Pak. J. Biotechnol. Vol. 16 (3) 169-174 (2019) pISSN: 1812-1837 eISSN 2312-7791 www.pjbt.org
http://doi.org/10.34016/pjbt.2019.16.3.25

ANTAGONISTIC ACTIVITY OF ACTINOMYCETES ISOLATED FROM KUALA LUMPUR SOIL SAMPLES AGAINST PATHOGENIC BACTERIA.

Abdullah Y. Al-Mahdi[1], Ebthag A. Mussa[2], Alabed Ali A. Alabed [3], Rasheed Abdulsalam [4], Nagi A. Al-haj[5].

[1]Abdullah Y. Al-Mahdi, Microbiology Department, Faculty of Medicine Lincoln University College, 47301, Petaling Jaya, Selangor, Malaysia. E. mail: 1drabdullahyahya@lincoln.edu.my. [2]Biotechnology Department, Faculty of Science, Lincoln University College, 47301, Petaling Jaya, Selangor, Malaysia. ebtihaj.almabrok@gmail.com. [3]Community Medicine Department, Faculty of Medicine, Lincoln University College, 47301 Petaling Jaya, Selangor, Malaysia. abed11k@gmail.com. [4]Faculty of Dentistry, Lincoln University College, 47301, Petaling Jaya, Selangor, Malaysia. dr_rashed68@yahoo.com. [5]Laboratory of Vaccine & Immunotherapeutic, Institute Bioscience, University Putra Malaysia (UPM), 43400 Serdang Malaysia. naji2005@gmail.com. Corresponding author: dr.microbiology2017@gmail. com

Article received 26.8.2018, Revised 19.9.2019 Accepted 24.9.2019

ABSTRACT

Background: The screening for novel antimicrobial agents continues in a rather overlooked hunting ground for many researchers. *Streptomyces* is the best-recognized genus of actinomycetes used in the screening approach for novel antibiotic-producing microorganisms. The work is aimed to isolate bioactive actinomycete strains and identify the most potent isolates. **Methods:** forty-one actinomycete strains were tested for their antimicrobial activity; out of them, 19 isolates showed a positive response. They were isolated from 18 different soil samples from different locations in Kuala Lumpur, Malaysia. Modified agar-streak, agar disc diffusion method (ADD) and agar well diffusion method (AWD) methods were used in both primary and secondary screening. Isolation media were applied, without antibiotic integration and pretreatment heat, which stimulated the growth of actinomycete isolates. Antagonistic actinomycetes were identified based on morpho-chemical properties using Probability Identification of Bacteria (PIB) software. **Results:** Starch casein agar was found to be selective for actinomycetes. The inhibition zone diameters were found to be larger on nutrient agar plates for bacterial targets. Out of 19 actinomycetes with antagonistic activity, 5 actinomycetes were chosen for secondary screening and further identification. Selected criteria's were based on a strong zone of inhibition against at least four tested bacteria, specifically targeted organisms, *E. coli* MTCC 740 and *S. aureus* MTCC 501. Four isolates were tentatively identified as; *Streptomyces violaceoniger, Streptomyces antibioticus, Streptomyces atroolivaceus* and *Streptomyces alboflavus*. **Conclusions**: Four actinomycete isolates of genus *Streptomyces* proved strong antibiosis activity against two Gram –ve and +ve important bacterial strains.

Keywords: Antagonistic, Atinomycetes, *Streptomyces*, PIB, *E. coli*, *S. aureus*, ADD, AWD.

