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Production, Structural Elucidation and Biological Activities of Bioactive Metabolite of Marine *Saccharomonospora viridis*

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Twenty seven of one hundred thirty five marine actinomycete isolates were producers. Analysis of the 16S rRNA gene of the most potent bioactive isolate showed a strong similarity with *Saccharomonospora viridis* AHK190. For the production of antimicrobial metabolites, nutritional and environmental conditions have been optimized. Maximum antibiotic production was obtained when galactose was used as a sole carbon source at a concentration of 4.5% (w/v) in the presence of 7% (w/v) aspartic acid with salinity level 3% (w/v) and K_2HPO_4 at 0.011%. Similarly initial medium pH of 8.0, incubation temperature of 55°C and incubation period of 72 hr were found to be the optimum. The chemical structure of this compound was elucidated on the basis of its spectroscopy data, elemental analysis and its molecular formula was found to be $C_{31}H_{34}O_4S_3$. The minimum inhibitory concentration of the pure compound against the tested bacteria was found to be in the range of 125-250 µg/ml and the minimum bacteriocidal concentration of the purified antibiotic was ranged between 250 and 500 µg/ml. For fungi, minimum inhibitory concentration was ranged between250-500µg/ml. The pure compound also found to have cytotoxic activity against human colon carcinoma with lethal concentration 50 of 24.2µg/ml.

Keywords: Marine, Thermophile, *Saccharomonospora viridis* Bioactive Biometabolite, Antimicrobial Activity, Cytotoxic Activity

The search for novel therapeutic agents for use in the pharmaceutical industry is driven by the need to combat the increase in the incidence of infection due to antibiotic resistant pathogens coupled with the search for antitumor and antiviral compounds (Eccleston *et al.*, 2008). The decreasing rate of discovery of novel drugs from established terrestrial sources has motivated the evaluation of new sources of chemically diverse bioactive compounds (Magarvey *et al.*, 2004).The increasing number of novel metabolites from marine microorganisms reported manually shows that marine habitats are a promising source of biotechnological commercially significant products (Blunt *et al.*, 2007). Marine actinomycetes have been traditionally recognized as a rich source for biologically active metabolite. Among the well characterized pharmaceutically relevant microorganisms, actinomycetes remain sources of novel therapeutically relevant natural products (Jensen and Fenical 2000).The majority of these compounds demonstrate one or more bioactivities, many of them developed into drugs for

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treatment of a wide range of diseases in human, veterinary and agriculture sector (Bernan *et al.*, 1997). Production of secondary metabolites by microorganisms differs qualitatively and quantitatively depending on the strains and species of microorganisms used as well as on their nutritional and cultural conditions (Lam *et al.*, 1989) and as fermentation moves into lower-value, higher-volume substrates, it becomes necessary to maximize the efficiency and minimize costs by using waste by-products to complete effectively with traditional high-value, low-volume compounds (John *et al.*, 2007).

In the present work, a marine thermophilic isolate S5, capable of producing a broad-spectrum antimicrobial agent against Gram positive, Gram negative bacteria and fungi, was grown on optimized culture medium to increase its bioactivity and in addition , the antimicrobial substance(s) was extracted and partial characterized.

Material and methods

Isolation and maintenance of the actinomycetes strains

During the course of screening for antibiotics from marine actinomycetes, a total of 135 actinomycete strains were isolated from Burj Al-Arab and El-Agamy salinases, in Alexandria, Egypt. Eleven sediment samples were taken at the depth of 5-15 cm into the sediment after the removal of approximately 5 cm of the sediment surface during March 2008. Actinomycetes were isolated using starch casein agar medium (SCA) (Okazaki and Okami, 1972) prepared with artificial sea water (Atlas, 1993) in different sodium chloride concentrations 2.4,5,7 and 9 % (wt/v) with addition of rifampin (5µg/ml) and nystatin (50µg/ml) in order to minimize bacterial and fungal contamination (Mincer *et al.*, 2005). The plates were incubated at 55ºC for one weak. The isolated actinomycetes were screened with regard to their potential to generate bioactive compounds. The most potent producer strain was selected and identified. The pure culture was maintained on SCA slants at 4ºC.

Identification of actinomycete strain

The identification of the most potent producer strain was carried out on the basis of morphological, cultural, physiological characteristics and the 16S rRNA gene sequencing.

Morphological, cultural and physiological characteristics

The morphological characteristics were studied by using cover slip method in which the culture was transferred to the base of cover slips buried in SCA medium and incubated at 55ºC for two days. The morphology of spore bearing hyphae with entire spore chain was then studied as described in Bergey's manual (Locci, 1989).The electron microscope study was conducted using a scanning and transmission electron microscope. The cultural and physiological characteristics of strain were studied in accordance with the guidelines established by The International Streptomyces Project (ISP) (Shirling and Gottlieb, 1966).

DNA isolation and manipulation

The genomic DNA of the locally isolated actinomycete strain was isolated according to Malacinski (2005).

Amplification and sequencing of the 16S rRNA gene

The 16 S rDNA gene was amplified by Polymerase Chain Reaction (PCR) using specific primers. The primers that was used to 16S rDNA sequencing were 243F with the sequence 5- GGATGAGCCC- GCGGCCA – 3 and A3R with sequence 5- CCAGCCCCAC – CTTCGAC–3 (Schwieger and Tebbe, 1998). The PCR mixture consisted of 30 Pico moles of each primer, 10 µg of chromosomal DNA, 200µM d NTPS and 2.5 units of Taq polymerase in 50 µl of polymerase buffer. The PCR was carried out for 30 cycles of 94ºC for 1min. 55ºC for 1 min. and 72º C for 2 min. After completion , a fraction of the PCR Mixture was examined using 1.5% agars gel in Tris – Boric acid – EDTA (TBE) buffer (pH 8.5), electrophoresis was carried out for 20 min. at 150v and the remnant mixture was purified using PCR purification kit (Qiagen , Germany). DNA sequences were obtained using an ABIPRISM 3700 DNA sequencer. The sequence was compared for similarity with the reference species of bacteria contained in genomic database banks, using the NCBI BLAST available at http: //www.ncbi- nIm-nih.gov (Duraipandiyan *et al*., 2010).

A phylogenic tree was constructed using MEGA with the neighbor-Joining method (WWW.megasoftware.net) .Bootstrap values were determined according to Felsenstein's method (Felsenstein, 1985).

Biological assay of antimicrobial activities

To determine the antimicrobial activities, the tested microorganisms used were those suggested by Kokare *et al*. (2003). The test bacteria cultures for bioassay were obtained from the American Type Culture Collection (ATCC) and the National Collection of Type Culture (NCTC). The test fungal cultures were obtained from the agriculture college, Ain Shams University, Cairo, Egypt.

