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ORIGINAL ARTICLE

Molecular and functional characterization of myxobacteria isolated from soil in India

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Abstract This study reports the isolation of myxobacteria from soil collected from plains in north India. Based on the morphology and 16S rDNA sequence, the isolated myxobacteria were identified as *Corallococcus* sp., *Pyxidicoccus* sp., *Myxococcus* sp., *Cystobacter* sp. and *Archangium* sp. The myxobacteria were functionally characterized to assess their ability to produce antibacterial and anticancer metabolites. The isolates were found to be functionally versatile as they produced extracellular bioactive molecules that exhibited high frequency of activities against *Bacillus cereus*, *Mycobacterium smegmatis*, *Enterobacter cloacae* and *Pseudomonas syringae*. The strains also showed cytotoxic activity against the human cancer cell lines of liver, pancreas, prostrate, bone and cervix. These results indicate the importance of isolating diverse strains of myxobacteria from unexplored habitats to find novel bioactive compounds. Moreover, the bioactive molecules explored in this study are predominantly hydrophilic compounds, obviating the limitations of solubility-related aspect of drug discovery.

Keywords Myxobacteria · Functional characterization · Antibacterial · Anticancer

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Introduction

For decades, microbes have been the major contributor to natural products for drug discovery and development. The microbial natural products being used as drugs are: antibacterial agents, such as the penicillins, cephalosporins, aminoglycosides, tetracyclines and various polyketides; cholesterol lowering agents, such as mevastatin and lovastatin; immunosuppressive agents, such as the cyclosporins and rapamycin; and anthelmintics and antiparasitic drugs, such as the ivermectins (Buss and Waigh 1995). The emergence of antibiotic-resistant pathogenic microorganisms and rapid development of resistance to chemotherapeutic drugs have necessitated the discovery of structurally diverse and mechanistically distinct antimicrobial and anticancer compounds. Most of the microbial bioactive compounds discovered so far originated from actinomycetes, but the focus is currently on the less exploited microbes as viable sources of secondary metabolites; viz., marine bacteria, cyanobacteria, endosymbionts and myxobacteria. Myxobacteria are aerobic Gram-negative, unicellular, rod-shaped δ-proteobacteria, distributed in a wide range of habitats including soil, bark of trees, decaying plant materials, and freshwater and marine environment (Ravenschlag et al. 1999; Reichenbach 1999; Garcia et al. 2009; Dawid 2000). They have a very large genome size compared to other bacteria, and a number of structurally unique biologically active secondary metabolites are produced by this group of bacteria (Wenzel and Muller 2009; Han et al. 2013; Reichenbach 2001). Many compounds isolated from myxobacteria act on unique cellular targets, which, at the time of their discovery, were not targeted by other secondary metabolites (Bode and Muller 2006; Herrmann et al. 2017).

Myxobacteria have been the source of several new antibacterials, e.g., thuggacins from *Sorangium cellulosum* (active against *Mycobacterium tuberculosis*), crocacin from *Chondromyces crocatus* (active against Gram-positive bacteria), indiacens from *Sandaracinus amylolyticus* (mildly inhibit Gram-positive and Gram-negative bacteria), disciformycins from *Pyxidicoccus fallax* (active against methicillin- and vancomycin-resistant *Staphylococcus aureus*), Coralmycins (potent activity against Gram-negative bacteria), etc. (Irschik et al. 2007; Kunze et al. 1994; Steinmetz et al. 2012; Surup et al. 2014; Kim et al. 2016). Several new antifungal compounds have also been reported from myxobacteria: pedein A and B, miuraenamides, aurafurons A and B and cyrmenins (Kunze et al. 2005, 2008; Iizuka et al. 2006; Sasse et al. 2003). A recent assessment of the antibacterial compounds form myxobacteria has revealed that most of these compounds are active against Gram-positive bacteria (Schaberle et al. 2014).

Several secondary metabolites produced by myxobacteria are promising anticancer agents. Epothilone, a paclitaxel mimetic, obtained from *Sorangium cellulosum* So ce56, has been approved for breast cancer treatment (Burris 2008). In 2007, ixabepilone, an analog of epothilone B, was approved by FDA for the treatment of metastatic or locally advanced taxane-resistant breast cancer (Tan and Toppmeyer 2008). Tubulysins, produced by *Archangium gephyra* Ar 315, *Angiococcus disciformis* An d48 and *Cystobacter* sp. SBCb004, are potent growth inhibitors compared to taxol, epothilone and vincristine and are effective in multidrug-resistant cell lines (Sasse et al. 2000; Steinmetz et al. 2004; Chai et al. 2010). Argyrin A and cruentaren A produced by *Archangium gephyra* and *Byssovorax cruenta*, respectively, are highly cytotoxic against various human tumor cell lines (Nickeleit et al. 2008; Kunze et al. 2007). Recently, nannocystin from *Nannocystis* sp. has been found to show activity against various cancer cell lines by targeting eukaryotic translation (Krastel et al. 2015).

