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Screening and Characterization of Marine Actinomycetes Isolated from Al-Khoms Shoreline

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ABSTRACT

The detection of new antibiotics and other bioactive microbial metabolites is a benefit step given the rate of the rising multi-drug resistant pathogenic microorganisms. This study aimed to isolate and screen the antimicrobial activity of gram positive bacteria named actinomycetes. The actinomycetes were isolated from near-shore marine sediment that were collected from three different depths at the Libyan Alkhoms coastline. A total of five out of twenty actinomycetes were isolated on different supported medium, whereas, Sabouraud Dextrose Agar was found to exhibit the antagonistic properties of the bioactive compounds produced by actinomycetes isolates. These isolates had shown a clear zone on *Mueller*-Hinton agar against target microorganisms were considered as actinomycetes labelled from $HSEH_1$ to $HSEH_{20}$. The antimicrobial properties of these isolates against (Klebcella pneumonia, Escherichia coli, Salmonella typhi, Staphylococcus aureus and Candida albicance were examined by primary screening using spot inoculating method. were examined by primary screening using spot inoculating method. The results that all the five selected isolates were according to biochemical testes found to show inhibitory activity against the target microbes. Generally $HSEH_{16}$ isolate showed the highest inhibitory activity against all target pathogenic microbes compared with other selected isolates which have activity however, with different ranges of inhibition zone. Thus, the isolate $HESH_{16}$ is the best among the other isolates considered to have a broad bacterial spectrum in this study. This isolate could be considered as potential antimicrobial properties of secondary metabolites against strains human pathogens and should be further studied for their human health.

فحص وتوصيف الفطريات الشعاعية البحرية المعزولة من شاطئ الخمس

هناء ونيس الفلاح

لقد أصبحت ظاهرة ظهور السلالات المقاومة للمضادات الحيوية وانتشارها في المستشفيات من المشاكل التي تواجه العلماء والباحثين. ومع اكتشاف المركبات الأيضية الثانوية الفعالة من بعض الأحياء الدقيقة في البيئة المحيطة من حولنا خاصة البيئة البحرية التي تساهم بشكل واضح لمنع ظهور هذه السلالات المقاومة للمضادات الحيوية. تهدف هذه الدراسة إلى عزل هذه مركبات الأيضية الثانوية الفعالة من البكتريا الموجبة لصبغة جرام (الاكتينومايسيتات) والتي عزلت من رمال شاطئ مدينة الخمس ليبيا, وبواقع ثلاث أعماق مختلفة, وباستخدام بيئات غذائية مختلفة. تم الحصول علي خمس عزلات من أصل عشرون عزلة رمز لها $HSEH_1$ إلي $HSEH_{20}$ أثبتت تأثيرها الفعال ضد الأحياء الدقيقة المرضة مثل الخميرة *Candida albicance*، والبكتريا (*Staphylococcus aureu*
Salmonella typhi, *Escherichia coli*، و *Klebcella pneumonia*) حيث كان وسط Sabouraud Dextrose Agar هو الوسط الغذائي الأفضل لإظهاره الخصائص التضادية للمركبات الحيوية الفعالة التي تنتجها عزلات الاكتينومايسيتات, في حين أنها أظهرت هذه العزلات منطقة تثبيط واضحة ضد هذه المرضات علي الوسط الغذائي *Mueller*- Hinton agar بطريقة نشر القرص. أثبتت نتائج هذه الدراسة أن العزلات الخمسة لها قدرة على تثبيط المرضات من خلال منطقة التثبيط التي حول كل مستعمرة وبرهنت أن أفضلها العزلة $HSEH_{16}$ وفقا للخواص والاختبارات البيوكيميائية ونشاطها الجرثومي الواسع ضد المرضات المستخدمة في هذه الدراسة. من خلال هذه الدراسة يتبين ضرورة مواصلة البحث مستقبلا في الخواص التضادية لهذه العزلات وخصوصا $HSEH_{16}$ وتعريفها، ومعرفة خواص المواد الفعالة التي تنتجها, لما لها من دور فعال ومهم يخدم عملية الحفاظ على صحة البشرية.