Bacterial strains include four reference strains; *Escherichia coli* (NCTC 10418/ATCC 10536), *Staphylococcus aureus* (NCTC 10788/ATCC 6538), *Bacillus cereus* (NCTC 7464/ATCC 10876) and *Salmonella typhimurium* (NCTC 12023/ATCC 14028); two clinical bacterial cultures: *Pseudomonas aeruginosa* and *Bacillus subtlis*. However fungal strains include *Aspergillus niger*, *Aspergillus flavus*, *Fuserium oxysporium*, *Trichoderma spp*. *Penicilium spp*. and *Candida albicans* as a yeast strain. The most potent producer strain was inoculated in SCA broth and incubated at 55 ºC. At the end of the incubation period, the antimicrobial activity was investigated by well diffusion method (Nedialkova and Naidenova, 2004).

Basal medium and inoculum preparation

To determine the optimal nutritional and cultural conditions for the growth and antimicrobial activities, starch casein broth medium was used. The optimum condition obtained for each factor was applied when studying the other factors. The most potent producer strain was grown on starch casein broth for 3 days at 55ºC. Spores where then harvested and homogenized in 0.5% tween 20 (0.2 O.D.) 0.5 ml of this suspension was used as inoculum for optimization experiments (Singh *et al*., 2009). After the incubation period, the broth was filtered using Whatman No.1 filter paper. Growth was determined as dry mycelial weight in a fixed volume of culture medium by drying the cell mass in oven at 70ºC overnight and expressed as growth dry weight mg/ 30 ml (Thakur *et al.,* 2009).

Optimization of the culture medium for production of bioactive biometabolite
Incubation period, temperature and pH

The effect of cultural conditions like different incubation temperature (35-60ºC), initial pH (6-9.2) and incubation period (1-6 days) on the growth and bioactive metabolite production were studied separately.

NaCl concentration

The effect of salinity on the growth and on the antimicrobial agent produced by the most potent producer strain was carried out by cultivating in various NaCl concentrations ranging from 0 to 20% in the basal medium (SCA) . The growth dry weight as well as antibiotic production in the term of mean diameter of inhibition zone was estimated.

Carbon and nitrogen sources

Thirty milliliters of the starch casein broth medium was distributed into each 100 ml Erlenmeyer flask and sterilized separately, glucose, arabinose , fructose , galactose, glycerol , lactose, maltose, mannitol , mannose , Meso-inisitol , starch , sucrose , xylose and raffinose were separately sterilized and added as carbon sources into the starch casein broth medium at concentration 1 % prior to inoculation . Various nitrogen sources such as Ammonium nitrate, ammonium sulphate, monosodium glutamate potassium nitrate, sodium nitrate and urea were provided separately into the basal medium at concentration equivalent to 2.0 g KNO_3 /L. A number of amino acids were also tested including , alanine , asparagine , aspartic acid, glutamic acid hydroxyl proline , leucine , methionine , phenylalanine , threonine, tyrosine , beef extract , peptone , lysine , pyridoxine , glycine , valine, proline , glutamine and cystine .

Extraction and purification of the bioactive compound

The crude bioactive compound produced in liquid culture medium by the most potent strain was extracted using solvent extraction method as recommended by El- Naggar *et al.* (2006). Thirteen different solvents and solvent systems were used in the extraction by shaking thrice with equal volume of each solvent (1:1) in a separating funnel. The solvent layer was collected and then evaporated in a rotary evaporator under vacuum to give 2.0 g compound. Purification of this crude compound was carried out by thin layer chromatography (TLC) technique on silica

gel (Merck ltd) using toluene: petroleum ether (3:1) , toluene : chloroform (3:1), toluene: chloroform (3:2), toluene : chloroform (1:1) , toluene: ethyl acetate (3:1), toluene: acetone (3:1), toluene : glacial acetic acid (3:1) as running solvent system. The TLC plates were prepared and spotted with the sample. Then, plates were developed by running the mobile phase and observed under UV light at 254 and 365 nm (Model, MOPEL ENF- 260 C New York, U.S.A). After visualization with ultraviolet light, spots was pooled, concentrated and analyzed using biological assay (Ilic *et al.*, 2005).

The pure fraction was then subjected to spectroscopic analyses : 300 MHZ 1H NMR (Varian Genini–300 MHZ spectrophotometer) , Elemental analysis , IR (Nicolet Is 10 FT- IR Spectrophotometer), U.V. absorption (U.V.-1600 spectrophotometer) . Mass spectrum (EI – MS) was recorded on HP MODEL GC MS – QPL000EX mass spectrometer (Shimoden) at 70 eV.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC was determined according to Haque *et al.,* (1996) by adding 1 ml standard inoculums (O. D. of 0.1 at 620 nm) of the test bacteria to 5 ml nutrient broth (NB) in different test tubes. Two fold serial dilutions of the active compound were added at the same time. MIC of bacterial test organisms was determined after 48 hr of incubation by visual comparison method (presence of turbidity due to growth). The MBC was determined by streaking on nutrient agar plated from tubes that showed growth inhibition. The concentration of extract in the well where was no growth on plate was considered as MBC.

Antifungal assay was done according to Aneja and Joshi (2009) by adding 100 µl of each two fold serially diluted tubes containing the active compound to wells in Sabaraud agar medium plates already seeded with 100 µl of standardized inoculums (0.1 O.D. at 350 nm) of the test fungal strains . All of the test plates were incubated aerobically at 30º C for 48 hr and observed for the inhibition zones. The lowest concentration of each extracts showing a clear zone of inhibition (>8 mm) (in triplicate), considered as the MIC, was recorded for each microorganism.

Antitumor activity of the pure antibiotic on human cell line

Antitumor activity was measured using a colorimetric cytotoxicity assay (Skehan *et al.*, 1990).

Statistical analysis

The means and standard deviations of the inhibition zones diameter and growth dry weight were calculated. Data were analyzed by one-way analysis of variance (ANOVA) for multiple comparisons among groups. Significant differences ($P< 0.05$) between the means were determined by the Least Significant Difference (LSD) test.

Results and Discussion

One hundred thirty five actinomycete isolates were isolated and screened for their antibacterial and antifungal activities. Isolate S5 of twenty seven isolates proved to be the most potent bioactive isolate (Table 1). It has the ability to utilize all tested carbon sources except xylose, produce melanin pigment when cultivated on ISP1, ISP6 or ISP7 and can tolerate up to 5% NaCl (Table 1). The morphological, physiological and cultural characteristics of S5 isolate were compared with known actinomycete species described in Bergy's manual of systematic bacteriology (Table 2&3). It was suggested that S5 isolates belong to *Saccharomonospora spp.*

TABLE1. Mean diameters of inhibition zones (mm) caused by 100µl of the actinomycete metabolite

