Scientific Inquiry and Review (SIR)
Volume 7 Issue 3, 2023
ISSN (P): 2521-2427, ISSN (E): 2521-2435
Homepage: https://journals.umt.edu.pk/index.php/SIR

Article QR

Title:	**Screening of Actinomycetes Isolated from Soil and their Antimicrobial Activity against Plant Pathogens**
Author (s):	Rahat Zamir[1,2], Sumaira Mazhar[1], Huma Shafique[1], Roheela Yasmeen[1]
Affiliation (s):	[1]Lahore Garrison University, Pakistan [2]Government College University, Lahore Pakistan
DOI:	https://doi.org/10.32350/sir.73.06
History:	Received: August 16, 2022, Revised: March 16, 2023, Accepted: July 10, 2023, Published: August 28, 2023
Citation:	Zamir R, Shafique H, Yasmeen R, Mazhar S. Screening of actinomycetes isolated from soil and their antimicrobial activity against plant pathogens. *Sci Inq Rev.* 2023;7(3):82–94. https://doi.org/10.32350/sir.73.06
Copyright:	© The Authors
Licensing:	This article is open access and is distributed under the terms of Creative Commons Attribution 4.0 International License
Conflict of Interest:	Author(s) declared no conflict of interest

A publication of
The School of Science
University of Management and Technology, Lahore, Pakistan

Screening of Actinomycetes Isolated from Soil and their Antimicrobial Activity against Plant Pathogens

Rahat Zamir[1,2], Sumaira Mazhar[1*], Huma Shafique[1] and Roheela Yasmeen[1]

[1]Department of Biology, Lahore Garrison University, Pakistan
[2]Government College University, Lahore Pakistan

ABSTRACT

Actinomycetes abundantly present in soil are renowned for various secondary metabolites production with a wide range of use in diverse fields of life. Many reports are available on the use of actinomycetes for the regulation of pathogenic microbes in plants. For this purpose, this study used thirteen Actinomycetes strains, which were isolated from the fields of Piplan (district Mianwali) Punjab, Pakistan. However, out of thirteen, only seven were found active for the production of both primary and secondary screening. The active seven actinomycetes (AW1, AW2, AW3, AW4, AM1, AC1, and AC2) were separated for their microbial potential against pathogenic strains (*Escherichia coli* and *Staphylococcus aureus*) isolated from the same agriculture soil. About 42.85% of isolated actinomycetes (three out of seven) showed antagonistic properties against *S. aureu* and *E. coli* in primary screening. Thus, it was noticed that the three strains (AW3, AM1, and AC1) had great antimicrobial potential, which showed the most promising results. Whereas in Secondary screening only AC1 showed the best result with ethyl acetate extract as compared to ethanol, methanol, and chloroform. Hence, It was concluded that actinomycetes isolates had great potential for antibacterial activity and they can be used in agriculture industry for the regulation of plant pathogens.

Keywords: Actinomycetes, antimicrobial, metabolites, piplan, primary screening, secondary screening

1. INTRODUCTION

Actinomycetes belong to a group of bacteria placed in order in Actinomycetales, having a distinguished taxonomic group in the bacterial domain; however, with fungi, they share certain morphological features [1]. They are aerobic, spore formers, and Gram-positive. In soil, they create thread-like filaments [2]. They display filamentous growth, producing substrate or aerial mycelium, occasionally coccoid to rod in shape [3].

* Corresponding Author: humashafique@lgu.edu.pk

Actinomycetes DNA has high G+C content in the range of 57-75% [4]. Out of eighteen main lineages of the bacterial domain, Actinomycetes are one of the prime taxonomic units [5]. They cultivate as hyphae and are characteristically capable of the production of soil earthy smell [4, 6]. According to [7] around 100 actinomycetes genera are found in soil and several species, which are primarily found as soil inhabitants.

However, reportedly in the previous literature, Actinomycetes existed as an appearing group of microorganisms, which were extensively dispersed in natural ecosystems around the world [8, 9]. Furthermore, the species had been found in a divergent aquatic environment containing deep ocean sediments [10–12].