INTRODUCTION

Several pathogenic microbes have developed resistance that causing risk to human life, so scientists are endeavouring ceaselessly to search for novel antibiotic compounds that can cure these infectious cusses. The need to create new bioactive compounds it may be a major example for the development of experimental drugs against resistant bacteria (Mohammadipanah and Wink, 2016) and (Vela Gurovic and Olivera, 2017). Approximately 23,000 of compounds of bioactive metabolites produced by microorganisms, while, 45% (10,000 compounds) of it produced by *Actinobacteria* and *Streptomyces* genes are constituted of 76% (7600 compounds) among this group of bacteria. (Berdy, 2012). *Actinobacteria* are prokaryotes Gram-positive bacteria with DNA having high guanine and cytosine (GC) content, with extremely changeable metabolic potential. The variety of the *Actinobacteria* metabolic is owing to their tremendously large genome, which has huge copy factors that manage gene expression, allowing them to enhanced to specific necessities (Shantikumar *et al*., 2006). The class of *Actinobacteria* contains five subclasses, 10 orders, 56 families and 286 genera (Ait Barka *et al*., 2016). They are extensively increase in soils, especially in arid, a little acidic soils, rich in organic matter and indicate high quantity of the soil microbial biomass (Saadoun *et al*., 2015). *Actinobacteria* are necessary microbes that make a variety of beneficial enzymes and secondary metabolites such as antibiotics, anticancer compounds and immunomodulators (Saadoun *et al*., 2015). It is well identified that microbial variety has not been competently explored and the considerable mass of prokaryotes from 90 to 99% in natural environments are as yet to be isolated (Harwani, 2013). Many natural ecosystem are still either unexplored and therefore can be considered a productive reserve for the isolation of inadequately studied microorganisms counting rare actinomycetes (Tiwari & Gupta, 2012). A lot of extremophilic bacteria are recognized to be of industrial interest as potential candidates for future biotechnological applications (Cayol *et al*., 2015). *Actinobacteria* are identified as biofactories of enzymes, with applications in the material, bio-refineries, food, pulp and paper, agriculture, detergent and pharmaceutical industries (Richa and Vivek, 2018). Arid habitats are among the most plenteous ecosystems with regard to the occurrence of new bacterial species (Mohammadipanah and Wink., 2016). In addition, *Actinobacteria* are one of the majority prolific makers of natural bioactive composites of Mediterranean sea sediment (Oskay *et al*., 2004) and (Tiwari *et al*., 2015). The present study was aimed to isolate and characterize different species of actinomycetes from different areas across Alkhoms beach. Biochemical examination to explore the potential of antimicrobial activity of these species against other microorganisms that are pathogenic to human was also carried out.

MATERIALS AND METHODS

Target bacteria *Staphylococcus aureus* ATCC 23235, *Escherichia coli* ATCC 25922, *Klebcella pneumonia* ATCC 43816, *Salmonella typhi* ATCC 6539, and *Candida albicans* ATCC 10231 as fungi were obtained from the culture collection of the Microbial laboratory, Faculty of science, Almergib University, AlKhoms, Libya, AlKhoms is located in northeast of Libya 32°38′59″N, 14°15′52″E, about 120 km from the capital city of Libya (Tripoli). These bacteria used for screening of antimicrobial activity

Sampling in present study was performed according to Mastumoto *et al*. (1998) with some modifications. Briefly, using sterile spatula, soil sediments were collected from three different depth (1m, 2m and 3m) and placed in sterile plastic bags. Samples were then transferred to microbiology laboratory for further processing as follows: each sample was subjected to air dry at room temperature (25°C) for 3-5days, and later processed for isolation of actinomycetes species.

