Research Article

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Isolation and identification of promising antibiotic-producing bacteria

https://doi.org/10.1515/chem-2022-0233
received September 18, 2022; accepted October 18, 2022

Abstract: Multiple stresses in waste dumpsite soils can drive antibiotic production as one of the strategies for survival. Bacteria are the most prolific producers of antibiotics. This study investigated the antibiotic production potential of bacteria isolated from Bahir Dar city municipal solid waste dumpsite (MSWDS). Bacteria were isolated from soil collected from the dumpsite on starch casein or nutrient agar. The isolates were carefully screened for antimicrobial activity against six pathogenic bacterial test strains. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined from cell-free metabolites of the most promising isolates. Isolates showing antimicrobial activity were identified using cultural and biochemical methods. A total of 143 distinctive colonies were obtained and tentatively identified to 13 bacterial genera. Twenty-six (18.18%) of the isolates (six Bacillus and 20 actinobacteria related) demonstrated antimicrobial activities at least against one of the tested bacterial strains. These isolates were related to two actinobacterial and 11 other bacterial genera. Seven out of 26 isolates showed a broad-spectrum of antibiotic activities. Two isolates, which showed a wide spectrum, were selected for the MIC and MBC tests against *Escherichia coli* and *Staphylococcus aureus*. The MIC and MBC of the isolates were recorded to be 250–500 μg/mL against the test strains. Bahir Dar city MSWDS contained a high incidence of antibiotic-producing bacteria. Strain level identification of the isolates and detailed characterization of the metabolites will give a good insight into the antimicrobial production potential in the waste dumpsite.

Keywords: actinobacteria, antibiotics, bacteria, Bahir Dar city, municipal solid waste

1 Introduction

Natural products of microbes and plants have been used for centuries as a source of antibiotics for the treatment of various infectious diseases [1]. In particular, those of microbial origin have been the most important sources of antibiotics and are currently in use to a large extent. Several groups of microbes, such as bacteria, fungi, and actinomycetes produce antibiotics to kill or inhibit other competitive microbes [2]. For centuries, these antibiotics have been used to treat several bacterial diseases. However, antibiotic-resistant pathogenic bacteria emerge at a high rate and this has been a major international health concern and threat for decades [3]. Primarily, antibiotic-resistant pathogenic bacteria result in high mortality and pose a serious public health burden [4]. Thus, continued efforts to search for new antimicrobial products that are effective versus resistant microbes are one of public health priority research areas in order to tackle the associated disease burden at the national and global levels [5].

Antimicrobial-producing bacteria have been isolated from various environments. However, soil is found to be the hot spot to easily retrieve antimicrobial-producing bacteria. Soil is a very heterogeneous habitat and is rich in diverse microorganisms [6]. There is also a high variation in biotic and abiotic conditions in soils that challenge the microbiota. Accordingly, soil microbes have to face challenges by developing strategies like

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the production of antimicrobials for survival [7]. As a consequence, the soil has been the primary source of antibiotic-producing microorganisms [8,9].

Similarly, municipal solid waste dumpsite (MSWDS) soils are potential sources of antibiotic-producing microbes. High levels of competition and the synthesis of extracellular products among microorganisms in solid waste dumpsites result from the pressures caused by the abundance of microorganisms in these environments. As a result, solid waste dumpsites have been identified as an important source of biotechnologically significant microorganisms, including antibiotic producers, and there is a high chance of detecting novel antibiotic producers in such an environment.

The local climate and the type of waste dumped in one area vary in other places, which influence microbial growth and survival strategies. This means that microbial structure can be affected by a wide range of circumstances, including geographical location, waste composition, and nutrient availability [10,11]. Moreover, bacterial antibiotic production capacity might differ greatly between locations and can shift dramatically over time. Thus, microbes need to be explored for their antimicrobial production potential from various locations and at different times because of their public health significance.

Despite the massive amounts of solid waste generated in municipalities in developing countries such as Ethiopia, there has been little research on antibiotic-producing bacteria from dumpsites. This study thus aimed to isolate potential bacteria that produce antimicrobials from Bahir Dar city MSWDS. The study demonstrated that MSWDS could be a potential source of antibiotics.

