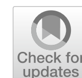
3 Biotech (2019) 9:167
https://doi.org/10.1007/s13205-019-1687-7

ORIGINAL ARTICLE



Taxonomic characterization and antimicrobial compound production from *Streptomyces chumphonensis* BDK01 isolated from marine sediment

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Received: 1 October 2018 / Accepted: 19 March 2019 / Published online: 4 April 2019
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Abstract

Streptomyces sp. isolated from marine sediment collected from Palk Strait, Bay of Bengal was investigated for its antagonistic potential. The isolate exhibited antimicrobial activity against selected bacterial strains of clinical importance such as *Staphylococcus aureus* MTCC 3160, *Bacillus pumilus* NCIM 2327, *S. aureus* (methicillin resistant), *Escherichia coli* MTCC 1698, *E. coli* (ESBL), *Shigella flexneri* MTCC 1457, *Proteus vulgaris* and *Enterobacter cloacae*. Phenotypic and molecular characterization ascertained the isolate BDK01 as *Streptomyces chumphonensis*. Media optimization with one variable-at-a-time strategy was attempted to identify the ideal concentrations of starch (5–15 g/l), casein (0.01–0.05 g/l), NaCl 1.0–3.0 g/l, pH (4.0–9.0 g/l), temperature (25–45 °C) and inoculum level (0.5–5 ml) towards achieving maximum antimicrobial compound production. Statistical optimization of production media was carried by establishing an 11 variables 17 run experiment through PB model which evinced starch, calcium carbonate, pH and inoculum concentration that highly influenced bioactive compound production. Spectral data of active ethyl acetate extract revealed the presence of various bioactive compounds such as Salicyl alcohol, *N*-phenyl benzamide. 6-Octadecenoic acid, (*Z*), 1,3,5-Cycloheptatriene. Antiproliferation activity of active fraction against MCF-7 Cell line exhibited IC_{50} value of 9.5 µg/ml. Overall, it is observed that the marine actinomycete *S. chumphonensis* BDK01 could be employable as promising strain for novel antimicrobial and cytotoxic metabolites.

Keywords Marine actinomycetes · Microbial diversity · Antimicrobial activity · *Streptomyces chumphonensis* strain BDK01 · Anticancer activity

Introduction

Marine habitats are potential and promising region unravelling novel biomolecules due to its diverse physical, chemical, and biological entities compared to terrestrial environment (Romano et al. 2017). Often, shallow and deep-water sediments are proven source for antimicrobial and antitumor compounds-producing microorganisms (Zheng et al. 2000; Gu et al. 2004; Kwon et al. 2006). Generally, Actinobacteria accounts for more than 7000 bioactive compounds as reported in the Dictionary of Natural Products. Among them, *Streptomyces* genus alone contribute for more than 80% of the actinobacterial compounds (Jensen et al. 2005; Bull and Stach 2007). Dalisay et al. (2013) found that 25% of *Streptomyces* isolated from marine environment showed antimicrobial activity, which increased when tested with seawater. On the other hand, multiple drug-resistant bacteria

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13205-019-1687-7) contains supplementary material, which is available to authorized users.

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and other clinical pathogens are evolving as big threat to human community leading to high morbidity and mortality especially in developing countries (Campfield et al. 2014). Hence, the present investigation was focused on discovery of novel bioactive compounds for the treatment of such burgeoning diseases.

Bay of Bengal is well known potential source for marine-derived bacteria rich in bioactive compounds (Peela et al. 2005; Arumugam et al. 2010; Saurav et al. 2013). Marine sediments of these regions predominantly contain the genus *Streptomyces*, which is reportedly active against a range of clinically important bacteria and fungi (Peela et al. 2005). Arumugam et al. (2010) reported an unusual metabolite 2-allyloxyphenol, with substantial antimicrobial activity from *Streptomyces* M1/7 isolated from the Sundarbans of Bay of Bengal. Similarly, a larvicidal compound, 5-(2,4-dimethylbenzyl) pyrrolidin-2-one, was found to be produced by *Streptomyces* VITSVK strain from Marakkanam coast of Bay of Bengal (Saurav et al. 2013). All such reports exposes enormous metabolites from this region that could serve as valuable source for exploring products of pharmaceutically importance.

In the present study, antimicrobial compound producing actinobacteria *Streptomyces chumphonensis* strain BDK01 was isolated from marine sediment sample collected from Palk Strait Region of Bay of Bengal. Taxonomic affiliation of the isolate was carried by incorporating various morphological, chemotaxonomic and molecular methods. Media used for antimicrobial compound production was optimized statistically through one variable-at-a-time strategy and Plackett–Burman model. Liquid–liquid extraction was employed for compound separation and subsequent distilled product was subjected to spectral studies to identify the biologically active compounds from fermented broth. Antiproliferative property of active ethyl acetate fraction was also investigated against MCF7 cell line through MTT assay.

Materials and methods

Sample collection and isolation of antagonistic *Actinobacteria*

Totally, 60 marine sediment samples were collected from 27 different locations of coastal Tamil Nadu and Kerala during pre-monsoon periods of 2012–2013. Few marine sediment samples were also collected from deep sea using the facilities extended by Centre for Advanced Studies in Marine Biology, Annamalai University. All the collected samples were dried in hot air oven at 55 °C for 30 min, serially diluted, and plated on starch casein agar and Actinomycete isolation agar (Himedia). The plates were incubated for 7–10 days under room temperature, and the matured colonies were sub-cultured on YEME agar. For genus level identification, the isolates were subjected to cultural, biochemical, physiological, and microscopic examinations according to Shirling and Gottlieb (1966) and Bergey's Manual of Systematic Bacteriology (Goodfellow et al. 2012). To analyze the antagonistic ability of the isolates, each strain was inoculated into starch casein broth and incubated in rotary shaker at 28 °C for 7 days. After fermentation, the supernatant was collected by centrifugation and the test bacterial strains (*Staphylococcus aureus* MTCC 3160, *Bacillus pumilus* NCIM 2327, *S. aureus* (methicillin resistant), *Escherichia coli* MTCC 1698, *E. coli* (ESBL), *Shigella flexneri* MTCC 1457 and *P. vulgaris*) were challenged against it (SM 1). The obtained results were recorded and those mean values were subjected to post hoc (Tukey's test) analysis. Based on comparative analysis amongst the isolates BDK01 from Dhanushkodi (Lat—09°09′07.29″; Lon—79°26′35.82″) was chosen for further studies.