It is also known that the Actinomycetes are producers of many secondary metabolites [13]. In actinomycetes, about 70–80% of secondary metabolites are produced by *Streptomyces* genera (almost 500 species); while other genera have less contribution, for example, *Micromonospora*, *Saccharopolyspora*, *Actinoplanes*, and *Amycolatopsis* [14]. They have a broad range of utilization within the agricultural, medical, and pharmaceutical industries. In medicine, secondary products are used as antibiotics, antitumors, antiviral, and anti-infection agents [15–17]. Furthermore, actinomycetes would manufacture plant growth promoters to boost plant growth and facilitate the institution of plants under stressed conditions [18, 19]. Secondary metabolites produced by actinomycetes facilitate other microbes by forming mutualistic and symbiotic interactions with them to protect their host from pathogenic microbes [20]. There is scarce data from fields of Piplan (district Mianwali) about the isolation of ascomycetes and their antimicrobial activity from the rhizospheric region of the crops. Therefore, the current study aimed to investigate the isolation of actinomycetes from (fields of piplan) and the use of these strains against pathogenic strains, which were isolated from crops such as maize (*Zea mays*), cotton (*Gossypium*), and wheat (*Triticum aestivum*). The current study would significantly highlight the applications of isolated actinomycetes for the control of plant pathogens strains.

2. MATERIALS AND METHODS

2.1. Collection of Soil Samples

Soil samples were collected from the rhizospheric region of the crops such as wheat (*Triticumaestivum*), cotton (*Gossypium*), and maize (*Zea mays*) for the isolation of actinobacteria.

The soil samples were sited in polythene bags (sterile) during the month of November 2017.

The samples were desiccated at ambient temperature for continuous 2 days and sieved. The sieved soils were then used for the actinomycetes isolation.

2.2. Isolation and Characterization of Actinobacteria

For isolation purpose, enrichment of Actinomycetes in the samples was carried out by physical [21] and chemical treatment [22]. Following physical and chemical treatment 1 gm of soil sample was serially diluted and spread on ISP media supplemented with 50 g/ml antifungal agent nystatin [23]. The isolated actinobacteria (13 strains) were sub-cultured and incubated for 7–12 days at 28°C. However, only seven selected colonies of actinobacteria were noticeably characterized both morphologically and biochemically [24].

2.3. Test Organisms

Pathogenic soil bacterial strains, such as *S. aureus* and *E. coli* were isolated from the same soil samples, which were used to assess the antagonistic activity of actinomycetes. Glycerol stock was prepared to store bacterial strains. It can be used by subculturing when required.

2.4. Primary Screening for the Antibacterial Activity of Actinomycetes

In primary screening, a modified cross-streak process was used to assess antimicrobial activity [25]. From each plate, an isolated colony of actinomycetes was picked and streaked on the modified nutrient agar surface.

A single streak of actinomycetes isolates was created for each isolate, which passed down the middle of the plate and incubated at 28°C for 5-7 days [26]. An upright ribbon growth of the actinomycetes was observed on the plates, followed by the inoculation within 24 hours old culture

pathogenic soil bacterial strains i.e. *E. coli* and *S. aureus*. Then, the plates were further incubated for 24 h at 37 °C [27]. The inhibition zone was measured and recorded after the incubation.

2.5. Secondary Screening for the Antibacterial activity of Actinomycetes

The Agar well diffusion process along with crude extracts of ethanol, ethyl acetate, chloroform, and methanol was used in the secondary screening of the isolate. Submerged fermentation bioactive compound production was done by Chaudhary et al. [2]. A conical 250 ml flask was taken with 50 ml of ISP broth, which was inoculated with Actinomycetes isolates. After the incubation was done at 30°C in a shaking incubator at 150-rpm rotation for continuous 7 days. When fermentation was done and centrifuged at medium twice for 10 min at 10,000 rpm to take out cells and debris and harvested them for fermented broth. Collected crude extracts were tested against test microorganisms (*S. aureus* and *E.coli*) via a method of agar well diffusion [28, 29]. Both test microorganism and cell concentrations were adjusted at 0.5 McFarland turbidity standards. A sterilized cotton swabs were used to inoculate the strains on nutrient agar plates. A sterilized micro tip (1000 µl) was used to bore wells in the plates. Then, the wells were poured with 20, 50, and 100 µl of each crude extract. The plates were incubated at 37°C for 24 h [2]. After incubation, the zone of inhibition was measured and noted for the record.