Actinomycetes spp were isolated from sediments as applied by Hayakawa *et al*. (2004). Briefly, about 50 gm of dried sediment samples were suspended separately in 200 mL of sterile

distilled water and shaked for 2 h on a mutual shaker. Sample suspension was then serially diluted (10^{-1} to 10^{-6}) and every dilution was spread on four different media namely Yeast Glucose Chloramphenicol Agar (YGCA) (Oxoid), Brain Heart Infusion Agar (BHIA) (Oxoid), Sabouraud Dextrose Agar (SDA) (Oxoid) and Tryptone Soya Agar (TSA) (Oxoid) in triplicates to evaluate the nutrient requirements of the actinomycetes isolates. The plates were incubated at 30°C for two weeks. The rising colonies were sub-cultured and transferred to slants without the antibiotic substance and reserved at 4°C until use.

The top five potent actinomycetes isolates selected from screening were characterized by morphological, cultural, biochemical experiments. Morphological and cultural characteristics includes gram staining, type of aerial hyphae, growth of vegetative hyphae, diffusible pigment and spore formation was observed. Biochemical tests including melanin pigmentation, casein, Voges-proskauer, citrate, urease and catalase tests were also performed by starch casein agar, DNase, starch hydrolysis, blood hydrolysis, amino acids hydrolysis, indole test, and motility test were performed and the results were observed. Actinomycetes isolates were identified based on Bergey's, (2000) of determinative bacteriology

Primary Screening of antimicrobial activity of isolates were performed by spot inoculating the isolates with circle diameter form about 8 mm on agar medium (Shomurat *et al* 1979). The pure actinomycetes isolates were spot on Mueller Hinton agar medium. All plates were incubated at 28°C for 24 hours. Clear zone around the bacterial isolates have demonstrated positive antimicrobial activity.

RESULTS AND DISCUSSION

Isolation f actinomycetes

Five out of twenty actinomycetes which isolated from three different depth 1m, 2m, 3m of seashore sediment named $HSEH_1$ $HSEH_5$, $HSEH_{13}$, $HSEH_{16}$. $HSEH_{18}$. These five actinomycetes isolates tested were growth on different four nutrients medium culture, showed activity against pathogenic microorganisms. Especially, on SDA, BHIA, YGCA **(**Table 1) which isolated from only 1m, and 2m depths of seashore.

Characterization of actinomycetes cultures

The morphological characterization of actinomycetes isolates. (Table 1) were carried out on the morphological features of each growing colonies were recorded followed by microscopic observation of gram-stained smear of each positive isolate. All the actinomycetes on SDagar texture of the actinomycetes colonies ranged from smooth to dry or powdery and was floccose and chalky, similar to those of the *Streptomyces* genus. The SDagar colonies showed shades of white, cream, grey, green, creamy, brown, yellow, pink, and red color, moreover produced brown, red, green pigments. The five isolates selected

Had white to grey aerial mycelia, while the color of their substratum mycelia showed strong variations. The information of biochemical and physiological characteristics (Table 2) these strains of actinomycetes isolated showed catalase, urease, oxidase positive, non motile.

Screening of Antimicrobial Activity:

Screening of antimicrobial activity was conducted based on spot inoculating method, as a result, there were five isolates of actinomycetes demonstrating antimicrobial activity against tests pathogenic bacteria. In this process, the bacterial colony will extract antimicrobial substances from the cell and then dissolve them into the medium. The efficiency of secondary metabolites can be seen by clear zone on agar medium in this test. Higher antimicrobial activity may also be demonstrated by the greater clear zone diameter. (Figs. 1 to 5) the five colonies of actinomycetes isolated from sediment have shown that all microorganism tests can inhibit growth. It was shown by producing a clear zone around the colonies of the five actinomycetes.

Table 1: Characteristic features of the five selected isolates.