Taxonomic characterization of BDK01 by polyphasic approach

Cultural characteristics of the potential isolate BDK01 was analyzed as suggested by Shirling and Gottlieb (1966). All the microscopic, metabolic, and physiological examinations, which include biochemical, carbohydrate utilization, and optimization of growth conditions, were carried out as per Bergey's Manual of Systematic Bacteriology (Goodfellow et al. 2012). For micromorphological profiling, the isolate was grown on starch casein agar by coverslip culture technique (Pridham et al. 1958) and the matured colonies were observed under scanning electron microscope JEOL JSM-5610 (Mitra et al. 2008). For whole cell sugar and amino acid investigation, the sample was prepared by the methods of Suput et al. (1967) and Becker et al. (1964), respectively. Furthermore, the cell hydrolysate was analyzed by thin layer chromatography (Staneck and Roberts 1974) against suitable standard sugars and amino acids. For cell wall Fatty acid methyl ester (FAME) investigation, sample was prepared using the standard protocol of MIDI (Sherlock Microbial Identification System, version 4.0) and analyzed through TRACE Ultra Ver: 5.0, MS DSQ gas chromatographic mass system (Thermo Fisher Scientific, Waltham, MA, USA) (Lu et al. 2013).

The extracted genomic DNA of isolate BDK01 was amplified for its 16S rRNA gene using the 27F 5′-GAG TTT GAT CCT GGC TCA G-3′—forward and 1530R 5′-GTT ACC TTG TTA CGA CTT-3′—reverse primers (Yukphan et al. 2004). The reaction volume of 25 µl consists of 1X Taq buffer contains 25 mM $MgCl_2$, 4 mM dNTPs, 0.5 IU Taq polymerase (Fermentas, Thermo Scientific, Vilnius, Lithuania), 10 pmol of forward and reverse primers and 50–100 ng template DNA. The PCR conditions included

initial denaturation at 94 °C for 10 min followed by 30 cycles of denaturation at 94 °C for 15 s, annealing at 55 °C for 15 s, extension at 72 °C for 1 min, and a final extension of 72 °C for 10 min using MyCycler™ Thermal Cycler (Bio-Rad). The purified PCR product was sequenced and the taxonomical affiliation of the isolate was assigned through BLAST search against GenBank database (http://www.ncbi.nlm.nih.gov). The phylogenetic tree was constructed for the isolate BDK01 with the closest identified type strains following pairwise and multiple sequence analysis by neighbour joining method. The phylogeny inference, maximum parsimony, and maximum likelihood methods were applied to assess dendrogram reliability and stability using MEGA 5.0 (Tamura et al. 2011).

Statistical optimization of medium for antimicrobial compound production

To select suitable media for antimicrobial compound production, three different media named as PM1 (Remya and Vijayakumar 2008), PM2 (Ilić et al. 2005), and PM3 (Badji et al. 2006) were prepared in 50% marine water (125 ml/500 ml flask). PM1 contained the following ingredients: soluble starch 10.0 g/l, casein 0.03 g/l, KNO_3 2.0 g/l, NaCl 2.0 g/l, K_2HPO_4 2.0 g/l, $MgSO_4$ $7H_2O$ 0.05 g/l, $CaCO_3$ 0.02 g/l, $FeSO_4$ $7H_2O$ 0.01 g/l, and pH 7.2. Another media PM2 contained glucose 2.0 g/l, yeast extract 3.0 g/l, NaCl 0.8 g/l, NH_4Cl 1.0 g/l, KCl 0.1 g/l, KH_2PO_4 0.1 g/l, $MgSO_4$ $7H_2O$ 0.2 g/l, $CaCl_2$ 0.04 g/l, marine water 50%, and pH 7.3. PM3 contained yeast extract 4.0 g/l, malt extract 10.0 g/l, dextrose 4.0 g/l, marine water 50%, and pH 7.2. All the three media were inoculated with 1% of 0.6 O.D (600 Å) inoculum of BDK01 and the flasks were kept in a rotary shaker at 28 °C for 7 days at 180 rpm. After incubation, the fermented broth was collected, centrifuged at 6000 rpm for 10 min, and supernatant was tested for antimicrobial activity against the selected test organisms by well diffusion method (Schillinger and Lücke 1989).

On the basis of zone formation, PM1 was selected as suitable media for production and subjected for statistical optimization, which includes one variable-at-a-time strategy and Plackett–Burman (PB) design through Design expert 8.0. One variable-at-a-time strategy was used to identify the effective concentrations and conditions of starch, casein, NaCl, pH, temperature, and inoculum concentrations on antimicrobial compound production. Those best productive conditions were subjected to PB design to find the significant factors that influence bioactive compound production. In addition, concentrations of other variables such as KNO_3, K_2HPO_4, $MgSO_4$ $7H_2O$, $CaCO_3$, and $FeSO_4$ $7H_2O$ were also included. With the selected low (−) and high (+) levels of variable concentrations (Table 1), a 11 factor 17 run experiment was generated through Design Expert 8.0 (Stat-Ease, Minneapolis, MN, USA). All experiments were conducted as triplicate and the collected data were analyzed at 5% significant level ($P < 0.05$), were considered to have substantial effect on the antimicrobial production. The predicted regression equation based on the results of Plackett–Burman model was as follows:

Table 1 Range of variables selected for Plackett–Burman design

Factor codes	Factors	Levels	
		− 1	+ 1
A (g/l)	Starch	5	15
B (g/l)	Casein	0.01	0.05
C (g/l)	KNO_3	1.0	3.0
D (g/l)	NaCl	1.0	3.0
E (g/l)	K_2HPO_4	1.0	3.0
F (g/l)	$MgSO_4$ $7H_2O$	0.01	0.1
G (g/l)	$CaCO_3$	0.01	0.03
H (g/l)	$FeSO_4 \cdot 7H_2O$	0.005	0.015
K	pH	4.0	9.0
J	Temperature	25 °C	45 °C
L	Inoculum concentration	0.5 mL	5 mL

− 1, minimum range of variables; + 1, maximum range of variables

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n, \quad (1)$$

where Y is the predicted response, β_0 is constant (intercept), β_1, β_2, to β_n are the coefficient of the variables and X_1, X_2, to X_n are the coded values of independent variables.

By applying the best obtained results of PB design, mass production was carried for 10 l in a series of 500 ml (1/4 working volume) conical flasks added with 6-mm diameter glass beads. After incubation, the culture was centrifuged (C-24 BL, REMI) at 6000 rpm for 10 min and supernatant was subjected for partial purification by liquid–liquid extraction.

