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EXPLORATION OF ANTIBACTERIAL AND ANTIPROLIFERATIVE SECONDARY METABOLITES FROM MARINE *BACILLUS*

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ABSTRACT

Antiproliferative secondary metabolites producing bacterial strain AVSC4 isolated from marine sediments was identified as *Bacillus flexus* based on 16S rRNA gene sequence analysis. Under the strategy of liquid- liquid extraction, the crude extract was obtained showed significant antibacterial activity against different clinical pathogens. 0.5% methionine and 0.4% NaCl act as inducers for maximizing the growth and antibacterial activity of strain at pH 7 and 40°C. Heptadecanoic acid and methyl hexadecanoic acid were identified as major and dominant secondary metabolites by GC-MS analysis and also showed significant antiproliferative activity against HT-29 (Human colorectal adenocarcinoma*) and* A-549 (Lung Cancer) with IC_{50} value 93.4 µg/ml and 50.04 µg/ml.

Keywords: Antibacterial, antiproliferative, A-549 cell line, *Bacillus flexus* AVSC4, GC-MS analysis, HT-29 cell line

INTRODUCTION

The antibacterial activities of long-chain unsaturated fatty acids have been well known for many years. Fatty acids function as the key ingredients of antimicrobial food additives which inhibit the growth of unwanted microorganisms **(Freese et al 1973)**. Besides normal fatty acids, fatty acid derivatives showing potent antimicrobial activities exist in nature and also mediate chemical defense against microorganisms **(Pfefferle et al 1996, Lopez and Gerwick 1988, Dellar et al 1996)**.
Marine bacteria have developed a complex biochemical and physiological systems which can adapt to extreme and unfavorable condition. They produce unique secondary metabolites which have shown significant applications in biotechnology and pharmaceutical industries **(Wenzel and Muller, 2005)**. Novel compounds so far isolated from marine organisms have been identified as antibiotics, anticancer enzymes and antimicrobial compounds examined for their pharmacological activity were not completely explored **(Jensen and Fenical, 1994; Pomponi, 1999)**. However it was only in the mid of 20th century, enormous interest has been shown by the scientists to explore oceans for biologically active compounds **(Proksh et al., 2002)**. Recent investigations showed that secondary metabolites produced by marine bacteria were potential drugs used to treat cancer, inflammations, **(Burgess et al., 1991; Bhatnagar and Kim, 2010)** bacterial, fungal, protozoan and viral infections **(Villa and Gerwick, 2010; Mayer et al., 2011)**. It is now well known that antibiotic resistance has become a global challenge, hence search of bioactive metabolites from marine environment is gaining more attention in recent years **(Ramachandran et al 2014)**.
Most of the bioactive compounds from the marine *Bacillus* are industrially worthy and have a history of safe usage. *Bacilli* are especially known for the production of a vast array of structurally distinct antimicrobial compounds, which include surfactin, iturin, fengycins and bacteriocins **(Stein, 2005)**. **Ravikumar et al, (2010)** reported that *B. thuringiensis and B. pumilus* of mangrove origin are potential antibacterial agents against human pathogenic bacteria. *B. subtilis* **(Jansen and Hirschmann, 1944)**, *B.* coagulans **(Hyronimus et al., 1998)** and *B. megaterium* **(Von Tersch and Carlton, 1983)** are not only capable of producing bacteriocins but also acts as biocontrol agents **(Wulff et al., 2002)**.
Optimization of the nutritional and culture conditions of the bacteria can enrich the fermentation profile including pH of media, incubation and temperature etc. Therefore optimal variables of physicochemical parameters are utmost important in increasing the production of bioactive compounds **(Nagar et al., 2012)**. *Bacillus* species are well known to produce unsaturated fatty acids, however a petite portion of work has done on their biological activities. 12- methyl tridecanoic (iso-C14), 14-methyl pentadecanoic (iso-C16), and 14-methyl hexadecanoic (anteiso-C,7) were some of the fatty acids reported from *B. subtilis* **(Kaneda, 1963)**. Bioactive compounds produced from the fermentation broth of marine *B. mojavensis* B0621A, displayed antifungal activity against a broad spectra of phytopathogens as well as cytotoxic activities against the human leukemia (HL-60) cell line with IC50 values of 100, 100, and 1.6 µM, respectively **(Ma, et al., 2010)**.
Exploration of potential antibiotics from marine microorganisms with low cost and less adverse effects has become essential biomedical research .Search for antibiotic producing marine organisms explored marine habitats, characterization and optimization of culture conditions for the exploration of novel secondary metabolites potential to antibiotic and anticancer is a continuous exercise. With this background the present research aimed to explore antibiotic and antiproliferative secondary metabolites of *Bacillus flexus* isolated from the Bay of Bengal from India and its identification, characterization and optimization of culture conditions.