Isolates code	Depth	Best Medium Isolate	Colony Colour	Margarine of Colony	Gram Staining	Pigment Production	Surface of Colony	Mycelium
$HSEH_1$	1m	BHIA	Red	Flat	G^+	Unproduced	smooth	Absent
$HSEH_5$	1m	YGCA	Brownish	Branched	G^+	yellow	Rough	Present
$HSEH_{13}$	2m	YGCA	White Chalky	Extensive	G^+	Red	smooth	Absent
$HSEH_{16}$	2m	YGCA	Green	Extensive	G^+	Browne	Rough	Present
$HSEH_{18}$	1m	SDA	Light yellow	Branched	G^+	Unproduced	smooth	Absent

Table 2. Biochemical characterization of the five actinomycetes isolated in the present study

Biochemical tests	**Actinomycetes isolates**				
	$HSEH_1$	**$HSEH_5$**	**$HSEH_{13}$**	**$HSEH_{16}$**	**$HSEH_{18}$**
Citrate test	-	-	-	-	+
Catalase test	-	+	+	+	+
Urease test	-	-	-	-	-
DNAse test	+	+	+	+	+
Casein test	+	+	-	-	-
Starch hydrolysis	-	-	-	-	-
Blood hydrolysis	-	-	-	-	-
Amino acids hydrolysis	+	+	+	+	+
Indole	+	+	+	+	+
Vogues proskauer	-	-	-	-	-
Motility	-	-	-	-	-
Gram staning	+	+	+	+	+

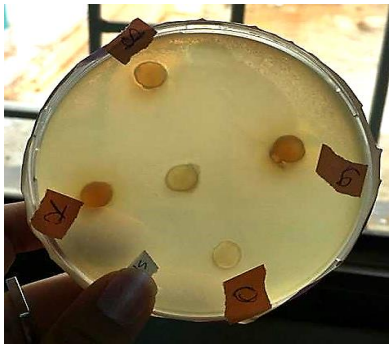

Figure 1. Inhibition zone of actinomycetes isolates against *staphylococcus aureus*

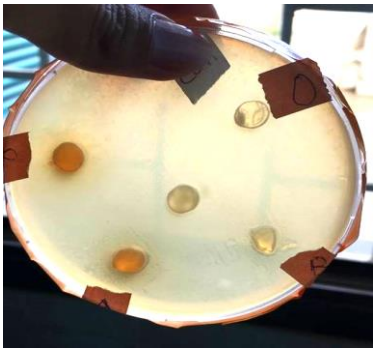

Figure 2. Inhibition zone of actinomycetes isolates against *Candida albicanse*

Figure 3. Inhibition zone of actinomycetes isolates against *Klebcella pneumonia*.

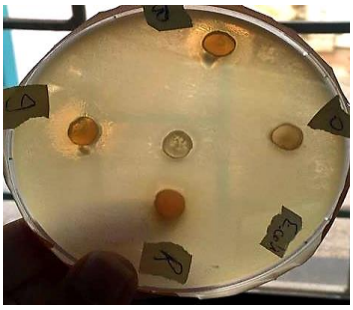

Figure 3. Inhibition zone of actinomycetes isolates against *E coli.*

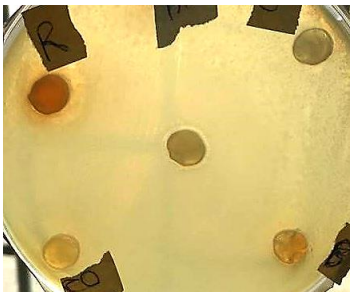

Figure 3. Inhibition zone of actinomycetes isolates against *Salmonella typhi*.

All the five actinomycetes isolated showed different inhibitory activities against target bacteria by spot inoculating method . *E.coli* was greatly inhibited by all actinomycetes isolated as shown by inhibitory zone greater than 21 mm while *K. pneumonia* was inhibited but to a lesser inhibitory effects (Table 4). The $HSEH_{16}$ isolate showed highest inhibition zone 33.8 mm, 32mm, 30mm, 27mm, 20mm against *E. coli, S. aureus, S. typhi , C. albicans, K. pneumonia* respectively. On the other hand, $HSEH_{18}$ isolate showed less inhibition zone with almost target bacteria compare with another isolates.

