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MOLECULAR IDENTIFICATION AND GROWTH INHIBITION OF SOME HUMAN PATHOGENIC BACTERIA ISOLATED FROM KING FAHAD GENERAL HOSPITAL, JEDDAH

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ABSTRACT

Antimicrobial properties of bacterial antagonist against human pathogenic bacteria have become a field of increasing importance in the medical sector. The present study has been carried out to identify the antimicrobial properties of actinomycetes bacteria against five human pathogenic bacteria viz., *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Escherichia coli* isolated from many wound swabs, from different service units of King Fahad General Hospital (KFGH), Jeddah. These isolated bacterial isolates were identified by using morphological and physiological characters along with molecular techniques. The antibacterial activities of five actinomycetes species along with Ampicillin (5µg/ml) as positive control were also determined against the multidrug resistant *S. aureus*, *S. pyogenes*, *P. aeruginosa*, *A. baumannii* and *E. coli* by using disc diffusion assay. Result of study revealed that among the tested five actinomycetes species, *Streptomyces* 5 (St 5) was highly effective against *P. aeruginosa*, *S. aureus*, *S. pyogenes* and *E. coli* while it showed weak activity against *A. baumannii*. As compared to this, *Streptomyces* 2, 3 and 4 showed moderate antibacterial activities while *Streptomyces* 1 showed the lowest activity. Further, all the tested *Streptomyces* extracts and Ampicillin were found weak against the multi-drug resistant *A. baumannii*. In conclusion, actinomycetes especially genus *Streptomyces* can be used as a safe and effective source against multidrug resistant bacteria.

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1 Introduction

Excess use of antibiotic, not only developed multidrug resistance in human pathogenic microorganisms. Indiscriminate uses of these drugs were leading to the selection of the bacterial pathogens, resistant to multiple antibiotics (Grasso et al., 2016). Resistance in bacteria against multiple drugs was due to the accumulation of multiple resistance genes against different drugs on the plasmids and/or the increase in gene expression that code for multidrug efflux pumps (Service, 1995, Nikaido, 2009). In addition to this, sometimes excess use of antibiotics are associated with various adverse effects such as hypersensitivity, immune-suppression and allergic reactions (Ahamed et al., 1998). Further, certain bacterial strains developed mechanisms or produced substances which block the action of antibiotics or change their target or ability to penetrate cells (Ali et al., 1995). Therefore, disease causing microbes that have become resistant to antibiotics are increasing public health problems and this forced scientists to search alternative to antibiotics which have good bactericidal activities.

Actinomycete metabolites have great bactericidal effects and therapeutic values against wide range of microorganisms. Further, antimicrobial activities of these actinomycetes are depending on the method of extraction. These metabolites can be used as alternative drugs with antibacterial or antifungal or antioxidant activities which protect the host from cellular oxidation (Singh et al., 2014). Zothan et al. (2017) reported that methanolic extract of *S. cyaneofuscatus* inhibited the growth of *E. coli*, *P. aeruginosa*, *Micrococcus luteus*, *S. aureus*, and *Candida albicans* with IC₅₀ ranged from 2.1-43.63 µg/ml. This antimicrobial activity may be due to the interaction of these metabolites with bacterial cell surface and causing structural changes, damaging, disturbing vital cell functions such as permeability, depressing the activity of respiratory chain enzymes, and finally leading to cell death (Demain, 1999, Ueda & Beppu, 2017).

The aim of the present study was to evaluate the antimicrobial activities of some actinomycetes against multidrug resistant bacteria, isolated from local hospital.

2 Materials and Methods

All the required chemicals for this study were purchased from Merck, Germany and Sigma–Aldrich, USA.