1. INTRODUCTION:

Novel antibiotic and other bioactive compounds lead molecules which discovered in pharmaceutical interest by using new bacterial secondary metabolite screening method is becoming increasingly fruitful. Know a day there is wide acceptance of microbes are practically unlimited sources of new secondary metabolism with many pharmaceutical therapeutic applications (Wang, *et al*., 2017). Out of them actinomycetes become a prominent position because of their diversity and had proven their ability to produce new substances which recognized as *Streptomyces*. They are aerobic, exospore forming Gram-positive bacteria which have DNA with a high G+C amount (69-73%). They form a branching substrate with aerial mycelia and widely distributed in soil (Al-Mahdi, 2006). In the past decades, the needs for drugs is increased to control new illness or resistant strains of pathogenic microorganisms which stimulated to look for unconventional new sources of bioactive metabolites. Soil is found to be an attractive field and great efforts have been accomplished worldwide aiming the isolation of novel products from soil microorganisms (Ali, *et al*., 2017). Culturing of novel bacteria would signify a unique and auspicious source for the discovery of novel bioactive secondary metabolites (Kanavade, 2003).

Microbial flora and fauna are an attractive source of new pharmaceutical therapeutic candidate compounds. Because of the massive biological diversity in the extreme area as a whole, it is increasingly predictable that a large number of novel bioactive substances exist in the soils. Soil micro flora have improved greatly genomic and metabolic diversity; efforts should be directed towards discovering extreme soil microorganisms as a source of novel bioactive compounds (Ali *et al*., 2017; Ningthoujam, *et al*., 2009).

Actinomycetes bacteria have been known for over a hundred years as sources of many bioactive compounds. For much of the time, they were observed as an interesting group of microbes with alliance to both bacteria and fungi. However, determination of

their fine structure and chemical component, initiated in the 1950s, proven their prokaryotic nature. They now constitute the order Actinomycetales and their removal from the mycologists, sphere of influence has been completed. Their change of status paralleled that of the blue-green algae to the cyanobacteria but it was accepted more rapidly and less acrimonious. It is not easy to give a short, accurate definition of actinomycetes. They are frequently described as bacteria which can form branching hyphae at some stage of their development. However, this characteristic is tenuous and it often requires imagination to believe in it (Okoro, *et al*., 2009). The exact composition and frontier of the order Actinomycetales are still open to investigation and modification by the application of new taxonomic techniques which have also led to improvements in the classification and identification of actinomycete genera and species. Despite their relegation to a single order of the kingdom Prokaryotes, their biological attributes, their importance to man and their history, actinomycetes have ensured that still generally studied as a group distinct from different bacteria (Al-Mahdi, *et al*., 2011). The aim of this research work to screen for new antimicrobial continues in a rather overlooked hunting ground, in the method of screening for novel antimicrobial-producing microorganisms

2.MATERIALS AND METHODS:

2.1 Soil samples: Soil were collected from 8 locations in Kuala Lumpur using hand-held scoops (100 g capacity); spoons (up to 300 g capacity) and shovels were used for sampling of surface soils. The upper layer of soil was removed to the desired depth with a clean spade and then using stainless-steel scoop, plastic spoon or shovel, a thin layer of soil from the area which encountered the spade was removed. The samples were transferred to an appropriate, labeled sample container with a sterile lab spatula (Ghadge and Patil, 2016).

2.2 Isolation of actinomycetes from soil samples: Actinomycetes was isolated by suspending 1 g of the collected soil in 10 ml sterile water, which was strongly shaken and then allowed to settle for 5 min. The supernatant was serially diluted then plated on Starch Casein Agar (SCA) of pH 7.5, followed by incubation at 37 °C for 14 days. The isolates were enumerated and selected for further study. The pH of the buffered medium was checked during incubation (Elamvazhuthi and Subramanian, 2013).

2.3 Antagonistic activity: All 41 isolated actionmycetes were tested for their antimicrobial activity by two techniques; ADD and AWD. The target bacterial cultures used were *Escherichia coli* (represent Gram-Negative bacteria) and *Staphylococcus aureus* (represent Gram-Positive bacteria) then compare with standard 4 antibiotics; Gentamycin (GE) 10ug, Streptomycin (S)10ug, Ampicillin (Amp)10ug and Tetracycline (Te) 30ug (Ali, *et al*., 2017).