Partial purification and identification of active compound

The supernatant was subjected to solvent extraction employing various solvents such as petroleum ether, butanol, chloroform, ethyl acetate, and methanol in a 250 ml flasks. Fifty mL of each solvent were mixed with equal volume of cell-free broth and left on a shaker for 60 min. Then, the solvent layer was separated, concentrated by distillation apparatus at 60 °C, and investigated against the test bacteria by well diffusion method. An extra well loaded with each pure solvent served as control against all test organisms. Obtained results were subjected to one sample t test to identify the significance of solvents in extraction process. Accordingly, ethyl acetate was identified as ideal solvent at the ratio of 1:4. Later, solvent phase was collected and distilled in a distillation flask at 60 °C (Parthasarathi et al. 2013). For partial

purification, the gummy crude compound was subjected to isocratic silica gel (60–120 mesh) column chromatography using ethyl acetate as mobile phase (Praveen et al. 2008). The active ethyl acetate fraction was subjected for spectral and mass analysis. The maximum absorbance (λ_{max}) spectra of the partially purified compound were analyzed between 800 and 200 nm in PerkinElmer Lambda 25 UV/Vis Spectrophotometer. Similarly, the functional group analysis of ethyl acetate fraction was carried out using Shimadzu IR Affinity FT-IR spectrophotometer (3500–600 cm^{-1}). Metabolic profiling of active fraction was done in gas chromatography mass spectrophotometer (PerkinElmer Clarus 500, Software: Turbomass ver 5.2.0) with the Capillary Column Elite-5MS (5% Phenyl 95% dimethylpolysiloxane). The oven program was set at 50 °C at 7 °C/min to 200 °C (3 min) @ 7 °C/min to 280 °C (15 min), and 1.0 µl (25 µg/ml) of crude sample was injected using helium (carrier gas) at the rate of 1 ml/min. The mass range of 40–600 amu was analyzed at 70 eV at the temperature of 200 °C.

Antiproliferative activity of the active ethyl acetate fraction against MCF 7 cell line

The human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), Pune, India, and grown in Eagles Minimum Essential Medium (EMEM) containing 10% foetal bovine serum (FBS). The monolayer cells were detached with trypsin—EDTA to make single cell suspensions, and the viable cells were diluted with medium containing 5% FBS to give final density of 1×10^5 cells/ml. Cell suspension (100 µl) was seeded into 96-well plate (10^3 cells/well) and incubated to facilitate cell attachment. After 24 h, the cells were treated with serial concentrations of the test samples (0, 3.12, 6.25, 12.5, 25, and 50 µg/ml), which were dissolved in dimethyl sulfoxide (DMSO). Furthermore, with appropriate control, the plates in triplicate were maintained for all concentrations. After 48 h of incubation, 15 µl of MTT (5 mg/ml) in phosphate-buffered saline (PBS) was added to each well and kept at 37 °C for 4 h. The medium with MTT was then flicked off and formed formazan crystals were solubilized in 100 µl of DMSO which was measured at absorbance of 570 nm using microplate reader. Mean and IC_{50} values were calculated by nonlinear regression analysis, and a graph was plotted between percentage of cell inhibition and Log_{10} concentration using GraphPad Prism software.

Results

Totally, 85 morphologically distinct actinobacterial strains were isolated from marine sediment samples on different media, which were subjected to phenotypic characterization to identify the genus. Among them, 34 (40%) isolates were *Streptomyces*, 15 (17.6%) were *Micromonospora*, 12 (14.1%) were *Saccharopolyspora*, 6 (7%) were *Actinopolyspora*, 7 (8.24%) were *Nocardia*, 2 (2.35%) *Nocardioides*, 5 (5.8%) were *Kitasatospora*, and 4 (4.71%) remained as 'unidentified'. The well diffusion assay of fermented broth had exposed 34 (40%) isolates for its antimicrobial activity against test and clinical bacterial strains. Among them, isolate BDK01, which is identified as *Streptomyces* sp., was splendid in antagonistic ability with enormous zone of inhibition and active against all employed test organisms.

Taxonomic characterization of the isolate BDK01

Colonies were circular and umbonate with ability to produce melanin on starch casein agar. The isolate showed excellent growth in Actinomycete isolation agar, Bennet's agar, nutrient agar, and ISP media except on ISP 5 and ISP 7. Pale orange colour aerial and dark brown colour substrate mycelia were the dominant growth features on all media. Isolate was identified as positive for hydrogen sulphide production, catalase, and oxidase, and negative for indole, methyl red, Voges–Proskauer, citrate, and urease. The strain had the ability to degrade starch, casein, and gelatin, and utilizing sugars like glucose and mannitol. Physiological examinations revealed that the strain can grow well between the pH 6.0 and 9.0 at 25–37 °C and optimal NaCl concentration was 1–5%. Scanning electron microscopic (SEM) studies revealed that aerial and substrate mycelia of the strain BDK01 were well developed without fragmentation. Oval, smooth surfaced rectiflexibiles long spore chain measured more than 5 µm in the length contains more than 10 spores per chain measuring 840.0 × 508.2 nm (Fig. 1). No sugar was diagnosed in the cell wall, whereas amino acid analysis revealed the presence of glycine and LL 2,6 diaminopimelic acid. FAME analysis indicated the presence of various straight and branched chain of iso and ante-iso fatty acids, mainly $C_{15:0}$ antesio (12-methyltetra decanoic acid), $C_{16:0}$ (hexadecanoic acid, methyl ester), and $C_{18:2}$ w6c (9,12-Octadecadienoic acid). BLAST analysis of BDK01 16S rRNA gene sequence revealed more than 23 *Streptomyces* strains were sharing 98–99% sequence homology (Fig. 2). In the phylogenetic analysis, BDK01 clustered with strain *S. chumphonensis* KK1-2^T (NCBI accession no: AB738401) carry 99% sequence homology. The 16S rDNA sequence of BDK01 was deposited in NCBI under the name of *S. chumphonensis* strain BDK01 (NCBI accession no. JX486779).