MATERIALS AND METHODS

A total of 15 soil samples were collected from sediment soils of Suryalanka, Andhra Pradesh, India. A preliminary screening medium composed of Beef extract, Peptone, NaCl and Agar were obtained from HiMedia Laboratories (Mumbai) Ltd. All the pathogenic strains were obtained from Microbial Type Culture Collection centre (MTCC). Human colorectal adenocarcinoma (HT-29) and Lung Cancer (A-549) cell lines obtained from the National Center for cellular Sciences (NCCS), Pune, India.

Screening, Isolation and identification of bioactive compounds producing strains

Bacillus flexus AVSC4 strain, producing potential antibacterial compounds **(Chandini et al., 2017)** was isolated and the Pure culture of the strain was maintained and periodically subcultured on nutrient Agar Medium in corresponding authors laboratory. Molecular identification of marine isolate AVSC4 was carried out by 16S rRNA partial gene sequencing. PCR amplification of 16S rRNA gene was done by using universal primers 27F(AGAGTTTGATCCTGGCTCAG) and 1492 R (GGTTACCTTGTTACGACTT) with the conditions (1min predenaturation at 94 °C, 30 cycles of denaturation for 30 seconds at 94 °C, 30 seconds annealing at 55 °C, 1 min extension at 72 °C and 10 min termination at 72 °C) as described **(Chalasani et al., 2015)**. The PCR product was sequenced at Macrogen South Korea and analysed with the GenBank database, National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov) using Basic Local Alignment Search Tool (blastn) tool. Phylogenetic tree was constructed with Neighbour joining method. Phylogenetic tree was constructed by comparing the sequence with GenBank database using Mega 5.0 software **(Tamura et al., 2011)**.

Antibacterial activity

Escherichia coli (MTCC 1696), *Klebsiella pneumonia* (MTCC4030), *Proteus vulgaris* (MTCC7299), *Salmonella typhi* (MTCC 8587), *Serretia marcescens* (MTCC2645) and *Staphylococcus aureus* (MTCC 3160) were used as target pathogens with Streptomycin and DMSO as positive and negative control. The test organisms were grown in nutrient broth. 24 hours old test organisms were inoculated by spreading pathogenic inoculum on NAM plates. 6-mm diameter wells were punched in the medium with a sterile borer. 60 µl of the crude extract of AVSC4 was introduced into each well and plates were incubated at 37 °C for 24-48 hours. After incubation, the diameter of each zone in millimeters was measured and results were recorded **(Balouiri et al., 2016)**. The experiment was performed in triplicates.

Growth characteristics evaluation and anti bacterial activity optimization

The isolated strain was transferred into flasks containing 50 ml of nutrient broth and incubated at 37 °C on a rotary shaker at 200 rpm. Optimization was carried out at different incubation periods (24, 48, 72, 96 and 120hrs), Temperature (25 °C, 30 °C, 35 °C, 40 °C, 45 °C), pH (1, 2, 3, 4, 5, 6, 7, 8 and 9) , NaCl concentration (0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%) , 0.5% Carbon sources (Glucose, Sucrose, Dextrose, Maltose, Lactose, Mannose, Fructose, Galactose, Starch, Glycerol and D-Arabinose) 0.5% nitrogen source (Sodium acetate, Peptone, Beef extract, yeast extract, Sodium nitrate, Ammonium sulphate, Urea) and 0.5% amino acids (Cysteine, Leucine, Methionine, Tryptophan, Glycine, Alanine and Proline) separately . The growth of the isolate was determined by measuring OD at 540 nm.