NaCl concentration (%)	Isolate code	Mean diameter of inhibition zone (mm) against the test microorganisms						
		Staph. Aureus	***Bacillus subtilis***	***Bacillus cereus***	***Aspergillus niger***	***Aspergillus flavus***	***Trichoderma* spp.**	***Penicillium* spp.**
2.4	B2	-	-	-	-	-	20.6	-
	B3	-	-	-	-	18.3	20.6	-
	B9	14.6	-	-	-	18.0	20.6	-
	B11	-	-	-	-	-	27.6	-
	C1	-	-	-	17.0	18.0	12.6	-
	A	21.6	-	-	-	-	17.6	-
5	G1	-	-	-	-	-	25.0	-
	G2	-	-	-	19.6	21.6	31.3	-
	I2	-	-	-	19.67	20.0	30.0	-
	C1	-	-	-	-	-	22.6	-
	C2	21.6	-	-	-	-	18.6	-
	C3	14.3	-	-	-	-	23.0	-
	B1	-	-	-	-	-	20.6	-
	I2	15.0	-	-	-	-	-	-
	S4	-	-	-	-	-	20.0	-
	S1	16.3	14.6	-	-	-	17.6	-
	S2	19.3	14.6	-	-	-	15.6	-
	S4	-	-	-	-	-	30.0	-
	S5	14.6	16.3	17.3	-	-	19.0	20.0
	F	15.6	15.3	17.6	-	-	17.0	20.0
	I1	16.0	15.3	15.3	-	-	-	-
	C1	-	16.0	16.3	-	-	-	-
	R1	-	14.3	14.6	-	-	-	-
7	R1	-	-	-	-	19.6	30.6	-
	A3	-	-	-	-	-	18.3	-
	B2	-	-	-	-	-	24.0	-
	H2	-	-	-	-	-	26.3	-

TABLE 2. Cultural characteristics of the isolate S5

Media	Growth	Arial mycelium	Substrate mycelium	Diffusible pigment
Glycerol asparagine agar ISP No.2	Abundant	Powdery-Velvet blue-grey	Very dark grey	Negative
Inorganic salt starch agar ISP N0.4	Abundant	Powdery-Velvet greenish blue	Very dark green	Negative
Oat meal agar ISP No.3	Good	Powdery grey-greenish blue	Very dark green	Negative
Yeast extract-Malt extract agar ISP No.2	Abundant	Powdery-Velvet bluish green	Black green	Negative
Nutrient agar	Abundant	Velvet light grayish blue	Light green	Negative
Czapek's agar	Abundant	Velvet deep grayish blue	Very dark blue	Negative

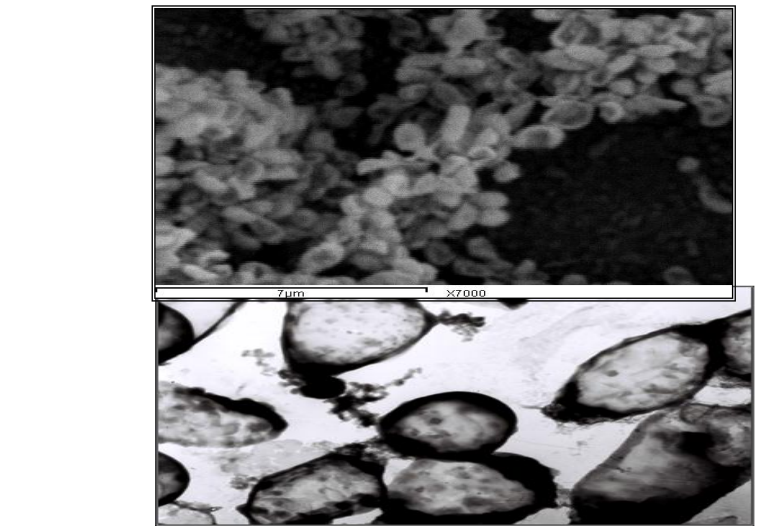
TABLE 3. Physiological and biochemical characteristics of the isolate S5

Parameters	Characteristics of
Gram reaction	+
Production of melanoid pigment	+
NaCl tolerance	≤7
Range of temperature for growth	33-57°C
Optimum temperature for growth	55
Range of pH for growth	6-10
Optimum pH for growth	8
Utilization of different carbon source:	
Glucose	+
Sucrose	+
Xylose	-
Meso-inositol	+
Galactose	+
Fructose	+
Lactose	+
Rhamnose	+
Mannose	+
Arabinose	+
Heat resistance of spores (100°C)	10 minutes
Catalase production	+
Oxidase production	+
Urease production	+
Hydrogen sulfide production	-
Nitrate reduction	-
Methyl red test	+
Vogues Proskauer test	-
Indole production	-
Citrate utilization	-
Starch hydrolysis	+
Gelatin liquefaction	+
Triple sugar iron	Alk/Alk
Casein hydrolysis	+
Hemolysis	+
Melanin production	+
Tyrosine decomposition	+
Cellulose decomposition	-

The scanning electron micrograph of the strain S5 revealed that the spores formed at the tips of simple unbranched sporophore of variable length in which spores are densely packed along with the hyphae and appeared as clusters (Fig. 1.a). Transmission electron microscope of the strain S5 revealed that, the spores are oval with a warty surface (Fig. 1.b). Single spores are observed only on the aerial mycelium either directly on the hyphae or on short sporophores.

The strain has got maximum 16S rRNA sequence homology with *Saccharomonospora viridis* AHK190. This 16S rRNA gene sequence analysis was carried out to elucidate the taxonomic position and relationships among closely related *Saccharomonospora* species. The alignment result (Phylogenetic tree) was graphically summarized in Fig. 1.c. Numbers at nodes indicate percentages of bootstrap support based on a neighbour –joining analysis of 1000 resample dataset: only values above 50% are given. Bar: 0.001 substitutions per nucleotide position.

a



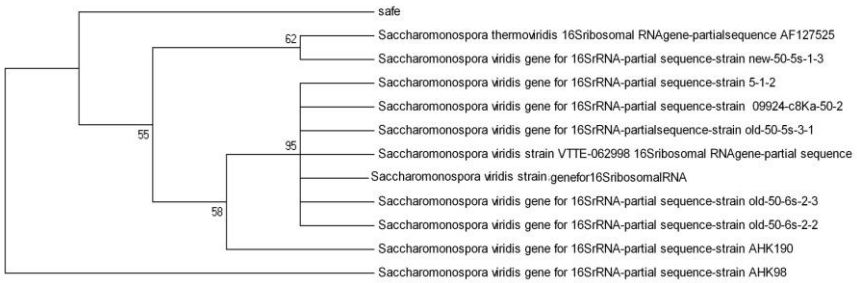


Fig. 1. Identification of the strain S5
(a) The surface morphology by SEM
(b) The surface morphology by TEM
(c) Neighbor-joining tree based on 16S rRNA sequences of isolated strain

The gene rRNA is the tool mainly used for molecular identification of bacteria (Boudemagh *et al*., 2005).The 16S rRNA sequence of the isolate S5 isolate was compared to the sequence of *Saccharomonospora* spp. in order to determine the relatedness to this *Saccharomonospora* spp., the most potent strain evidenced on 100% similarity with *Saccharomonospora viridis* AHK190.