2.1 Collection and isolation of bacterial isolates:

Sterile cotton swabs, moistened with sterile saline, were used to obtain samples from different patient wounds at King Fahad General Hospital, Jeddah, Saudi Arabia. Preliminary experiments were carried out to select antibiotics resistant bacteria, which have

resistant against at least 3 most commonly used antibiotics. Wound samples were collected in two swabs, among these, one swab was used for Gram staining and the other one was inoculated on Mac-conkey and blood agar plates for isolating the different bacterial pathogens. Isolated *Acinetobacter baumannii* was identified by culturing this bacterium on Leeds *Acinetobacter* medium (Hardy Diagnostics, USA, catalog no. G261). The inoculated plates were incubated at 37°C overnight and examined for growth. Isolated colonies were identified on the basis of morphological characteristic and biochemical tests including Catalase, Oxidase, Urease, Indole, Methyl Red, Voges Proskauer and Citrate Utilization tests as described by Koneman et al., (2005). The pure cultures of the tested bacteria were maintained on Nutrient agar slants at 4°C and in Glycerol Broth (16 ml glycerol + 84ml nutrient broth) at -70°C.

2 Molecular identification of the bacterial isolates

For PCR, required DNA templates were obtained from overnight bacterial cultures that were collected, re-suspended in 200 ml of sterile distilled water, and boiled for 1 minute (Usein et al., 2009). Species specific gene uid A for *E. coli*, *ecfX* for *Pseudomonas aeruginosa*, *oxa-51* for *A. baumannii* and 16S rRNA for *Staphylococcus aureus* and *Streptococcus pyogenes* were used for the identification the detection particular bacterium (Clifford et al., 2012). Primers for uidA (*E. coli*) were prepared as according to the method described by Moyo et al. (2007), while *oxa-51* (*A. baumannii*) primers were according to Brown & Amyes (2005), and *ecfX* primers (*P. aeruginosa*) were according to Clifford et al. (2012). The universal 16S rRNA primers were prepared as according to Tork et al., (2016). PCR performed in the Takara thermal cycler (Takara, Tokyo, Japan) and the PCR products were separated by using 1.5% agarose in Tris–acetate–EDTA buffer at 100V and visualized. O'Range Ruler™ 100+500 bp DNA Ladder, ready-to-use was included in each run.

2.3 Isolation of actinomycetes

The five Actinomycete isolates were obtained from Department of Botany, Faculty of Science, Tanta University, Egypt. These collected isolates were identified as *Streptomyces exfoliatus* (St 1) and *S. niveus* (St 2) as per guide line given by Agwa et al. (2000), while *S. anulatus* SM 21 (St 3) and *S. coelicolor* SM 1 (St 4) were identified as guideline given by Aly et al., (2011). Further, *S. exfoliates* LP10 (St 5) was identified as method given by Aly et al. (2012).

2.4 Estimation of Antimicrobial activity by agar well diffusion method

Standard agar well diffusion method was carried out to detect the activity of Actinomycete filtrates against some selected

pathogenic bacterial isolates (Cheesbrough, 2000). Each actinomycete isolate was cultured in 50 ml of starch nitrate broth for 2 days at 25°C and healthy cells were collected and used to inoculate in 50 ml of production broth medium, composed of (g/l) 10 g glucose, 1.0 g K₂HP0₄, 1.0 g MgSO₄, 7H₂O, 1.0 g NaCl, 1.2 g NH₄NO₃, and 2.0 g CaCO₃ (Agwa et al., 2000). After 7 days, cell free culture filtrate was collected for each organism and extracted with the same volume of methanol (V/V). The methanol extract was dried, dissolved in DMSO and the antibacterial activities were determined on nutrient agar on which 100 µl of the overnight suspension of each bacterial pathogen was spread. Using sterile cork borer (8 mm), agar well was made and filled with 50µl of the prepared actinomycete extract in DMSO or 5µl (5 mg/ml) of the standard antibiotic (positive control) under aseptic conditions. Then, the plates were kept in refrigerator for 2 hours before incubation to permit diffusion of the extract and incubated at 37°C for 24 hr, this was followed by the examination of antibacterial activity (diameter of inhibition zone, mm). Duplicate plates were used for each tested bacterial pathogen.