Extraction of crude

AVSC4 was grown in optimized fermentation medium (NAM supplemented with 0.5% Peptone, 0.5% Beef extract, 0.4% Sodium chloride and 0.5% Glucose) at pH 7.0 and 30 °C for 96 h on a rotary shaker at 200 rpm for four days. After 96 hours of incubation, the culture was harvested and centrifuged at 10,000 rpm for 20 min at 4 °C and supernatant was collected. An equal volume of ethyl acetate was added to the collected supernatant and vigorously shaken for 30-40 min. The organic layer was fractionated with a separating funnel. The extraction was repeated twice with equal volume of ethyl acetate and collected organic layer was evaporated to dryness in a rota evaporator under reduced pressure. The extracted pellet was dissolved in DMSO and used for further investigation **(Zheng et al., 2014)**.

Antiproliferation activity

Crude extract of AVSC4 was assessed for in vitro cytotoxicity by MTT assay. Doxorubicin was used as a standard. 96 well plates were loaded with 100 µl media at a density of 10,000 cells per well and grown for 24 h. The cells were then exposed to different concentrations (10 to 200 µg/ml) of the test compounds for 48 h. 10 µl of MTT solution (5 mg/ml in PBS) was added to each well (90 µl of the media) and incubated for four hours at 37 °C. After incubation, 200 µl of DMSO was added to each well and the absorbance was measured at 570 nm. The mean % of cell viability in relation to untreated cells was estimated from data of triplicates **(Venkanna et al., 2014)**. The percentage growth inhibition was calculated using the formula:

$$\% \text{ inhibition} = 100 \frac{\text{(control-treatment)}}{\text{control}}$$

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The ethyl acetate crude extract of AVSC4 was analyzed on mass spectrum of GC-MS (GCMSQP2010, SHIMADZU) by applying the database of National Institute standard and Technology which includes more than 62000 patterns **(Basa'ar et al., 2017)**. The chromatogram obtained exhibited the availability of fifteen active principles. The name, retention time, molecular formula and percentage area of expected compounds were tabulated in (Table 2).

Statistical analysis

Experiments were conducted in triplicates and results were statistically analyzed by single ANOVA with Turkeys HSD pair wise and Duncan's Multiple comparisons using XL STAT 2018 Version-1.49342 software.

RESULTS

Isolation and identification of bioactive compound producing bacteria

In this study bacterial strains isolated of 15 soil samples collected from different regions of Bay of Bengal at a distance of one meter, 23 bacterial strains showed antibacterial activity of which AVSC4 strain was observed as one of the potential strain. 16S rRNA gene sequence of AVSC4 showed similarity with *Bacillus flexus* Cl16 and *Bacillus* sp. JDMASP51 strain and deposited in GEN BANK, NCBI as *Bacillus flexus* AVSC4 with GenBank accession no. MG878436.

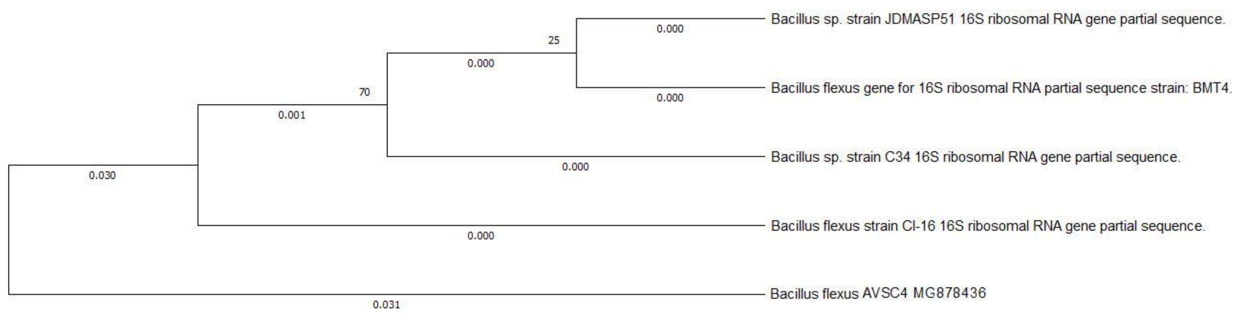

Figure 1 Phylogenetic analysis of 16S rRNA partial gene sequence of *Bacillus flexus* AVSC4