The results showed that the highest bioactive biometabolite production and growth (expressed as the dry weight of mycelium) of *Saccharomonospora viridis* strain were obtained after 72 hr-incubation (Fig. 2). *Saccharomonospora viridis* strain showed a narrow range of incubation temperature for relative good growth and antibiotic production. The highest growth as well as biometabolite production was obtained at 55 °C (Fig. 3). In the term of its optimum temperature for growth, this organism appears to be thermophile in nature. The rapid growth rate of thermophilic actinomycetes and the reduction in contamination due to the elevated growth temperature make these organisms particularly useful for industrial fermentation (Tendler and Burkholder, 1960) rather than bioactive mesophiles that take more time (Sujatha *et al.*, 2005 and Singh *et al.*, 2009; Thakur *et al*. 2009 andArasu *et al.*, 2009).

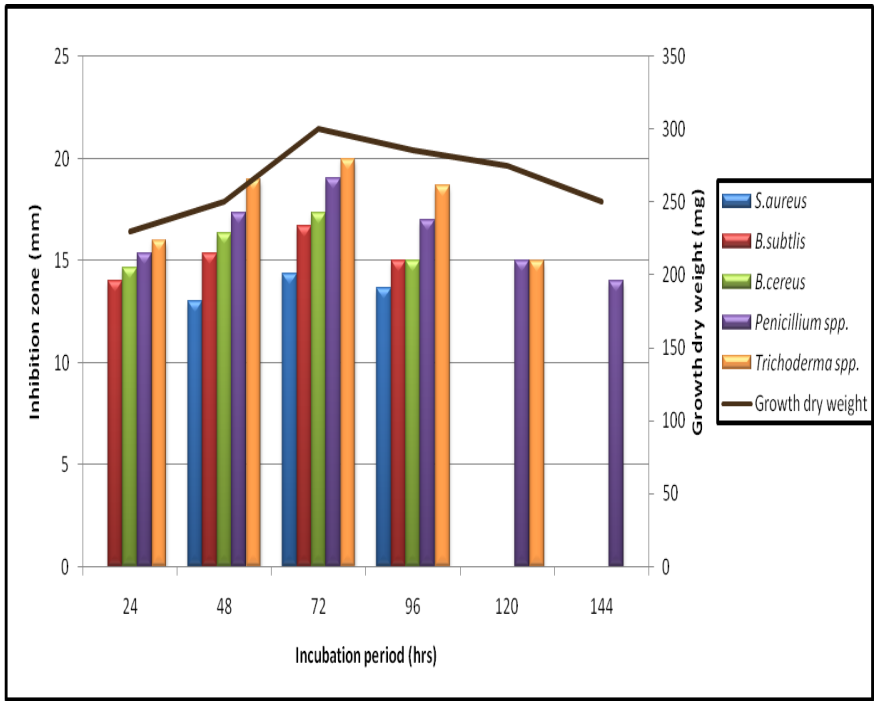


Fig.2. Time course of growth and antibiotic production by *Saccharomonospora viridis*

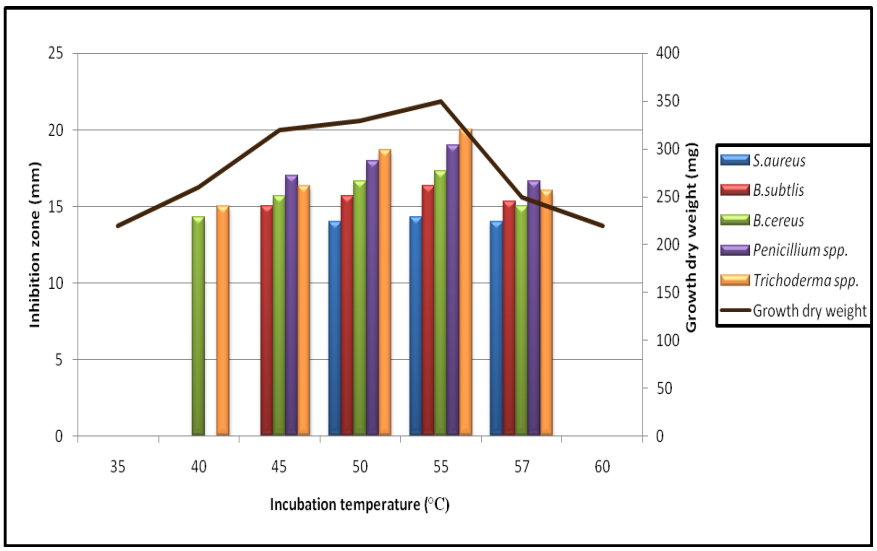


Fig. 3. Effect of incubation temperature on the growth and antibiotic production by

Saccharomonospora viridis

Bioactive metabolite production was found to be optimum at pH 8, while growth was increased up to pH 9, (Fig. 4) This suggests that this strain is an alkalophilic actinomycete. This result is comparable with some *Streptomyces* species recorded to produce antibiotics at alkaline pH (Basilio *et al.*, 2003; Vasavada *et al.*, 2006 and Singh *et al.*, 2009).

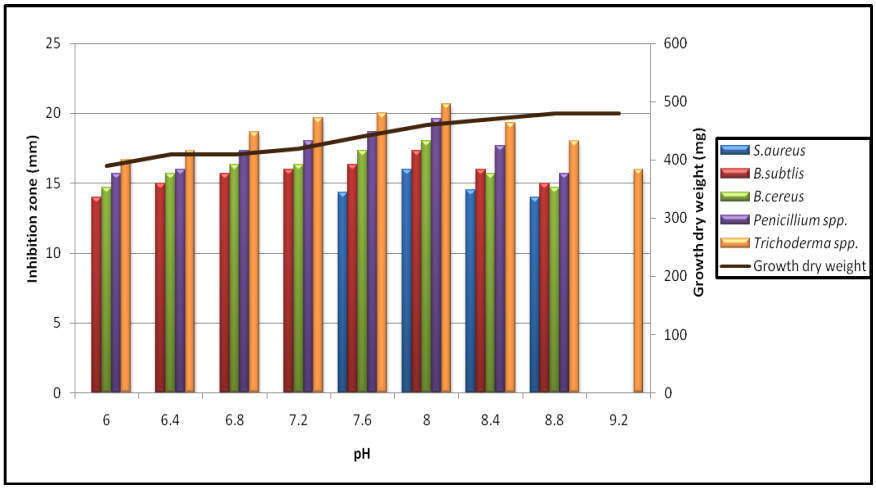


Fig. 4. Effect of initial pH on the growth and antibiotic production by *Saccharomonospora viridis*

Thakur *et al*, (2009) reported that, the production of antimicrobial agent as well as growth by *Streptomyces* sp. 201 was maximum at pH 7.5. Arasu *et al.* (2009) found that, the maximum antibiotic produced by *Streptomyces* sp. ER1-3 was obtained at pH 7.0 while Yu *et al.* (2008) found that the best pH for antifungal antibiotic production by *Streptomyces rimosus* MY02 was 6.0.

From our results, the environmental factors like incubation period, incubation temperature and pH were found to have profound influence on antibiotic production by *Saccharomonospora viridis* strain as surveyed by other investigators (Srinivasan *et al.*, 1991 and Sujatha *et al.*, 2005).