Table 3. Growth inhibition zone of Actinomycetes isolation against tested organisms

Pathogenic bacteria	Actinomycetes isolates				
	$HSEH_1$	$HSEH_5$	$HSEH_{13}$	$HSEH_{16}$	$HSEH_{18}$
S. aureus	22 ± 0.2	26 ± 0.7	15± 0.4	32 ± 0.7	14 ± 0.7
E. coli	24.5±0.4	24± 0.4	22 ± 0.3	33 ± 0.7	21±0.4
S. typhi	29.3±5.1	23 ± 0.4	13 ± 0 .3	30 ± 0.7	15 ± 0.4
K.pneumonia	12 ± 0.4	7.5±3.4	16.5±0.7	20 ± 0.5	17 ± 0.1
C. albicans	24.4±0.4	25 ± 0.4	25 ± 0.3	27 ± 0.3	19 ± 0.7

DISCUSSION

Actinomycetes are the most important prokaryotes bacteria responsible for the production of about half of the discovered bioactive secondary metabolites including antibiotics (Yuan, *et al* 2010; Miyadoh, 1993; Berdy, 2005) They are the most important resource of medically significant antibiotics. therefore, the microbial natural products still appear to be the most bacteria that shows potential resources for rising future antibiotics. In the present study actinomycetes were isolated from sediment samples collected from different depth of sea shore of alkhoms, for the isolation of effective and broad-spectrum antibiotic producing actinomycetes. Out of the total 20 isolates, five isolates showed good activity in primary screening (Pickup *et al*, 1993; Singh *et al*, 2006). Based on primary screenings, five potent antibiotic producers exhibiting broad spectrumactivity were selected and studied in biochemical details. All the five isolates grew well on most of the three media tested specially on SD agar. Antifungal compounds producing by, $HSEH_1$, $HSEH_5$, $HSEH_{13}$, $HSEH_{16}$, $HSEH_{18}$ isolates against *C. albicance* with (24mm, 25mm, 25mm, 27mm, 19mm) respectively which they previously demonstrated by (Raytapadar & Paul, 2001) when were characterized and identified actinomycetes genes *Streptomyces* and demonstrated to exhibit greatest antibiotic production via primary test. The marine ecosystem is a richest in these species for effective research to obtain antibiotics which provided by actinomycetes in various locations (Abo-Shadi *et al*, 2010).

Here, we found that different depths of sea shore sediment is a good region of biodiversity and has been adequately acceptable due to vast microbial diversity. The results showed that the five isolates were able to exhibit the extracellular compounds against test microorganisms by primary test in this study.

Actinomycetes isolates as $HSEH_{1,}$ $HSEH_{5,}$ $HSEH_{13,}$ $HSEH_{15,}$ $HSEH_{18}$ were obtained via preliminary screening of marine actinomycetes shown that only 5 (25%) out of the total 20 marine isolates had the ability to produce secondary metabolites which exhibited antimicrobial activity against the tested pathogenic bacteria. These bacteria used as an environmental and standard pathogen. Moreover, reported as a worldwide problem is the appearance of multidrug resistant pathogens, which cause serious problems in hospitals, long-stay residential centre and in the community. There is an increase of changing the sensitivity of the bacteria to antibiotics, (WHO 2002). While some isolates showed poor antimicrobial activity, most isolates did not exhibit antimicrobial activity. This was because these isolates did not produce antimicrobial compounds at sufficient levels to restrict the growth of test organisms. Antimicrobial activity of marine isolates has been importantly marked in a number of studies (Lemos *et al*, 1985; Dopazo *et al*,1988; McCarthy *et al*, 1994). The results showed that bacteria isolated $HSEH_{16}$ exhibited antimicrobial activity against all target pathogenic bacteria which may exhibit other metabolic abilities (Lee *et al*, 2010 & Hong *et al*, 2011). This is in concurrene with a previous study that reported marine sediments as rich sources of natural compounds with a wide range of biological activity (Derosa *et al*, 2003). This might be due to the carotenoid contents produced by marine actinomycetes as demonstrated by Marit *et al* (2010). These carotenoids are also responsible for the pigments which appear orange, yellow or red and pink among the marine actinomycetes obtained in this study. On the basis of cultural and morphological properties, all five isolates were classified in the genus *Streptomyces*. Previous works on novel antibiotics reported that a high proportion of organisms possessing antimicrobial activity belong to the genus *Streptomyces* (Demain & Sanchez, 2009; Ceylan, 2008). The antimicrobial compound derived from actinomycetes strains isolated in current study will be useful in developing antibiotics against drug-resistant bacteria.