1) Agar Disc Diffusion Method (ADD): the actionmycete isolates were streaked on Starch Casein Agar (SCA) of pH 7 and incubated at 37 °C for 7 days. After 7 days, the tested organisms were seeded in Nutrient Agar (NA) of pH 7 for bacteria. Agar discs 6.0mm of actinomycetes was transferred to the surface of NA plates. There were further incubated at 37°C for 24 hours. Antimicrobial activity was noted in terms of the zone of inhibition around the agar disc of isolated actinomycetes (Zhu, *et al*., 2011; Kumar, *et al*., 2011).

2) Agar Well Diffusion Method (AWD): all actinomycete isolates were tested for antimicrobial activity of their culture supernatant. The sterile Starch Casein broth (SCB) (15 ml) pH 7 was dispensed in bumper tubes inoculated with 1 ml actinomycetes spore suspension (1x 106 spores ml^{r}) and incubated in shaker incubator at 37 °C (200 rpm) for 10 days. After incubation the broth culture was centrifuged then supernatants screened for antimicrobial activity by agar well diffusion method (Jiang, *et al*., 2013). The NA plates were prepared and seeded as described under 1. Wells of 6.0 mm were made with sterile cork-borer of 6.0 mm and filled with cultures supernatant of acidophilic actinomycetes and incubated. Zone of inhibition was recorded as described under 1 (Jeffrey, *et al*., 2007).

2.4 pH effect on the growth of actinobacterial isolates: The selected isolates were grown on Starch Casein Agar (SCA) of pH 7.2, 7.0, and 10.0. to minimize changes in pH of the medium, SCA was buffered to desired pH using KH_2PO_4 buffer. Required volumes of sterile 1 N HCl or 1 N NaOH were added to the agar medium after autoclaving to adjust the pH to desired value. The group of actinomycetes depends upon the growth on pH 7.2 were isolated (Wang, *et al*., 2017).

2.5 Characterization of bioactivity actionbacterial isolates: To study growth, morphological and biochemical characters were recorded of the bioactivity actinobacterial isolates. All isolated actinomycetes were grown on following different media: (1) Starch Casein Agar (SCA) (Nawani, 2002), (2) Nutrient Agar (NA) (Qais, *et al*., 2016), (3) Czapeck's Dox-Thom Agar (CDTA) (Patel, *et al*., 2014) and (4) Potato Dextrose Agar (PDA) (Almahdi, *et al*., 2011) of pH 7.2.

The inoculated plates were incubated at 37°C for 7-14 days and record the colony characters, sporulation and pigmentation under the microscopy (Pro-

mnuan, *et al*., 2009). The isolates were subjected to slide culture and characters records for actinomycetes identifications. All morphological and biochemical characters of bioactive isolates were used to identify the actinobacteria by Probability Identification of Bacteria (PIB) software (Almahdi, *et al*., 2012).

3. RESULTS

Forty-one actinomycetes isolates (non- bioactivity and bioactivity) were isolated from all target location site as following; Kelana Garden (Ke) with 14 isolates (34%) and 6 bioactive isolates. Ampang Garden (Am) with 4 isolates (10%) and 4 bioactive isolates. Kl Garden (Kl) with 3 isolates (8%) and 3 bioactive isolates. Petaling Jaya Garden (Pe) with 3 isolates (7%) and 1 bioactive isolate. Genting Highlands (Gh) with 5 isolates (12%) and 2 bioactive isolates. Finally, Putra Jaya Garden (Pj) with 12 isolates (29%) and 3 bioactive isolates as shown in Figure 1.

