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Isolation, screening and antibiotic profiling of marine Actinomycetes extracts from the coastal of Peninsular Malaysia

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Abstract : In the last few years, Actinomycetes have been extensively studied in several underexplored environments, niche, and extreme habitats in various regions across the globe. Yet, there is no report regarding isolation of actinomycetes from the coastal waters of Malaysia. Given that marine environmental conditions are extremely different from the terrestrial environment, it is logical to opine that marine actinomycetes might be potentials of producing novel bioactive compounds of invaluable medical values. This study aimed at collecting and screening marine sediments from the coastal areas of Johor, Penang and Melaka coastal areas of the peninsular Malaysia. The antibiotic activities of the isolates were tested against *Escherichia coli, Staphylococcus aureus, Vibrio cholera, Vibrio parahaemolyticus, Aeromonas hydrophillia and Pseudomonas aeruginosa*. Fifty samples were collected from these locations and screened for actinomycetes, out of which 11 isolates coded as M1,M1, SDJ-1, SDJ-2,P1, P2,P3, SDJ-8, SDJ-9 and SDJ-10 were obtained on starch-casein agar media, However, out of the 11 isolates, only four isolates showed significant antibacterial activity against both gram-positive and gram-negative bacteria. Further studies were carried out with the most active isolate, SDJ-10. The results showed thatStarch casein Broth (SCB), 28°C and pH 7 were themost suitable for SDJ-10. The production of antibiotics began after 24 h reached maximum at 72 h. Ethyl acetate: methanol at the ratio of 6:4 were found to be the best for TLC using silica gel.Seventy two compounds were detected from the ethyl acetate extract of isolate SDJ-10 using GC-MS analysis while 4 peaks were detected in HPLC analysis. Based on the findings of this study, it was concluded that the actinomycete isolated in this study has the potential to be developed as effective antibiotic.
Key words : Antibacterial, bioactive compound, HPLC,GC-MS, Marine actinomycetes.

Introduction

The marine environment which covers about 70% of the earth's surface also have 95% of its tropical biosphere representing 34 of the 36 phyla of life and thisoffers fascinating variations in biodiversity higher than

that of the terrestrial environment[8].The marine ecosystem produces variety of natural products from verse structural classes that exhibit activity against several disease targets[8]. Marine organisms are known to be a rich source of structurally innovative and biologically active metabolites of verse advantages[13]. Recently, studies have also suggested that some bioactive compounds isolated from marine organisms have anti-microbial, anti-fungal or anti-inflammatory and other beneficial pharmacological activities[3,5]

Actinomycetes on the other hand are Gram positive bacteria normal existing in soils and sea sediments. The name ‘Actinomycetes’ was derived from the Greek words ‘aktis’ meaning “a ray” and ‘mykes’ meaning “fungus”. These two organisms form the initial observation of their morphology. They were originally considered to be an intermediate group of organism between bacteria and fungi, which are now recognized as prokaryotic organisms[16]. Actinomycetes are now known as antibiotic producers, being the source of three quarters of all known products. They are highly prolific and can produce numerous antibiotics and other class of biologically active secondary metabolites. They cover about 80% of total antibiotic products[16].

It is now indisputable that new therapeutic agents, notably antibiotics, are urgently needed to mitigate the ever increasing spread of antibiotic resistant pathogens which are already causing life threatening diseases undermining the viability of health care systems [23]. Owing the verse potential of actinomycetes, increasing attention is currently being given to its isolation and characterization from poorly researched habitats in the thrust of discovering new natural products that can be developed as newer effective therapeutic agents[4].Actinomycetes are gram-positive prokaryote with high guanine plus cytosine content of higher than 55% [24]. in their DNA. This organism has been recognized as sources of several secondary metabolites, antibiotics, and bioactive compounds that influence microbial growth[8]. They have filamentous nature, branching pattern, and conidia formation, which are not different from those of fungi[5].

Media optimization has been shown to be a crucial factor in the production of bioactive compounds, seeing that proper nutritional composition guarantees the expression of actual genes and triggers the essential metabolic pathways [1]. It is upon this background that media optimization has been shown to play a key role in isolation of new organisms through culture [3].

Even though several studies have documented the potentials of actinomycetes as antibiotic producers from different regions across the globe, such studies are limited in Malaysian coastal waters. We hypothesized that actinomycetes isolated from Malaysian coastal waters could be a potential of generating newer antibiotics that could be effective against even some of the multi-drug resistant bacteria in our environments. In view of the large geographic variation, there is huge variation in sea sediments and their contents in Malaysia coastal waters and hence it is probable that the distribution of antibiotic producing actinomycetes could alsodiffer from those isolated in other regions of the world. This study was conducted to screen the antibiotic producing actinomycetes from different locations in Malaysia coastal waters

Materials and Methods

Collection of marine sediments

Samples of marine sediments used in this study were collected using core sample from the coastal regions of Johor,Penang and Melaka in Malaysia. The sampled sediments were of varying colours ranging from brown to black and were of sandy texture

Isolation of SDJ-10

Actinomycetes were isolated using soil dilution plate technique on starch nitrate agar medium, starch casein agar medium, Chitin agar medium and glycerol glycine agar medium. The plates were incubated for 15 days prior to determination of the number colonies. All the media used contain 50% sea water which were supplemented with Nystatin 50 µg/mL and Nilidixidic acid 20 µg/mL for the purpose of inhibiting bacterial and fungal contamination respectively. Selected colonies were further purified using streak plate technique on starch casein agar slants.

Screening for microbial activity

Antimicrobial activity of the isolates were preliminarily evaluated using cup plate method[6] against selected bacteria species. These bacteria included *Escherichia coli, Staphylococcus aureus, Vibrio cholera, Vibrio parahaemolyticus, Aeromonas hydrophillia and Pseudomonas aeruginosa*. Following the preliminary screening of the isolates for their antimicrobial activities, the most active isolates were selected for further evaluation.

Selection of suitable medium

The test media selected were marine broth (MB), nutrient broth (NB), Tryptic soy broth (TSB) and Starch casein Broth (SCB). The seed inoculum for each of the medium were transferred at 10% level into 100 mL of the medium contained in 25o mL conical flask. The seeded flasks were incubated in shaker incubator at 200rpm for 5 days at 28 ^{o}C.