Although myxobacteria are an attractive source of new chemical class of compounds, they have not been fully exploited in the quest for new bioactive compounds, because their isolation and purification are tedious. The fact that they are very sensitive to mechanical stress and are prone to lysis make their handling very difficult (Reichenbach and Dworkin 1992). The ability of myxobacteria to synthesize a particular compound is specific to a strain, which makes myxobacteria an attractive proposition for bioprospection (Reichenbach and Hofle 1999). In view of their immense potential as a source of natural compounds having new chemical structures with unique mechanism of action and no report on functional diversity of myxobacteria from Indian habitats, the present study was undertaken to investigate myxobacteria to assess them as source of novel pharmaceutically active molecules.

Our study shows that myxobacteria isolated from soil in India inhibit *Bacillus cereus*, *Mycobacterium smegmatis* and Gram-negative bacteria (*Pseudomonas syringae* and *Enterobacter cloacae*) suggesting their potential to be exploited as a source of antimicrobial compounds. The extracted molecules also showed antiproliferative activity against the cancer cell lines of cervix, liver, pancreas, prostate and bone. Most of the compounds tested in laboratories fail to make it to the clinic because of bioavailability limitations. To address this constraint, in this study, we focused on the assessment of bioactive potential of water-soluble compounds secreted by myxobacteria.

Materials and methods

All chemicals and media components were procured from HiMedia, Sisco Research Laboratory, Lobachemie, Genei-Merck or Sigma-Aldrich. All plastic wares and glass wares were purchased from HiMedia, Tarsons or Borosil.

Isolation, purification and taxonomy of myxobacteria

Myxobacteria were isolated by baiting technique from the soil samples collected near decaying plants and animals in the plains of north India (Reichenbach and Hofle 1999). *Escherichia coli* and/or autoclaved baker's yeast was streaked on cycloheximide (100 µg/ml) incorporated water CleriGel plate as three parallel streaks, cross streak or dot. Cycloheximide (100 µg/ml)-treated soil samples were placed on *E. coli*/autoclaved yeast and incubated at 30 °C for 3–20 days. For purification, colony morphology and/or fruiting bodies were viewed under stereomicroscope to confirm the identity of myxobacteria according to the criteria indicated by Reichenbach and Dworkin (1992). The fruiting bodies and cells from the edge of the swarms were picked up and streaked on CY agar (g/l: Casitone, 10; yeast extract, 1; $CaCl_2 \cdot 2H_2O$, 1; agar 20) or SP agar (DSZM medium 222) and incubated at 30 °C. This step was repeated till the isolates were obtained in pure culture. Thereafter, the isolates were maintained on SP medium.

Molecular characterization of myxobacteria

Genomic DNA isolation

Myxobacteria were grown in SP medium at 30 °C for 48 h on a rotary shaker. Genomic DNA was extracted using HiYield™ Genomic DNA Mini Kit (Real Biotech Corporation, Taiwan).

PCR amplification of 16S rDNA

Genomic DNA of myxobacterial isolates was used as template for PCR amplification of 16S *rRNA* gene with bacterial universal forward primer 8-27F (5′-AGAGTT TGATCCTGGCTCAG-3′) and reverse primer 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR reaction mixture (50 µl) contained 5 µl of 10× Taq reaction buffer (Genei, Bangalore, India), 0.2 µM each primer, 200 µM each dNTP, 20 ng of template DNA and 1.5 U Taq DNA polymerase (Genei, Bangalore, India). PCR amplification was carried out using MyCycler (Biorad). Thermal cycling conditions were as follows: (1) initial denaturation (2 min at 95 °C), (2) five cycles of denaturation (95 °C for 45 s), annealing (45 °C for 45 s) and primer extension (72 °C for 45 s), (3) five cycles of denaturation (95 °C for 45 s), annealing (50 °C for 45 s) and primer extension (72 °C for 45 s), (4) 30 cycles of denaturation (95 °C for 45 s), annealing (55 °C for 45 s) and primer extension (72 °C for 45 s), (5) final extension step at 72 °C for 10 min. The amplified product was resolved by agarose electrophoresis, and the fragment was excised out from the gel and purified by HiYield™ Gel/PCR DNA Kit (Real Biotech Corporation, Taiwan).