Statistical optimization and production of antimicrobial compound

Among the three different media employed, PM1 (Table 2) was observed as an ideal media for antimicrobial compound production. The one variable-at-a-time strategy reveals the

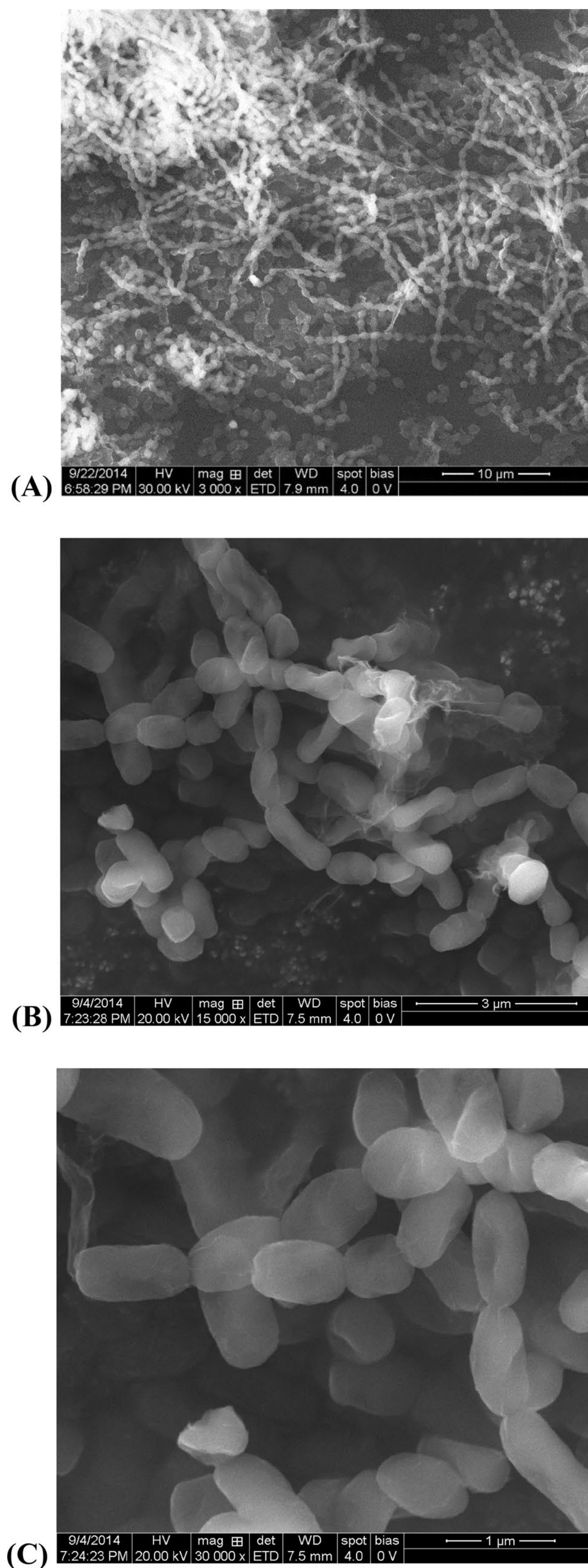

Fig. 1 Electron micrographs of BDK01 with different magnification (**a** ×3000, **b** ×15,000, **c** ×30,000)

effective concentrations of selected variables on antimicrobial compound production (Fig. 3). The variables such as starch, casein, sodium chloride, temperature, pH, and inoculum concentrations expressed antimicrobial compound in all ranges served with difference in production volume. Based on the results of One variable-at-a-time strategy Plackett–Burman experimental model (Table 3) was attempted, where the eighth run produced maximum amount of antimicrobial compound followed by eleventh run. ANOVA test revealed F value of 21.39 and a low probability value of 0.0047, thereby implying that the model is significant (Tables 4, 5). The p values of A, G, K, and L were significant as they are less than 0.05, whereas other values were greater and not significant (Li et al. 2008). The predicted R^2 value 0.9832 variability in response, is in reasonable agreement with the adjusted R^2 value 0.9408. The lack of fit value 0.4545 for the design is insignificant, suggested that the obtained data were good in fit. The Pareto chart indicates that the pH (K), calcium carbonate (G), starch (A), and inoculum concentration (L) were highly influencing the antimicrobial compound production (Fig. 4). Particularly, calcium carbonate and pH were influencing the production positively, whereas starch and inoculum concentration were influencing negatively. The antimicrobial compound production could be predicted with the regression equations derived through Plackett–Burman model as follows:

$$\begin{aligned} Y = {} & 16.33 + 2.16A + 0.555B - 0.555C - 0.111D \\ & - 0.222E + 0.833F - 3.222G + 0.0555H \\ & + 0.944J - 4.055K + 1.611L, \end{aligned} \tag{2}$$

where Y was the response, whereas A, B, C, D, E, F, G, H, J, K, and L were the coded values of starch, casein, KNO_3, NaCl, K_2HPO_4, $MgSO_4$ $7H_2O$, $CaCO_3$, $FeSO_4$ $7H_2O$, pH, temperature, and inoculum concentration, respectively.

Extraction and spectral identification of potential bioactive compound

Among the five different solvents studied for solvent extraction, ethyl acetate ($P \leq 0.001$) was found to be the best for extraction of antimicrobial compounds (SM 2). Ethyl acetate fraction (25 µg/ml) was potentially active against 16 reference bacterial and fungal strains there was no activity against *Aspergillus niger* (SM 3). Antiproliferative activity against MCF7 cell line by MTT assay resulted in significant IC_{50} value of 9.5 µg/ml (Fig. 5). Maximum peak value was observed at 324.30 nm and 273.12 nm in UV–Vis spectrum analysis. Absorption peak 273.12 indicated possible presence of polyene compounds (Ilić et al. 2005). Multiple sharp and broad peaks were observed between 3500 and 600 cm^{-1} in FT-IR indicated the presence of functional groups such