In our study for screening of bioactive marine thermophilic actinomycetes, NaCl is the major constituent of sea water. A kinetic study of growth and antibiotic production with varying concentrations of this salt revealed that 3% NaCl was found to be significantly the optimum for antibiotic production while, 5% NaCl was the optimum for growth, Fig. 5. This result was consistent with the result of Vasavada *et al*. (2006) who found that, antibiotic production by salt-tolerant and alkalophilic *Streptomyces sannanensis* was optimum at 3% NaCl with slight decrease at 5% concentration.

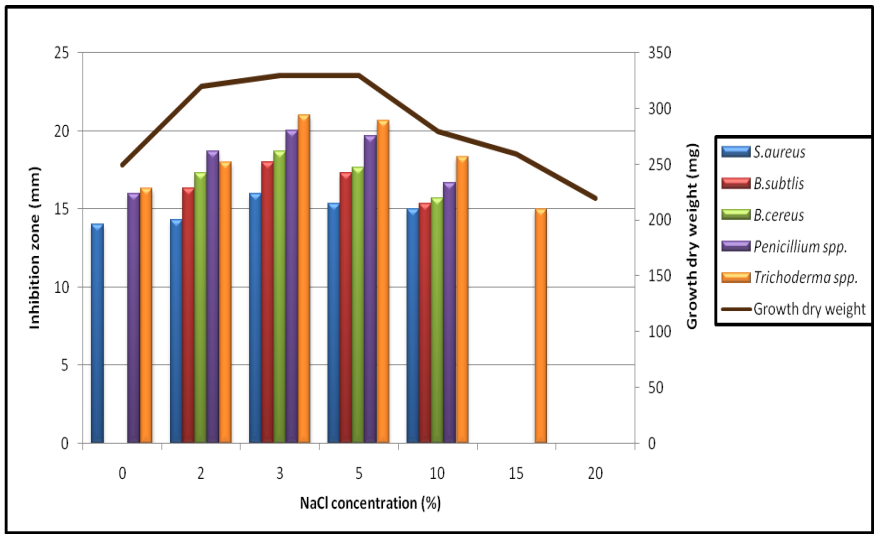


Fig. 5. Effect of NaCl concentration on the growth and antibiotic production by *Saccharomonospora viridis*

Results presented in Fig. 6 explored the effect of 14 carbon sources on the antibiotic production by *Saccharomonospora viridis* strain. D-galactose proved to be an excellent carbon source for bioactive metabolite and starch was for the growth. This finding was compatible with that obtained by Basak and Majumdar

(1973) who found that, D-galactose was proved to be an excellent carbon source for Kanamycin formation by *Streptomyces kanamyceticus. Streptomyces* sp.201 produced antibiotic and growth optimally with mannitol as a sole carbon source (Thakur *et al.*, 2009). It was also found that starch and Meso-inositol provided maximum growth, This may be due to these carbon sources are utilized rapidly for the synthesis of cellular material so that little would be available as carbon and energy source for antibiotic(s) synthesis. Galactose may be utilized less rapidly and thus it is available during the phase of antibiotic(s) production (Basak and Majumdar, 1973). All of 14 carbon sources used in this study were sterilized by ether to avoid denaturation and caramelization of some carbon sources (Thakur *et al.*, 2009).

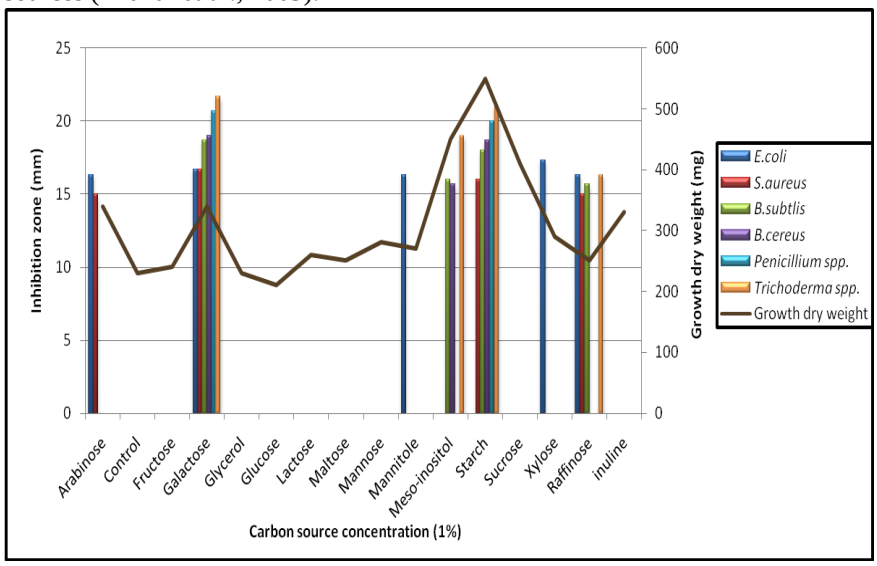


Fig. 6. Effect of different carbon sources on the growth and antibiotic production by *Saccharomonospora viridis*

The medium used for testing effect of different nitrogen sources on the antibiotic production contained the basal medium plus 3% NaCl and 4.5% galactose. The amino acids and inorganic nitrogen compounds were employed at a concentration equivalent to 2g KNO_3/L. The results of nitrogen source utilization were shown in Fig. 7. The maximum significant antibiotic production was obtained in the culture containing aspartic acid as nitrogen source. Highest significant antibiotic yield and maximum growth were obtained in a synthetic medium containing aspartic acid at 7% concentration as a nitrogen source. This result agreed with the finding of Singh *et al.* (2009) who found that aspartic acid provided the highest biomass as well as antimicrobial agent by *Streptomyces tanashiensis* strain A2D.

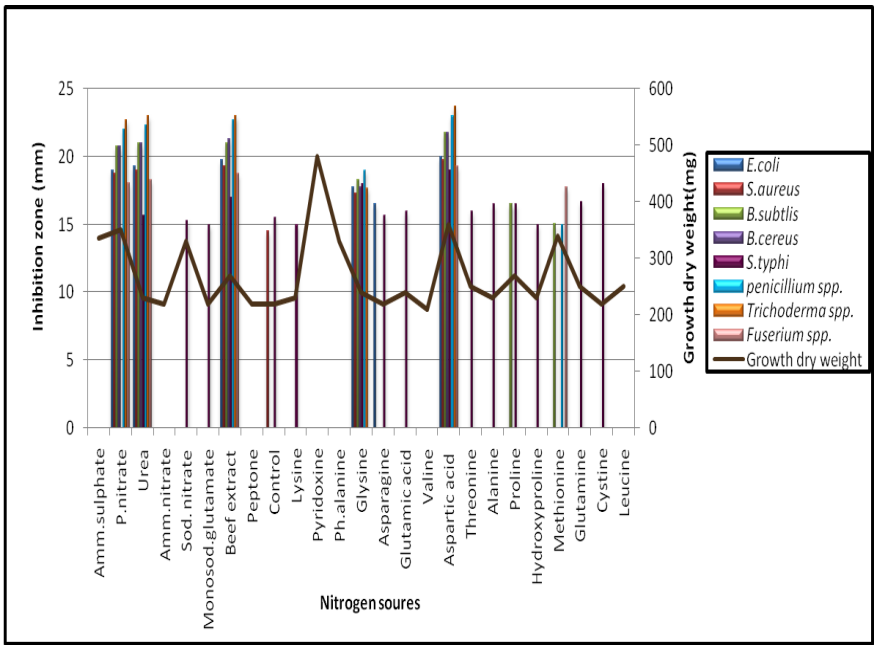


Fig. 7. Effect of different nitrogen sources on the growth and antibiotic production by *Saccharomonospora viridis*