16S rDNA sequence analysis

16S rDNA was sequenced at Bioserve Biotechnology Pvt. Ltd., Hyderabad, India, and the identification of the isolates was confirmed on the basis of 16S rRNA gene sequence using the Ribosomal Database Project (RDP; http://rdp.cme.msu.edu) (Cole et al. 2014). Multiple sequence alignment and phylogenetic analysis were performed using neighbor-joining (N-J) method with 1000 bootstrapping replications of the MEGA 6.06 software package (Tamura et al. 2013).

Preparation of extract

A seed culture was prepared by growing myxobacteria isolates in 10 ml CY broth at 30 °C for 36–48 h in an incubator shaker. To prepare extract for evaluating the antibacterial properties, 5 ml of seed culture was inoculated into 500 ml CY broth and incubated at 30 °C at 180 rpm for 72 h. Thereafter, the culture supernatant was incubated with 2% (w/v) Amberlite XAD16 N (Sigma) at 30 °C for 16 h. The resin was extracted with 200 ml of methanol at 30 °C for 12 h at 180 rpm. The extract was dried on a vacuum rotary evaporator (Buchi) at 40 °C. The dried powdered extract was suspended in water at 0.25 mg/ml, incubated at 30 °C overnight and centrifuged at 10,000 rpm; the supernatant was designated as water extract (WE). The insoluble residual matter was dissolved in 250 µl DMSO and designated as DMSO extract (DE).

To prepare extracts for checking the anticancer activity, myxobacteria were grown in 300 ml of CY broth at 30 °C for 36 h in an incubator shaker. Thereafter, 300 ml of ethyl acetate was added to each flask and extraction was carried out at 30 °C for 2 h at 180 rpm. The upper organic phase thus obtained was separated and concentrated on a rotary evaporator and suspended in water as above.

Water-soluble fractions of the extracts were sterilized through a 0.22 µ filter and stored at 4 °C till further use.

Antimicrobial activity

The antibacterial activity was evaluated by agar plate diffusion assay. The extracts from the isolated myxobacteria were screened for antibacterial activity against *Bacillus cereus* MTCC 430, *Enterobacter cloacae* MTCC 509, *Pseudomonas syringae* MTCC 1604 and *Mycobacterium smegmatis* MTCC6. 100 µl of each WE or 50 µl of each DE was added to the wells (5 mm) on Mueller–Hinton (MH) agar plate seeded with 100 µl of activated test cultures. The plates were incubated at 30 °C for 24 h and the zones of inhibition around the bacterial extracts were measured and compared with the negative control (water for WE and 10% DMSO for DE). Kanamycin (100 µg/ml) was included as positive control in the assay.

In vitro cytotoxicity against human cell lines

Human cancer cell lines (HepG2, HeLa, MIA PaCa-2, MG-63 and PC-3) were procured from NCCS, Pune, India. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) with 10,000 U penicillin, 10 mg streptomycin and 25 µg amphotericin B per ml at 37 °C in a CO_2 incubator (5% CO_2; 90% RH).

In vitro cytotoxicity of different isolates was determined by sulforhodamine B (SRB) assay. 3×10^3 cells were added to each well of 96 well plates and allowed to attach and grow for 24 h. 10 µl of WE was added to each well and the plate was incubated for 48 h. Cells were also incubated with 10 µM doxorubicin (DOX) as positive control. The cells were then fixed with 50 µl of ice-cold 50% (w/v) trichloroacetic acid at 4 °C for 1 h (Skehan et al. 1990). To the washed and air dried plate, 100 µl SRB (0.057% w/v SRB in 1% acetic acid solution) solution was added to each well and the plate was incubated at room temperature for 30 min. The unbound SRB solution was removed by washing with 1% acetic acid solution and plates were air dried. 200 µl of 10 mM Tris base solution (pH 10.5) was added to each well and after shaking on a gyratory shaker for 5 min, the optical density was measured at 510 nm

using a microplate reader (Vichai and Kirtikara 2006). Inhibition of cell growth was calculated as: % cell growth = (OD of treated cells/OD of control) × 100; % cell inhibition = 100 − % of cell growth. Cytotoxicity was also checked on Chinese hamster ovary (CHO) cells to assess the specificity of extracts on cancer cells.