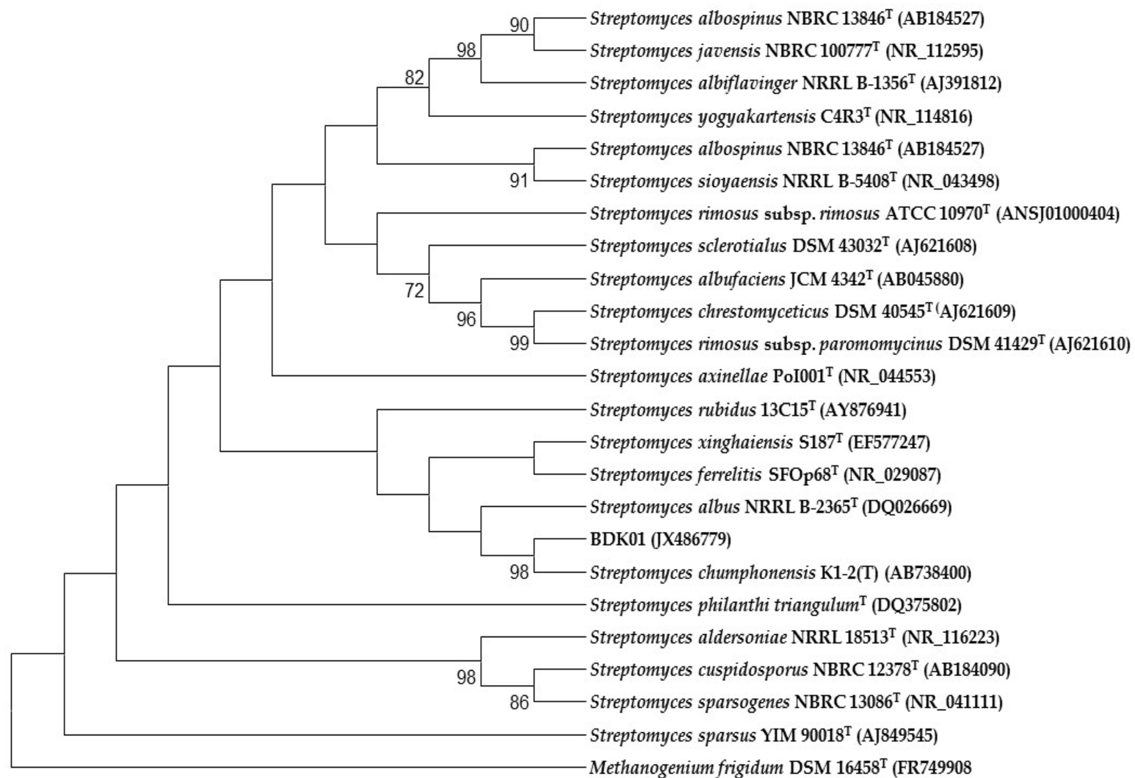

Fig. 2 Phylogenetic tree of isolate BDK01 based on 16S rRNA gene sequences aligned to their closest type strains. Bootstrap values above 50% (1000 replicates) are shown at branches. *Methanogenium frigidum* used as an out-group

Table 2 Antimicrobial efficacy of *S. chumphonensis* BDK01 cultured on different production media

Test organism	PM1	PM2	PM3
Staphylococcus aureus MTCC 3160	24.0 ± 1.52	21.6 ± 0.57	14.3 ± 1.52
Bacillus pumilus NCIM 2327	21.6 ± 0.57	17.6 ± 1.15	10.3 ± 1.15
Escherichia coli MTCC 1698	32.3 ± 0.57	28.0 ± 1.73	12.6 ± 0.57
Shigella flexneri MTCC 1457	20.0 ± 1.73	17.6 ± 0.57	13.6 ± 1.15

The values (average of triplicate) are diameter zone of inhibition in mm

PM1 Remya and Vijayakumar (2008), *PM2* Ilić et al. (2005), *PM3* Badji et al. (2006)

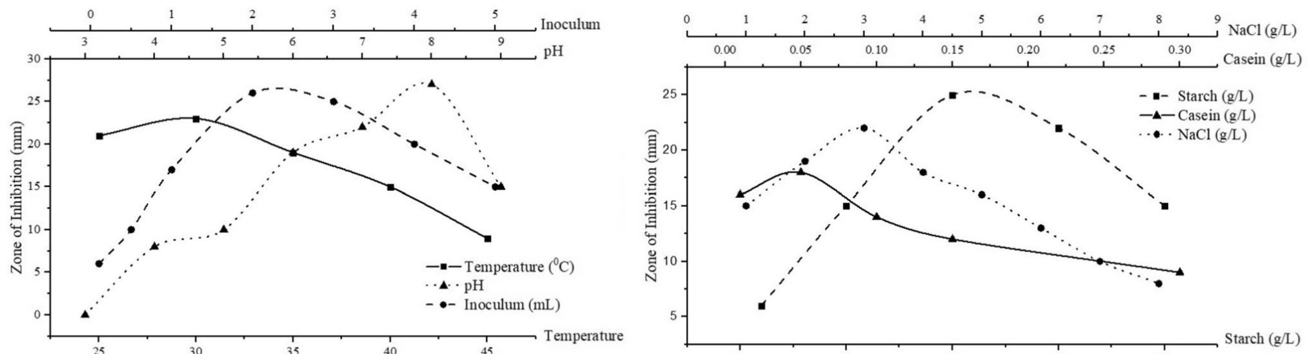

Fig. 3 Effect of changes in different parameters such as temperature, pH, inoculum, starch, casein and NaCl concentrations on antimicrobial compound production by *S. chumphonensis* BDK01 analysed by one variable-at-a-time strategy

Table 3 Experimental design and results of Plackett–Burman design for antimicrobial compound production

Run	Variables											Antimicrobial compound production	
	A	B	C	D	E	F	G	H	J	K	L	Actual	Predicted
	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l	–	°C	ml	(mg/50 ml)	(mg/50 ml)
1	15	0.05	1	3	3	0.1	0.01	0.005	4	45	0.5	16.667 ± 3.786	16.6667
2	5	0.05	3	1	3	0.1	0.03	0.005	4	25	5	16.333 ± 1.528	16.3333
3	15	0.01	3	3	1	0.1	0.03	0.015	4	25	0.5	16.667 ± 1.528	16.6667
4	5	0.05	1	3	3	0.01	0.03	0.015	9	25	0.5	14.333 ± 1.528	14.3333
5	5	0.01	3	1	3	0.1	0.01	0.015	9	45	0.5	12.333 ± 2.517	12.3333
6	5	0.01	1	3	1	0.1	0.03	0.005	9	45	5	10.333 ± 2.082	10.3333
7	15	0.01	1	1	3	0.01	0.03	0.015	4	45	5	11.000 ± 1.000	11.0000
8	15	0.05	1	1	1	0.1	0.01	0.015	9	25	5	30.667 ± 1.528	30.6667
9	15	0.05	3	1	1	0.01	0.03	0.005	9	45	0.5	10.000 ± 1.000	10.0000
10	5	0.05	3	3	1	0.01	0.01	0.015	4	45	5	13.333 ± 0.577	13.3333
11	15	0.01	3	3	3	0.01	0.01	0.005	9	25	5	26.000 ± 3.000	26.0000
12	5	0.01	1	1	1	0.01	0.01	0.005	4	25	0.5	18.333 ± 3.512	18.3333
13	10	0.03	2	2	2	0.055	0.02	0.01	6.5	35	2.75	16.667 ± 2.517	16.9333
14	10	0.03	2	2	2	0.055	0.02	0.01	6.5	35	2.75	19.000 ± 2.646	16.9333
15	10	0.03	2	2	2	0.055	0.02	0.01	6.5	35	2.75	15.333 ± 3.215	16.9333
16	10	0.03	2	2	2	0.055	0.02	0.01	6.5	35	2.75	16.333 ± 5.033	16.9333
17	10	0.03	2	2	2	0.055	0.02	0.01	6.5	35	2.75	17.333 ± 5.132	16.9333