Our results indicated the dependence of the antibiotic synthesis on the medium constituents. In fact, it has been shown that the nature of carbon, nitrogen sources, and sodium chloride strongly affect antibiotic production in different organisms. The medium developed in the present study to obtain high yield of bioactive metabolite by *Saccharomonospora viridis* strain has the following composition (g/l): galactose, 45.0; casein, 0.3; aspartic acid, 7.0; NaCl, 30.0; $CaCO_3$, 0.02; $FeSO_4$, 0.001; Yeast extract, 2.0; $MgSO_4.7H_2O$, 7.0; $MgCL_2.6H_2O$, 5.3; KCl, 0.7 and $CaCL_2$, 0.1.

Up to date, still new antibiotics are isolated from extremophilic actinomycetes (Lu *et al.*, 2009). In this work, the producing antibiotic isolates were screened to select the most potent isolate with the best environmental and nutritional conditions for antibiotic production.

Purification, structural elucidation and biological activities of the bioactive metabolite

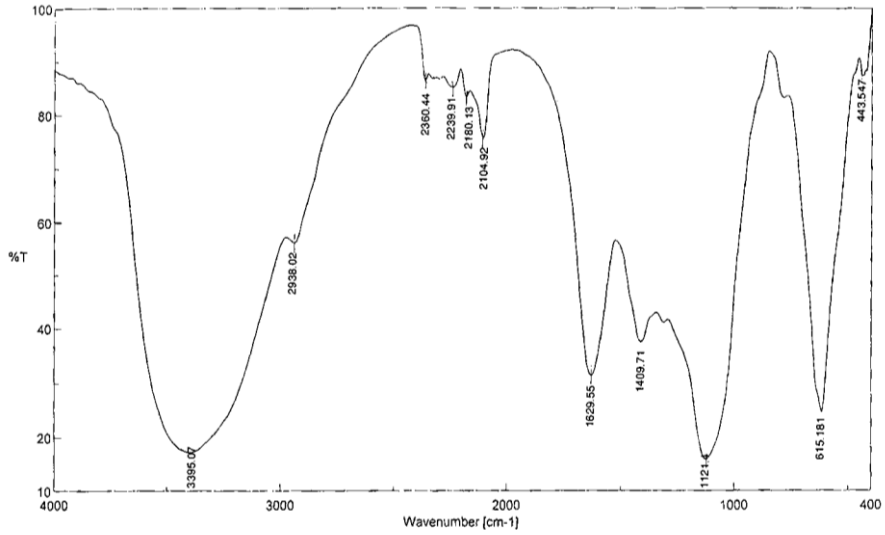
The fermentation process was carried out for 3 days at 55°C using modified starch casein broth medium. 5-liter total volume filtered was conducted followed by centrifugation at 5000 p.m. for 20 min. The clear filtrate was extracted using toluene which is the most appropriate solvent for antibiotic extraction. The

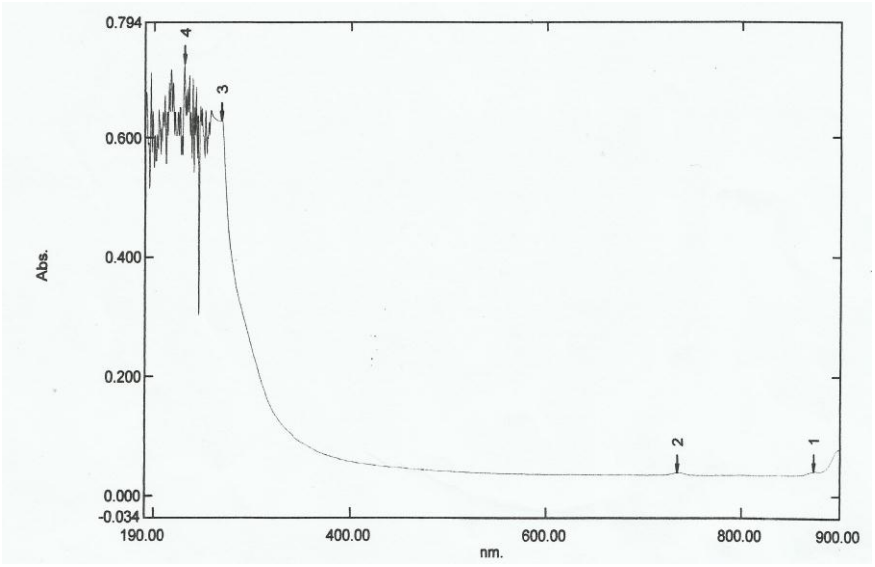
organic phase was concentrated using vacuum at 40°C to obtain 2g the crude compound. It was carried out by thin layer chromatography and found that, the spot given by toluene as a running solvent was circular with R_f value 0.31.The fluorescence color of the spot was dark orchid. In an attempt to establish the chemical structure of this antibiotic produced by *Saccharomonospora viridis* strain, Physical properties of purified bioactive biometabolite was brown in color , crystalline, soluble in DMSO, sparingly soluble in water insoluble in ethanol, methanol, toluene, butanol, diethyl ether and dimethyl formamide.

Spectral studies such as FT-IR, UV, ^{1}HNMR, ^{2}HNMR and Mass spectrum were performed. The infrared spectrum of the pure compound evidenced a diagnostic peak at 3396.08 cm-1, which is indicative of OH alcoholic group. However, the peak at 1629.81 cm-1 was assigned to carbonyl (C=O) group of the ketone functional group. The peak appeared at 1145.21cm-1 was assigned to thione (C–S) group. Finally, the peak appeared at 617.06 was assigned to aliphatic C–H bend (CH3 attached to aliphatic chain). Twelve libraries were searched for IR matches (Georgia state crime Lab sample libraries, HR Nicolet sampler libraries1-6, Aldrich condensed phase sample libraries 1&2, Hummel polymer sample libraries and Sigma biological sample libraries 1&2) but didn't give significant matches(>50%) (Fig. 8a).

The UV visible spectrum of the purified biometabolite that dissolved in DMSO indicates the presence of conjugated structure with absorptions at λmax=268 and 230 nm (Fig. 8.b). The ^{1}H NMR and ^{2}HNMR spectra of the pure compound (Fig.9. a & b) confirm the presence of alcoholic OH which was observed at 6.076-6.449 ppm. Methyl group protons were observed at 1.271& 1.231ppm. The aliphatic protons appeared at 3.651& 4.90, 5.125 and 3.512 ppm were assigned to CH (methine), CH_2 (ethylene) and CH_2 (methylene) respectively.