3. RESULTS AND DISCUSSION

In the last few years, actinomycetes seemed extensively studied in various undiscovered locations in distinct parts of the globe (including Pakistan). However, there are no reports of isolation of actinomycetes from, District: Mianwali Punjab (Pakistan). Therefore, attempts have been made to isolate actinomycetes from this undiscovered area to find new species. From Soil samples, 7 actinomycetes strains (out of 13) were isolated and labeled as Actinomycete wheat 1 (AW1), Actinomycete wheat 2 (AW2), Actinomycete wheat 3 (AW3), Actinomycete wheat 4 (AW4), Actinomycete maize 1 (AM1), Actinomycete cotton 1(AC1), and Actinomycete cotton 2 (AC2). In the current study overall, 53.8 % isolates of actinomycetes were found to be gram-positive organisms and the results were similar with Subhan et al. [30] who identified 55 % isolates of actinomycetes as gram-positive. Furthermore, two pathogenic bacterial

strains (*E.coli* and *S. aureus*) were also isolated, which were characterized both morphologically and biochemically (see Table 1).

Table 1. Morphological and Biochemical Characteristics of Bacterial Strains Isolated from Agricultural Soil

Sr. No.	GS	S	M	C	Sp	F	C	O	In	MR	VP	Cit	Nit	U	H_2S
S. aureus	+	Cocci	-	-	-	-	+	-	-	+	+	+	+	+	-
E. Coli	-	Rods	+	-	-	+	+	-	+	+	-	-	+	-	-

Note. GS: Gram Staining; S: Shape; M: Motility; C: Capsule; Sp: Spore; F: Flagella; C: Catalase; O: Oxidase; In: Indole Production; MR: Methyl Red; VP: Voges-Proskauer; C: Citrate Utilization; Nit: Nitrate Reduction; U: Urease; H_2S: H_2S Production

The morphological characterization of all the seven isolates showed dry and rough to the powdery and smooth texture of isolates. Moreover, the colonies were chalky white, dry, nodular, and sticky on isolation agar of actinomycetes. The morphological and biochemical characteristics suggested that the isolates were representative of genus Actinomycetes (see Table 2). The characteristics of the genus were also in line like the previous findings of Gurung et al. [31] and Anwar et al. [32].

Table 2. Biochemical Characteristics of Actinomycetes Bacterial Strains

Biochemical Test	Isolated strains of Actinomycetes						
	AW1	AW2	AW3	AW4	AC1	AC2	AM1
Catalase	+	-	+	+	+	+	+
Citrate Utilization	+	-	+	+	+	+	-
Urease	+	+	-	-	+	-	+
Methyl Red	+	+	+	+	+	+	+
Voges-Proskauer	-	+	+	+	-	-	-
Melanin Production	+	+	+	-	+	-	+
H_2S Production	-	-	+	-	-	-	-
Nitrate Reduction	+	+	+	+	+	-	+
Motility	-	-	-	-	-	-	-
Starch Hydrolysis	+	+	+	+	+	+	+

Note. AW1: Actinomycete wheat 1; AW2: Actinomycete wheat 2; AW3: Actinomycete wheat 3; AW4: Actinomycete wheat 4; AM1: Actinomycete maize 1; AC1: Actinomycete cotton 1 and AC2: Actinomycete cotton 2

In the current study, a soil sample was collected from the surface and rhizospheric region of crops at the depth and it has been found that as a result of appropriate pH and water condition in depth, actinomycetes

quantity at depth is quite higher than the surface of the soil and similar findings were also reported Basavaraj et al. [33].

For antimicrobial potential, strains of actinomycetes were assessed. By perpendicular streak method, primary screenings were performed [29]. Firstly, the perpendicular streak method (primary screening) was used to test actinomycetes strains and it was concluded that three (AW3, AM1, and AC1) out of seven strains (AW1, AW2, AW3, AM1, AC1, AW4, and AC2) (see Table3) showed activity against test *S. aureus* and *E. coli* (gram-positive and gram-negative bacteria). This broad-spectrum activity of actinomycetes may be attributable to the production of more than one antimicrobial compound, which compels the strains more effectively against the both tested bacteria [31]. An overall 42.5% activity of actinomycetes (three out of seven strains) was recorded and founding was in close agreement with Seipke et al. [34] who stated 45 % of isolates; whereas Remya and Kumar [35] reported that 47 % of isolates showed broad-spectrum activity in the primary screening. However, a lower antimicrobial activity of actinomycetes of 38 % (51 out of 134 actinomycetes) were compared to this study, which was reported by Sharma et al. [36]. Similarly, findings of low antimicrobial activity of actinomycetes were reported by [37, 38]. Moreover, Belyagoubi et al. [39] reported a very high level of 72.86 % of antimicrobial activity of actinomycetes as compared to this study's findings.