Table 4 ANOVA of the fitted quadratic polynomial model and significant test

Source	Sum of squares	Degrees of freedom	Mean square	*F* value	*P* > *F*
Model	436.667	11	39.697	21.3936	0.0047 (significant)
Residual	8.69	5	1.74		
Lack of fit	1.271	1	1.271	0.68475	0.4545 (not significant)
Pure error	7.422	4	1.856		
Cor total	445.359	16			

SD 0.36, R^2 0.9832, *C.V.* 8.25%, *Adj.* R^2 0.9373, *Mean* 16.50, *Adeq Precision* 17.349

as C=C alkenes, C=O group of acids, C≡C alkynes, –C–H stretch (alkane H), and O–H (hydrogen bond, intermolecular, polymeric association). GC–MS results of active ethyl acetate extract indicated the presence of more than 44 different possible compounds, of which 18 compounds were already reported for several biological activities. Salicyl alcohol was in maximum proportion (16.32%) followed by *N*-phenylbenzamide (10.73%). Other important biologically potential compounds such as 6-octadecenoic acid, (*Z*) (8.12%), 1,3,5-cycloheptatriene (3.29%), 2-pyrrolidinone (0.083%), and nomifensine acetate (0.48%) were also observed. Other bioactive compounds with complete volatile metabolic profiling are presented in Table 6.

Table 5 Regression analysis of the second-order polynomial model for optimization of antimicrobial compound production

Factor	Code	Estimate	Mean error	*F* value	*P* value
Intercept		16.33			
Starch	A	2.16	56.33	30.35	0.0053
Casein	B	0.55	3.70	1.99	0.2306
KNO_3	C	−0.55	3.70	1.99	0.2306
NaCl	D	−0.11	0.14	0.07	0.7915
K_2HPO_4	E	−0.22	0.59	0.31	0.6022
$MgSO_4.7H_2O$	F	0.83	8.33	4.49	0.1014
$CaCO_3$	G	−3.22	124.59	67.14	0.0012
$FeSO_4.7H_2O$	H	0.05	0.03	0.01	0.8945
pH	J	0.94	10.70	5.76	0.0742
Temperature	K	−4.05	197.37	106.36	0.0005
Inoculum concentration	L	1.61	31.14	16.78	0.0149

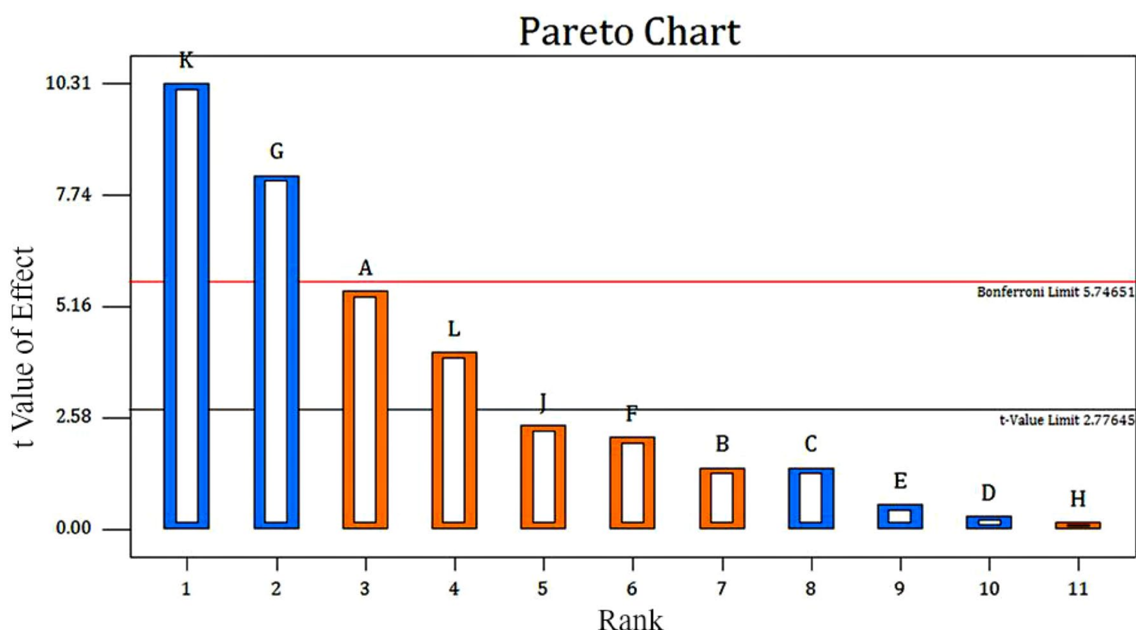

Fig. 4 Pareto chart showing effects of factors above and below the 'Bonferroni Limit' (absolute significance) and '*t*-value Limit' (line of significance) on antimicrobial compound production. A (Starch), B (Casein), C (KNO_3), D (NaCl), E ($KHPO_4$), F ($MgSO_4$ $7H_2O$) (F), G ($CaCO_3$), H ($FeSO_4$·$7H_2O$), K (pH), J (temperature) and L (inoculum concentration). Blue and orange bars indicates positive and negative effects, respectively

Discussion

Marine *Actinobacteria* are well known for its bioactivities, such as antimicrobial and cytotoxic activities as evinced by many previous investigations (Sujatha et al. 2005; Remya and Vijayakumar 2008; Suthindhiran and Kannabiran 2010; Parthasarathi et al. 2013). In the present study, 85 morphologically distinct actinobacteria, dominantly *Streptomyces* (40%), were isolated from sediment samples collected from coastal Tamil Nadu and Kerala. Post hoc (Tukey's test) analysis of results obtained from well diffusion assay showed that the isolate BDK01 had excellent activity against the test pathogens based on average mean performance value.

