Antibacterial metabolite prospecting from Actinomycetes isolated from waste damped soils from Thika, central part of Kenya

Abebe Bizuye1,2*, Christine Bii3, Gatebe Erastus4, Naomi Maina2,5
1Department of Biology, College of Natural and computational Sciences, University of Gondar, Gondar, Ethiopia
2Molecular and Biotechnology, Pan African University Institute of Basic Sciences, Innovation and Technology, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
3Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya
4Kenya Industrial Research Development and Innovation, Nairobi, Kenya
5Department of Biochemistry, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

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Objective: To evaluate antibacterial activity of the metabolites produced by actinomycetes isolates isolated from waste damped soil in Thika.

Methods: Soil samples were collected randomly from selected waste damping sites and composite soil samples were prepared. Composite soil samples were pre-treated with dry heat and CaCO3. 0.1 mL of soil suspension from 10^-5 serial diluted composite soil sample spread on selective media for selective growth and isolation. The primary and secondary screenings and evaluation of antibacterial active isolates were done by streak plate and well diffusion assay, respectively.

Results: 29 (23.2%) isolates showed antibacterial activity during primary screening. From these, isolate KGT22 showed 30 ± 0 mm, 31.3 ± 0.6 mm, 30 ± 0 mm and 36 ± 1 mm inhibition zone against E. coli ATCC 25922, S. boydii, S. typhi and V. cholerea, respectively. Isolate KDO24 showed antibacterial activity against both MRSA (16.25 ± 0.50 mm) and E. coli (26.5 ± 0.58 mm). Supernatants from 11 (37.93%) isolates showed antibacterial activity during secondary screening. Supernatant from BML45, KGT31 and PLS 34 showed better inhibition zone (17 ± 1 mm) against E. coli ATCC25922, S. boydii and S. typhi, respectively.

Conclusions: Therefore, antibacterial activity result showed that soils collected from Thika waste damping sites are potential sources of antibacterial producing isolates.

1. Introduction

Actinomycetes (Actinobacteria) are unicellular Gram-positive prokaryotes, filamentous, aerobic bacteria having DNA with high G+C composition[1]. They are widely found in natural environments and widely distributed groups of soil microorganisms[2]. Garden rhizosphere soil[3,4], mangrove forest soil[5], virgin soil[6], agriculture field soil[7], acidic soil[8], compost soil[3] are just list of a few terrestrial places where actinomycetes are found.

Actinomycetes produce secondary metabolites (organic acids, enzymes, antibiotics or antimicrobial compounds) that are industrially, biotechnologically and environmentally valuable compounds. Previous studies have shown that antimicrobial compounds produced by actinomycetes have antiviral[9,10], antifungal and antibacterial[3,5,6] activity.

The current challenge in the treatment of infectious disease is the occurrence of drug resistant in both Gram-negative and Gram-positive bacteria pathogens worldwide. Escherichia coli, Vibrio cholerae, Salmonella enterica, Enterobacter spp. and Staphylococcus aureus are some of multi drug resistant developing bacterial[11]. Moreover, Salmonella species, Shigella species, Vibrio species and E. coli are frequently occurring drug resistant bacteria in East Africa region[12]. In this region, the status of antibacterial agent research against these antibacterial resistant bacteria is inadequate.
In addition to this, majority of the ecological niches of this region still remain unexplored yet for the purpose of searching novel groups of actinomycetes for novel antibacterial discovery.

Thus, the need for effective antibacterial compounds from actinomycete isolated from potential soils that were not researched before is high priority. However, searching of antibacterial compound from actinomycetes isolated from waste damped soil from Thika area has not been investigated. Waste damping areas may be potential niches for actinomycetes due to availability of diverse nutrients. Thus, the main purpose of this study was to isolate actinomycetes from Thika waste dump sites and to determine antibacterial activity of them against selected bacteria pathogens using streak plate method (direct challenge of isolates against pathogens) and well diffusion assay using supernatants from the isolates.