Temperature and Ph

The optimum temperature for the productivity and growth of the strain SDJ-10 was determined through inoculation of the organism with starch casein broth and incubated at 200, 240, 280, 320, and 38°C for 3 days on a shaker incubator at 200 rpm[7,10]. Following the incubation period, growth and production of antibiotic were determined as described above. Optimum pH was similarly determined via adjustment of the pH of starch casein broth to varying pH values such as 5, 6, 7, and 8. The pH values were adjusted using 0.1N NaOH and 0.1n HClprior to sterilization[7,10]. The incubation was performed at 28°C for 72 h at 200 rpm on a shaker incubator. Growth and production of the antibiotic were determined as described above.

Time course study of activity by shake flask fermentation

Starch casein broth was employed in preparing the seed inoculum. Three 250 ml flasks were used. To each of the flask 100 mL starch casein broth of pH 7 were added and the flasks were seeded with 48 h seed inoculum at 10%concentration. The flasks were then incubated at 28°C in shaker incubator at 200 rpm. The study was performed for 6 days. For each day, 10 mL of the culture was withdrawn for the determination of the packed cell volume (PCV) using centrifuging at 4000 rpm for 20 min. The supernatants were collected and subsequently subjected to agar diffusion assay using cup plate technique against *Staphylococcus aureus* to evaluate antibiotic production[6,21].

Thin layer chromatography and bioautography

The obtained concentrated ethyl acetate extracts were subjected to TLC using ethyl acetate:hexane at the ratio of 6:4. Direct bioautography using *Staphylococcus aureus* as marker organism were performed for localization of antimicrobial substances[17,22].

Column chromatography

The concentrated ethyl acetate layers were loaded on to the column and were then eluted using dichloromethane while the polarities of the mobile phases were gradually increased via addition of ethyl acetate[16]. All fractions obtained were individually checked for their purity using running thin layer chromatogram along with crude extract as reference in a different lane.

Screening of Bacterial metabolite extracted with GC-MS

Isolate SDJ10 which was selected based on its performance, was placed in a conical flask containing 500 mL TSB broth, and incubated in a shaker incubator at 250 rpm and the temperature regulated to 28 ±2°C for 7 days. Following the 7 days incubation, the staling substances were subjected to filtration through filter paper. Following filtration, the mycelial mat was carefully collected and crushed manually, using methanol in pestle and mortar. The crushed product was vortexed for 30 min. after which it was centrifuged at 20,000 xg. The supernatant was harvested while the compounds present in the filtrates were analysed by GC-MS.This technique enables the detection of key compounds having antimicrobial activity. From the Streptomyces extracted by ethyl acetate,10 µl was directly inoculated into the port of injection of gas chromatograph(Agilent Technologies 7890A GC system) straightly combined with a mass spectrometer system (Agilent Technologies

5975C inert MSD with Triple-Axis Detector).The gas chromatography was operated on an Agilent DB-5MS UI GC column, 30 m x 0.25 mm, with 0.25µm film thickness of 5%-phenyl methyl polysiloxane. Helium was employed as the transportation gas. The temperature command was adjusted with an initial temperature of 50 ^{o}C for 2 minutes, then 6 °C/min to 280 °C for 10 min with a flowing rate of 1 mL/min while the Run Time was 50.333 min. The MSD Chemstation was employed to determine each of the crests in the raw GC chromatogram. Library analysis was conducted for each crests utilising National Institute of Standards and Technology NIST/EPA/NIH version 2.0.All outcomes were computed in one crest table.

HPLC (High Performance Liquid Chromatography)

HPLC was employed for further isolation and identification of active compound from the active fraction. The HPLC system employed an elution solvent with water to acetonitrile ratio of 8:2. The sample was run in 60 minutes at a flow rate of 1 mL/minute. Briefly, 20 µl of samples were inoculated into Apollo C18 5µ column (length 250 mm, ID 4.6 mm, Alltech) and the compounds present were detected at 215 nm with a UV detector. The HPLC products of each peak were pooled and concentrated before being assayed against the selected pathogens. The method of analysis employed in this study was according to the USP 34 (United State Pharmacopeia). Column L1 was employed for the analysis of samples and the mobile phase was also prepared accordingly.

Results And Discussion

A preliminary survey of bioactive actinomycetes from marine sediment collected from coastal areas of kualalingii, kuala Selangor, kukup, and ketam was conducted. During the period of the survey, a total of 11 actinomycetes were isolated and these isolates were coded as P1, P2, P3, SDJ-1, SDJ-2, M1, M5, M7, SDJ-5, SDJ9 and SDJ-10. The antimicrobial activities of these isolates were analysed.

The marine environment has been a potential source of natural products from numerous structural classes exhibiting activity against several disease targets [5]. The sensitivities of six bacteria species against actinomycetes isolated from the four locations are shown in Table 1. Among the 13 isolates, isolates B1, A1, SDJ-9 and SDJ-10 exhibited significant antibacterial activity and none of the isolate was without some levels of antibacterial activity as they were all observed to have varying degrees of antibacterial activity. This findings agrees with the findings reported in other related studies [2,8,17].The utilization of marine actinomycete extracts as antibiotic as observed in this study corroborate with the findings reported in an earlier studies [3,17]. where marine sediments were collected from the coastal areas were screened for antibacterial and antifungal activity. However, among the isolates with significant antibacterial activity, isolate SDJ-10 exhibited the highest antibacterial activity and the highest antibacterial activity was against *Streptococcus aureus*. This finding was in accord with the observations reported in an earlier studies[3,17]. where actinomycete isolates were observed to have highest antibacterial activity against *S. aureus*. Based on the high antibacterial activity observed in isolate SDJ-10, it was subsequently selected for further investigations.