2 Materials and methods

2.1 Study area description

This work was carried out at Bahir Dar City, located in northwest Ethiopia, some 565 km far from Addis Ababa, the country's capital. The study site, which is the solid waste located in Bahir Dar city is 11° 32′ 28.5″ and 11° 32′ 37″N latitude and 37° 23′ 15″ and 37° 23′ 24″E longitude. It is located around 7 km west of the city at a height of 1,790 m above sea level and has approximately 22 ha. The average annual temperature ranges from 13.5 to 27.7°C, and the average annual precipitation is around 1,500 mm, with 54% of the precipitation falling in July and August, when monthly precipitation may exceed 250–300 mm.

More than 98.8 tons of trash are produced every day in Bahir Dar, Ethiopia. The garbage comes from a variety of sources like homes (54%), businesses (24.2%), institutions (17%), and street cleaners (3.56%) [12]. Due to the "unrestricted" disposal technique, wastes including old prescription medications were discovered strewn around the dumping site during sampling.

2.2 Sampling sites and sample collection

In order to get a representative sample of the trash dumped at Bahir Dar's MSWDS, three locations inside the dump were chosen at random. Once the area was cleared of debris, a hole was bored to a depth of 4–6 cm and nine soil samples, each weighing 50 g, were gathered using a sterile spatula and put in ziplock polythene bags [13]. The samples were collected in three rounds each month from February to April 2021. Once the soil samples were obtained, they were placed in a cool box and sent to the Bahir Dar University Microbiology Laboratory for examination.

2.3 Isolation and identification of bacteria

Isolation and identification of bacteria were conducted based on the procedures in a previous study by Sitotaw et al. [14]. The bacteria were extracted from soil samples using a serial dilution method. In each cycle of sampling, 5 g of soil was collected from each location and combined to create a single representative sample. One gram of the homogenized soil sample was combined with 9 mL of sterile normal saline solution (0.850% NaCl). The test tube was whirred for 1 full minute to create the suspension. Serial dilution was made to obtain 10^{-5}–10^{-9} dilutions, from which 0.1 mL of the suspension was spread on starch casein agar for actinobacteria isolation (HiMedia, India) or nutrient agar for the isolation of other bacteria (Merck, Germany) using bent glass spreaders. The dilutions were tested on triplicate plates, which were then incubated at 28°C for 3 days (for actinobacteria) or for 24–48 h (for other bacteria). The streak plate technique was used to collect and purify colonies with varying morphologies on their various substrates. All of the pure isolates were stored in nutritional broth (Merck, Germany) at 4°C for future study and characterization. Standard colony characteristics and standard biochemical tests were

conducted to identify the isolated strains to the genus level [15].

2.4 Standardization of the inoculum for antibiotic production

To make a 0.5 McFarland standard, we mixed together 0.50 mL of 0.048 mol/L (1.1750% w/v) dehydrated barium chloride solution with 99.50 mL of 0.18 mol/L (1% v/v) sulfuric acid (H_2SO_4). Notably, the turbidity standard solution was aliquoted into test tubes. The absorbance at 625 nm for the 0.5 McFarland Standard was between 0.08 and 0.10. The standard solution was kept in an airtight tube at room temperature to avoid loss of concentration due to evaporation and light. The turbidity standard tube was thoroughly mixed with a vortex mixer to provide a consistent turbid look before comparison with the bacterial suspension [16].

2.5 Inoculum preparation and inoculation procedures for antibiotic production

After an overnight incubation, 5 mL of a bacterial culture (0.5 McFarland) was suspended in nutritional broth (HiMedia, India) and incubated at 37°C for 4 h. A sterile cotton swab was used to adjust the turbidity, and the inoculum was spread evenly throughout the agar medium by rotating the plate by 60° [17].