2. Materials and methods

2.1. Study area and sampling sites description

The study area was carried out in Thika industrial and waste dumping areas, found in Thika district located in Kiambu County. Its location has 3°53’ and 1°45’ south latitudes and 36°35’ and 37°25’ east longitudes[13]. Thika is an industrial town, 42 km away from northeast of Nairobi, Kenya. Wastes from industries were damped temporally around the industry compound. Kiganjo (Kang’oki) waste damping area receives both the industrial and municipal wastes. The selected sampling sites for the present study were BIDCO Africa Ltd waste disposal site (BAL), BIDCO Africa Ltd waste damping site (BAD), Poly Sack Ltd waste damping site (PLS), Bakex Millers Ltd waste dumping site (BML), Kiganjo waste damping site one (KDO), Kiganjo waste damping site two (KDT), Kiganjo waste damping site three (KGT). The control site selected was Mangu’ shrubs rehizosphere site (MRS). The geographical location of different soil sampling sites is described in Figure 1.

2.2. Composite soil preparation, analysis and pre-treatment

Nine plots were prepared per site in 16 m² area using GPS coordinate points as a reference point. Soil samples from each plot were collected aseptically from 0-20 cm depth[14] at depth of 0-5 cm, 5-10 cm, 10-15 cm[15]and 15-20 cm[16]. Four composite soil samples were prepared based on depth per site and the samples were packed using sterile polyethylene bag[5,15,16] to minimize soil moisture lose. From 8 sites 32 composite soil samples were prepared, packed and transported to Jomo Kenyata University of Agriculture and

![Figure 1. The Geographical location and representation of the soil sample sites in Thika district, Kenya (prepared by Temitope Idowu). Key: 1 (BAL), 2 (PLS), 3 (BAD), 4 (BML), 5 (KDO), 6 (KDT), 7 (KGT) and 8 (MRS).](image-url)
2.3. Isolation of actinomycetes from composite soil samples

Serial dilution and spread of soil suspension on starch casein agar plate was done according to Bizuye et al.[3], Sudha and Hemalatha[4], and Azira et al.[5] with minor modification. 10 gram of pre-treated composite soil samples were mixed with 90 mL sterilized saline water to make 10⁻¹ stock dilution. Serial dilution was done up to 10⁻⁸ and 0.1 mL suspension from 10⁻⁸ was taken and spread on starch casein agar media supplemented with 100 µg/mL cyclohexamide and 1 µg/mL penicillin (experimental group). Experimental group and negative control (media without soil sample) in duplicates were incubated at 28 °C for 2 weeks and the numbers of colonies were recorded. Different colonies per sample from the plates were subcultured in to starch casein media for growth and the recovered isolates were temporarily preserved at 4 °C for further use.

2.4. Bacterial pathogen suspension preparation and standardization

A total of six test (five Gram-negative and one Gram-positive) bacterial pathogens used for this study were obtained from Kenya Medical Research Institute (KEMRI). These include Escherichia coli ATCC25922, Salmonella typhi, Shigella boydii, Vibrio cholerae, extended spectrum beta-lactamase (ESBL) producing E. coli and Methicillin resistance Staphylococcus aureus (MRSA). Except E. coli ATCC25922, other test pathogens used for this study were clinical isolates. Test bacterial suspension preparation and standardization was done according to Ataee et al.[21]. The test bacterial pathogens were streaked on Muller Hinton agar plate and incubated at 37 °C for 24 h to get pure colonies. Two to three colonies were taken and put in to test tube containing 3 mL sterile water and mixed well. By adding a colony or adding sterile water, each bacterial pathogen suspension was standardized using McFarland standard (0.5%) to use for antibacterial activity screening.

2.5. Primary screening of antibacterial producing actinomycetes

The primary screening was done according to Bizuye et al.[3] and Azira et al.[5] with some modification. The recovered (125) isolates were subcultured in to starch casein broth for three days growth at 28 °C. Each 3 days old isolate was streaked on starch casein plate media horizontally and were incubated at 28 °C for 8 days with negative control (starch casein plate). E. coli ATCC25922, S. typhi, S. boydii and V. cholerae suspensions were streaked perpendicular to the grown actinomycete isolate on starch casein plate and incubated at 37 °C for 24 h.