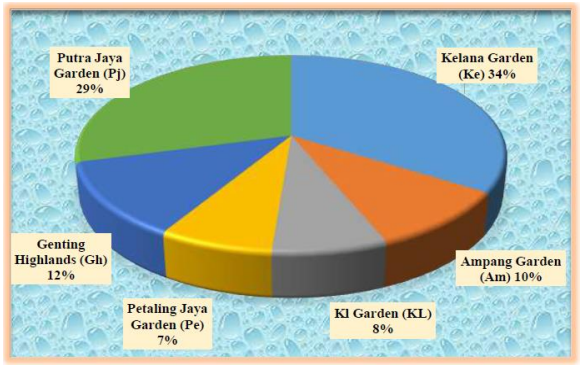

Figure 1: Location sites with no. of isolates (%).

3.1 Antagonistic activity against pathogenic bacteria: All the isolated actinomycetes were tested for antagonistic activity, by agar disc diffusion method (ADD). Antimicrobial activity was studied against Gram-negative and Gram-positive bacteria. The cultures bacteria used were *Escherichia coli* MTCC 740, as Gram-ve target bacteria and *Staphylococcus aureus* MTCC 501 as Gram+ve target bacteria. Out of 41 isolates only 19 (46%) isolates show activity against target bacteria. Out of 19 bioactive isolates 4 (21%) isolates show antimicrobial activity against *E. coli* MTCC 740 and 3 (15%) isolated actinomycetes show antimicrobial activity against *S. aureus* MTCC 501.

Out of 19 actinomycete isolates with antimicro-bial activity 6 (31.5%) isolates from location site no 1 (Kelana Garden Ke) only one show activity against *S. aureus* MTCC 501. From location site no 2 (Ampang Garden Am) only 3 (21%) isolates show activity against *S. aureus* MTCC 501. Location sites no 3 (Kl Garden Kl) 2 (10.2%) one (5%) isolates show activity against *E. coli* MTCC 740. Location site no. 4 (Petaling Jaya Garden Pe) with only one (5%) isolates show antimicrobial activity against *E. coli* MTCC 740. For location sites no. 5 (Genting Highlands Gh) only 2 (10.5 %) isolates show activity against *E. coli* MTCC 740. Last location sites no. 6 (Putra Jaya Garden Pj) only 3 (15.5 %) isolates show activity but only one against *E. coli* MTCC 740 and 2 isolates show activity against *S. aureus* MTCC 501 out of 12 isolates in this locations. These results are presented in Table 1. The data shown in Table 1 only for antibacterial activity other information not shown.

Table 1: Antimicrobial activity against targets pathogenic bacteria.

Soil samples no	Isolates no	Zone of inhibition by actinomycetes isolates (cm)		Total/%
		E. coli MTCC 740	*S. aureus* MTCC 501	
1	5	-	-	6 (31.5%)
	6	-	-	
	7	-	-	
	8	-	-	
	12	-	-	
	14	-	1.5	
4	22	3.5	-	1 (5%)
5	25	3.1	-	2 (10.5)
	29	-	-	
6	30	2.2	-	3 (15.5%)
	39	-	1.5	
	41	-	3.0	
*4 soils	*19 (100%)	*4 (21%)	3 (15.5%)	100 %

From antagonistic activity of all isolates; out of 19 isolates were select 4 isolates with maximum antagonistic activity (maximum zone of inhibition in NA for bacterial targets) one isolates from each location sites as shown in Figure 2.

Antagonistic activity for important isolates as following; from location sites no 1 the maximum antagonistic zone show with isolates no 14 which show 1.5 cm zone of inhibition with *S. aureus* MTCC 501 as shown in Plate 1 (A, B & C). From location site no 2 there is no antagonistic zone show with all target bacteria.

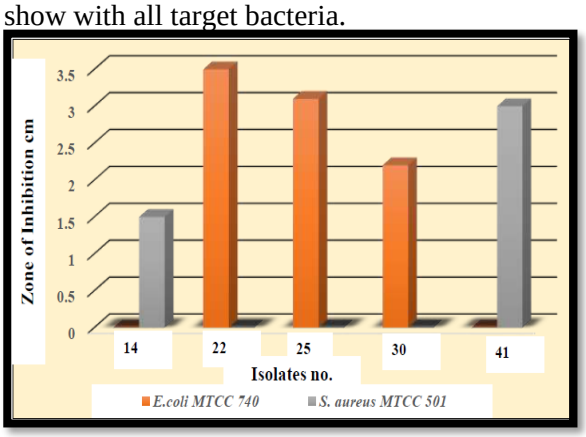

Figure 2: Zone of inhibition of 5 strong antagonistic isolates.