2.6 Primary screening for antibiotic production

The inhibitory metabolite-producing abilities of the isolates were preliminarily screened *in vitro* against test bacterial strains by employing a transverse pattern on agar plates. Those isolates were horizontally streaked to test for antibiotic production at the diameter of the Muller-Hinton Agar (MHA) medium (Accumix, India) and incubated at 28 ± 2°C for 24–48 h.

Following incubation, bacterial strains, namely *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Streptococcus pyogenes* (ATCC® 19615™), *Enterococcus faecalis* (ATCC® 51299™), and *Pseudomonas aeruginosa* (ATCC 27853) were scattered vertically (at a 90° angle), close to the screened isolates. Next, the plates were then kept at 37°C for 24 h. It was shown that there were zones of inhibition between the antibiotic-producing isolates and the test organisms that were thought to generate antibiotics [18].

2.7 Antibacterial compound production

Isolates showing antimicrobial activity in the preliminary screening were further tested in a small-scale submerged fermentation state. Two hundred milliliters of starch casein or nutrient broth were dispensed into separate 500 mL Erlenmeyer flasks. A loopful of 7-day-grown actinomycete isolates and 24 h grown other bacterial isolates were inoculated in the respective broths. The cultures were then put on a shaker at 200 rpm and room temperature for 10 days (for actinomycetes) and 3 days (for other bacteria). After 10 days, the contents of the flasks that had been incubated were filtered by use of Whatman No. 1 filter paper. The culture filtrates were added to an equal amount of ethyl acetate (1:1) and shook hard for 1 h. A separator funnel was used to separate the aqueous phase from the solvent phase, which is thought to contain an antibiotic compound (Assistant, Germany). A rotary evaporator was then used to concentrate the antibiotics in the ethyl acetate phase [19].

2.8 Antibacterial activity test

The filtered cell-free extracts of each isolate were selected for antibacterial activity using the disc diffusion method. The inoculum was prepared as described earlier. After adjusting the turbidity, bacterial strains, viz., *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 700603), *S. pyogenes* (ATCC® 19615™), *E. faecalis* (ATCC® 51299™), and *P. aeruginosa* (ATCC 27853) were swabbed on sterile MHA medium (Accumix, India) using a sterile cotton swab, and left for 5–10 min. Sterile Whatman paper No. 1 discs having a 6 mm diameter were immersed in each cell-free extract for 30 min [20]. Discs treated with the cell-free extracts and the selected standard antibiotic disc were applied in triplicate on pre-inoculated MHA medium and left for 15–20 min to allow the diffusion of the metabolite, then incubated at 37°C for 24 h without inverting the plates. After incubation, the zone of inhibition (mm) around each disc was measured and recorded. Gentamicin standard disc (GN, 10 µg) was used as a

positive control, and a disc immersed in cell-free culture that was not inoculated was used as a negative control.

From cell-free metabolites of the most promising isolates, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined. Two bacterial test strains, one from Gram-positive (*S. aureus*) and another from Gram-negative (*E. coli*) were selected. The cell-free metabolite that was extracted and concentrated was dissolved in nutrient broth (2,000 µg/mL). Next, from this stock solution, a two-fold dilution was made ranging from 1,000 to 2 µg/mL [21]. For the assay, an equal amount of nutrient broth (1 mL) was added to 11 test tubes. The different amounts of the extract and 0.1 mL of the standardized inoculum of the bacterial test strains were added into the ten test tubes. In one of the 11 test tubes, 0.1 mL of distilled sterilized water was used as a negative control, and all the 11 test tubes were incubated at 37°C for 18–24 h. After the incubation period had passed, the MIC value was calculated by examining the progression of the bacterial growth in the test tube. A total of 0.1 mL was dispensed from the test tubes that exhibited no turbidity and showed no signs of growth to cover the MHA plates. After incubation at 37°C for 24 h, the MBC was determined by observing the colonies. All experiments were done in triplicate.

2.9 Data analysis and interpretation

The antibacterial activities of the isolates were evaluated by measuring the diameter of the inhibition zone in millimeter. The data collected were analyzed using descriptive statistics and reported as mean ± SD after three repeats of the experiment. The results were then presented in tables and figure.