Table 3. Primary Screening against Bacterial Isolates

Bacterial Isolate	Actinomycetes and zone of inhibition (mm)						
	AW1	AW2	AW3	AW4	AM1	AC1	AC2
Staphylococcus aureus	10.5	0	10.5	0	20.5	20.6	0
Escherichia coli	0	10.5	20.5	0	10.5	20.8	0

Table 4. Secondary Screening Results

Bacterial Isolate	Actinomycetes		
	AW3	AM1	AC1
Staphylococcus aureus	-	-	+
Escherichia coli	-	-	+

For secondary screening, three actinomycetes (AW3, AM1, and AC1) isolates were selected based on the primary screening (see Table 4). The Agar well method was used to perform secondary screening of active

isolates [28, 29]. A finding of secondary screening was conclusive. In all three actinomycete isolates only Actinomycetes AC1 showed great potential for antimicrobial activity as compared to those strains, which exhibit activity in primary screening but did not give antimicrobial activity in secondary screening. The reason behind this is maybe when growing on a solid medium the modification in the morphology of actinomycetes (filamentous mycelia) while in liquid broth (fragmenting mycelia) [28] and mostly actinomycetes are poor fermenters. Actinomycetes release active compounds, which became inactive or stick to the liquid medium component or in broth they modified chemically is also the possibility [31, 40].

Table 5. Zone of Inhibition in mm with Ethyl Acetate Extract

Bacterial Isolate	Zone of inhibition (mm)		
	20µL	50 µL	100 µL
Staphylococcus aureus	3.1	5.5	12
Escherichia coli	2.9	5.1	10

Different solvents were used for the isolation of antimicrobial metabolites including ethyl acetate, ethanol, chloroform, and methanol from the broth. This method is called a solvent extraction method. Only ethyl acetate extract of actinomycetes AC1 exhibited antimicrobial potential against *Staphylococcus aureus* and *Escherichia coli*. The recorded activity of AC1 against *Staphylococcus aureus* was measured by inhibition zone as 3.1, 5.5 and 12 mm with 20, 50, and 100 mg/L concentrated extracts of ethyl acetate respectively. However, the recorded activity of AC1 against *E.coli* was measured by inhibition zone as 2.9, 5.1, and 10 mm with 20, 50, and 100 mg/L concentrated extracts of ethyl acetate respectively (Table:5). It was noticed in the present study, that fermented broth with other solvents did not extract any antimicrobial metabolite as no inhibition zone was observed with any concentration. Gurung et al. [31] findings with other solvents suggest the failure of metabolite extraction may be due to the existence of polar functional group in a secondary metabolite that form metabolite soluble in water and insoluble in solvent, the use of inappropriate solvents and inadequate shaking of the mixture.

3.1. Conclusion

The current study showed that isolates of Actinomycetes, found in Piplan soil of Mianwali, had the potential to use it as sources for the novel

antibacterial compounds against pathogenic microorganisms of soil. Isolate AC1 exhibited the highest activity in secondary screening against both gram-positive and gram-negative test bacteria. However, further work in this research is required about AC1 for its practical application in this field.

REFERENCES

1. Hirsch CF, Christensen DL. Novel method for selective isolation of actinomycetes. *Appl Environ Microbiol*. 1983;46(4):925–929. https://doi.org/10.1128/aem.46.4.925-929.1983.
2. Chaudhary HS, Yadav J, Shrivastava AR, Singh S, Singh AK, Gopalan N. Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India). *J Adv Pharma Technol Res*. 2013;4(2):118–123. https://doi.org/10.4103%2F2231-4040.111528
3. Adegboye MF, Babalola OO. Taxonomy and ecology of antibiotic producing actinomycetes. *Afr J Agric Res*. 2012;7(15):2255–22561. https://doi.org/10.5897/AJARX11.071
4. Boroujeni ME, Arijit D, Prashanthi K, Sandeep S, Sourav B. Enzymatic screening and random amplified polymorphic DNA fingerprinting of soil streptomycetes isolated from Wayanad district in Kerala, India. *J Biol Sci*. 2012;12(1):43–50. https://doi.org/10.3923/jbs.2012.43.50
5. Jeffrey LS. Isolation, characterization and identification of actinomycetes from agriculture soils at Semongok, Sarawak. *Afr J Biotechnol*. 2008;7(20): 3697–3702
6. Sprusansky O, Stirrett K, Skinner D, Denoya C, Westpheling J. The bkdR gene of Streptomyces coelicolor is required for morphogenesis and antibiotic production and encodes a transcriptional regulator of a branched-chain amino acid dehydrogenase complex. *J Bacteriol*. 2005;187(2):664–671. https://doi.org/10.1128/jb.187.2.664-671.2005
7. Yamamoto Y, Kouchiwa T, Hodoki Y, Hotta K, Uchida H, Harada KI. Distribution and identification of actinomycetes lysing cyanobacteria in a eutrophic lake. *J Appl Phycol*. 1998;10:391–397. https://doi.org/10.1023/A:1008077414808