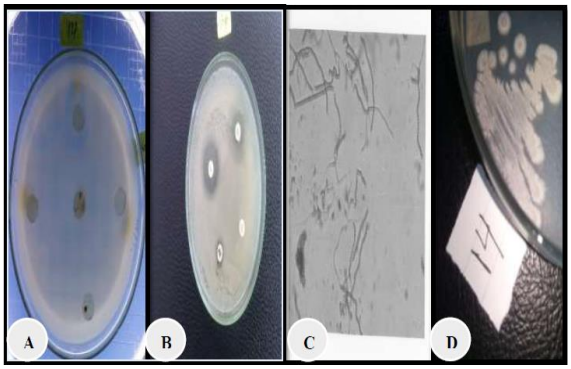

Plate 1: Maximum zone of inhibition for isolates no. 14 against S. aureus MTCC 501; A grow on SCA, B sensitivity with antibiotics, C under micro-scopy and D grow on SCA.

Location sites no 3 the maximum antagonistic zone show with isolates no 21 which show 2.6 cm zone of inhibition with *E. coli* MTCC 740 only as shown in Plate 2.

Antagonistic activity for isolates from location sites no 4 the maximum antagonistic zone show with isolates no 22 which show 3.5 cm zone of inhibition with *E. coli* MTCC 740 as shown in Plate 3 (A, B, C & D).

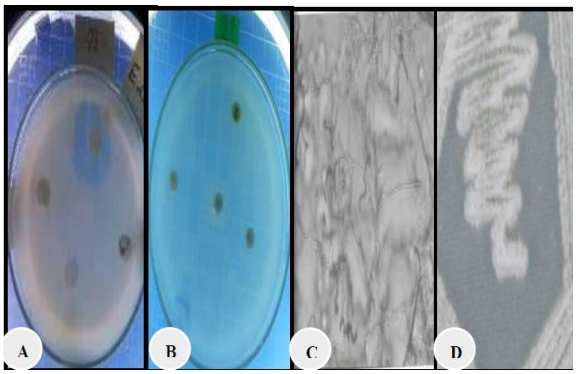

Plate 2: Maximum zone of inhibition for isolates no. 22 against *E. coli* MTCC 740; A bioactivity on SCA, B sensitivity with antibiotics, C under micro-scopy and D grow on SCA.

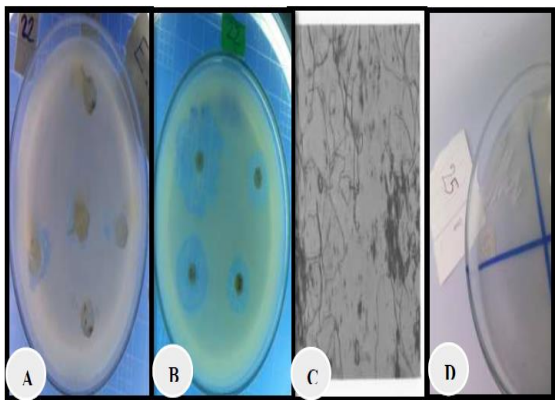

Plate 3: Maximum zone of inhibition for isolates no. 25 against *E. coli* MTCC 740; A bioactivity on SCA, B sensitivity with antibiotics, C under microscopy and D grow on SCA.

Location sites no 5 the maximum antagonistic zone show with isolates no 25 which show 3.1 cm zone of inhibition with *E. coli* MTCC 740 as shown in Plate 4 (A, B, C and D). Antagonistic activity for isolates from location sites no 6 the maximum antagonistic zone show with isolates no 41 which show 3.5 cm zone of inhibition with *S. aureus* MTCC 501 as shown in Plate 4 (A, B, C & D).