Optimization studies for growth and antibacterial compound production

Impact of optimization on growth and antibacterial activity of AVSC4 was analyzed at different physical and chemical factors such as incubation period, temperature, pH, salinity, carbon sources, nitrogen sources and amino acids. Antibacterial activity of AVSC4 and its correlation with growth was studied against E.coli. Highest antibacterial activity was observed at 96 hours and 120 hours of incubation at 35 °C and 40 °C, pH 7 and pH 8, 0.4% NaCl. Maximum antibacterial activity at 40 °C indicates thermo stability of AVSC4 isolate and adaptability to culture conditions by expressing maximum antibiotic activity at pH 7 and pH 8 followed by 0.4% and 0.5% NaCl. In order to bring culture conditions to recommended laboratory parameters, 40°C, pH 7 and 0.4% NaCl were opted and further investigation of impact of chemical factors (carbon, nitrogen sources and amino acids) have been analyzed in the ethyl acetate extract recovered from the isolate grown in optimized conditions (40 °C, pH 7, 0.4% NaCl). 96 hrs and 120 hrs incubation periods have shown maximum antibacterial activity in presence of Glucose, Sucrose and Dextrose (Carbon Sources), Beef extract (Nitrogen source) and Methionine (Amino acid). Based on the observations, the formulated culture media composition is glucose 0.5%, beef extract 0.5%, methionine 0.5%, NaCl 0.4% at 40 °C, pH 7.0 and 96 hours of incubation.

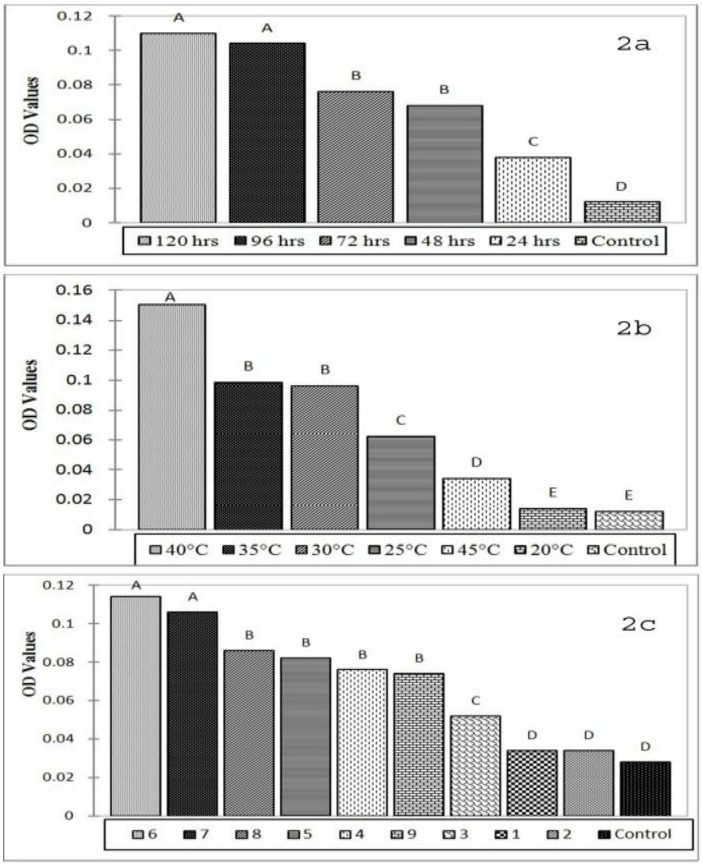

Figure 2 Effect of incubation period, temperature, pH on growth of *Bacillus flexus* AVSC4.