3 Results

3.1 Characterization and identification of bacterial isolates

In the present study, 143 distinct colonies were isolated and characterized based on colony characteristics and standard biochemical tests. Based on a series of other biochemical assays, isolates were tentatively identified as one of the 13 bacterial genera, namely *Streptomyces*, *Actinomyces*, *Bacillus*, *Staphylococcus*, *Micrococcus*, *Pseudomonas*, *Klebsiella*, *Citrobacter*, *Proteus*, *Escherichia*, *Enterobacter*, *Salmonella*, and *Shigella*. Isolates related to *Staphylococcus* and *Streptomycetes* species were the most dominant amongst the bacterial and actinobacterial isolates, respectively (Figure 1).

3.2 *In vitro* screening and evaluation of bacteria for antibiotic production

Six out of the 73 bacterial and 20 out of 70 actinobacterial isolates were shown to have antibiotic production potential against bacterial test strains (Table 1), and all of the

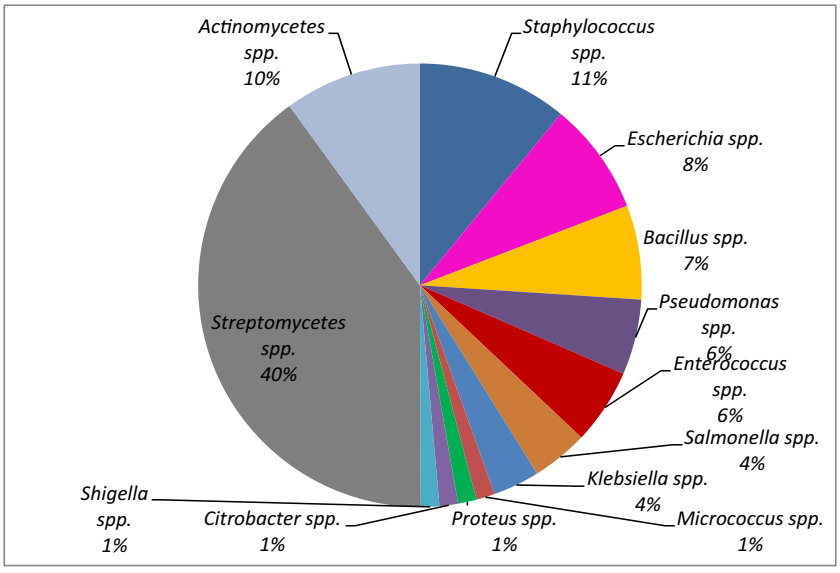

Figure 1: Percentage of the isolated bacteria from Bahir Dar city MSWDS, 2022.

Table 1: Primary screening of bacterial isolates for antimicrobial production versus selected pathogenic bacterial strains from Bahir Dar City MSWDS, Ethiopia, 2022

Isolates*	Gram-positive			Gram-negative		
	S. aureus (ATCC® 25923)	*S. pyogenes* (ATCC® 19615™)	*E. faecalis* (ATCC® 51299™)	*E. coli* (ATCC® 25922)	*P. aeruginosa* (ATCC® 27853)	*K. pneumoniae* (ATCC® 4352)
B5	+	NT	NT	+	+	+
B7	+	NT	NT	—	—	—
B9	+	NT	NT	+	—	—
B15	+	NT	NT	—	—	—
B16	+	—	+	+	—	—
B24	+	—	—	—	+	—
A2	+	—	+	—	—	—
A4	+	+	+	+	—	—
A7	+	—	—	—	—	—
A10	+	—	+	—	+	—
A11	—	—	+	—	—	—
A15	—	+	+	—	—	—
A23	+	—	+	—	—	—
A31	+	—	—	+	—	—
A37	—	—	—	—	+	—
A40	—	—	—	+	—	—
A42	+	—	—	+	—	—
A44	+	—	—	—	—	—
A46	+	—	+	+	+	+
A48	—	+	+	+	—	—
A49	+	—	—	—	+	—
A51	—	—	—	+	—	—
A59	—	+	—	—	—	—
A60	—	+	—	—	—	—
A63	—	+	—	+	—	—
A68	+	—	—	—	+	—

Keys: + denotes presence of inhibition zone; — denotes no clear zone; *only those isolates that showed antimicrobial activities are presented here.

bacterial isolates belong to *Bacillus* spp., while the actinobacterial isolates were related to *Streptomycetes* and *Actinomycetes* species.