8. Singh R, Dubey AK. Diversity and applications of endophytic actinobacteria of plants in special and other ecological niches. *Front Microbiol*. 2018;9:e1767. https://doi.org/10.3389/fmicb.2018.01767

9. Tandale A, Khandagale M, Palaskar R, Kulkarni S. Isolation of pigment producing actinomycetes from soil and screening their antibacterial activities against different microbial isolates. *Int J Curr Res Life Sci*. 2018;7(6):2397–2402.

10. Arifuzzaman M, Khatun MR, Rahman H. Isolation and screening of actinomycetes from Sundarbans soil for antibacterial activity. *Afr J Biotechnol*. 2010;9(29):4615–4619.

11. Ayoubi H, Mouslim A, Moujabbir S, et al. Isolation and phenotypic characterization of actinomycetes from Rabat neighborhood soil and their potential to produce bioactive compounds. *Afr J Microbiol Res*. 2018;12(8):186–191. https://doi.org/10.5897/AJMR2017.8761

12. Kirby BM, Roes-Hill ML, Cary SC, Burton SG, Tuffin IM, Cowan DA. Actinobacterial Diversity Associated with Antarctic Dry Valley Mineral Soils. In: de Bruijn FJ, ed. *Handbook of Molecular Microbial Ecology II: Metagenomics in Different Habitats*. Wiley-Blackwell; 2011:125–33. https://doi.org/10.1002/9781118010549.ch13

13. George M, Anjumol A, George G, Hatha AM. Distribution and bioactive potential of soil actinomycetes from different ecological habitats. *Afr J Microbiol Res*. 2012;6(10):2265–2271. https://doi.org/10.5897/AJMR11.856

14. Berdy J. Bioactive microbial metabolites. *J Antibiot*. 2005;58(1):1–26. https://doi.org/10.1038/ja.2005.1

15. Chen Y, Zhou D, Qi D, Gao Z, Xie J, Luo Y. Growth promotion and disease suppression ability of a Streptomyces sp. CB-75 from banana rhizosphere soil. *Front Microbiol*. 2018;8:e2704. https://doi.org/10.3389/fmicb.2017.02704

16. Gallagher KA, Fenical W, Jensen PR. Hybrid isoprenoid secondary metabolite production in terrestrial and marine actinomycetes. *Curr Opinion Biotechnol*. 2010;21(6):794–800. https://doi.org/10.1016/j.copbio.2010.09.010

17. Igarashi Y. Screening of novel bioactive compounds from plant-associated actinomycetes. *Actinomycetologica*. 2004;18(2):63–66. https://doi.org/10.3209/saj.18_63

18. Bonjar GS. Broadspectrim, a Novel Antibacterial from Streptomyces sp. *Biotechnology*. 2004;3:126–130.

19. Srivastava S, Patel JS, Singh HB, Sinha A, Sarma BK. Streptomyces rochei SM 3 induces stress tolerance in chickpea against Sclerotinia sclerotiorum and NaCl. *J Phytopathol*. 2015;163(7-8):583–592. https://doi.org/10.1111/jph.12358

20. Seipke RF, Kaltenpoth M, Hutchings MI. Streptomyces as symbionts: An emerging and widespread theme? *FEMS Microbiol Rev*. 2012;36(4):862–876. https://doi.org/10.1111/j.1574-6976.2011.00313.x

21. Seong CN, Choi JH, Baik KS. An improved selective isolation of rare actinomycetes from forest soil. *J Microbiol*. 2001;39(1):17–23.

22. Oskay M. Comparison of Streptomyces diversity between agricultural and non-agricultural soils by using various culture media. *Sci Res Essays*. 2009;4(10):997–1005.