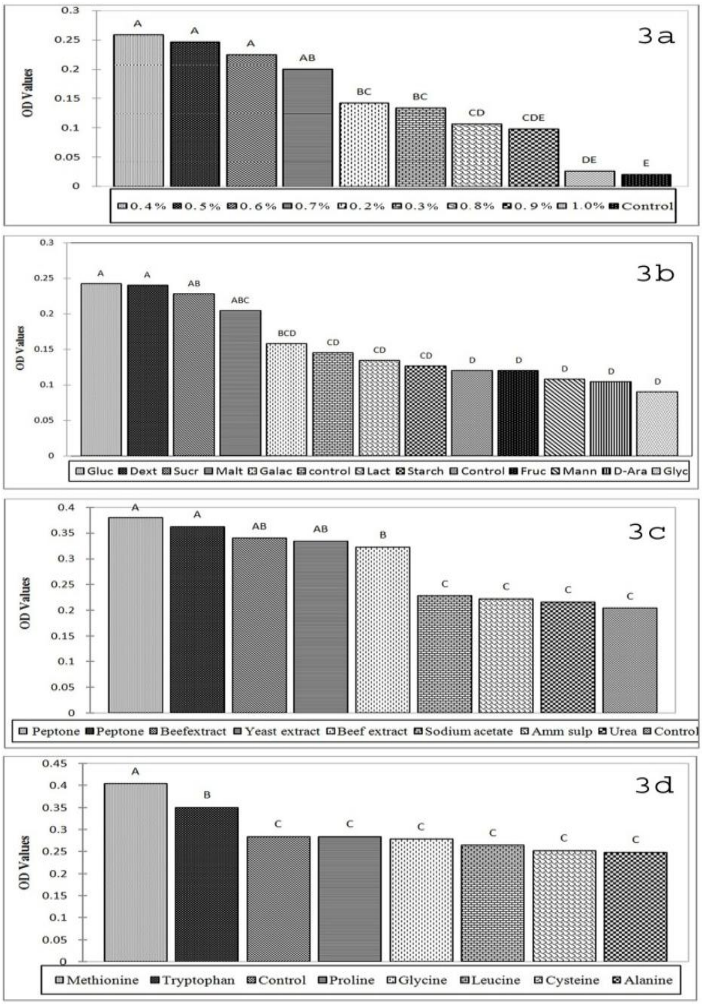

Figure 3 Effect of salinity concentration, carbon sources, nitrogen sources and amino acids on growth of *Bacillus flexus* AVSC4.

Table 1 Antibacterial activity for optimized broth against human pathogen *Escherichia coli*

Temperature	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
25 °C	-	-	-	-	+
30 °C	-	-	-	++	++
35 °C	-	-	+	++	++
40 °C	-	-	+	+++	+++
45 °C	-	-	-	-	+
pH					
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	+	+
7	-	-	-	+++	+++
8	-	-	-	+++	+++
9	-	-	-	-	-
Salinity concentration					
0.2%	-	-	-	-	-
0.3%	-	-	-	-	-
0.4%	-	-	+	+++	+++
0.5%	-	-	++	+++	+++
0.6%	-	-	+	++	++
0.7%	-	-	-	+	+
0.8%	-	-	-	-	-
0.9%	-	-	-	-	-
1.0%	-	-	-	-	-
Carbon sources					
Glucose	-	-	-	+++	+++
Sucrose	-	-	-	+++	++
Dextrose	-	-	-	+++	+++
Maltose	-	-	-	++	++
Lactose	-	-	-	++	+
Mannose	-	-	-	+	+
Fructose	-	-	-	+	++

Galactose	-	-	-	+	+
Starch	-	-	-	+	+
Glycerol	-	-	-	-	-
D arabinose	-	-	-	+	+
Nitrogen sources					
Peptone	-	-	-	++	++
Beef extract	-	-	-	+++	+++
Yeast extract	-	-	-	++	++
Sodium acetate	-	-	-	-	-
Ammonium sulphate	-	-	-	-	-
Urea	-	-	-	-	-
Sodium nitrate	-	-	-	-	-
Amino acids					
Cysteine	-	-	-	++	++
Leucine	-	+	+	+	+
Methionine	-	-	-	+++	+++
Tryptophan	-	-	-	++	+++
Glycine	-	-	-	+	+
Alanine	-	-	-	+	+
Proline	-	-	+	++	+

*Absence of zone indicated as (-), Minimum zone of inhibition (+), Moderate zone of inhibition (++), Maximum zone of Inhibition (+++).