2.6. Antibacterial activity screening of potential isolates against MRSA and ESBL E. coli by streak plate assay

29 isolates that were showed antibacterial activity during primary screening was also streaked on the plate containing starch casein agar and incubated for 8 days at 28 °C. After 8 days incubation, the 18 h old suspensions of clinical MRSA and ESBL E. coli was streaked perpendicular to each isolates and incubated for 24 h at 37 °C. The antibacterial activity was determined by measuring zone of inhibition.

2.7. Comparison of number of active isolates in relation to incubation period during secondary screening

Secondary screening was done by well diffusion assay using supernatants from starch casein broth cultured isolates that was taken at different day incubation. Isolates (29) showed antibacterial activity during primary screening were selected for secondary screening. Five millilitre of 3 days old seed culture from each active isolates was added in to a flask containing 50 mL sterile starch casein broth and incubated for 13 days at 28 °C. The supernatant from each isolate (experimental group) and starch casein broth (negative control) was collected from centrifuged (at 10 000 r/min for 10 min) cultures taken at 8, 9, 10 and 13 days old. Each pathogen (E. coli ATCC25922, S. typhi, S. boydii and V. cholerae) was swabbed on a sterile Muller Hinton agar. Eighty micro-litres supernatant from isolates, streptomycin (positive control) and starch casein broth (negative control) was added on each well on Muller Hinton agar. The triplicates were done and incubated for 24 h at 37 °C. The zone of inhibition result in millimetre was recorded.

2.8. Data analysis

Data collected from the experiments were analyzed using descriptive analysis by excel and SPSS version 20 and the mean ± SD were calculated. The value of the moisture content, pH, total count of CFU/g of collected soil sample, the distributions of recovered isolates were analyzed using Microsoft excel. The mean ± SD value of inhibition zone of antibacterial activity against selected bacterial pathogens was compared. These comparisons were performed using One-way ANOVA ranked with Tukey’s multiple range tests with descriptive analysis by SPSS version 20. The differences were tested on P < 0.05 (95% probability level) and all statistical values at P < 0.05 are statistically significantly different.

2.9. Ethical consideration

The locations for collection of composite soil samples were waste
disposal and damping sites that were not involved any endangered or protected species, so it was not needed strict and specific permission. The topology, land structure and the plant and animal diversity of the places were not affected by using appropriate sample collection procedure.

3. Results

3.1. Sampling site description, composite soil sample analysis and colony counting

BAL and BAD sampling site has sandy soil with oiled waste dumped and sandy soil with sewage sludge waste disposal area, respectively. BML and PLS sampling sites has loam soil with wheat husk and sandy soil with ashes come from burned oily waste from the industry, respectively. On the other hand, different types of wastes that were transported from different industries and from the town were dumped on Kiganjo (Kangoki) area. KDO, KDT and KGT sites were randomly selected soil sampling sites in this waste damping area. KDO site has dark grey sandy soil with paper bags, wigs and paper wastes damped on it. Sandy soils with charcoal and ashes are the description of KDT site. KGT site has grey sandy soil with oiled slugs, broken glasses, avocado peels damped on it. On the other hand, MRS site has sandy and sticky soil in the shrubs.

A total of 32 composite soil samples were collected from 8 soil sampling sites. As the result indicated, the moisture content, pH and number of CFU/g of composite soil were varied among eight sampling sites. The highest moisture content was recorded from KDT site (48.65 ± 7.26%). Acidic soil was from both BAD (5.74 ± 0.37) and BML (5.95 ± 0.29), while alkaline soil was from KDT site (9.16 ± 0.24). The highest average colony count (16.75 ± 7.32×10^6 CFU/g) was recorded from KDO site. However, no growth was observed from BAD site (Table 1).