3 Results and Discussion

In wound infections, many aerobic and anaerobic bacteria are found which lead to morbidity or prolonged hospitalization (Bowler et al., 2001). From last few decades, the emergence of antibiotic resistance in pathogenic isolates of human pathogenic bacteria is a dangerous threat to worldwide public health. This became more serious in case of Gram-negative bacteria such as *A. baumannii*, *E. coli* and *P. aeruginosa* and Gram-positive *S. aureus* which were associated with pus and wound infections, due to extensive prescription and inadequate dose of antibiotics (Rice, 2006, Misic et al., 2014). Rapid spread of multidrug resistant bacteria poses a serious threat to public health due to the limited treatment options and the decrease in the discovery of new classes of antibiotics (Iredell et al., 2016).

Bacterial identification is very important to reduce morbidity and mortality in patients. Good identification of the bacterial pathogens improves treatments options and lead to successful therapy (Barenfanger et al., 1999). In this study, five human pathogenic bacteria viz., *Staphylococcus aureus*, *Streptococcus pyogenes*, *P. aeruginosa*, *A. baumannii* and *E. coli* were isolated from wound of regular visiting patients of KFGH and identified by using conventional as well as molecular identification techniques. Among the isolated microorganisms, 16S rRNA primer was used for the identification of *S. pyogenes* and *S. aureus* while *ecfX*, *uidA* and *bla* oxa-51 were used for identification of *P. aeruginosa*, *E. coli* and *A. baumannii* respectively (Figures 1 and 2). The results of bacterial identification by using 16S rDNA are in agreement with the findings of Weisburg et al. (1991). Similar findings was reported

by Zhang et al. (2014) those who reported that predominance of *E. coli*, *S. aureus*, and *P. aeruginosa* in pus samples from patients with severe intra-abdominal infection. In another study, *S. aureus* was identified as the dominant bacterial species from wounds and this was followed by *P. aeruginosa*, *P. mirabilis*, *E. coli* (Lorrot et al., 2014). *Acinetobacter baumannii* is a nosocomial pathogen which affects critically ill patients and has increased importance. Further, Aly et al. (2014) recorded resistance nature of *A. baumannii* against various commonly used

Figure 1 A: Agarose gel electrophoresis of PCR product of amplified *uidA* gene (623 bp). Lane M: Marker, Lane 1 positive control, Lane 2-5: positive strains for *uidA* gene. B: Agarose gel electrophoresis of PCR product of amplified *ecfX* gene. Lane M: Marker, Lane 1: positive control for *ecfX* gene, Lane 2-3 positive strains for *ecfX* gene.

Figure 2 A: Agarose gel electrophoresis of amplified PCR product of *oxa-51* gene B: PCR product 16S rRNA gene. Lane M: Marker, Lane 1 positive control, Lane 2-5: positive strains for 16S rRNA gene.

Figure 3 The effect of methanol extracts of the five tested *Streptomyces* species on *S. aureus* (A), *E. coli* (B) and *S. pyogenes* (C).

Figure 4 The selected *Streptomyces* isolate (St 5) under light microscope (A), scanning electron microscope (B) and on starch nitrate agar (C).

antibiotics. There is an evidence that carbapenemase gene was naturally occurred in *A. baumannii* (Al Masoudi et al., 2013).

Actinomycetes have highly significant roles in drug discovery and have provided bioactive secondary metabolites with interesting activities such as antimicrobial, antiviral and anticancer. An antimicrobial activity of actinomycete extracts varies with the tested bacterial isolates and the used pathogens (Bruntner et al., 2005, Bhavé et al., 2013). In this study, methanolic extracts of the five *Streptomyces* species were found effective against the tested resistant bacteria (Figure 3) and *S. exfoliates* (St 5) was the most effective one with highest inhibition zone against *P. aeruginosa* (27 mm), *E. coli* (25 mm), *A. baumannii* (18mm), *S. pyogenes* (25 mm) and finally *S. aureus* (24 mm) as shown in Table 1. The growth and morphology of the *S. exfoliates* were shown in Figure 4. From past seven decades, the antibiotics obtained from actinomycetes have many successes. Actinomycetes are a sustained mine of new antibiotics with many mode of action that