Antibacterial activity of isolate AVSC4 against different clinical pathogens at 96 hours

Antibacterial activity was analysed using crude Ethyl Acetate – extract of AVSC4 grown in formulated culture broth against five clinical pathogens (Fig 4). The Crude extract of AVSC4 has shown maximum inhibitory activity against *K.pneumonia* (16.8±0.2mm), *E.coli* (15.0±0.1mm), *S.typhi* (12.8±0.2mm), *P.vulgaris* (12.6±0.5), *S.aureus* (10.5±0.1mm) and *S.marcescens* (09.0±0.2mm).

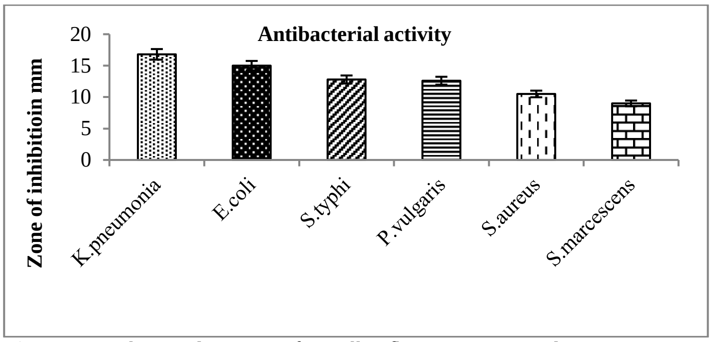

Figure 4 Antibacterial activity of *Bacillus flexus* AVSC4 crude extract against clinical pathogens.

GC-MS analysis

The plethora of compounds present in AVSC4 was identified by GC-MS analysis. GC-MS chromatogram of the ethyl acetate extract of AVSC4 recorded 15 peaks indicating the presence of the many antimicrobial bioactive metabolites (Table 2). Out of 15 peaks separated in GC- MS chromatogram, the eighth peak is the highest peak (1060268) and the maximum percentage area (29.04%) followed by the seventh peak (8.53 area). Based on NIST normal database seventh and eighth peaks were identified as methyl haptadecanoic acid and methyl hexadecanoic acid. Earlier reports on GC- MS of other organisms revealed that this compound could be potential antibacterial and anticancer secondary metabolites.

Table 2 Secondary metabolites identified in ethyl acetate extract of *B. flexus* AVSC4 by GC-MS analysis.

Peak	R.Time	I. Time	F.Time	Area	Area%	Height	Height %	A/H	Base M/Z	Name
1.	1.255	1.233	1.283	266113	0.24	240929	0.49	1.1 0	44.95	Isopropyl Alcohol
2.	7.124	7.075	7.183	5203545	4.62	3179971	6.52	1.64	73.90	Octanoic acid, methyl ester
3.	10.1 16	10.067	10.192	6204093	5.51	3602768	7.39	l.72	73.90	Decanoic acid, methyl ester
4.	13.163	13.100	13.217	6835299	6.07	3168019	6.50	2.16	73.90	Dodecanoic acid, methyl ester
5.	16.264	16.200	16.317	7131082	6.34	3363665	6.90	2.12	73.90	Methyl tetradecanoate
6.	18.791	18.742	18.850	4334974	3.85	2055790	4.22	2.11	54.95	9-Hexadecanoic acid, methyl ester
7.	19.075	19.008	19.133	9602810	8.53	4378122	8.98	2.19	73.90	Methyl hexadecanoate
8.	20.406	20.300	20.467	32683937	29.04	11060268	22.69	2.96	73.90	Heptadecanoicacid,methyl ester
9.	21.225	21.167	21.258	4139376	3.68	1931201	3.96	2.14	66.95	9,12-0ctadecadienoic acid methyl ester
10.	21.309	21.258	21.375	8313627	7.39	3353778	6.88	2.48	55.00	8,11,14-Docosatrienoic acid methyl ester
11.	21.604	21.542	21.658	6534579	5.81	2967387	6.09	2.20	73.90	Octadecanoic acid, methvl ester
12.	23.904	23.842	23.958	6256714	5.56	2831739	5.81	2.21	73.90	Eicosanoicacid, methyl ester
13.	25.769	25.708	25.817	3832079	3.40	1721294	3.53	2.23	54.95	13- Docosenoic acid, methyl ester
14.	26.021	25.958	26.075	5971181	5.30	2634750	5.40	2.27	73.90	Methyl docosanoate
15.	27.979	27.917	28.042	5255761	4.67	2264677	4.65	2.32	73.90	Tetracosanoic acid,methyl ester