23. Taechowisan T, Peberdy JF, Lumyong S. Chitinase production by endophytic Streptomyces aureofaciens CMUAc130 and its antagonism against phytopathogenic fungi. *Ann Microbiol*. 2003;53(4):447–462.

24. Ali B, Sabri AN, Ljung K, Hasnain S. Quantification of indole-3-acetic acid from plant associated Bacillus spp. and their phytostimulatory effect on Vigna radiata (L.). *World J Microbiol Biotechnol*. 2009;25:519–526. https://doi.org/10.1007/s11274-008-9918-9

25. Holt JG. *Bergey's manual of systemic bacteriology*. UK: Cambridge University Press; 1989.

26. Hemashenpagam N. Purification of secondary metabolites from soil actinomycetes. *Int J Microbiol Res*. 2011;3(3):148–156.

27. Bizuye A, Moges F, Andualem B. Isolation and screening of antibiotic producing actinomycetes from soils in Gondar town, North West Ethiopia. *Asian Pacific J Trop Dis*. 2013;3(5):375–381. https://doi.org/10.1016/S2222-1808(13)60087-0

28. Budhathoki S, Shrestha A. Screening of actinomycetes from soil for antibacterial activity. *Nepal J Biotechnol*. 2020;8(3):102–110. https://doi.org/10.3126/njb.v8i3.33664

29. Wahab A, Shumaila S, Subhan SA, Ali ST, Mujahid TY. Isolation and identification of actinomycetes isolated from Karachi soil and screening of antimicrobial compounds. *Int J Curr Res*. 2015;7(2):12760–12765.

30. Subhan SA, Wahab A, Mujahid TY, Abbas T, Khan I, Idrees S. Screening of actinomycetes from indigenous soil for production of extracellular metabolites. *Int J Curr Res*. 2015;7:12078–12083. https://doi.org/10.1016/S2222-1808(13)60087-0

31. Gurung TD, Sherpa C, Agrawal VP, Lekhak B. Isolation and characterization of antibacterial actinomycetes from soil samples of Kalapatthar, Mount Everest Region. *Nepal J Sci Technol*. 2009;10:173–182. https://doi.org/10.3126/njst.v10i0.2957

32. Anwar S, Ali B, Sajid I. Screening of rhizospheric actinomycetes for various in-vitro and in-vivo plant growth promoting (PGP) traits and for agroactive compounds. *Front Microbiol*. 2016;7:e1334. https://doi.org/10.3389/fmicb.2016.01334

33. Basavaraj KN, Chandrashekhara S, Shamarez AM, Goudanavar PS, Manvi FV. Isolation and morphological characterization of antibiotic producing actinomycetes. *Trop J Pharma Res*. 2010;9(3):231–236. https://doi.org/10.4314/tjpr.v9i3.56282

34. Seipke RF, Kaltenpoth M, Hutchings MI. Streptomyces as symbionts: an emerging and widespread theme? *FEMS Microbiol Rev*. 2012;36(4):862–876. https://doi.org/10.1111/j.1574-6976.2011.00313.x

35. Remya M, Vijayakumar R. Isolation and characterization of marine antagonistic actinomycetes from west coast of India. *Med Biol*. 2008;15(1):13–19.

36. Sharma D, Kaur T, Chadha BS, Manhas RK. Antimicrobial activity of actinomycetes against multidrug resistant Staphylococcus aureus, E. coli and various other pathogens. *Trop J Pharma Res*. 2011;10(6):801–808. https://doi.org/10.4314/tjpr.v10i6.14

37. Pandey B, Ghimire P, Agrawal VP. Studies on the antibacterial activity of the Actinomycetes isolated from the Khumbu region of Nepal. *J Biol Sci*. 2004;23:44–53.

38. Rana S, Salam MD. Antimicrobial potential of actinomycetes isolated from soil samples of Punjab. India. *J Microbiol Exp*. 2014;1(2): 63–68. https://doi.org/10.15406/jmen.2014.01.00010

39. Belyagoubi L, Belyagoubi-Benhammou N, Jurado V, et al. Antimicrobial activities of culturable microorganisms (actinomycetes and fungi) isolated from Chaabe Cave, Algeria. *Int J Speleol*. 2018;47(2):189–199. https://doi.org/10.5038/1827-806X.47.2.2148

40. Aedms H, Sheir DH, Eldewany AI. Production of antimicrobial agent from marine bacteria Isolated from Mediterranean. *Aust J Basic Appl Sci*. 2011;5(5):121–128.