Table 1: Sensitivity of Different Microorganism Against of Actinomyces Isolates

Isolates	Zone of inhibition (mm)					
	E. coli	*S. aurous*	*V. cholera*	*V. para*	*A. hydrophilia*	*P.aeruginosa*
P1	-	8	-	9	-	-
P2	9	10	-	10	-	-
P3	-	-	-	-	-	8
SDJ-1	-	7	-	8	10	-
SDJ-2	9	-	-	7	-	9
M1	-	11	11	8	-	-
M5	-	9	10	-	-	-
M7	8	-	8	-	-	-
SDJ-5	8	12	-	-	8	-
SDJ-9	-	8	13	17	12	-
SDJ-10	8	19	15	21	16	9

The period or rather time of incubation of actinomycete isolates have been shown to be influence the growth of the organism [3,20]. The growth of the isolate in this study was expressed as packed cell volume (PCV) following methods previously described [3]. The supernatants of the cultures of the isolate SDJ-10 on four both media were tested for their activity against 18 h culture of *Staphylococcus aureus* using the cup plate technique (Figure 1). The zone of inhibition of the isolates from the four media were measured following incubation at 37°C for 18 h. The growth of SDJ-10 isolate expressed as PCV was observed to be maximum following 120 h of fermentation in all the four media tested even though SCB peaked at 72 of fermentation. However, isolate SDJ-10 culture on SCB medium exhibited the highest level of growth followed by TSB and NB and the least growth was observed from MB media. This shows that the maximum antibiotic production could only be obtained towards the end of the fifth day of fermentation. This implied that media have influence on the growth of actinomycete and this observation agrees with the findings in recent studies [1,20]. where media were reported to influence the growth rate of actinomycete isolates. While an earlier study [3] reported maximum antibiotic product at the 3rd and 4th day of fermentation, in this study, the maximum antibiotic activity was observed on the fifth day of fermentation even with the different media employed. The variation observed in the period of maximum antibiotic production could be associated with the media used. While in the study, MB, NB, TSB and SCB were used, corn steep liquor were used in the said study. The origin of the isolate may equally play a crucial role. Based on these results, the SCB medium appeared to be the most suitable medium for shake flask fermentation of SDJ-10 isolate and this finding corroborate with the findings document in a previous related study [3].