CONCLUSION

This study confirms that the actinomycetes isolates have the potential to be active as resources of new antimicrobial compounds against human resistant pathogenic microorganisms. The current study found that Alkhoms sea shore is a good region of biodiversity of microbial community and has been effectively suitable for the development of secondary bioactive metabolites, such as antibiotics. Additional molecular and chemotaxonomic research will be carried out to expose the biotechnological capacities and pharmaceutical applications of these strains.

REFERENCES

Abo-Shadi M., Sidkey N.M., and Al-Mutrafy A.M. (2010). Antimicrobial agent producing microbes from some soils rhizosphere. *Journal of American Science*. 6(10), 915–925.

AitBarka E., Vatsa P., Sanchez L., Gaveau-Vaillant N., Jacquard C., Klenk H-P., Clément C., Ouhdouch Y., and Van Wezel G.P. (2016). Taxonomy, physiology, and natural products of Actinobacteria. *Microbiology and molecular biology reviews*. 80:1- 43.

Berdy J. (2005). Bioactive microbial metabolites. *Journal of Antibiotic*. 58: 1-26.

Berdy J. (2012). Thoughts and Facts About Antibiotics: Where We Are Now And Where We Are Heading. *Journal of Antibiotics*. 65: 385-395.

Bergey[s] manual of determinative bacteriology, (2000). *Actinomyceteles* 9th edition.

Cayol J.L., Ollivier B., Alazard D., Amils R., Godfroy A., Piette F., and Prieur D (2015). The Extreme Conditions of Life on the Planet and Exobiology. Environmental

Microbiology *Fundamentals and Application Microbial Ecology* pp. 353-394.

Ceylan O., Okmen G., and Ugar A. (2008). Isolation of soil Streptomyces as source of antibiotics active against antibiotic-resistant bacteria, *Eurasian Journal of BioSciences*. 2:73-82.

Demain A. L., and Sanchez S. (2009). Microbial drug discovery: 80 years of progress, *Journal of Antibiotic.* 62:5-16.

Derosa S., Mitova M. and Tommonero G. (2003). Marine bacteria associated with sponges as a sources of cyclic peptides. *Biomolecular Engineering* 20: 311-316.

Dopazo C.P., Lemos M.L., Lodeiros C., Bolinches J., Barja J. and Toranzo A. E. (1988). Inhibitory activity of antibiotic producing marine bacteria against fish pathogens. *Journal of Applied Bacteriology* 65: 97-101.

Harwani D. (2013). Biodiversity of Rare Thermophilic Actinomycetes Inthegreat Indian Thar Desert: An overview in doam. *Journal of Pharmaceutical Research*. 3: 934-939.

Hayakawa M., Ishiwwa K., and Nonomurha H. (2004). Distribution of rare actinomycetes in Japanese soils. *Journal of Fermentation and Bioengineering*. 66:367–373

Hong, S., Ryu, H. W., Kim, J. & Cho, K. S. 2011. Rhizoremediation of diesel contaminated soil using the plant growth promoting rhizobacterium *Gordonia* sp. S2RP-17. *Biodegradation* 22:593-601.

Lee D. W., Lee J. M., Seo J. P., Schumann P., Kim S.J. and Lee S.D. (2010). *Phycicolagilvus* gen. Nov., an actinobacterium isolated from living seaweed. *International Journal of Systematic and Evolutionary Microbiology.* 58:1318-1323.

Lemos M. L., Toranzo A. E. and Barja J. L. (1985). Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. *Microbial Ecology.*11:149-163.