kills the pathogens without harming the host. Further, erythromycin, tetracyclines, aminoglycosides, daptomycin, tigecycline are the most common antibiotics which obtained from various actinomycetes. Among the identified bioactive compounds that have been obtained so far from microbes, 45 % are produced by actinomycetes while 38 % by fungi and 17 % by unicellular eubacteria (Mahajan & Balachandran, 2012). Result of study revealed that all the tested methanolic extracts of *Streptomyces* have good antibacterial activity against all tested bacteria except for *A. baumannii* and among the tested microbes, maximum antibiotic activity was reported against *P. aeruginosa* and *S. pyogenes* while in case of conventional antibiotic, maximum activity of Ampicillin was reported against *S. pyogenes* with inhibition zone of 19 mm. The antimicrobial ability of *Streptomyces* might be referred to their effect on the cell wall of the bacteria which finally causing the destruction of cell wall and death of bacteria. Also, the extracts interact with the building elements of the outer membrane and might cause structural

Table 1 The antibacterial activity (diameter of inhibition zone mm) of the methanolic extracts of five actinomycete isolates against some bacterial pathogens

Tested actinomycetes	Zone of inhibition (mm) $\pm$ SEM in various bacterial isolates				
	<i>P. areuginosa</i>	<i>A. baumannii</i>	<i>E. coli</i>	<i>S. pyogenes</i>	<i>S. aureus</i>
<i>Streptomyces</i> 1	14 $\pm$ 2.1	9 $\pm$ 1.0	9 $\pm$ 2.0*	18 $\pm$ 0.8	14 $\pm$ 0.9
<i>Streptomyces</i> 2	25 $\pm$ 1.7*	9 $\pm$ 1.1	18.3 $\pm$ 1.1	25 $\pm$ 1.9	19 $\pm$ 0.1
<i>Streptomyces</i> 3	20 $\pm$ 1.5*	9 $\pm$ 2.3	8 $\pm$ 1.0*	19 $\pm$ 1.1	19 $\pm$ 2.9
<i>Streptomyces</i> 4	14 $\pm$ 2.3	11 $\pm$ 1.0	19 $\pm$ 2.4	24 $\pm$ 2.5	14 $\pm$ 2.9
<i>Streptomyces</i> 5	27 $\pm$ 1.6*	18 $\pm$ 1.1*	25 $\pm$ 2.4*	25 $\pm$ 2.6	24 $\pm$ 2.0
Ampicillin (control)	17 $\pm$ 1.6	11 $\pm$ 1.0	17 $\pm$ 2.1	19 $\pm$ 1.5	15 $\pm$ 1.3

*: Significant results compared to control

changes; degradation and finally cell death. Also, antibacterial activities of the extracts could be due to the susceptibility of pathogens cell wall and toxicity in addition to the change in membrane potential, inhibition of ATP syntheses and levels leading to collapse of all biological process (Cui et al., 2012). Similar results were reported by Srinivasan et al. (2009) who reported strong antibacterial activity against Gram-positive than the gram-negative bacteria, this may be due to the structure differences in cell wall structure, where Gram negative has outer membrane which block the penetration of antibiotics and plant extracts and making them resistant against various antibiotic substances. Extract of *Streptomyces* 5 was more effective as compared to other methanolic extracts of actinomycetes and it is equally effective against all the tested microbes. Further, moderate antibacterial activities were recorded for the methanolic extracts of *Streptomyces* 2, *Streptomyces* 3 and *Streptomyces* 4 while *Streptomyces* 1 showed the least antibacterial activity. Antibiotics from actinomycetes may affect essential processes in bacterial cell wall biosynthesis and change bacterial structures and functions. Antibiotics essentially target cell membrane protein translation, RNA transcription, DNA replication and synthesis (Ueda & Beppu, 2017). Glycopeptides are a class of drugs produced by Actinomycetes bind to the dipeptide D-alanyl-D-alanine of cell wall of Gram-positive bacteria preventing the addition of new units to the peptidoglycan and inhibiting the peptidoglycan synthesis (Demain, 1999, Ueda & Beppu, 2017).

In conclusion, the antibiotics from actinomycetes must be intensively studied, their sources, structures, activities, and mode of actions and further research is required to use these safe and effective extracts in alternative medicines.

Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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