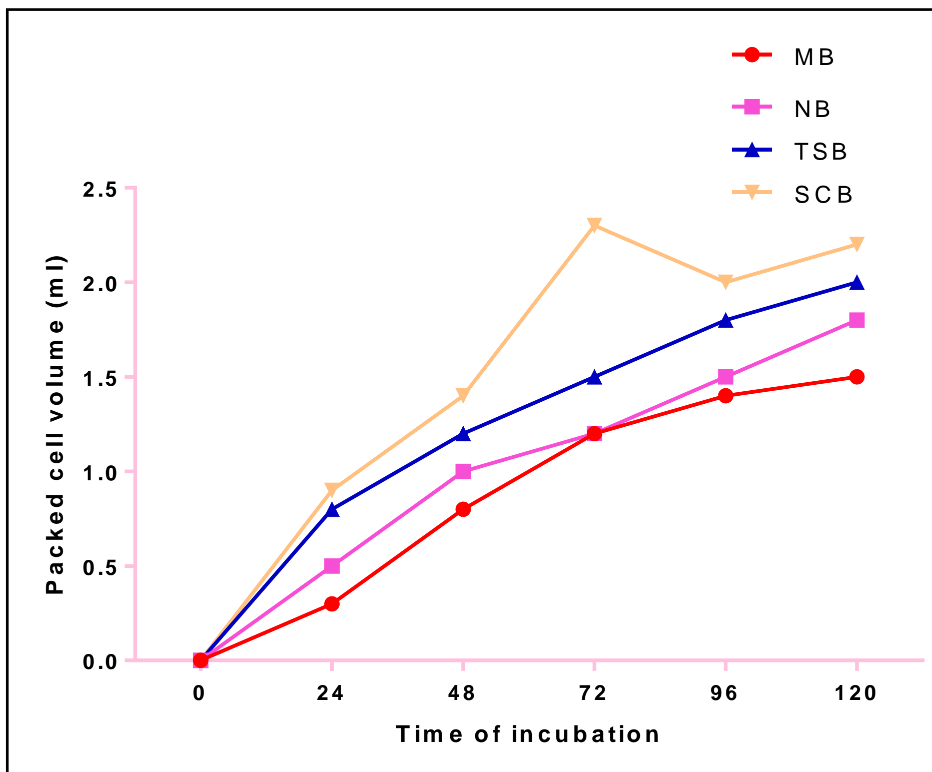

Figure 1: Growth and antibacterial activity of SDJ-10 isolate on different media

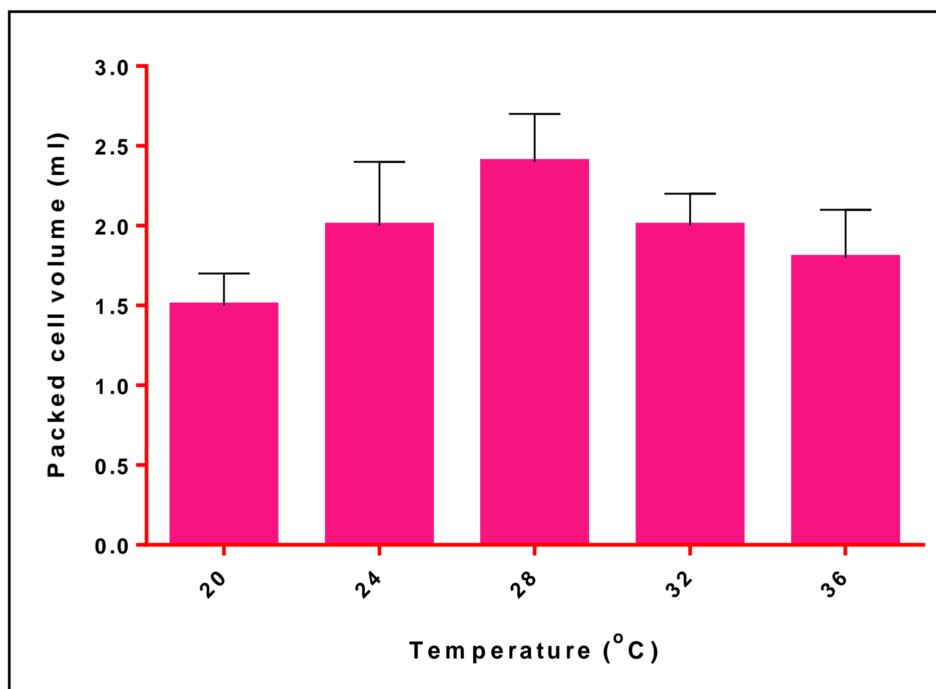

Figure 2: Growth of SDJ-10 in different temperature

The optimum temperature for maximum growth of SDJ-10 was evaluated by studying their PCV across varying degrees of temperature ranging from 20 to 36 °C(figure 2). From the temperature optimization tests, it was found that the optimal temperature essential for growth of SDJ-10 isolate was 28 °C and the least were growth were found to be at 20 °C and 36 °C. This result agrees with the report in a recent study [3,20] where the temperature of 28 oC was reported to be the optimum temperature for growth of actinomycete isolate. At the temperature of 24 °C and 32 °C however, average growth, relatively higher than those of 20 °C and 36 °C were observed which similarly corroborate with the findings documented in a recent study [20]. Isolate SDJ-10 strain was found tohave maximal growth.

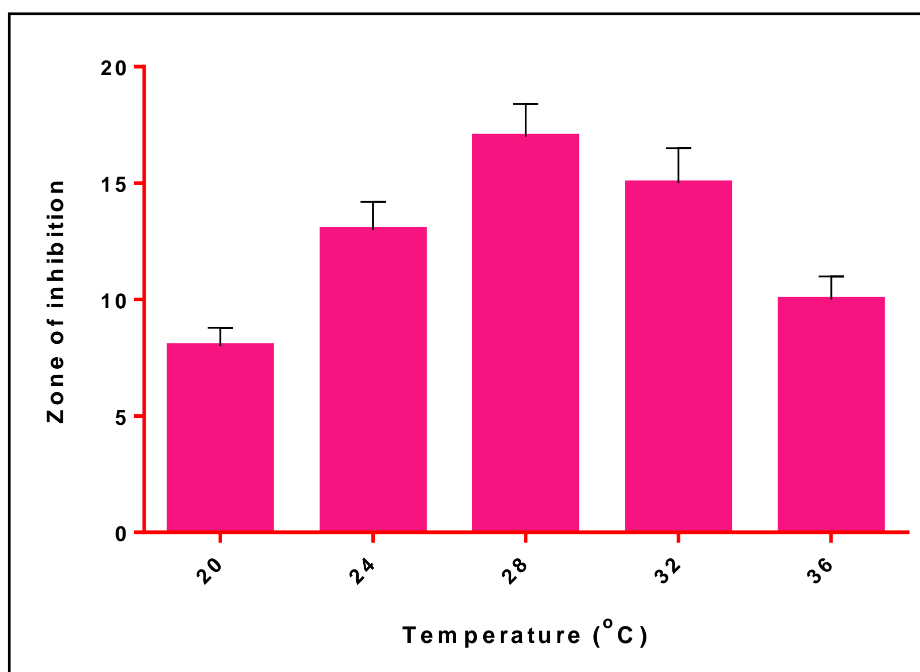

Figure 3: Zone of antimicrobial activity of sdj-10 against s. aurous in different temperature

Temperature has been shown to influence the growth and production of antibiotics by actinomycetes[3,19,20]. In this study, the zone of inhibition of SDJ-10 isolate across varying degrees of temperature ranging from 20 to 36 °Cwas evaluated and it is presented in figure 3. From the temperature optimization tests, it was found that the optimal temperature for optimal antibiotic production of the SDJ-10 isolate was 28 °C. This was observed to be the same with the optimal temperature for growth of the SDJ-10 isolate as shown in figure 2 above. Isolate SDJ-10 strain was found to have maximal growth and antibiotic production at 28 °C. This findings does not agree with the findings reported in a previous related study [19] who reported that the optimal temperature for the growth of *Streptomyces rochei* was 30 oC but agrees with the reports in other studies[3,20] where the optimum temperature for the growth of actinomycete was reported to be 28 °C. Moderate antibiotic production were observed at temperatures of 24 and 32 °C while lowest antibiotic production was found at temperatures of 20 °C and 36 °C. This finding corroborate with the findings reported in other earlier studies[19,20] where moderate growth was reported at the temperature of 22 °C and 25 °C. This shows that the optimal temperature for antibiotic production of SDJ-10 isolate is likely to be 28 °C. The antibiotic production of the isolate was measured using zone of inhibition, a method previously reported in other related studies[3,20] to correspond to the growth of the isolate.

The effect of pH on the growth of actinomycete has documented in previously related studies [3,19]. The optimal pH for the growth of the isolate using varying pH ranging from 5 to 9 is presented in figure 4. The pH of the culture medium was found to affects growth of the isolate significantly. The optimum pH for maximal growth of SDJ-10 isolate was found to be the pH of 7. Other related studies [3,19] have similarly documented that the optimal pH for the growth of actinomycete is the pH of 7. Moderate growth which were significantly less compared to the growth at pH 7 were observed at pH of 6 and 8 while the least growths were found at the pH of 5 and 9. This findings corroborate with the findings reported in a previous related study [19] where pH of 6, 8 and 9 were reported to support moderate growth of actinomycete. From the results obtained, it can be deduced that the pH 7 was the most suitable pH for growth of isolate SDJ-10.