Mass spectrometry of the extracts

Extracts were fractionated by HPLC performed on an Agilent 6410 LC/MS–MS instrument (Agilent Technologies, USA) equipped with a column Agilent ZORBAX Eclipse XAD C18, (4.6 × 100 mm) eluted at 0.5 ml min flow rate. The mobile phase consisted of solvent A (water with 0.01% formic acid) and solvent B (acetontrile). A gradient program was used as follow: 0–8 min, 5–70% B; 8–12 min, 70% B; 12–13 min, 70–5% B; and 13–15 min, 5% B. The effluent from the column was diverted to the ESI interface of a 6410B triple quad LC/MS system (Agilent Technologies, USA.

The analysis was performed using an electrospray ionization (ESI) source in positive and negative modes. The operation conditions were as follows: scan range of 50–1800 amu, ion source temperature 300 °C, nebulizer 50 psi, gas flow 11 L/min, capillary voltage 3000 and a step size of 0.1 amu. Agilent Mass Hunter software (version B.04.00) was used for data acquisition and processing. Data were manually sorted to list such information as the retention time, m/z values for adduct ions.

Statistical analysis

All the experiments were carried out in triplicate and the values were represented as their mean ± standard deviation.

Results

Taxonomy of the isolated myxobacteria

Fifty myxobacterial strains were isolated from pure culture from various soil samples in India. These isolates were identified by observing the morphology of vegetative cells, swarms and fruiting bodies. The isolates that had identical colony/swarm morphology or fruiting bodies from the same soil sample were discarded to avoid replication of the isolated strains. Among these myxobacteria, nine isolates showing potent inhibitory activities against the test microorganisms and human cancer cell lines were selected for molecular characterization. The amplified 16S rDNA of the selected isolates was sequenced and compared with the 16S rDNA sequence of myxobacteria represented in the RDP database (Table 1). Using MEGA6, a phylogenetic tree was generated by comparing the amplified 16S rDNA sequences with the 16S rDNA sequences of the myxobacterial type strains (Fig 1). All the isolated myxobacteria belong to two families (Cystobacteraceae and Myxococcaceae) in the suborder Cystobacterineae within the order myxococcales. The isolated myxobacteria belonged to five genera: *Corallococcus*, *Pyxidicoccus*, *Cystobacter*, *Archangium* and *Myxococcus*. The swarm morphology of the purified myxobacterial strains is shown in Online Resource 1.

Antimicrobial activity of myxobacteria

The results in Table 2 show that seven of the isolated myxobacterial strains examined in this study exhibited antimicrobial activity against one or more of the test microorganisms. The extract from S104 and S145 showed broad-spectrum antibacterial activity and inhibited all the test organisms. WE and DE of GNDU 172 showed appreciable activity against *B. subtilis* and *E. cloacae*, whereas S225 did not exhibit inhibitory activity against any of the test organisms. The DE of both S213 and S223 exhibited a similar spectrum of antibacterial activity, being active against *B. cereus*, *P. syringae* and *M. smegmatis*, whereas S223 WE in addition strongly inhibited *B. cereus*. WE and DE of the isolate S229 showed potent inhibitory activity against *B. cereus* and *P. syringae*, respectively. The DE of the isolate S233 was specifically active against *M. smegmatis* (Fig. 2).

Anticancer activity of myxobacteria

Out of the selected isolates, eight exhibited antiproliferative activity against one or more cancer cell lines, viz., cervical (HeLa), HepG2 (liver), MG-63 (osteosarcoma), MIA PaCa-2 (Pancreatic) and PC-3 (prostate) tested in this study (Table 3). The extract from S145 was active (more than 50% growth inhibition) against all tested cell lines except HepG2 (liver) cell line. S145 showed 58, 53, 60 and 86% inhibition of HeLa, MG-63, MIA PaCa-2 and PC-3 cell lines, respectively. GNDU172 and S225 produced antiproliferative agents against MIA PaCa-2 (88 and 72% inhibition, respectively) and PC-3 (59 and 67% inhibition, respectively) cell lines. The extract of S199 showed inhibition of 76% against PC-3 and 54% against HeLa cell line. S213 and S223 produced potent anticancer agents against MIA PaCa-2 (more than 80% inhibition) and PC-3 (64 and 85% inhibition, respectively). S229 showed 57% inhibition of MG-63 in addition to being active against MIA PaCa-2 and PC-3 exhibiting 73 and 86% inhibition, respectively. The isolate S233 was active against most of the tested cell lines where it exhibited 61, 53, 84 and 76% activity against