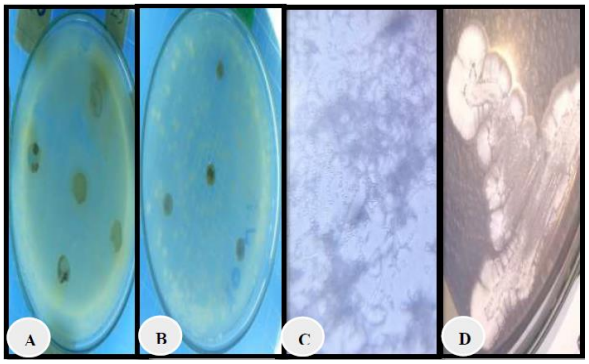

Plate 4: Maximum zone of inhibition for isolates no. 41 against *S. aureus* MTCC 501; A bioactivity on SCA, B sensitivity with antibiotics, C under microscopy and D grow on SCA.

3.2 Identification of Bioactive Isolates: Isolate no. 14 was *Streptomyces violaceoniger*. The spore chains were spirales. The spore chains were gray. Diffusible and melanin pigments were not produced. Antimicrobial activity against *Candida albicans* was observed. The isolate was lipolytic and grow at 45°C. Isolate was utilized cysteine, phenylalanine, histidine and valine as a nitrogen source and utilized inositol and raffinose, as a carbon source.

Isolate no. 22 was identified as *Streptomyces atroolivaceus*. The spore chains were rectiflexibiles. The spore mass was gray to white. Diffusible and melanin pigments were not produced. Antimicrobial activity against *E. coli* and *Candida albicans* was observed. The isolate was lipolytic and utilized phenylalanine and cysteine as a nitrogen source. Utilized sucrose and raffinose as a carbon source which recorded.

For isolate no. 25 which identified as *Streptomyces antibioticus*. The spore chains rectiflexibiles and spirales. The spore mass was gray. None of the isolates produced diffusible and melanin pigments. Most of the isolates tolerated 7% NaCl, not grown at 45°C and utilized meso-Inositol as a carbon source. Antimicrobial activity against *E. coli*, *Candida albicans* and *Aspergillus niger* was observed.

Isolate no. 41 was identified as *Streptomyces alboflavus*. The spore chains were straight. The spore

mass was white. Diffusible and melanin pigments were not produced. Isolate was utilized alanine, glycine, and valine as a nitrogen source and utilized arabinose, fucose, raffinose, inositol, mannitol and glutamate as a carbon source. Isolates no 21 and 39 identified as *Bacillus sp*. which out of our study about actinomycetes.

4.DISCUSSIONS

Four actinomycetes were selected for antagonistic antibacterial antibiotics production. Production of antibiotic was influenced by media components and cultural condition, such as; pH, temperature and type of inoculum and amount of inoculum. Such conditions are known to affect antibiotic production and these fermentation conditions differ from organism which recorded in our study and mention by Breza-Boruta and Paluszak, (2016).

Form of inoculum was found to influence antibiotic production by *Streptomyces* sp. Single cell inoculum of 72hrs was found suitable for maxi-mum antibiotic production. At low pH *Streptomyces sp.* was found to produce unicellular forms. Antibiotic condition such as pH, temperature and target type were found to affect antibiotic activity which is produced by actinomycetes. The optimum pH for antibiotic activity was 5.0 in this investigation. Wu, *et al*., (2009) found that change in pH of the antibiotic produce new substances which affect antibiotic activity. Effect of pH on stability of antibiotics more than that of temperature for all anti-biotics. The activity unstable at high pH, because the side chains of antibiotics getting inactivated due to change in pH from the normal value and the maximum antimicrobial activity at pH 7.0 and at pH 5 the activity is unstable in all of antibiotics. The antibiotics which were unstable at high temperature because of side chain of antibiotics getting inactivated at this temperature and those antibiotics produced by actinomycetes isolated from acidic soils.

