Isolation of antimicrobial producing Actinobacteria from soil samples

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Abstract
Emergence of multidrug resistant bacteria has made the search for novel bioactive compounds from natural and unexplored habitats a necessity. Actinobacteria have important bioactive substances. The present study investigated antimicrobial activity of Actinobacteria isolated from soil samples of Egypt. One hundred samples were collected from agricultural farming soil of different governorates. Twelve isolates have produced activity against the tested microorganisms (S. aureus, Bacillus cereus, E. coli, K. pneumoniae, P. aeruginosa, S. Typhi, C. albicans, A. niger and A. flavus). By VITEK 2 system version: 07.01 the 12 isolates were identified as Kocuria kristinae, Kocuria rosea, Streptomycyes griseus, Streptomycyes flaveus and Actinobacteria. Using ethyl acetate extraction method the isolates culture’s supernatants were tested by diffusion method against indicator microorganisms. These results indicate that Actinobacteria isolated from Egypt farms could be sources of antimicrobial bioactive substances.

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1. Introduction
Bacteria have so far been the most promising resource for antibiotics in the past decades and will undoubtedly remain an important resource of innovative bioactive natural products in the future. Approximately 45% of bioactive compounds obtained from microbes were produced by Actinomycetes (Berdy, 2005). Actinomycetes remain the most economically and biotechnologically useful microbes, producing 80% of the world’s antibiotics, mostly from the genera Streptomycyes and Micromonospora (Pandey et al., 2004).

Many vitamins, antibiotics, enzymes and siderophores obtained by Actinomycetes have pharmaceutical, veterinary, agricultural and clinical applications (Koehn and Carter, 2005; Kekuda et al., 2010; Naine et al., 2011), in addition to antitumor and wound healing properties (Janardhan et al., 2012; Jiao et al., 2013).

Since the discovery of antibiotics, bacterial resistance to these drugs has continued to evolve. Thus, we are witnessing more and more multiresistant bacteria that pose a serious public health problem. The present investigation aimed to study antimicrobial activity of Actinobacteria isolated from soil samples of Egypt.

2. Materials and methods

2.1. Sample processing and isolation of Actinomycetes

One hundred soil samples collected from Egyptian governorates were dried in room temperature for 24 h then ground and sterilized as recorded by Saadoun et al. (1999). The samples were inoculated on actinomycetes isolation agar and incubated at 30°C for 5–7 days. The Gram positive mycelial shape bacilli were identified (Holt et al., 1994; Anderson and Wellington, 2001).

2.2. Screening of antimicrobial activities of pure isolates

Preliminary screening for antibiotic activity of the isolates was done by using perpendicular streak method on Mueller Hinton agar medium (Shomura et al., 1980).
2.3. The indicator organisms

Gram Positive bacteria: *S. aureus* (NCTC 7447 0.0), *Bacillus coagulans*, Gram Negative: *E. coli* (NCTC 10416 0.0), *Klebsiella pneumoniae* (NCIMB 9111 0.0), *P. aeruginosa* (ATCC 10145 0.0), *Salmonella Typhi*, *Candida albicans*, *A. niger* and *A. flavus* were kindly supplied from Department of Microbiology Faculty of Veterinary Medicine Cairo University.

2.4. Extraction of bioactive substances

The potential Actinobacteria was subjected to submerged state fermentation method to produce crude extracts (Westley et al., 1979). Antimicrobial activity of the crude extracts was estimated among the indicator microorganisms.

3. Results

3.1. Occurrence of Actinobacteria among the examined soil samples

As shown in Table 1 a total of 12 isolates out of 100 soil samples were identified as Actinobacteria. All isolates were Gram positive aerobic bacilli slow growing small white to creamy colonies Fig. 1. By VITEK 2 system version: 07.01 the isolates were identified as *Kocuria kristinae* (1), *Kocuria rosea* (3), *Streptomyces griseus* (4), *Streptomyces flavolus* (2) and Actinobacteria (2).

3.2. Antimicrobial activities of the isolates

The isolates were screened for their antibacterial activity on Mueller Hinton agar medium using streak-plating technique Fig. 2. The zone of inhibition among the *Actinobacteria* isolates against the indicator microorganisms was recorded as shown in Tables 2 and 3.

4. Discussion

Actinomycetes are widely distributed in the nature and have the ability to produce many biologically active substances like antibacterial, antifungal, antiviral, anti-parasitic, herbicides, pesticides, antioxidant and antitumor. The present study was performed to study the antimicrobial activity of *Actinobacteria* isolated from soil. Actinomycetes have provided many industrially important bioactive compounds (Kumar and Jadera, 2016). One hundred farming soil samples collected from Egyptian Governments were investigated. The samples were growing on Actinomycetes isolation agar for isolation and identification of Actinobacteria. All isolates (n = 12) were Gram positive aerobic slow growing bacteria (Anderson and Wellington, 2001) with earthy odor. The isolates were identified as *Kocuria kristinae*, *Kocuria rosea*, *Streptomyces griseus*, *Streptomyces flavolus*, and Actinomycetes using VITEK 2 system.

The twelve isolates exhibited a broad spectrum of antimicrobial activity. Twenty isolates (13.30%) out of 150 *Actinomycetes* examined by Rahman et al. (2011) had antimicrobial activity against the test microorganisms. Also Dehnad et al. (2010) investigated the bioactivity of *Streptomyces* isolates from Iran. Arifuzzaman et al. (2010) recorded 20 isolates (36.36%) showed activity against the test bacteria.

### Table 1

| Governorates | No. of samples | No. of positive samples |
|--------------|----------------|------------------------|
| Alexandria   | 3              | -                      |
| El Gharbia   | 15             | 2                      |
| El Monofia   | 55             | 8                      |
| El Sharkia   | 5              | -                      |
| El Menia     | 2              | -                      |
| El Behira    | 5              | -                      |
| El Dakahlia  | 4              | -                      |
| Kafr El Shak | 5              | 2                      |
| Matrouh      | 6              | -                      |
| Total        | 100            | 12                     |
These identified isolates showed antimicrobial activity against the test organism and the range of inhibition zones was between 2.5 and 10.2 mm. Strain 1 showed the largest inhibition zone in B. cereus, while strain 11 was weakly active, and showed the least inhibition zone in Klebsiella pneumoniae. All strain could not inhibit E. coli, all strain showed moderate to high activity in B. cereus. One strain only showed inhibition zone in Klebsiella pneumoniae. Gram-negative strains were highly resistant and were consistent with the known susceptibility differences among the target organisms (Basilio et al., 2003).

Also, 4 isolates had activity against Candida albicans, 2 against A. niger and 2 against A. flavus. Out of 287 actinomycetes 166 isolates were found antagonistic to Candida albicans, while 164, 134 and 132 actinomycetes showed antagonistic properties against A. niger, M. gypseum and Trichophyton sp., respectively (Jain and Jain, 2003).

The actinomycete isolated by Dhurumaduari et al. (2008) had antimicrobial activity. Ouldouch et al. (2001) recorded that 32 out of 320 actinomycete strains isolated from several Moroccan habitats showed strong activity against yeast, molds and bacteria.

We can conclude that the soil samples are rich source of Actinobacteria which exhibit a wide spectrum antimicrobial agent. Further investigations are needed to identify the strain at molecular level and to determine the active metabolites of these isolates.

5. Conclusion

We can conclude that the soil samples are rich source of Actinobacteria which exhibit a wide spectrum antimicrobial agent.

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