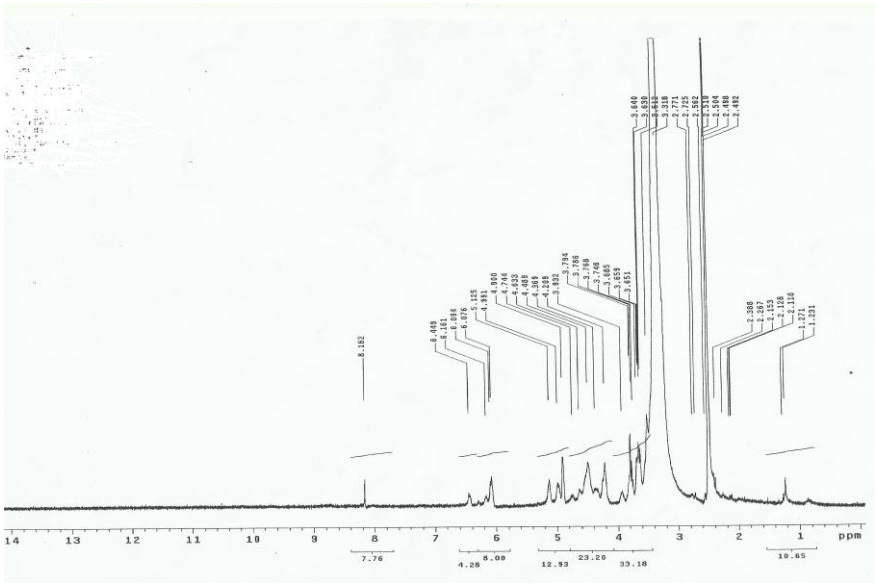
The molecular formula of the antibiotic was deduced as $C_{31}H_{34}O_4S_3$ based on the results of elemental analysis (Anal. Cal. for $C_{31}H_{34}O_4S_3$: C, 65.69%; H, 6.05%; S, 16.97%; O, 11.29; found C, 69.3%; H, 5.61%; S, 15.18%). The electron impact (EI) mass spectrum confirmed the molecular weight of biometabolite was 567 (Fig. 10.a). According to the described chemical assignments obtained from the IR, UV, ^{1}HNMR, MS and elemental analysis, this compound is not identical with similar antibiotics described in literatures. Its probable structure and IUPAC name is given in Fig. 10. b.



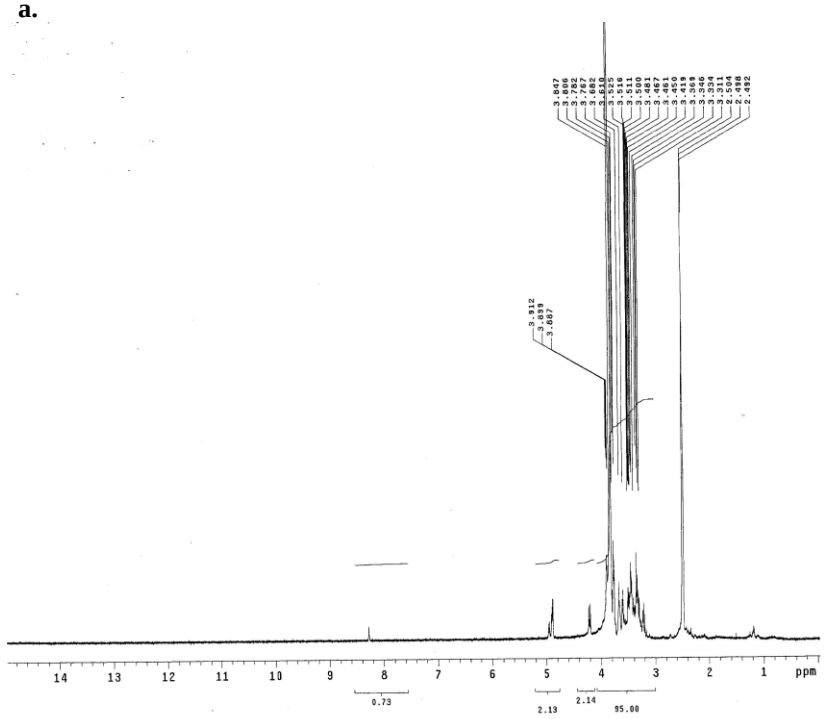


b.

Fig. 8 a. Fourier Transform Infra-redi spectroscopy spectrum, b. Ultraviolet (UV) spectrum (DMSO) of the purified biometabolite

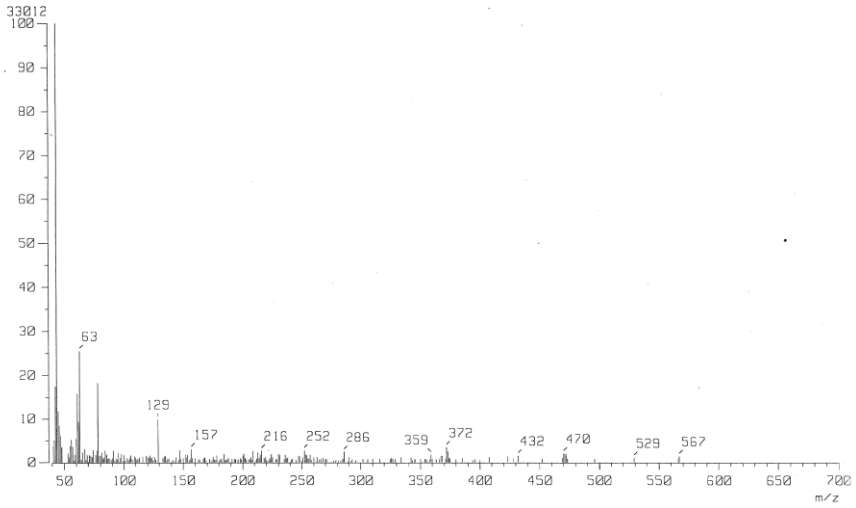


a.

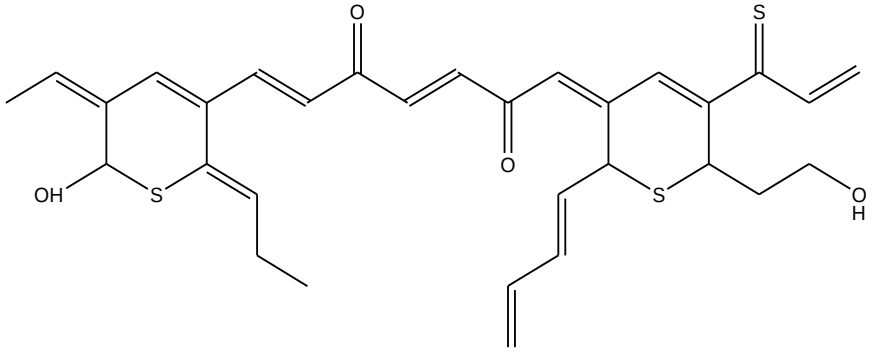


b.