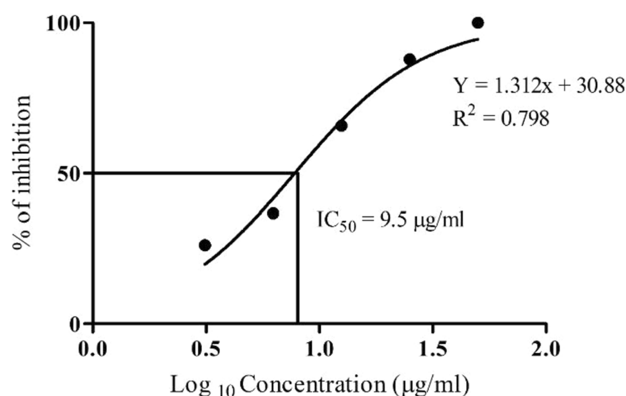

Fig. 5 Graphical representation of antiproliferative activity of ethyl acetate fraction of *S. chumphonensis* BDK01 against MCF-7 cell line MTT assay

The isolate BDK01 was subjected to polyphasic taxonomic characterization for species level identification. Morphological, microscopical, biochemical, carbohydrate utilization, and physiological examination suggested that the strain BDK01 could be a *Streptomyces* species (Shirling and Gottlieb 1966; Goodfellow et al. 2012). Lack of cell wall sugar and the presence of LL isomeric form of 2,6 diaminopimelic acid is the unique taxonomic feature of type I cell wall. The cell wall fatty acid methyl esters of *S. chumphonensis* KK1-2^T, a strain which is phylogenetically related to the isolate BDK01, were investigated by Phongsopitanun et al. (2014). The strain contains C15:0 anteiso, C16:0 iso, and C15:0 iso in cell wall, whereas the isolate BDK01 of present investigation contains C15:0 antesio, C16:0, and C18:2 w6c, which indicates that the chemical composition of these two strains had subtle variation. Phylogenetic analysis revealed that the isolate BDK01 was closely related to strain *S. chumphonensis* KK1-2^T (NCBI accession no: AB738401) (Fig. 1). A recently reported study, *S. chumphonensis* KK1-2^T was isolated from marine sediments of Chumphon Province, Thailand (Phongsopitanun et al. 2014). Hence, it is strongly believed that the strain BDK01 is a marine habitant.

Among 3 media compared, PM1 showed the maximum appropriateness for antimicrobial compound production than

Table 6 Characteristics of the peaks obtained by GC–MS analysis of the ethyl acetate fraction of *Streptomyces chumphonensis* strain BDK01

RT	Peak name	MF	MW	%Peak area	Activity reported
3.71	1,3,5-Cycloheptatriene	C_7H_8	92	3.29	Antibacterial (Trust and Bartlett 1975)
5.56	Butanoic acid, 3-methyl	$C_5H_{10}O_2$	102	0.54	Antibacterial and antifungal (Huang et al. 2011)
8.73	Phenol	C_6H_6O	94	1.26	Antibacterial, fungal, antiviral, anticancer, antipyretic, antioxidant (Dr. Dukes); antiseptic and disinfectant (Prescott et al. 2002)
9.49	Benzaldehyde, 2-hydroxy-	$C_7H_6O_2$	122	1.68	Antibacterial (Friedman et al. 2002) and antifungal (López et al. 2002)
9.94	Acetophenone	C_8H_8O	120	0.20	Antibacterial, antifungal (Dr. Dukes); anti-inflammatory hypnotic activity (The Merck Index 2002)
10.29	Phenol, 2-methyl-	C_7H_8O	108	0.10	Antimicrobial preservative, disinfectant (Rowe et al. 2009)
10.79	2-Pyrrolidinone	C_4H_7NO	85	0.08	Anticancer, antioxidant (Thangam et al. 2013)
11.15	Phenylethyl Alcohol	$C_8H_{10}O$	122	0.25	Antibacterial (Corre et al. 1990)
14.24	2-Methoxy benzyl alcohol	$C_8H_{10}O_2$	138	0.17	Potent antifungal active against yeasts, mold and more active against Gram-positive than Gram-negative bacteria (Tayung et al. 2011)
15.14	Salicyl alcohol	$C_7H_8O_2$	124	16.32	Antibacterial and antifungal (Harun et al. 2005), anticancer (Ghasemzadeh et al. 2012; Vejselova and Kutlu 2015)
16.15	1-Tetradecene	$C_{14}H_{28}$	196	2.22	Antibacterial and antifungal (Tayung et al. 2011)
18.81	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	1.30	Antioxidant and anticancer (Varsha et al. 2015); antimicrobial and anti-inflammatory (Arockia et al. 2014)
19.77	3-Eicosene, (E)-	$C_{20}H_{40}$	280	3.79	Antibacterial, antifungal (Hsouna et al. 2011)
23.24	3-Eicosene, (E)-	$C_{20}H_{40}$	280	3.20	Antibacterial, antifungal (Hsouna et al. 2011)
24.55	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.70	Antioxidant (Dr. Dukes)
27.29	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.26	Antifungal, antioxidant, hypocholesterolemic, nematicide, anti-androgenic flavour, haemolytic, 5-Alpha reductase inhibitor, potent antimicrobial agent, antimalarial and antifungal. (Hsouna et al. 2011; Hema et al. 2011; Pietro et al. 2010)
28.12	Nomifensine acetate	$C_{18}H_{20}N_2O$	280	0.48	Antidepressant (Ulrich and Wolfgang 1974)
29.36	Benzamide, *N*-phenyl-	$C_{13}H_{11}NO$	197	10.73	Antiviral (Ji et al. 2013)
32.65	6-Octadecenoic acid, (*Z*)	$C_{18}H_{34}O_2$	282	8.12	Antimicrobial activity (Gheda et al. 2013), anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, hypocholesterolemic, 5-alpha reductase, inhibitor, anemiagenic insectifuge, flavour (Basu et al. 2013)
32.88	9-Hexacosene	$C_{26}H_{52}$	364	3.34	Antinociceptive, anti-inflammatory activity (Githinji et al. 2012)
34.00	Longifolene-(V4)	$C_{15}H_{24}$	204	0.47	Antiproliferative (Murugesan et al. 2013)

RT retention time, *MF* molecular formula, *MW* molecular weight, % percentage, *Dr. Duke's* U.S. Department of Agriculture, Agricultural Research Service, Dr. Duke's Phytochemical and Ethnobotanical Databases

PM2 and PM3 (Table 2). This agrees with the investigation of Vijayakumar et al. (2012), who reported the elevated antimicrobial activity of *Actinobacteria* when grown in medium containing starch. PM2 containing glucose failed to produce quantifiable amount of antimicrobial compound. Similar findings were observed by Gupte and Kulkarni (2002) assuming that the complex polysaccharides were the best constituent for production media than any other monosaccharides. While the readily available carbon source like glucose is utilized for microbial growth, complex carbohydrate like starch was assimilated slowly for growth and compound production (Remya and Vijayakumar 2008; Durairaj and Ramasamy 2013).