3.2. Distribution of recovered isolates with respect to sites

A total of 125 isolates were recovered from 7 sites where the largest number of isolates were recovered from KDO [56 (45%)]. Except from BAL site, all other five sites had greater number of recovered isolates when compared to control (MRS) site. From a total of 125, 29 (23.2%) isolates showed antibacterial activity against more than one selected pathogens where all of active isolates were recovered from six [PLS, BML, KDO, KDT, KGT and MRS (control)] sites. The greater number of active isolates were recovered from both PLS [8 (28%)] and KDO [8 (28%)] sites when compared to other sites and control or MRS site [3 (10%)]. According to this experiment there was no active isolate recovered from BAL site. From this result, we can conclude that the numbers of active isolates were varied from site to site (Figure 2).

3.3. Distribution of recovered isolates and active isolates with respect to sampling depth

From a total of 125 isolates recovered from 7 sampling sites at different depth, the greater number of isolates [35 (28%)] were recovered from 5–10 cm depth when compared to other depths.

Table 1

| Sampling sites | Soil type with major dumped wastes | GPS coordinates (o) | Latitude | Longitude | MC (%) | pH | Average CFU/g (10^6) | 28°C |
|----------------|-----------------------------------|---------------------|----------|-----------|--------|----|---------------------|------|
| BAL            | Sandy with oiled sludge           | -1.051134           | 37.086341| 23.90 ± 1.88 | 8.12 ± 0.23 | 1.38 ± 0.95 |
| PSL            | Sandy with ashes                 | -1.050989           | 37.105572| 16.44 ± 1.88 | 7.71 ± 0.20 | 1.88 ± 0.25 |
| BAD            | Sandy with sludge                | -1.051035           | 37.083528| 15.71 ± 6.17 | 5.74 ± 0.37 | 0.00 ± 0.00 |
| BML            | Loam with wheat husks            | -1.053114           | 37.086125| 14.33 ± 6.89 | 5.95 ± 0.29 | 3.00 ± 2.16 |
| KDO            | Sandy with wigs, paper bags      | -1.075395           | 37.108762| 33.23 ± 3.32 | 8.16 ± 0.15 | 16.75 ± 7.32 |
| KDT            | Sandy with charcoal              | -1.075283           | 37.109494| 48.65 ± 7.26 | 9.16 ± 0.24 | 1.63 ± 1.25 |
| KGT            | Sandy with sludge                | -1.079382           | 37.112864| 26.28 ± 3.06 | 7.70 ± 0.16 | 1.25 ± 0.50 |
| MRS            | Sandy and sticky                 | -1.076685           | 37.046068| 22.13 ± 0.51 | 7.60 ± 0.29 | 1.50 ± 1.73 |
| Starch casein  | media (Negative control)         |                     |          | 0.00 ± 0.00 |        |                 |

CFU/g: Colony forming unit per gram of soil, MC: Moisture content, α: Degree.
According to the result, the active isolates were found in all depth but the number varied from depth to depth. The greater number of active isolates [9 (31%)] were obtained from a depth of 10–15 cm when compared to other depths. From these result we can conclude that the active isolates were found from 0–20 cm depth range (Figure 3).

3.4. Comparison of bioactivity of isolates against selected pathogens during primary antibacterial screening

The antibacterial activity of active isolates against selected bacterial pathogens were compared and described using the inhibition zone measured in millimetre. The activity of selected isolates against selected pathogens using streak plate assay were depicted (Figure 4).

There was a significance difference ($P < 0.05$) of inhibition zone among the bioactivity of 29 active isolates against one or more selected bacteria. The highest bioactivity against $E. coli$ ATCC25922 was recorded by KGT22 (30 ± 0.0 mm) when compared to MRS 44 (2.0 ± 0.0 mm) from control site. In addition, KGT22 active isolate also showed highest anti-$S. typhi$ (30.0 ± 0.0 mm) and anti-$V. cholerae$ (36.0 ± 1.0 mm) when compared to others. Moreover, $S. boydii$ was more strongly inhibited by KGT22 (31.3 ± 0.6 mm) followed by BML44 (26.3 ± 0.6 mm), PLS11 (25.3 ± 0.6 mm) and PLS32 (25.0 ± 0.0mm (Table 2).