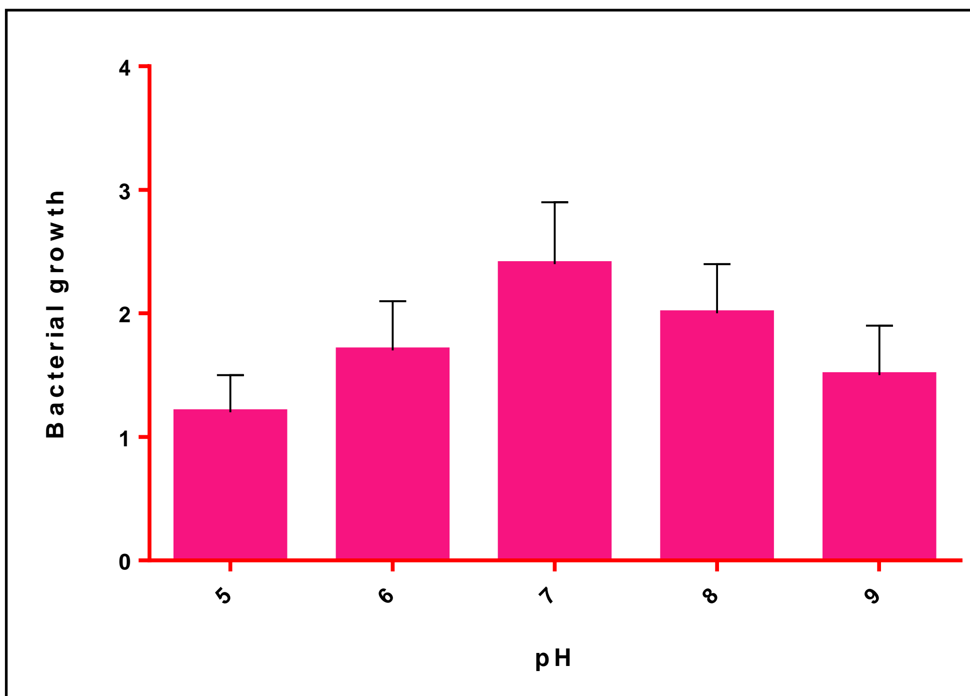

Figure 4: Growth of SDJ-10 in different pH

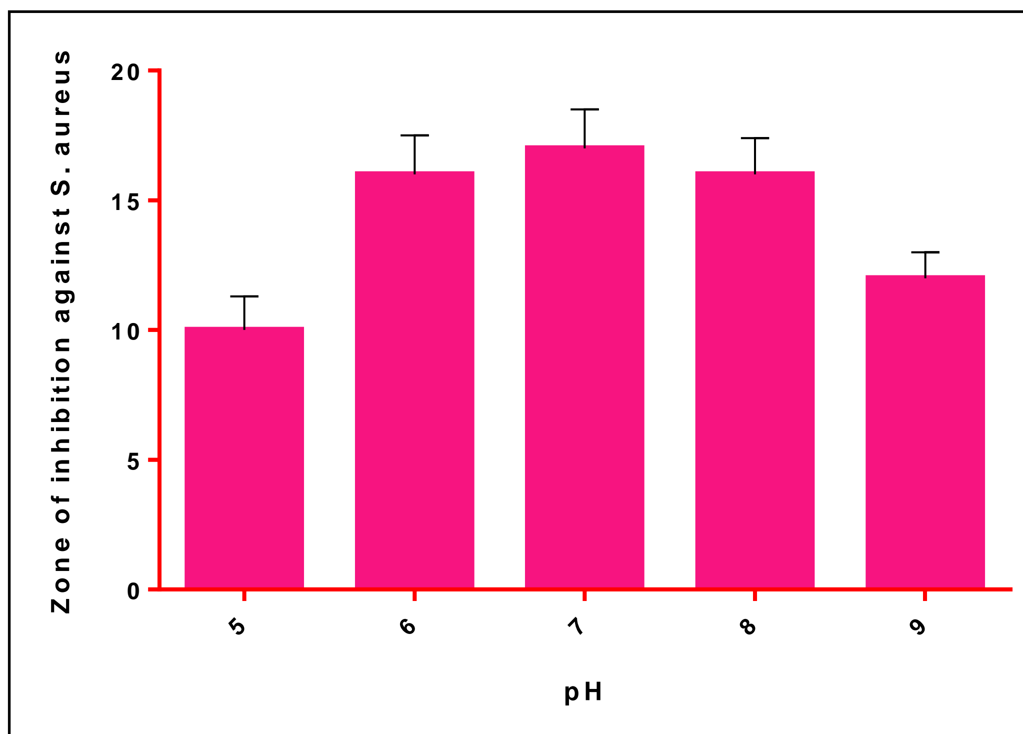

Figure 5: Zone of antimicrobial activity of SDJ-10 against staphylococcus aureus in different pH

Actinomycetes have been recognized as sources of several secondary metabolites, antibiotics, as well as bioactive compounds that have varying degrees of influence on microbial growth [5,17]. In this study, the mean antibiotic activity of the SDJ-10 isolate against *Streptococcus aureus* under variations of pH ranging from 5 to 9 is shown in figure 5. Analysis of the results showed that the mean optimum pH for the antibacterial activity of SDJ-10 isolate against the *S. aureus* was the pH of 7. The result of ours completely agree with the findings reported in other related studies [3,19] where the pH of 7 was reported to be the optimum pH for the growth of actinomycete isolates. The antibacterial activity of SDJ-10 isolate against the *S. aureus* at the pH of 6 and 8 were observed not to be significantly different from those at pH 7. However, at the pH of 5 and 9, the antibiotic activity of SDJ-10 against *S. aureus* declined significantly compared to the other pH evaluated. This shows that pH has influence of the rate of antibiotic production of SDJ-10 against *S. aureus*as observed in a previous related study [19].

The mean culture filtrate of SDJ-10 that were subjected to solvent extraction using solvents of increasing polarity including dichloromethane, hexane and ethyl acetate is shown in Table 2. Analysis of the results showed that the ethyl acetate extract demonstrated the highest activity compared to the dichloromethane, hexane extracts. However, the antibiotic activity of hexane extract was higher compared to that of dichloromethane and this finding corroborate with the findings in other earlier studies[3,20]where ethyl acetate was reported to provide the best separation compared to other solvents. Amazingly, the left over broth following ethyl acetate extraction showed negligible antibiotic activity against *S. aureus* when tested using the cup plate technique. It can therefore be deduced that the antibiotic was primarily extracted from the culture filtrate. Thus, based on the antibiotic activity of the ethyl acetate, it was selected for the extraction of the remaining culture filtrate.