The *in vitro* antibacterial activities of cell-free extracts from the isolates were further evaluated using the disk diffusion assay, as shown in Table 2. In this investigation, antimicrobials produced by the bacterial isolate showed variable zones of inhibition against Gram-positive and Gram-negative test strains. The inhibition zones of the extracts by some isolates were even higher compared to the positive control (Table 3).

As shown in Table 2, the extracts of the selected isolates have shown antibacterial activities against the six test strains with maximum zones of inhibition (in mm) 25.0 ± 1.0, 24.3 ± 1.5, 25.6 ± 1.0, 26.0 ± 1.0, 25.0 ± 2.0, and 24.7 ± 1.5 against *S. aureus*, *K. pneumoniae*, *E. coli*, *S. pyogenes*, *P. aeruginosa*, and *E. faecalis*, respectively.

Table 2: Determination of MIC and MBC (µg/mL) of the crude extracts from selected isolates of Actinomycetes

Isolates	MIC		MBC	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
A4	250	500	250	500
A46	250	250	500	500

3.3 Determination of MIC and MBC

Actinomycete isolates designated as A4 and A46, which inhibited 4 and 5 of the six test strains, respectively, were selected to determine their MIC and MBC. The MIC and MBC of crude extracts from the isolates against test strains are indicated in Table 2. The MIC of the crude extract from both isolates was 250 µg/mL against

Table 3: Antimicrobial activities of bacteria (mean ± SD of the clear zone in mm) isolated from Bahir Dar city MSWDS against selected pathogenic bacterial strains $n = 3$, 2022

Cell-free extracts and positive control	Zone of inhibition in mm					
	Gram-positive			Gram-negative		
	S. aureus	*S. pyogenes*	*E. faecalis*	*K. pneumoniae*	*E. coli*	*P. aeruginosa*
B5	13.26 ± 0.25	NT	NT	6.16 ± 0.25	11.66 ± 0.57	12.40 ± 0.10
B7	12.46 ± 0.15	NT	NT	—	—	—
B9	12.76 ± 0.37	NT	NT	—	10.56 ± 0.66	—
B15	13.23 ± 0.37	NT	NT	—	—	—
TB16	24.0 ± 1.0	—	22.0 ± 1.0	—	20.0 ± 1.0	—
TB24	22.0 ± 1.0	—	—	—	—	24.3 ± 1.5
A2	11.3 ± 2.1	—	17.0 ± 1.0	—	—	—
A4	22.7 ± 1.5	23.0 ± 1.0	23.0 ± 1.0	—	23.0 ± 1.0	—
A7	13.3 ± 1.5	—	—	—	—	—
A10	23.0 ± 1.0	—	23.0 ± 1.0	—	—	22.0 ± 1.0
A11	—	—	24.0 ± 1.0	—	—	—
A15	—	24.0 ± 1.0	24.3 ± 1.2	—	—	—
A23	24.0 ± 1.0	—	24.7 ± 1.5	—	—	—
A31	23.3 ± 0.6	—	—	—	23.3 ± 1.5	—
A37	—	—	—	—	—	16.3 ± 14.2
A40	—	—	—	—	23.0 ± 1.5	8.0 ± 13.9
A42	23.0 ± 1.0	—	24.3 ± 1.2	—	22.3 ± 1.5	—
A44	23.3 ± 1.5	—	—	—	—	—
A46	25.0 ± 1.0	—	24.0 ± 1.0	24.3 ± 1.5	24.7 ± 1.2	25.0 ± 2.0
A48	—	23.7 ± 1.5	23.0 ± 1.0	—	24.0 ± 1.0	—
A49	24.3 ± 1.5	—	—	—	—	24.7 ± 1.2
A51	—	—	—	—	25.6 ± 1.0	—
A59	—	25.0 ± 2.2	—	—	—	—
A60	—	26.0 ± 1.0	—	—	—	—
A63	—	24.0 ± 1.0	—	—	24.3 ± ± 1.5	—
A68	24.3 ± 1.5	—	—	—	—	24.0 ± 1.0
GN (10 µg)	25.13 ± 0.23	25 ± 0.34	26 ± 0.11	21.60 ± 0.36	23.40 ± 0.10	19.80 ± 0.20
NC	—	—	—	—	—	—