3.5. Antibacterial activity screening of potential isolates against MRSA and ESBL E. coli by streak plate assay

Antibacterial activity of 29 active isolates also tested against MRSA and $E. coli$. From these, only 5 (17.2%) isolates showed antibacterial activity against clinical MRSA and $E. coli$ and the bioactivity showed significantly difference ($P < 0.05$) among active isolates. Isolate KDO24 showed highest inhibition zone against MRSA (16.25 ± 0.50 mm) and $E. coli$ (26.5 ± 0.58 mm) when compared to others. Isolate KDO24 and KGT12 showed bioactivity against both MRSA and $E. coli$ (Table 3).
The supernatants from 29 isolates were taken at different periods of incubation and tested antibacterial activity against selected pathogens by well diffusion assay. The antibacterial active isolates against one or more pathogens and susceptible pathogens are only described on Table 4 and Figure 6.

Table 3

| Isolates | MRSA | ESBL E. coli |
|----------|------|--------------|
| PLS32    | 14.5 ± 0.58b | 0.0 ± 0.01  |
| BML25    | 13.75 ± 1.50c | 0.0 ± 0.01  |
| BML44    | 8.0 ± 1.83c  | 0.0 ± 0.01  |
| KDO24    | 16.25 ± 0.50c | 26.5 ± 0.58  |
| KGT12    | 10.5 ± 0.58c  | 4.0 ± 0.82   |

Control: Media and clinical pathogen. Values are means ± SD. The outcomes not sharing a common superscript letter (a < b < c < ... ) in the same column are significantly different at P < 0.05.

3.6. Comparison of number of active isolates in relation to incubation period during secondary screening

The supernatants from 29 isolates were taken at different period of incubation and tested antibacterial activity against selected pathogens. Here only active isolates, susceptible pathogens and the antibacterial producing incubation period were described. From a total of 29 active isolates, supernatants taken from 8–13 days old culture of 11 (37.93%) isolates showed antibacterial activity against one or more selected pathogens. As antibacterial activity of the supernatant taken from 8 days old culture showed the highest number of isolates were active against E. coli ATCC25922 [11 (37.93%)] and S. typhi [6 (20.7%)] when compared to others incubation period. However, at 9 day incubation period, the highest number of active isolates against S. boydii [6 (20.7%)] were recorded when compared to other incubation periods (Figure 5). These results indicate that the number of incubation days influence the antibacterial activity of the supernatant.

3.7. Comparison of antibacterial activity of supernatants from selected incubation period of isolates during secondary screening

The antibacterial activities of the supernatant from 29 isolates were tested against selected pathogens by well diffusion assay. The antibacterial active isolates against one or more pathogens and susceptible pathogens are only described on Table 4 and Figure 6.

Table 4

| Isolates | E. coli ATCC25922 | S. boydii | S. typhi |
|----------|-------------------|-----------|---------|
| PLS13    | 15 ± 1.0b         | 0 ± 0.0   | 0 ± 0.0 |
| PLS31    | 16 ± 1.0c         | 0 ± 0.0   | 14 ± 1.0c |
| PLS34    | 15 ± 1.0d         | 0 ± 0.0   | 17 ± 1.0d |
| PLS41    | 16 ± 1.0d         | 0 ± 0.0   | 16 ± 1.0d |
| PLS44    | 14 ± 0.0d         | 0 ± 0.0   | 17 ± 1.0f |
| BML45    | 17 ± 1.0d         | 15 ± 0.0  | 0 ± 0.0  |
| KDO19    | 17 ± 1.0d         | 16 ± 1.0d | 0 ± 0.0  |
| KDO38    | 17 ± 0.0d         | 15 ± 1.0d | 0 ± 0.0  |
| KGT32    | 20.3 ± 0.6d       | 24 ± 1.0d | 24.7 ± 0.6d |

Broth: Negative control, 0: No activity observed. Values are means ± SD. The outcomes not sharing a common superscript letter (a < b < c < d ... ) in the same column are significantly different at P < 0.05.