Table 1 Molecular and morphological characteristics of the isolated myxobacteria

S. no.	Isolate ID (GenBank accession no.)	Closest related match in RDP	% Identity	Morphological features
1	S104 (JX316830)	*Corallococcus coralloides* DSM 52497	100	Colonies: pink swarm; fruiting bodies: pink corals
2	S145 (JX316838)	*Corallococcus coralloides* DSM 52499	97.9	Colonies: pink swarm; fruiting bodies: reddish-brown granules
3	GNDU 172 (KJ152124)	*Pyxidicoccus fallax* DSM 14698	98.9	Colonies: pink swarm; fruiting bodies: not observed
4	S199 (KM25773)	*Myxococcus fulvus* NBRC 100067	98	Colonies: pink swarm; fruiting bodies: not observed
5	S213 (KP178620)	*Cystobacter violaceus* DSM 14727	96.5	Colonies: mucilaginous violet swarm; fruiting bodies: not observed, only cell aggregates were observed
6	S223 (KP178626)	*Cystobacter minus* DSM 14751	96.9	Colonies: mucilaginous pink; fruiting bodies: not observed, rounded cell aggregates were observed
7	S225 (KM978082)	*Pyxidicoccus fallax* DSM 14698	99.3	Colonies: pink swarm; fruiting bodies: not observed, cells formed rounded aggregates
8	S229 (KM978086)	*Cystobacter badius* DSM 14723	97.4	Colonies: pink swarm; fruiting bodies: pink and spherical
9	S233 (KM978088)	*Archangium gephyra* DSM 2261	98.1	Colonies: pink swarm; fruiting bodies: not observed

HeLa, HepG2, MIA PaCa-2 and PC-3 cell lines respectively.

Discussion

There is a persistent requirement for new bioactive compounds so that the demand for new natural products in therapy can be met. Myxobacteria have great potential to contribute to the pool of natural products for drug discovery. Myxobacteria were isolated from soil collected near decaying plants and animals from plains in north India by *E. coli* baiting method, because it has been reported to be a productive isolation method for soil myxobacteria (Gaspari et al. 2005). The isolates were identified by swarm morphology or fruiting bodies and subjected to molecular characterization by 16S rDNA sequencing. A phylogenetic tree was constructed based on the sequences of the 16S rDNA of the isolated myxobacteria, strains showing the highest similarity with the isolated myxobacteria and the type strains from the database which showed that isolates were distributed within the suborder Cystobacterineae. Though the isolates S104 and S145 showed the greatest similarity in the RDP database to *C. coralloides*, S145 resembles *C. exiguus* based on the morphology of its fruiting bodies. Stackebrandt and Pauker (2005) have reported that *Corallococcus* spp., *C. coralloides* and *C. exiguus*, share high 16rRNA gene sequence similarity and can be distinguished based on their fruiting bodies. The isolates included in this study that belong to the same genera showed differences in the functional characteristics with respect to their ability to produce bioactive metabolites. The isolates obtained in pure cultures exhibited inhibitory activities against *M. smegmatis*, *B. cereus* and Gram-negative bacteria. Five out of the nine myxobacterial isolates selected for functional characterization produced inhibitory agents against Gram-negative test bacteria (*E. cloacae* and *P. syringae*), whereas six isolates showed activity against *M. smegmatis*. An assessment of the available antibiotic compounds till date has revealed that 30% of these inhibit only Gram-positive bacteria and only 1.5% of the compounds exhibit activity specifically against Gram-negative bacteria (Berdy 2005). Studies on myxobacteria have revealed that they secrete natural products that are inhibitory predominantly to Gram-positive bacteria, whereas very few myxobacterial isolates show inhibitory activity against Gram-negative bacteria (Schaberle et al. 2014). On the contrary, more than 50% of myxobacteria isolated from Israel by Gaspari et al. (2005) synthesized compounds showing inhibitory activity against Gram-negative bacteria, suggesting that myxobacteria have the capability to meet the medical need for compounds acting on Gram-negative pathogens. The antibacterial spectrum of myxobacterial isolated in this study is different from the inhibitory activities reported earlier for the same genera. Corallopyronins, reported from *C. coralliodes*, do not inhibit Gram-negative bacteria, whereas the extract reported in this study from S104 showed potent activity against *E. cloacae* and weak activity against *P. syringae* (Schaberle et al. 2014). The comparison of the m/z of the peaks in mass spectra of the water-soluble fraction of methanol extracts from isolates S104 and S145 with the bioactive compounds synthesized by *C. coralloides* listed in the Dictionary of Natural Products (http://dnp.chemnetbase.com) did not identify any known compounds, suggesting that myxobacteria isolated in this study