Key: – = no observed inhibition zone; NT = not tested; GN = gentamicin; NC = negative control (a disc immersed in cell-free culture which was not inoculated).

S. aureus. However, the MIC of the crude extract against *E. coli* varied between the isolates. Similarly, the MBC of the crude extract from A4 was lower (250 µg/mL) against *S. aureus* compared to *E. coli* (500 µg/mL). The MBC of crude extract from A46 was the same for both test strains (Table 2). Both isolates were identified as *Streptomycete* species. It is to be noted that the MIC and MBC values were similar for the triplicate samples.

4 Discussion

The emergence of antibiotic-resistant bacteria at an alarming rate poses the biggest threat to global health, and as a result, new antibiotic discoveries have always been among the top priority research areas. A study by Sitotaw et al. [14] in the same area (Bahir Dar City MSWDS) revealed a high prevalence of antibiotic-resistant bacteria. With this view, this study was conducted in order to provide a contribution of scientific knowledge by isolating potential antibiotic-producing bacteria from a solid waste dumpsite in Bahir Dar city. In this study, high antibiotic production potential was observed among bacterial isolates retrieved from Bahir Dar City MSWDS.

The isolates belong to several genera of bacteria, among which, *Staphylococcus*, *Escherichia*, *Bacillus*, *Pseudomonas*, and *Enterococcus* species were the most frequently encountered groups. Similarly, actinobacterial isolates were related to *Streptomycetes* (80%) and *Actinomycetes* (20%) species. The recovery of members of these genera from the dumping site was also

reported in the previously conducted studies in Ghana [8], Kenya [22], India [23], and Nigeria [24]. Besides these genera, Chetan et al. [23] isolated *Serratia*, *Arthrobacter*, *Streptococcus*, *Corynebacterium*, and *Aeromonas* species from the solid waste dumpsite. Moreover, Song'oro et al. [22] isolated *Vibrio cholerae*, *Enterobacter*, *Serratia*, *Shigella*, *Salmonella*, *Providencia*, *Yersinia*, and *Morganella* species from the waste dumpsite soil. The compositions of the wastes that were dumped, the physicochemical characteristics of the soil at the dumpsite, as well as the geographic and seasonal considerations among the study areas may determine the type of bacteria recovered from waste dumpsite. This suggests that a diverse community of soil bacteria may develop at a dumpsite due to the environmental variation present there [25].

The presence of human-associated bacteria in the waste dumpsite is clearly linked to the wastes of human origin [26]. The majority of the bacterial isolates related to *Staphylococcus*, *Pseudomonas*, *Klebsiella*, *Citrobacter*, *Proteus*, and *Escherichia* isolated in this study were reported earlier [7,9,27,28] as potential pathogens from the dumping site, which is in agreement with the results of this finding. Furthermore, Williams and Hakam [29] isolated *Bacillus* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *S. aureus*, and *Streptococcus* spp. from dumpsites in Port-Harcourt metropolis, Nigeria. The presence of these potential pathogens reported in previous and current investigations may be attributed to the disposal of complex wastes from various sources in the municipal waste dumping site [26].

