococcus* and *Corallococcus* strains, BNEM1 and SFEC2

Two of the most active isolates were identified on the basis of morphological and physiological approaches and 16S rRNA gene sequencing. Morphological identification was confirmed by microscopic and macroscopic examination. The strain BNEM1 was a Gram-negative rod with slender vegetative cells, spherical myxospores and yellow fruiting bodies. The swarm colony was unpigmented or lightly yellow. Observed structures led to the identification of *Myxococcus xanthus*. The strain SFEC2 was Gram-negative with slender vegetative cells, orange and brown fruiting bodies forming ridges, and spherical myxospores were bizarrely coralloid. Based on these features, this strain was identified as *Corallococcus coralloides*. Fruiting bodies and swarms of these strains are shown in Fig. 1. Myxobacteria taxonomy was earlier associated only with morphology, which is adequate in differentiating between genera [30]. Nowadays, the identification of myxobacteria up to the species level is commonly derived from 16S rRNA gene analysis. The PCR reaction of the 16S rRNA gene sequence from the tested strains showed excellent congruence between morphological characteristics and 16S rRNA data.

The 16S rRNA genes were compared by BLAST analysis, together with the morphology of structures typical for myxobacteria, in order to identify the tested isolates. A phylogenetic analysis at the species level is shown in Fig. 2. A phylogenetic comparison of evaluated sequences with available sequences in the NCBI BLAST tool confirmed phylogenetic position with morphology identification. Both isolates were identified to the species level; isolate BNEM1 had 99% similarity with *Myxococcus xanthus* and isolate SFEC2 with *Corallococcus coralloides*. Sequences were deposited in GenBank under the accession numbers KX669224 for BNEM1 and KX669225 for SFEC2.

Test strips that determine enzyme reactions, namely API ZYM, resulted in the enzyme patterns for *Corallococcus* and *Myxococcus* strains. However, according to [31], the features offered by these strips are not helpful in distinguishing members of the genera *Myxococcus* and *Corallococcus*, because *Myxococcus* strains displayed the same reactions as are described for *Corallococcus* species. Our results confirmed this observation. The reactions on the API ZYM strips by tested strains were reasonably similar to the reactions given by [32] for the genus *Corallococcus* and

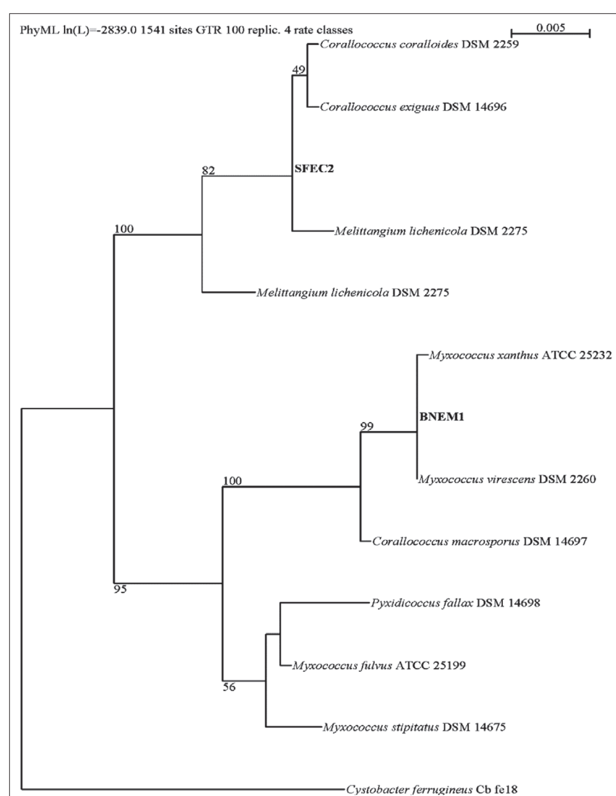

Fig. 2. Phylogenetic tree of two the most active strains based on 16S rRNA gene sequences.

Myxococcus, which suggest a close relationship. Both strains showed similar enzymatic activities; highly positive reactions were confirmed for alkaline phosphatase, esterase-lipase, lipase, leucine arylamidase, valine arylamidase, and phosphatase acid (Table 3). Results from morphological and molecular methods strengthened their position in the *Myxococcales* order.

Antibiotic resistance was tested against 13 antibiotics. Strain BNEM1 was resistant to gentamycin, hygromycin, polymyxin, spectinomycin, cephalosporine, and bacitracin; strain SFEC2 was found to be resistant to ampicillin, gentamycin, hygromycin, polymyxin, kanamycin, cephalosporine, and bacitracin. Some growth was determined on medium with the addition of fusidic acid. The results of growth of *Myxococcus xanthus* and *Corallococcus coralloides* in the presence of various antibiotics was similar to [32]. The growth response of the BNEM1 and SFEC2 strains to different temperatures was tested at 20, 30, 37 and 42°C on VY/2 agar. The optimum growth temperatures for strains were near 30°C, like most myxobacteria, and neither strain could grow at 37 and 42°C. The pH optimum was seen at pH 6.0-7.0.

The present findings highlight the significance of myxobacterial strains as potential sources of a broad spectrum of antimicrobial agents. These findings have important implications for the discovery of potent antibiotics from myxobacteria.

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