PB model evinced that the variables such as pH (K), calcium carbonate (G), starch (A), and inoculum concentration (L) were influencing the antimicrobial compound production (Fig. 4). Earlier, Sathiyanarayanan et al. (2014) proposed that pH is a significant variable in antimicrobial compound production by *Streptomyces* sp. MAPS15. Besides, other earlier findings also suggested that minerals such as KNO_3, NaCl, K_2HPO_4, $MgSO_4$, $CaCO_3$, and $FeSO_4$ had influenced antimicrobial compound production either positively or negatively (Guo et al. 2012; Sathiyanarayanan et al. 2014). In the present study, the R^2 value of antimicrobial compound 0.9832 indicates that the model is significant to enhance the production (Tables 4, 5). Even, a regression model having an R^2 value higher than 0.90 was considered as a high correlation factor (Chen et al. 2009). The standard error counter plot was very circular and flat, indicating that the design is very significant. Conclusively, PB design identified the

significant factors that influence antimicrobial compound production. The obtained variables through central composite design may be utilized to identify the precise medium for enhanced production of antimicrobial compounds.

Ethyl acetate was concluded as best solvent for extraction of antimicrobial compounds (SM 2) (Parthasarathi et al. 2012; Vijayakumar et al. 2012). Nithyanand et al. (2011) reported, besides ethyl acetate chloroform also good for bioactive compound extraction. Ethyl acetate with polarity index 4.4 was immiscible in water phase which had no effect on test pathogens making it as the best solvent for extraction of antimicrobial compounds.

Results of UV and FT-IR were compared with the Bordoloi et al. (2001), Wu et al. (2007), and Dhanasekaran et al. (2008) to identify peak points and their functional group which indicated the possible presence of various biologically active functional groups. GC–MS analysis disclosed more than 18 different compounds with various biological activities, whereas rest of the compounds was not. Significantly, salicyl alcohol in vast quantity (16.32%), was repeatedly reported for antibacterial and antifungal activities (Harun et al. 2005).

Salicyl alcohol or Saligenin is a phenolic alcohol usually produced as a derivative by hydrolysis of salicin. Generally, this compound is a derivative of benzyl alcohol with substitution of an OH group at the ortho position. In FT-IR analysis of the current investigation, peak value of 3327 indicates the presence of O–H stretching frequencies. Saligenin is used as multipurpose drug that can act as analgesic and antipyretic agents. Earlier, the antimicrobial activity of salicyl alcohol against 20 bacterial strains were studied by Harun et al. (2005). Results showed promising antagonistic effects against *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter* sp, and *S. aureus*, which are in highly accordance with current investigation. Ghasemzadeh et al. (2012) and Vejselova and Kutlu (2015) reported the anticancer potential of salicylic acid against MCF7 cell line and A549 human lung adenocarcinoma, respectively. Another study carried by El-Shemy et al. (2007) suggested that salicyl alcohol, contains antitumor activity, which can eliminate immature white blood cells through apoptosis associated DNA damage. Moreover, salicyl alcohol can induce resistance to multiple antibiotics in bacteria, notably the quinolone group of antimicrobial agents (Cohen et al. 1993).

The second major constituent *N*-phenylbenzamide (10.73%), was already reported for strong antiviral activity against *Enterovirus* (Ji et al. 2013), Hepatitis C Virus and Enterovirus 71 (strain SZ-98) (Jiang et al. 2015). Another study carried by Pasha et al. (2008) suggested that the compound is highly active against a range of Gram-positive and Gram-negative bacteria. Besides, antimalarial and antifungal properties of *N*-phenylbenzamide was endorsed by various investigators (Niewiadomy et al. 2001; Ertan et al. 2009; Desai et al. 2011).

Another biologically active compound 6-Octadecenoic acid, (*Z*) was detected in the GC–MS spectrum. Earlier, Raghad and Jalill (2014) found that the compound 6-Octadecenoic acid extracted from *Calendula officinalis* leaves widely known for its ability to suppress Human epidermoid larynx carcinoma (Hep-2). The compound has several bioactivities such as antimicrobial (Gheda et al. 2013), anti-inflammatory, antiandrogenic, anticancer, dermatitigenic, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge, and flavouring agents for food/pharmaceutical industries (Basu et al. 2013). 9-Hexacosene known for antinociceptive and anti-inflammatory activities (Githinji et al. 2012) was also reported in considerable quantity. Another major constituent 1,3,5-Cycloheptatriene (3.29%) commercially called Triprolidine, is an antibacterial compound, which is currently in usage (Trust and Bartlett 1975).

Overall, many bioactive compounds were detected in GC–MS profile of ethyl acetate extract recovered from *S. chumphonensis* BDK01 fermented broth. Although many compounds are not reported for any biological activities, certain structurally related compounds have significant activities. Hence, we strongly believe that the *S. chumphonensis* strain BDK01 could be a novel source for several new antimicrobial compounds.

American National Cancer Institute recommends that any crude compound IC_{50} value less than 30 µg/ml could be a promising anticancer drug (de Oliveira et al. 2016). The MTT assay of the current study revealed that the compounds present in the crude could be a potential drug against cancer cells as it expressed the IC_{50} value of 9.5 µg/ml (Fig. 5). The GC–MS analysis revealed the presence of many compounds in various concentrations with different antagonistic effects against microbial and cancer cell lines. Especially the phenolic compounds (Dr. Duke's Phytochemical and Ethnobotanical Databases), 2-pyrrolidinone (Thangam et al. 2013), 2,4-bis(1,1-dimethylethyl) phenol (Varsha et al. 2015), 6-octadecenoic acid (*Z*) (Basu et al. 2013), and longifolene-(V4) (Murugesan et al. 2013) were also present in considerable quantity. Consequently, the present investigation confirms that the crude extract contains many anticancer compounds at various concentrations, which could be a promising agent to control the cancer proliferation. Moreover, anticancer analysis against other transformed cell lines could reveal potential information about the compounds.

Conclusion

Our study on isolation, taxonomic characterization, and identification of bioactive compounds from marine Actinobacteria from coastal Tamil Nadu and Kerala resulted in the discovery of *S. chumphonensis* BDK01. The strain BDK01 was phylogenetically closer to identified as *S. chumphonensis* KK1-2^T, a novel species recently reported by Phongsopitanum et al. (2014) and yet to be studied for any bioprospecting potential. Hence, to our knowledge, the present study serve as a first report for antimicrobial activity in this species. The isolate *S. chumphonensis* strain BDK01 exhibited significant antagonistic activity against test bacterial and fungal strains. Moreover, the crude compound displayed remarkable IC_{50} value of 9.5 µg/ml against MCF-7 (Breast Cancer) cell line, which demonstrate its activity to combat cancer cell proliferation. Optimization of production media was done statistically towards the maximization of antimicrobial compound production. Metabolic profiling of the active compounds was reported through spectral studies. Further exploration of *S. chumphonensis* strain BDK01 will throw light on its pharmacological potential which could benefit drug industries.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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