Most of 4 isolates produce strong antibacterial antibiotics were tested for their activity against *E. coli* MTCC 740 and *S. aureus* MTCC 501. All four acidophilic actinomycetes producers belonged to *Streptomyces* genera. Three isolates produce antibiotic was found to be having wide range of activity against *E. coli* MTCC 740 as; *Streptomyces violaceoniger*, *Streptomyces atroolivaceus* and *Streptomyces antibioticus*, one isolate produce antibiotic was found to be having wide range of activity against *S. aureus* MTCC 501 this result like research done by Wang, *et al*., (2017).

Conclution:

Four actinomycete isolates of genus acidophilic *Streptomyces* proved strong antibiosis activity against two Gram–ve and +ve important bacterial strains. Maximum number of antimicrobial compound-producing acidophilic *Streptomyces sp*. remain undiscovered in the environment. These actionbacterial resources should receive more study in research and for the development of novel antibacterial, antiviral, antifungal, antitumor and antiplant pathogen agents. Modified medium for isolation of acidophilic *Streptomyces* is required to discover more acidophilus. Modeling of different biodiversity aspects and baseline data of each aspect. The enzymes and antibiotics production by acidophilic *Streptomyces* from acidic soils were given excellent results in this study.

5. Current and Future Developments: Current development to focus our studies to determine the interaction of antibacterial antibiotics which compare it with new generation antibiotics. For future developments to study the mechanism of acidophilic *Streptomyces* in different habitats, especially, their reaction with heavy metals, detergents and other inorganic compounds and the genetic aspects of this acidophilic *Streptomyces* need to be studied in detail.

6. Limitations: Due to the retrospective nature of our study, there was a limitation of describing the various types of soil samples, morphological and biochemical characteristics of the actinomycetes and pathogenic bacteria usage.

Conflict of interest disclosure: There is no conflict of interest.

5. ACKNOWLEDGEMENTS

The authors received the financing for the implementation of this work from Biotechnology Department, Faculty of Science, Lincoln University College, Kelana Jaya, Selangor, Malaysia. We thank all authors for the grammar review of the manuscript.

REFERENCES:

Ali Mohammed Abdullah Bawazir, Ahmed M. Al-Haddad, Abdullah AL-Mahdi and Manjula Shantaram, Actinomycetes from Mountains of Hadhramout–Yemen. Int. J. Curr. Microbiol. App. Sci. 6(8): 3521-3530 (2017).

Al-Mahdi Y. Abdullah, Ahmed, A. Albana, Nissrin, A. Almeklafi and Saeed, M. Al-Galibi, Bioactivity of some Actinomycetes isolated from the Yemeni soils. Thamar University Journal of Natural and Applied Sciences A(4); 113-123 (2011).

Al-Mahdi Y. Abdullah, Qaees Y. M. Abdullah, Anas A. Al-Mahbashi, Abdulrahman A. Homed, Salwa H. Al-Kaeat and Saeed M. Al-Galibi, Inhibition of Mycoparasitic growth by filamentous nonpolyne chitinolytic producing bacteria. International Conference on Medical, Biolo-

gical and Pharmaceutical Sciences (ICMBPS Bangkok 2012).

Breza-Boruta, B. and Paluszak, Z., The antagonis tic activity of Actinomycetes of *Streptomyces* genus in relation to *Trichoderma koningii*. *Journal of Ecological Engineering* **17 (1)**: 106–113 (2016).

Elamvazhuthi, P. and Subramanian, M., Antagonistic Activity of Actinomycetes from Jeypore paddy soils against selective phytopathogenic fungi. *J. Mod. Biotechnol*. **2:** 66–72 (2013).