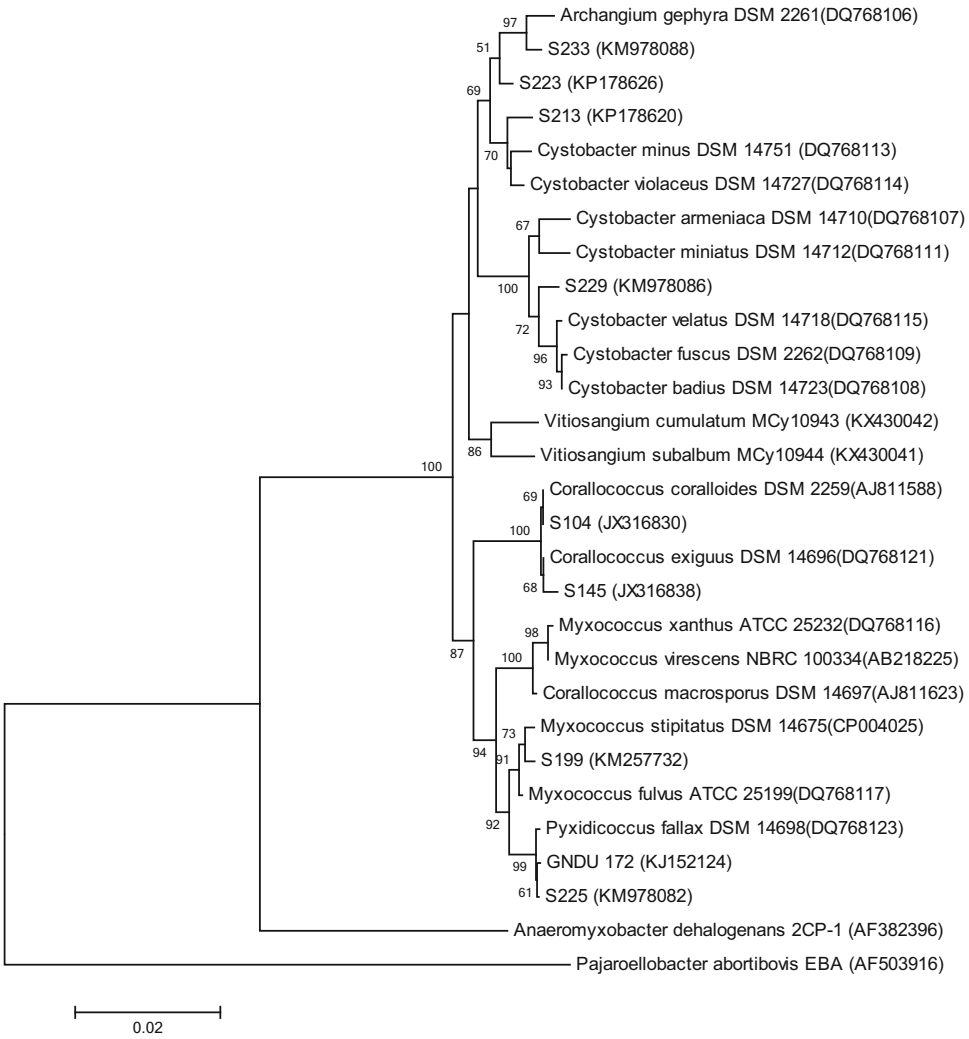

Fig. 1 Phylogenetic tree of the isolated myxobacteria constructed using the neighbor-joining method based on 16S rRNA gene sequences aligned to their closest type strains. Phylogeny was tested with 1000 bootstrap replications (accession numbers are mentioned in *parenthesis*)

Table 2 Antibacterial activities of myxobacteria isolates

S. no.	Isolate ID	*B. cereus* MTCC 430	*E. cloacae* MTCC 509	*P. syringae* MTCC 1604	*M. smegmatis* MTCC 6
1.	S104 (WE)	1.83 ± 0.2	1.63 ± 0.1	0.67 ± 0.1	2.47 ± 0.2
	S104 (DE)	1.03 ± 0.1	–	–	0.82 ± 0.1
2.	S145 (WE)	2.40 ± 0.1	1.07 ± 0.1	0.68 ± 0.1	2.47 ± 0.1
	S145 (DE)	0.97 ± 0.1	–	–	0.72 ± 0.1
3.	GNDU172 (WE)	2.33 ± 0.1	0.83 ± 0.1	–	–
	GNDU172 (DE)	2.23 ± 0.1	1.17 ± 0.2	–	–
4.	S213 (WE)	–	–	–	–
	S213 (DE)	0.97 ± 0.1	–	0.72 ± 0.1	2.07 ± 0.1
5.	S223 (WE)	1.43 ± 0.1	–	–	–
	S223 (DE)	1.00 ± 0.1	–	0.77 ± 0.1	0.72 ± 0.1
6.	S229 (WE)	1.03 ± 0.1	–	–	–
	S229 (DE)	0.93 ± 0.1	–	–	2.23 ± 0.2
7.	S233 (WE)	–	–	–	–
	S233 (DE)	–	–	–	0.98 ± 0.2