The predominance of *Bacillus* species in the dumpsite soil can be accounted for different factors. *Bacillus* species possess a broad spectrum of physiological capacities, secrete a variety of extracellular enzymes, survive in extreme physical and chemical environments due to their endospores, and create metabolites with antagonistic effects on other microbes [30]. These traits allow the organism to flourish in a wide variety of settings and endure adverse situations, such as the selection pressure that pollution places on some types of soil bacteria. Similarly, regarding, *Pseudomonas*, it is a common genus that can be found in a variety of environments [28,30].

The results obtained from the primary screening step indicated that 26 out of 143 isolates showed antibacterial activity against both Gram-negative and Gram-positive test bacteria. The isolates demonstrating antimicrobial activities belong to *Bacillus*, *Streptomycetes*, and *Actinomycetes* species, with some differences in cultural characteristics. As presented in Table 2, seven isolates exhibited broad-spectrum antibacterial activity against both Gram-positive and Gram-negative tested bacteria. These diverse *in vitro* antagonistic features may be due to the multiple modes of action of the isolates against test strains. Actinobacteria are well recognized for their potential in the production of antibiotic compounds [31,32]. Similarly, a considerable proportion (28.6%) of the actinomycete isolated in this study showed antimicrobial activity against one or more test bacterial strains. However, this proportion is lower compared to previously reported data. For instance, a higher proportion was reported by Sapkota et al. [17] and Chaudhary et al. [5].

The findings of this study are also in line with several other scientific reports where *Bacillus* spp. is known to produce more than 800 bioactive secondary metabolites, some of which are used as pharmacological agents [28].

Among all the screened bacterial isolates, maximum inhibitions with broad activity against Gram-positive as well as Gram-negative bacterial strains were shown by isolates A4 and A46, as shown in Table 2. This suggests that these isolates may possess diverse mechanisms of action in combating and eliminating pathogenic bacterial strains.

Isolate B5 (*Bacillus* spp.) has also shown a wide spectrum of inhibition. Comparable inhibition of these pathogens by a *Bacillus* spp. was also reported by Ramachandran et al. [33]. A lower inhibition zone, than that was recorded in this study, by some *Bacillus* species against common pathogens was also reported by Prashanthi and Shreevatsa [34]. This may reflect, in part, a better understanding of variations in the strains of antibiotic-producing bacteria with their diverse bioactive secondary metabolites due to geographical variations and available nutrients. The results of the present findings proved that *Bacillus* species have the potential to produce a variety of bioactive secondary metabolites against a wide spectrum of microbial growth in different conditions.

The MIC and MBC values of the crude extract from the A4 culture were the same. In most cases, MIC is lower than MBC. However, there are reasons for MIC to be equal to MBC. This may depend on the organism and the mode of action of the antimicrobial agent. For example, if the agent becomes more toxic, MIC and MBC will approach and even equal each other, and vice versa. Another possible reason may be that the peptide/product may not be soluble in the nutrient broth and give a wrong value of MIC, which can be solved by adding more buffers and less culture medium. The MIC and MBC values of the crude extract in this study were lower than those reported by Gurung et al. [35], who documented the MIC values of

1,000 µg/mL. The variations in the MIC and MBC values could be attributed to the concentration process of the extract and the nature of the solvent used [35], or variations in the test organisms used and several other parameters [36].

5 Conclusion

The present study further confirmed that *Streptomycetes*, *Actinomycetes*, and *Bacillus* species that inhabit waste dumpsite soil are important sources of antibiotics against Gram-positive and Gram-negative bacterial strains. Further purification and production optimization will give more insight into the real application of the metabolites.

Acknowledgments: The authors would like to extend their sincere appreciation to the Researchers Supporting Project, King Saud University, Riyadh, Saudi Arabia for funding this work through project number (RSP2022R457). We highly acknowledge Amhara Public Health Institute for providing the bacterial test strains.

Funding information: This work is financially supported by King Saud University, Riyadh, Saudi Arabia through project number (RSP2022R457).

Author contributions: All authors designed the project, carried out the experiments, analyzed the data, drafted and edited the manuscript, and read and approved the final manuscript.

Conflict of interest: The authors declare that there are no conflicts of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: All data used to support the finding are included within the article.

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