Errakhi, R., Lebrihi, A. and Barakate, M., In vitro and in vivo antagonism of Actinomycetes isolated from Moroccan Rhizospherical soils against *Sclerotium rolfsii*: A causal agent of root rot on Sugar Beet (*Beta vulgaris L.*). *Journal of Applied Microbiology*. **107**: 672–681 (2009).

Ghadge, S. and Patil, R., Antagonistic Activity of Actinomycetes isolated from soil samples of satara district, Maharashtra, India against selected human pathogens. *I.J.A.B.R.* **6(2)**; 188-194 (2016).

Jeffrey, L.S.H., Sahilah, A.M., Son, R. and Tosiah, S., Isolation and screening of actionmycetes from Malaysian soil for their enzymatic and antimicrobial activities. *J. Trop. Agric. and Fd. Sc*. **35(1)**; 159–164 (2007).

Jiang Y., Han L., Chen X., Yin M., Zheng D., Wang Y., Qiu S. and Huang X., Diversity and bioactivity of cultivable animal fecal actionbacteria. *Adv Appl Microbiol*. **3**:1-13 (2013).

Kanavade, V.L., Use of bio-industrial waste for production of microbial biomass with potential in environmental management. Ph.D. Thesis, University of Pune, India (2003).

Kumar, J.S., Santhanam, P. and Selvam, M.M., Antimicrobial Activity of Actinomycetes Isola ted from the Western Ghats of Tamil Nadu. *International Journal of Pharma and Bio Sciences* **2(1):** 42-49 (2011).

Nawani, N.N., Diversity of chitinases of bacterial origin. Ph.D. Thesis. University of Pune. Pune. India (2002).

Ningthoujam, S.D., Sanasam, S., Tamreihao, K. and Nimaichand, S., Antagonistic activities of local Actinomycete isolates against rice fungal pathogens. *African Journal of Microbiology Research* **3(11):** 737-742 (2009).

Okoro, C.K., Brown, R., Jones, A.L., Andrews, B.A., Asenjo, J.A., Goodfellow, M. and Bull, A.T., Diversity of culturable actinomycetes in hyper-arid soils of the Atacama Desert, Chile. *Antonie van Leeuwenhoek* **95**: 121-133 (2009).

Patel, J.D., Parmar, M., Patel, P., Rohit, P., Ramesh, T., Ansari, P., Bhattacharya, B., Vyas, D., Kumar, V., Sahay, N.S. and Singh, P.K., Dynamism of antimicrobial activity of actionmycetes-A case study from undisturbed microbial niche. *Adv. Microbiol*. **4**: 324-334 (2014).

Promnuan, Y., Kudo, T. and Chantawannakul, P., Actinomycetes isolated from beehives in Thailand. *World J Microbiol Biotechnol*. **25**: 1685-1689 (2009).

Qais, Y.M. Abdullah, Maher, A. Al-Maqtari, Ola, A.A. Al-Awadhi and Abdullah, Y. Al-Mahdi, New records of Streptomyces and non-Streptomyces Actinomycetes isolated from soils surrounding Sana'a high mountain. *International Journal of Research in Pharmacy and Biosciences* **3(3):** 19-31 (2016).

Wang, D.; Ren, H., Erihemu, A. and Zheng, Z., Isolation, identification and antagonistic activity evaluation of Actinomycetes in barks of nine trees. *Arch. Biol. Sci*. **69(2)**; 345-351 (2017).

Wu, Y., C. Lu, C., Qian, X., Huang, Y. and Shen, Y., Diversities within genotypes, bioactivity and biosynthetic genes of endophytic actionmycetes isolated from three pharmaceutical plants. *Curr. Microbiol*. **59**: 475-482 (2009).

Zhu, W., Xue, Q., Cao, Y., Xue, L., Shen, G. and Lai, H., Distribution and characteristics of soil antagonistic actinomycetes on northern slope of Taibai Mountain, Qinling. *Chinese J. Appl. Ecol*. **22**: 3003-3010 (2011).