Marit H. S., Kjell D. J., Geir K. A., Svein V., Trond E.E. and Per B. (2010). Isolation and characterization of marine pigmented bacteria from Norwegian Coastal waters and screening for carotenoids with UVA-Blue light absorbing properties. *The Journal of Microbiology* 48(1):16-23.

Matsumoto D., Takeuchi S., Andayani S., Kouznetsova N., and Krupp D. (1998). The contribution of Individualism-collectivism to cross-national differences in display rules. *Asian Journal of Social Psychology*. 1:147-165.

McCarthy S. A., Johnson R. M. and Kakimoto D. (1994). Characterization of an antibiotic produced by *Alteromonas luteoviolacea* Gauthier 1982, 85 isolated from Kinko Bay, Japan. *Journal of Applied Bacteriology* 77: 426-432.

Miyadoh S. (1993). Research on antibiotic screening in Japan over the last decade: a producing microorganisms approach, *Actinomycetol*. 7:100-106.

Mohammadipanah F. and Wink J. (2016). Actinobacteria from Arid and Desert Habitats: Diversity and Biological Activity. *Frontiers in Microbiology* 6:1541.

Oskay M., Tamer A.Ü. and Azeri C. 2004. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. *African Journal of Biotechnology*. 3: 441-446.

Pickup K.M., Nolan R.D. and Bushell M.E. (1993). A method for increasing the success rate of duplicating antibiotic activity in agar and liquid cultures of Streptomyces isolates in new antibiotic screens, *Journal of Fermentation and Bioengineering*. 76: 89-93.

Raytapadar S. and Paul A.K. (2001). Production of an antifungal antibiotic by *Streptomyces aburaviensis* (1DA-28), *Microbiology Research*. 155:315-323.

Richa S. and Vivek S. (2018). The Role of Actinobacteria in the Production of Industrial Enzymes. *New and Future Developments in Microbial Biotechnology and Bioengineering* pp. 165-177.

Saadoun I., Al-Joubori B. and Al-Khoury R. (2015). Testing of Production of Inhibitory

Bioactive Compounds by Soil *Streptomycetes* as Preliminary Screening Programs In UAE For Anti-Cancer and Anti-Bacterial Drugs. *international journal of current microbiology and applied sciences*. 4(3):446-459.

Shantikumar L., Baruah I. and Bora T.C.(2006). Actinomycetes of loktak habitat:Isolation and Screening for Antimicrobial Activities.*Biotechnology*. 5:217-221.

Shomurat T., Yoshida J., Amano S., Kojina, M. and Niida T. (1979). Studies on actinomycetal producing antibiotics only in agar culture. I. Screening taxonomy and morphology productivity relationship of *Streptomyces halstedii*, strain SF. *Journal of. Antibiotics*, 32: 427-435.

Singh L.S., Baruah I. and Bora T.C. (2006). Actinomycetes of Loktak and Habitat: Isolation and screening for antimicrobial activities. *Biotechnology*. 5:217-221.

Tiwari K. and Gupta R.K. (2012). Rare Actinomycetes: A Potential Storehouse For Novel Antibiotics. *Critical reviews in biotechnology*. 32, 133-142.

Tiwari K., Upadhyay D.J. and Mösker E. (2015).Culturable bioactive actinomycetes from the Great Indian Desert. *Annals of Microbiology*. 65(4), 1901-1914.

Valli, S., Suvathi, S. S., Aysha, O. S., Nirmala. P., Vinoth Kumar, P., and Reena, A. 2012. Antimicrobial potential of Actinomycetes species isolated from marine environment. *Asian pacific journal of tropical biomedicine*;2:469-73.

Vela Gurovic M.S. and Olivera N. L. (2017). Antibacterial producing actinomycetes from Extra Andean Patagonia. *Journal of Arid Environments* pp.1-4.

World Health Organisation, WHO. (2002). Tuberculosis Fact Sheet. No. 104.

Yucel S. and Yemac M. (2010). Selection of *Streptomyces* isolates from Turkish Karstic Caves against antibiotic resistant microorganisms. *Pakistani Journal of Pharmacutical Science*. 23:1– 6.