Fig. 9 a. [1]HNMR spectrum (DMSO) , b. [2]HNMR spectrum (DMSO-D2O) of the purified biometabolite



a.



b.

(1Z, 3E, 6E)-1-(2- ((E)-buta-1, 3-dienyl)-6-(2-hydroxyethyl)-5-(prop-2 enethioyl)-2H-thiopyran-3 (6H)-Ylidene)-7-((5Z, 19Z)-5-ethylidene-5,6-dihydro-6-hydroxy-2-propylidene-2H-thiopyran-3-yl) hepta-3, 6-diene-2,5 dione

Fig. 10 a. Mass spectrum (EI-MS) of the purified biometabolite, b. Proposed structure of the active biometabolite from and its IUPAC name

Determination of the antimicrobial activities

The antimicrobial activities (MIC and MBC) of the pure bioactive metabolite compound are shown in Table 4. It inhibited the growth of both gram-positive and gram-negative bacteria as well as fungi. This was recorded by Nedialkova and Naidenova (2004) and Duraipandiyan *et al.* (2010) that screening the

antimicrobial properties of actinomycetes strains. Chandransekar *et al*. (2015) found that the lowest minimum inhibitory concentrations were 125 µg/mL against *S. flexneri, B. subtilis, M. luteus* and *P. vulgaris* respectively

Table 4. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the pure bioactive substances against bacteria and fungi

Test bacteria	Gram reaction	MIC (µg/ml)	MBC (µg/ml)	MBC/MIC
E.coli	-	125	250	2
P.aeruginosa	-	125	250	2
S.typhi	-	125	250	2
S. aureus	+	125	250	2
B.subtlis	+	250	500	2
B.cereus	+	125	500	4

Test fungi	MIC (µg/ml)
A. niger	250
A. flavus	250
Fuserium spp.	500
Penicillium spp.	250
Trichoderma spp.	500

Antitumor activity of the pure antibiotic on human cell line

The survival fraction of colon (HCT116) cell line was plotted against the different concentrations (5–50µg/ml) of the pure compound (Fig. 11). The concentration of the pure antibiotic that reduced survival of carcinoma cell line of colon to 50% was 24.2 µg/ml. Olano *et al*., 2009 reported that secondary metabolites of actinomycetes may possess antitumor activities.

Chandransekar *et al*. (2015) recorded that the ethyl acetate extract showed cytotoxic activity against A549 lung adenocarcinoma cancer cell line. It showed 72% activity at the dose of 1000 µg/mL with IC50 value of 600 µg/ mL.

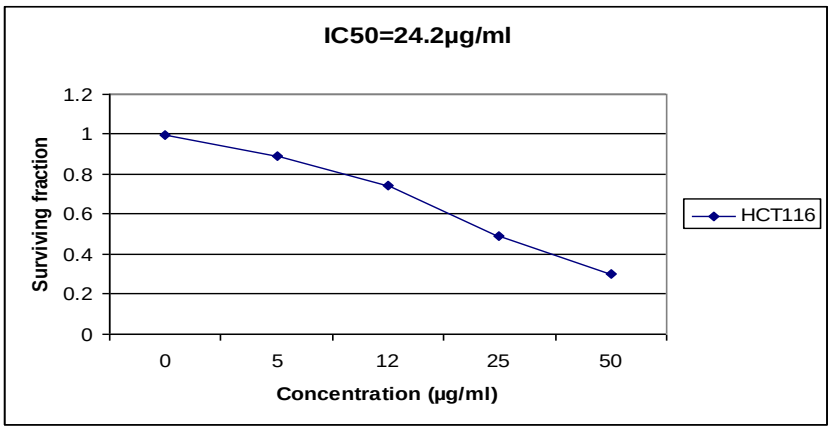


Fig. 11. Potential antitumor activity of the pure antibiotic on human cell line HCT116 (colon carcinoma) with LC50 value

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إنتاج وتكهن التركيب الكيميائى والانشطة البيولوجية للمادة الحيوية المنتجة من الاكتينوميسيتات السكارومونوسبورا فيريدزالبحرية

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أجريت هذه دراسة على الأكتينوميسيتات البحرية المحبة لدرجة الحراره المرتفعة والتي تم عزلها من ثلاث مناطق مختلفة بالملاحات بمحافظة الإسكندريه- مصر، واشتملت عد وعزل سلالات ميكروبية نشطة حيوياً . وجدت 27 عزلة لها نشاطات مضادة للميكروبات المختبرة ، أن العزلة S5 هى أكثر العزلات فاعلية ضد كلا من البكتيريا والفطريات . أظهرت الدراسات الجزيئية باستخدام تحليل الوحده الصغرى (16S) من الحمض النووي الريبونيوكليك أن درجة التشابه قويه (100٪) بين هذه العزله وبين السكارومونوسبورا فيريدز.

وقد اثبت الظروف البيئية والغذائية المثلى أسفرت عن أعلى انتاج للمادة الحيوية يمكن تلخيصها على النحو التالى ، درجة التحضين 55° م ، فترة تحضين 72 ساعة ، أفضل أس هيدروجينى هو 8 ، ظروف الإهتزاز لمدة 72 ساعة وبسرعة 180 لفه في الدقيقة ، تركيز 3٪ من كلوريد الصوديوم ، الجالاكتوز كمصدر للكربون أعلى إنتاج للمادة الحيوية بتركيز 4,5 ٪ ، حمض الأسبارتك كمصدر نيتروجين هو الأمثل لإنتاج المضاد الحيوي ، واستخدام ثنائي فوسفات البوتاسيوم كمصدرللفسفور وكان تركيز 0,11 ٪ هو الأمثل إنتاج المضاد الحيوي ، تم انتاج المادة الحيوية في نطاق معملي بإستخدام البيئة الغذائية المثلى وتنقيتها ومعرفة التركيب الكيميائى بالتحاليل الكيميائية الدقيقة مثل نسبة الكربون والاوكسجين والهيدروجين والكبريت وكذلك التحاليل الطيفية والتي شملت الأشعه تحت الحمراء والأشعه الفوق بنفسجية والرنين المغناطيسي والطيف الكتلي وتحليل العناصر ووجد ان الصيغه الجزيئيه هي $C_{31}H_{34}O_4S_3$

تم دراسة النشاطات المضادة للميكروبات في صورة أقل تركيز مثبط وأقل تركيز قاتل للبكتيريا ،وكذلك تأثيره على الفطريات الخيطية ، وكان أقل تركيز مثبط للبكتيريا والفطريات المختبرة يتراوح بين 125و500 ميكروجرام /مل وكان أقل تركيز قاتل للبكتيريا يتراوح بين 250و500 ميكروجرام /مل .

وجد أيضا أن هذا المضاد الحيوي النقي له نشاط سام للخلايا ضد خلايا سرطان القولون في الإنسان (HCT116) وكانت قيمة LC50 هى 24و2 ميكروجرام/مل.