Values shown as diameter of inhibition in centimeter

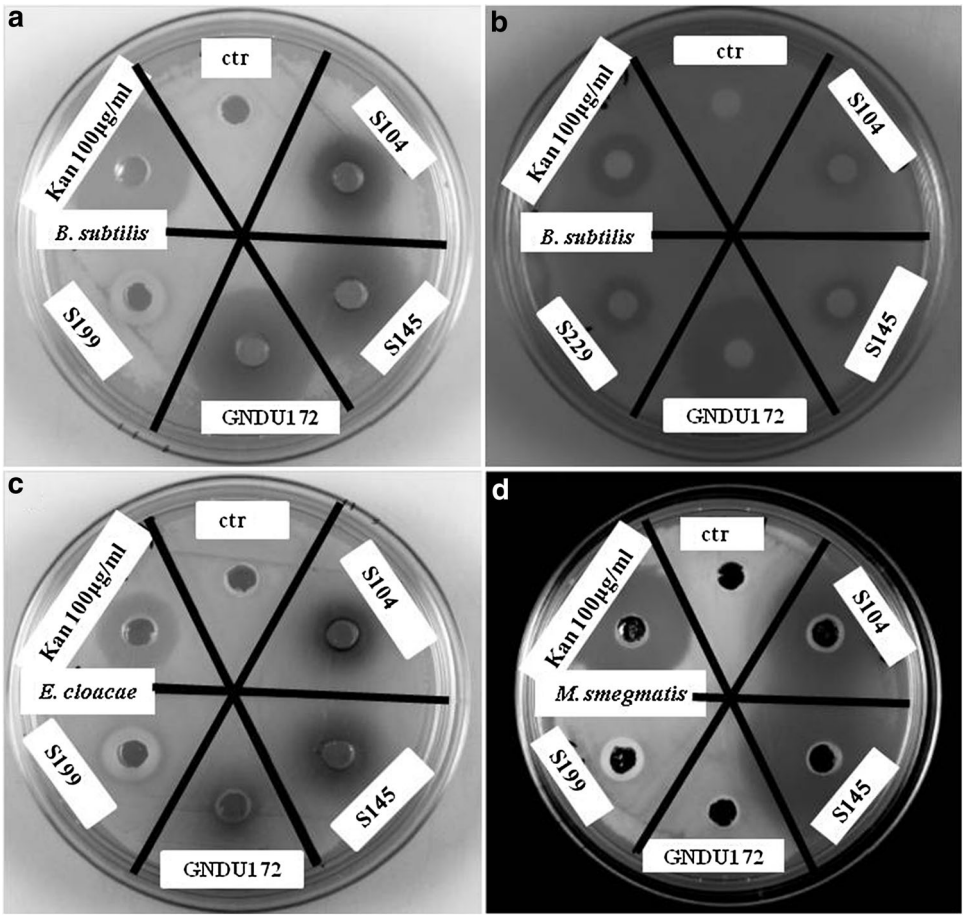

Fig. 2 Antimicrobial activity producing isolates showing zone of inhibition against **a** *B. subtilis* WE, **b** *B. subtilis* DE, **c** *E. cloacae* WE, **d** *M. smegmatis* WE. Kanamycin (kan) as positive control and 10% DMSO or water as negative negative control (ctr) was included in each plate

Table 3 In vitro cytotoxicity of myxobacterial isolates against human cancer cell lines