Table 2: Antibacterial activity of the fractions of SDJ-10 culture filtrate in different solvent

DICHLOROMETHANE	**7**
HEXANE	**15**
ETHYL ACETAT	**21**

The use of thin layer chromatography of the for the separation of the actinomycete ethyl acetate extract has documented in several studies [3,17]. In this study, the concentrated ethyl acetate extract was further subjected

to TLC analysis using three mobile phases which include dichloromethane: methanol in the ratio of 4:1, ethyl acetate: methanol in the ration of 6:4 and hexane :methanol in the ratio of 4:1. Following air-drying, the plates were then visualized using iodine vapour. Among the three mobile phases, the plate developed using ethyl acetate: methanol ratio was found to produce the best separation (Figure 6). The observed optimum separated induced by the ethyl acetate: methanol corroborate with the findings reported in an earlier related study [3]. Based on this result, the plate was further subjected to bioautography to localize antibacterial compounds.

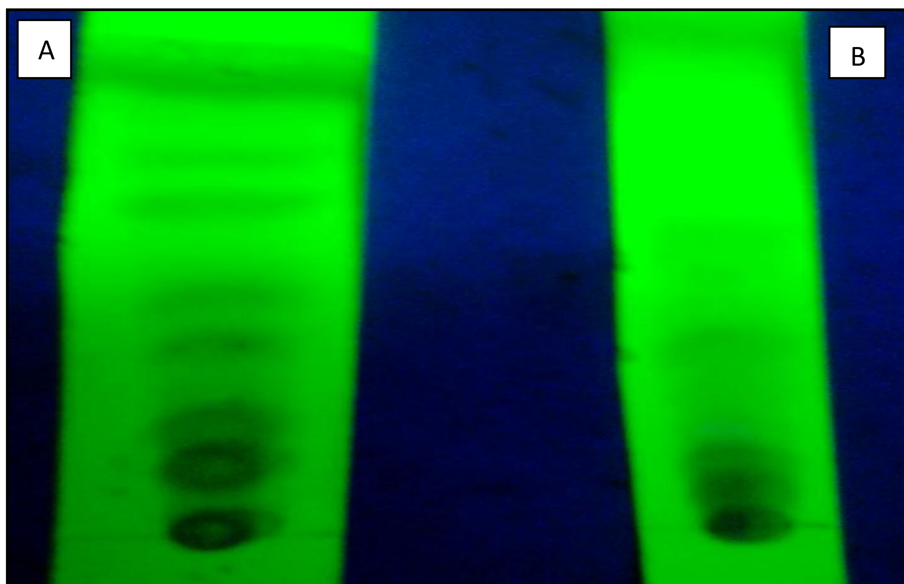

Figure 6: TLC of ethyl acetate extract (A)and hexane extract (B) of culture filtrate of SDJ-10

Figure 7: Morphological characteristics of the SDJ10 isolate

Morphological characteristic of colonies of actinomycetes have been used in its identification [3,12]. The SDJ-10 isolate was plated on starch casein agar medium and incubated at 37°C. The morphological appearance of the isolate showed a Gram positive, filamentous organism with smooth colony, whitish mycelium and substrate mycelium. The colonies were creamy on the reverse side. The isolate exhibited optimum growth under aerobic conditions at temperature 28 °C and at pH 7. These morphological features observed corroborate with the morphological description documented to be characteristic of actinomycete[12].

Table 3: GC – MS analysis of SDJ10 extract

R.T	Compound Name	M.W	Formul ae	Area %	Co. Na.	Activity
28.85	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$	0.97	Fattt acid	Cytotoxic
22.36	5-Octadecene, , E)-	252	$C_{16}H_{32}$	2.30	long-chain fatty acid	Stronger sexual characters
20.78	Phenol, 2,4-bis, 1,1dimethylethyl	206.32	$C_{14}H_{22}O$	20.2	Organic compound	Antimicrobial
36.23	Hexacosane	226.45	$C_{26}H_{54}$	0.41	Alkane	Antimicrobial
28.74	Eicosane	280	$C_{20}H_{42}$	0.32	Chain fatty acid	Antimicrobial
18.16	Tetradecanoic acid	228.38	$C_{14}H_{28}O_2$	0.29	Fatty acid	Cancer reventive
25.99	Pentadecanoic acid	242	$C_{15}H_{30}O_2$	1.54	Fatty acid	Antimicrobial
27.70	hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	0.21	Fatty acid methylester	Antioxidant
29.39	Heptadecanoic acid	270	$C_{17}H_{34}O_2$	0.73	Fatty acid	Antimicrobial
37.32	Bis, 2-ethylhexyl) phthalate	390.56	$C_{24}H_{38}O_4$	7.36	Organic compound	Antitoxicity and antioxide
26.13	-Nonadecene	266	$C_{19}H_{38}$	1.04	long-chain fatty acid	Anti-fungal activity
40.74	Squalene	410	$C_{30}H_5$	0.44	Organic compound	Antimicrobial and Antioxident
31.24	Octadecanoic acid	284	$C_{18}H_{36}O_2$	1.46	Fatty acid	Antimicrobial