The antibacterial activity of the supernatants from 11 (37.93%) isolates showed significantly difference (P < 0.05) between them and the control. The antibacterial activity of supernatants from isolate BML45 (17 ± 1 mm), KDO19 (17 ± 1 mm) and KDO38 (17 ± 0 mm) showed greater inhibition against E. coli ATCC25922 when compared to supernatants from other isolates but smaller than when compared to streptomycin (20.3 ± 0.6 mm). The greater inhibition zone against S. boydii was recorded by supernatants from both isolate KGT31 (17 ± 1 mm) and KGT32 (17 ± 0 mm) when compared to other supernatant activity but smaller than streptomycin.
(23.7 ± 0.6 mm). The antibacterial activity of supernatant from isolate PLS34 (17 ± 1 mm) and PLS44 (17 ± 1 mm) showed the highest activity against *S. typhi* when compared to other supernatant activity but smaller than when compared to streptomycin (24.7 ± 0.6) (Table 4).

4. Discussion

The emergency of antibacterial resistant bacteria is a cause for concern. It is the basic challenge for effective treatment of infectious disease throughout the globe including the continent of Africa. *E. coli*, *Salmonella* sp., *Shigella* sp. and *Vibrio* sp. are some of frequently occurring drug resistant groups of Gram-negative bacteria in East Africa region[12]. Thus, searching of effective antibacterial product from actinomycetes isolated from unexplored environments of soil is required. Identifying and selecting such soil sampling areas in local districts may lead to getting of such biotechnologically and pharmaceutically valuable producing isolates. Thika waste damping sites are unexplored area for these purposes.

The present study showed that 23.2% antibacterial active isolates were recorded in Thika waste damped sites. This indicated that the frequency of getting antibacterial isolates in the present study area was greater than 16.6%[3] and smaller than when compared to 36.4%[6] and 55.7%[5] finding. This variation occurred may be due to difference in geographical location of the sampling sites and sample pre-treatment methods. However, from present study 23.2% isolates showed either anti-*E. coli* ATCC25922, *S. boydii*, *S. typhi* and or *V. cholerae* activity which was better than when compared to none of them showed anti-*E. coli* ATCC25922[6], 12.8% of active isolates showed anti-*E. coli* ATCC25922[5] and 16.6% isolates showed anti-*E. coli* and anti-*S. typhi* ATCC9289 activity[3]. Our study made difference by indicating potential waste damping sites for isolation of antibacterial producing isolates and contributing more biologically active isolates in this study area. Therefore, the present study fills the research gap on searching of antibacterial producing isolates in these particular areas against Gram-negative bacteria pathogens.

The present study showed that some of isolates were showed good antibacterial activity against these pathogens and others were not. The antibacterial activity against *E. coli* (26.5 ± 0.58 mm), *S. boydii* (31.3 ± 0.6), *S. typhi* (30 ± 0 mm), *V. cholerae* (36 ± 1 mm) and MRSA (16.25 ± 0.5 mm) was best results recorded from the present study. As the previous study showed that the highest antibacterial activity was 30 ± 2 mm against *E. coli* ATCC25922 and 32 ± 2
isolates as well as identification of selected isolates at species level. These differences may be due to diverse types of active isolates isolated from different natural environments.

According to the present result, Thika industrial waste damping soils have potential isolates that show effective antibacterial activity against selected pathogens. And the number of active isolates that showed antibacterial activity varies from site to site. Thus, this study confirmed that soil samples collected from waste damping sites in Thika have a potential antibacterial producing isolates. However, further research is under progress for functional and chemical characterization of antibacterial compounds and synthesis of antibacterial silver nanoparticle using metabolites from potential isolates as well as identification of selected isolates at species level.

Conflict of interest statement

We declare that we have no conflict of interest.

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