Antiproliferation activity

The ethyl acetate extract of *Bacillus flexus* AVSC -4 showed IC $_{50}$ value of 50.04 μg/ml against A594 cell line and 93.4 against HT-29 cell lines. Highest percentage inhibition in cell proliferation of A-549 cancer cells was observed compared with HT-29 (Table 3). The crude extract is effective against HT-29 cell lines. Table 3 shows the increase in viability percentage in a dose dependent manner.

Table 3 IC_{50} Values of AVSC4 extract and Doxorubicin against HT-29 and A-549 cell lines

Conc. (µg/ml)	HT-29 Cell Line at 570nm	Average-Blank	% Viability	IC_{50} (µg/ml)	A-549 Cell Line at 570nm	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.93 ±0.002	0.928	48.996		0.844±0.002	0.844	41.969	
75	1.005±0.002	1.003	52.956		0.92±0.002	0.92	45.748	
50	1.074±0.002	1.072	56.599		1.011±0.002	1.011	50.273	
25	1.121±0.001	1.118	59.028		1.073±0.002	1.073	53.356	50.041
10	1.187±0.001	1.185	62.566		1.195±0.002	1.195	59.423	
5	1.215±0.002	1.213	64.044	93.4	1.221±0.002	1.221	60.716	
Untreated	1.898±0.0005	1.894	100		2.013±0.002	2.011	100	
Blank	0.004±0.0005	0			0.002±0.0005	0		

DISCUSSION

Presence of *Bacillus* in marine habitat with potent antibiotic properties is gaining interest in recent years. **Ramasubburayan et al.,(2014)** reported that *B. pumilus, B. licheniformis, B. subtilis, B. mojavens* and *B. firmus* are some of marine bacterial strains with antibiotic potential. *B. flexus* APGI, an active epibiotic bacterium of marine origin was extensively studied for maximizing the potential antibiotic property **(Ramasubburayan et al., 2014)**. Present study was aimed to characterize a marine bacillus AVSC4 for its antibiotic potential. AVSC4 was identified as *B.flexus* AVSC4 with accession no MG878436.

Acclimatization of AVSC4 to neutral pH (pH 7) from native alkaline habitat (Fig 2c), thermal and osmotic stability by showing growth maxima at 40°C(Fig 2b) and 4% NaCl (Fig 3a) is a positive indication to the isolate for exploration of novel bioactive secondary metabolites . Earlier reports also revealed that *B. flexus* APGI required 40°C for enhancing growth and secondary metabolite biosynthesis **(Ramasubburayan et al., 2014)**. *B. subtilis* has shown higher antimicrobial activity in the range of pH 7.0 and pH 8.0 **(Muaaz et al., 2007)** and other marine *Bacillus* sp showed potential production of antimicrobial metabolite at pH 8.0 **(Awais et al, 2008)**. Our results also revealed that the isolate AVSC4 showed potential antibacterial activity after 96hrs and 120 hrs of incubation period at 40°C, pH 7 and 8, salinity 0.4% and 0.5% in correlation with growth (Table 1). However 40°C, pH 7 and 0.4% NaCl have been selected in formulation of media.