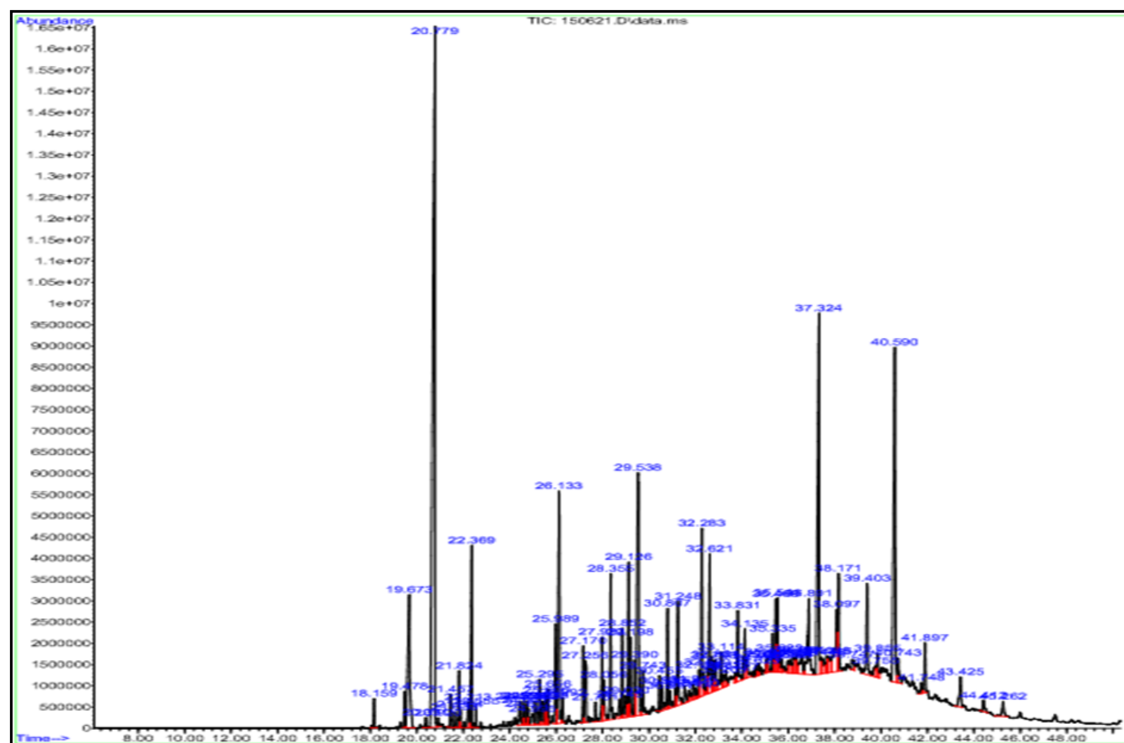

Fig 8: GC-MS chromatogram of the ethyl acetat SDJ10 extracs

GC-MS analysis of SDJ10 extract

The use of GC-MS (figur 8, table 3) for the identification of compounds present in actinomycete ethyl acetate extract based on the peak areas, molecular weight and molecular formula has been performed recently[14,15]. The peak areas have been verified to be directly proportional to the quantity of the compound present in the active band [14].In this study, GC-MS analysis used to identify 72 compounds based on peak area percentage, retention time, molecular formula and molecular weight.The biological activities of the major

constituent's compound of the extract are given in Table 3. The major constituents were n-Hexadecanoic acid (28.85R), 5-Octadecene(E 22.36R), Phenol, 2,4-bis, 1,1dimethylethy (20.78R), Hexacosane (36.23R), Eicosene (28.74R), Tetradecanoic acid (18.16R), Pentadecanoic acid (25.99R), hexadecanoic acid, methyl ester (27.70R), Heptadecanoic acid (29.39R). Bis, 2-ethylhexyl) phthalate (37.32R), Nonadecene (26.13R), Squalene (40.74R) and Octadecanoic acid (31.24R). This findings corroborrate with reports in recent related studies where the presence of 16 [14]and 19 [15]compounds in Streptomyces compounds were detected respectively.Additiontially, the presence of theotherminute peaks in the spectrum is an indication that the extract could contain other unidentified chemical compounds. The major constituents alone or in combination with minor constituents might be responsible for the antibacterial activity. This also reveals the potentials imbeded in marine streptomyces as a source of newer and more effective antibiotic agent [15,18,23].

HPLC Analysis

High performance liquid chromatography is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying and purifying the individual components of the mixture[11]. In the current study,the active compound in extract SDJ10 isolate showed many peaks in HPLC graph, with retention times of 0.25 min, 1.77 mins, 2.88 mins, 3,63 mins, 4.42 mins and 5.63 mins(Figure 9). This findings corroborate with the findings reported in a recent study [11], where only a peak was observed at the rention time of 4.779 minutes in HPLC analysis of.streptomyces isolate extract.

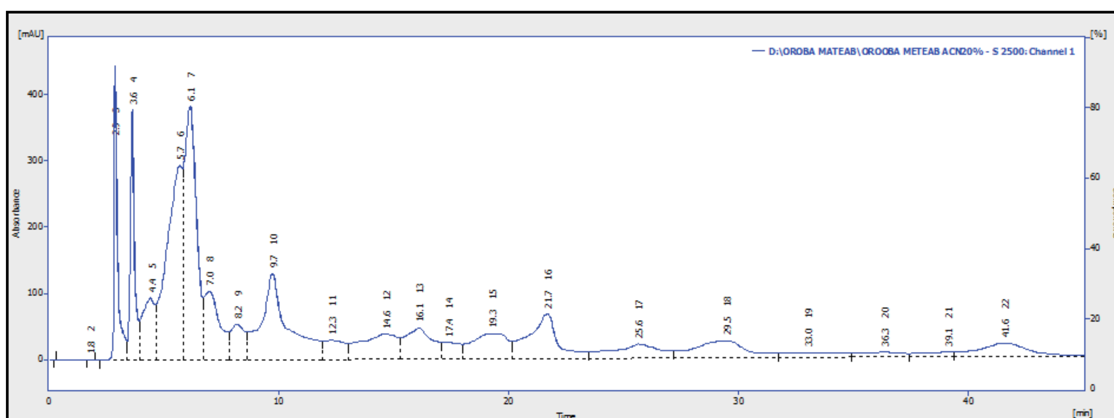

Fig 9. Analysis HPLC chromatogram of SDJ10 extract

Conclusion

The antibiotic activity of the actinomycete obtained in this study is interestingly encouraging, seen that it was activive against numerous bacterial agent coupled with the ease with which it can be cultivated. Based on the findings of this study, it is obvious that isolate SDJ-10 has the potential to be developed as an effective antibiotic agent.The study has also demonstrated the best optimal growth conditions for SDJ10 isolate which include the optimum growth temperature of 28°C and the optimum pH for growth (pH 7).Although the antimicrobial agentfound in this study could notbe declared as completely new antibiotics, the potential of finding newer and more effective antibiotics in Malaysia coastal waters is very high owing to its wide biodiversity.