S. no.	Isolates	Growth inhibition (%) Human cancer cell lines				
		HeLa (cervix)	HepG2 (liver)	MG-63 (bone)	MIA PaCa-2 (pancreas)	PC-3 (prostate)
1.	S145	58 ± 1.6	46 ± 2.1	53 ± 0.8	60 ± 1.8	86 ± 1.4
2.	GNDU172	31 ± 1.2	28 ± 1.8	45 ± 2.9	88 ± 1.2	59 ± 0.9
3.	S199	54 ± 2.1	34 ± 1.9	48 ± 2.0	17 ± 1.9	76 ± 1.8
4.	S213	28 ± 0.8	24 ± 0.5	44 ± 1.2	84 ± 0.7	64 ± 1.5
5.	S223	48 ± 3.1	22 ± 0.9	41 ± 1.3	86 ± 0.65	86 ± 0.7
6.	S225	27 ± 1.6	24 ± 0.9	36 ± 2.5	72 ± 1.3	67 ± 0.7
7.	S229	42 ± 0.9	15 ± 2.1	57 ± 1.1	73 ± 1.0	86 ± 0.6
8.	S233	61 ± 1.3	53 ± 0.8	48 ± 1.5	84 ± 1.1	76 ± 0.5
9.	DOX (1×10^{-5} M)	55 ± 0.7	29 ± 0.9	31 ± 1.0	70 ± 0.8	67 ± 0.9

Cytotoxicity of the extracts against CHO cell line was found to be insignificant

produce novel bioactive metabolites (Online Resource 2). This is the first report of the inhibitory activity of *Pyxidicoccus* sp. (GNDU172) against *E. cloacae*. Two molecules, gulmirecins and disciformycins, reported from *P. fallax* are active only against staphylococci (Schieferdecker et al. 2014). Our isolates S213 and S223; identified as *Cystobacter* sp., showed weak activity in DE against *P. syringae*. On the contrary, Baumann et al. (2014) reported cystobactamids from *Cystobacter* sp. that efficiently inhibited the growth of Gram-negative bacteria *E. coli* and *Acinetobacter baumannii*. Another light- and oxygen-sensitive secondary metabolite with no aqueous solubility,

Roimatacene, synthesized by *Cystobacter ferrugineus* Cb G35 inhibits Gram-negative bacteria (Schaberle et al. 2014). We have used the extracts stored at 4 °C over a time period of more than 3 months and did not encounter any decrease in the activity of the compounds (data not shown). It is possible that the isolates in this study are novel strains that produce previously unknown metabolites having new chemical structures that can be developed into potent lead structures against Gram-negative pathogens. A study on the secondary metabolome of *M. xanthus* has suggested that intraspecific screens of species of myxobacteria are likely to yield novel secondary metabolites (Krug et al. 2008). These compounds can be attributed to the orphan biosynthetic pathways discovered in the genomes of myxobacteria (Gross 2007).

The isolated myxobacteria produced anticancer compounds that showed high frequency of inhibitory activity against the human cancer cell lines of the pancreas and prostate. The extracts of some isolates inhibited more than one cancer cell line tested in this study. It is possible that the compounds produced by these bacteria target a common underlying mechanism of inhibition of growth of cancer cells. This study reports for the first time the anticancer activity of secondary metabolites from *C. exiguus*. *Pyxidicoccus* sp. in this study produces compounds that are inhibitory to pancreatic (MIA PaCa-2) and prostate (PC-3) cancer cell lines, whereas a secondary metabolite from *P. fallax* has been associated with inhibition of leukemic cells by Schieferdecker et al. (2015). In the present investigation, the extract from *M. fulvus* (S199) exhibited the inhibition of PC-3 (prostate cancer) and HeLa (cervical cancer) cells which have not been documented in the cytotoxicity profile of the metabolite isolated from *M. fulvus* in a study by Ahn et al. (1999). The anticancer peptides, tubulysins, which are very effective against drug-resistant cancers, have been isolated from *Cystobacter* sp. and *Archangium* sp. but these peptides have no aqueous solubility and require the development of targeted delivery efforts (Murray et al. 2015). In this study, the extracts from *Cystobacter* spp. (S213, S223 and S229) and *Archangium* sp. (S233) that exhibit anticancer activity constitute water-soluble compounds and are unlikely to be previously described tubulysins. Horstmann et al. (2011) reported inhibition of cervix and prostate cancer cell lines by secondary metabolites of *Cystobacter* sp. and *Archangium* sp. and in this study MG-63 (bone), and MIA PaCa-2 (pancreatic) cancer cell lines were also inhibited by extracts from these myxobacteria.

It is evident from the results of this study that the isolated myxobacteria isolated from Indian habitats can contribute novel compounds to the pool of natural products for therapy against pathogenic microbes and human cancers. Further studies are being focused on the production and isolation of bioactive molecules, to determine their chemical nature and to deduce the mechanism of action for antibacterial and anticancer activities.

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Compliance with ethical standards

Conflict of interest The authors declare no commercial/financial conflicts of interest.

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