Bacillus flexus APGI showed a consistent increase in antibacterial activity in the broth amended with galactose, fructose whereas mannitol recorded the higher degree of antibacterial activity **(Ramasubburayan et al., 2014)**. *Bacillus subtilis* also effectively utilize mannitol as the best carbon source for promising antibacterial activity **(Todorova and Kozhuharova, 2010)**. In contrast, our *Bacillus flexus* AVSC4 represented higher growth and potential antibacterial activity in the broth amended with glucose, sucrose and dextrose under optimized conditions of 40°C, pH 7 and 0.4% NaCl. As carbon sources play a major role in production medium which acts as source of energy and production of bioactive metabolites glucose a simple monosaccharide has been chosen as specific carbon source.

B. flexus AVSC4 has shown maximum growth in broth supplemented with peptone (Fig 3c) and maximum antibacterial activity in Beef extract (Table 1). Growth and antibacterial activity of *B. flexus* AVSC4 was reported maximum in methionine (Fig 3d). Earlier reports suggested meat extract and yeast extract were the best nitrogen sources for *Bacillus* sp. towards achieving maximum antibacterial activity **(Nishanth Kumar et al., 2012)**. In order to induce the synthesis and production of precursors, 0.5% beef extract and 0.5% methionine were selected as inducers in our formuated medium. Though the isolate AVSC4 showed maximum growth and antibacterial activity after 96hrs and 120 hrs of incubation periods, in order to shorten the length of bioprocessing and to avoid allosteric inhibition 96 hrs of incubation has been standardized.

GC-MS analysis revealed the chemical composition of this extract which included heptadecanoic acid, was found to have the highest area. Earlier research reports show that fatty acids produced from bacteria exhibited antibacterial activities **(Choi and Jiang, 2014)** and as the biological labelling **(Zhang et al., 2009)**. 9-12 Octadecenoic acid and Octadecanoic acid methyl ester were ninth and eleventh compounds which function as the antiacne, antieczemic, antihistaminic, anti-inflammatory, cancer preventive, hepatoprotective, hypocholesterolemic, insectifuge, nematicide, 5-alpha- eductase inhibitor antiandrogenic, antiarthritic, anti-coronary and insectifuge **(Kalaivani et al., 2012)**.

In earlierbstudies *Bacillus flexus* 47 MM isolated from Pensacola Bay has shown no significant antibacterial activity against *B. subtilis, P. aeruginosa* and *S. aureus* but, have shown anticancer activity against Pancreatic cancer cell line (PANC-1) and the multidrug- resistant ovarian cancer cell line NCI/ADR **(Christensen and Martin, 2017)**. Significant antibacterial activity of ethyl acetate extract of bacillus flexus AVSC4 grown in formulated media against five test organisms *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Salmonella typhi*, *Serretia marcescens* and *Staphylococcus aureus* (Fig 4) indicate that AVSC4 synthesized potential bioactive secondary metabolites under optimized culture conditions.

Bacillus thuriengiensis S13 and *Bacillus flexus* S15 exhibited a significant inhibition activity on A-549 lung cancer cell lines with IC 50 value of 120.39 µg/ml and 133.27µg/ ml. Marine *Bacillus sp* isolated from the Egyptian saline habitat produced bioactive compounds which have cytotoxic activity on hepatocellular carcinoma with IC50 (218 µg ml-1) **(Salma M Abdelnasser et al 2017)**. Ethyl acetate extract of B.flexus reported here could able to inhibit growth (Human colorectal adenocarcinoma cell line HT-29) at IC 50 value of 93.4 µg/ml and (Lung cancer cell line A-549) at IC 50 value of 50.04 µg/ml (Table 3).

CONCLUSION

Bacillus flexus AVSC4 is a marine bacteria with potent antibacterial activity. Acclimatization to mesophilic condition, thermal and osmotic stability of the isolate are positive characteristics for the exploration of novel bioactive secondary metabolites of pharmaceutical significance. Presence of saturated fatty acids, antibacterial and anticancer activities of the crude extract of the isolate grown in formulated culture broth reveal that glucose, beef extract and methionine are potential inducers and play a pivotal role for the synthesis of novel bioactive secondary metabolites. Future studies on purification of active principles and mechanism of action would be required to elucidate potential usefulness of *Bacillus flexus* AVSC4.

Conflict of interest statement: No conflict of interest.

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