Acknowledgment

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References

1. ALAM, M. T., MEDEMA, M. H., TAKANO, E. & BREITLING, R.(2011). Comparative genome-scale metabolic modeling of actinomycetes: the topology of essential core metabolism. *FEBS letters,* 585**,** 2389-2394.
2. ATTA, H. M. & AHMAD, M. S.(2009). Antimycin-A antibiotic biosynthesis produced by Streptomyces sp. AZ-AR-262: taxonomy, fermentation, purification and biological activities. Aust J Basic Appl Sci 3: 126–135.
3. ATTIMARAD, S., GAVIRAJ, E., NAGESH, C., KUGAJI, M. & SUTAR, R. (2012). SCREENING, ISOLATION AND PURIFICATION OF ANTIBIOTIC (S) FROM SCREENING, ISOLATION AND PURIFICATION OF ANTIBIOTIC (S) FROM MARINE ACTINOMYCETES. *International Journal of Research in Ayurveda & Pharmacy,* 3.
4. BREDHOLT, H., FJÆRVIK, E., JOHNSEN, G. & ZOTCHEV, S. B. (2008). Actinomycetes from sediments in the Trondheim fjord, Norway: diversity and biological activity. *Marine drugs,* 6**,** 12-24.
5. CHAUDHARY, H. S., YADAV, J., SHRIVASTAVA, A. R., SINGH, S., SINGH, A. K. & GOPALAN, N.(2013). Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India). *Journal of advanced pharmaceutical technology & research,* 4**,** 118.
6. DUBEY, R. & MAHESHWARI, D.(2002). *Practical microbiology*, S. Chand.
7. GESHEVA, V., IVANOVA, V. & GESHEVA, R.(2005). Effects of nutrients on the production of AK-111-81 macrolide antibiotic by Streptomyces hygroscopicus. *Microbiological Research,* 160**,** 243-248.
8. GUL, W. & HAMANN, M. T. (2005). Indole alkaloid marine natural products: An established source of cancer drug leads with considerable promise for the control of parasitic, neurological and other diseases. *Life sciences,* 78**,** 442-453.
9. ISHIDA, N., MIYAZAKI, K., KUMAGAI, K. & RIKIMARU, M. (1965). NEOCARZINOSTATIN, AN ANTITUMOR ANTIBIOTIC OF HIGH MOLECULAR WEIGHT. ISOLATION, PHYSIOCHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITIES. *The Journal of antibiotics,* 18**,** 68-76.
10. JAMES, P., EDWARDS, C. & DAWSON, M. (1991). The effects of temperature, pH and growth rate on secondary metabolism in Streptomyces thermoviolaceus grown in a chemostat. *Microbiology,* 137**,** 1715-1720.
11. JEYARAMAN, T. (2015). ISOLATION, SCREENING AND CHARACTERIZATION OF POTENT MARINE STREPTOMYCES SP. PM 105 AGAINST ANTIBIOTIC RESISTANT PATHOGENS. *Asian Journal of Pharmaceutical and Clinical Research,* 8**,** 439-443.
12. KUMAR, P. S., RAJ, J. P. P., DURAIPANDIYAN, V. & IGNACIMUTHU, S.(2012). Antibacterial activity of some actinomycetes from Tamil Nadu, India. *Asian Pacific journal of tropical biomedicine,* 2**,** 936-943.
13. MA, B.(1988). Vitamins and fine chemicals from Microalgae. Borowitzka, MA and Borowitzka, LJ, Cambridge University Press.
14. NANDHINI, S. U.(2015). Gas chromatography-Mass spectrometry analysis of bioactive constituents from the marine Streptomyces. *Asian Journal of Pharmaceutical and Clinical Research,* 8**,** 244-246.
15. NARENDHRAN, S., RAJIV, P., VANATHI, P. & SIVARAJ, R.(2014). Spectroscopic analysis of bioactive compounds from Streptomyces cavouresis kuv39: Evaluation of antioxidant and cytotoxicity activity. *International Journal of Pharmacy and Pharmaceutical Sciences,* 6**,** 319-322.
16. OHSHIMA, T., TAKADA, H., YOSHIMURA, T., ESAKI, N. & SODA, K. (1991). Distribution, purification, and characterization of thermostable phenylalanine dehydrogenase from thermophilic actinomycetes. *Journal of bacteriology,* 173**,** 3943-3948.
17. PANDEY, B., GHIMIRE, P. & AGRAWAL, V. P.(2004). Studies on the antibacterial activity of the Actinomycetes isolated from the Khumbu Region of Nepal. *J. Biol. Sci,* 23**,** 44-53.
18. PARTHASARATHI, S., SATHYA, S., BUPESH, G., SAMY, R. D., MOHAN, M. R., KUMAR, G. S., MANIKANDAN, M., KIM, C. & BALAKRISHNAN, K.(2012). Isolation and characterization of antimicrobial compound from marine Streptomyces hygroscopicus BDUS 49. *World J Fish Mar Sci,* 4**,** 268-77.
19. REDDY, N., RAMAKRISHNA, D. & RAJA GOPAL, S.(2011). A morphological, physiological and biochemical studies of marine Streptomyces rochei (MTCC 10109) showing antagonistic activity against selective human pathogenic microorganisms. *Asian J Biol Sci,* 4**,** 1-14.

20. 20.SENGUPTA, S., PRAMANIK, A., GHOSH, A. & BHATTACHARYYA, M.(2015). Antimicrobial activities of actinomycetes isolated from unexplored regions of Sundarbans mangrove ecosystem. *BMC microbiology,* 15**,** 1.
21. 21.SHOMURA, T., GOMI, S., ITO, M., YOSHIDA, J., TANAKA, E., AMANO, S., WATABE, H.-O., OHUCHI, S., ITOH, J. & SEZAKI, M. (1987). Studies on new antibiotics SF2415. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities. *The Journal of antibiotics,* 40**,** 732-739.
22. 22.STANECK, J. L. & ROBERTS, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Applied microbiology,* 28**,** 226-231.
23. 23.SUDHA, S. & MASILAMANI SELVAM, M. (2013). In vitro cytotoxic activity of Bioactive metabolite and crude extract from a new Actinomycete Steptomyces avidinii strain SU4. *Int J Pharm Pharm Sci,* 5**,** 612-6.
24. 24.TALBOT, G. H., BRADLEY, J., EDWARDS, J. E., GILBERT, D., SCHELD, M. & BARTLETT, J. G. (2006). Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clinical Infectious Diseases,* 42**,** 657-668.
25. 25.VENTURA, M., CANCHAYA, C., TAUCH, A., CHANDRA, G., FITZGERALD, G. F., CHATER, K. F. & VAN SINDEREN, D.(2007). Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. *Microbiology and Molecular Biology Reviews,* 71**,** 495-548.

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