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Inhibition of *Staphylococcus aureus* and its biofilm by the metabolites of endophytic *Streptomyces* sp. ADR1

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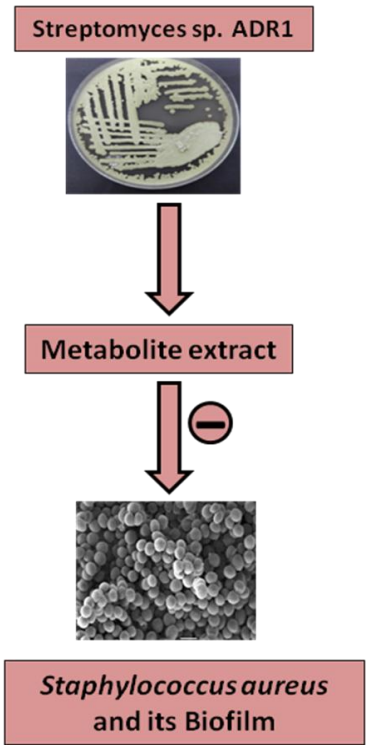
Graphical Abstract



Abstract

Staphylococcus aureus is a gram positive, tissue colonizer pathogen in humans. It is known for its tendency to build up biofilm which is a major cause of antibiotic resistance. To overcome this problem, there is an urgent requirement to discover novel antimicrobial compounds against new bacterial targets and drug resistance. In this direction the actinobacteria inhabiting special niche like plant tissues can be promising agents for novel compounds against methicillin sensitive and resistant *S. aureus* (MRSA).

The ethyl acetate extract of Streptomyces sp. ADR1 is found to be a strong inhibitor of various *Staphylococcus* sp. and its resistant strain MRSA with very low MIC_{90} values; <31.25 µg/ml. The extract was found to inhibit biofilm formation as well as preformed biofilms of *S. aureus* and MRSA to a significant extent.

Introduction

Infectious diseases have been posing greater threats to human health due to fast evolving resistance to drugs. Among various factors that contribute to drug resistance, formation of biofilm by the pathogen is an important one. A common bacterial infection involving S*taphylococcus* spp. may present life threatening situation due to its ability to acquire drug-resistance against the present antibiotics and also the ability to form biofilms, which confer even greater resistance to antibiotics. Similar observations have been made in case of other infectious diseases, for example, candidiasis or

pseudomonas infections. This has caused an increase in mortality and morbidity (Tang et al., 2010). The occurrence of resistance in *Staphylococcus* spp. against methicillin and β-lactam has also been seen to rise in the past few years (Seal et al., 2003). Strains of *Staphylococcus* spp. are known to possess various defense mechanisms against the antibiotics which include enzymatic inactivation of drug, entrapment of antibiotics within the cell and formation of biofilms. Some virulence determinants like extracellular toxins (hemolysins, leukotoxins and enterotoxins), enzymes (proteases and coagulases) and *S. aureus* surface proteins, help the pathogen to defeat the host immune response leading to the onset of infection (Zecconi et al., 2013). Biofilms are the aggregation of bacterial community attached to a substratum (living or non living). It plays a key role in the persistence of bacterial infection that may lead to a deleterious consequence (Rabin et al., 2015). Biofilms alone account for approximately 80% of the human infections (Romling et al., 2012). Biofilms can be formed on both living (Respiratory tract, eyes, urinary tract, teeth gums etc.) as well as abiotic (orthopedic prostheses, artificial cardiac valves, coronary stents, intravascular and urinary catheters, neurosurgical, cochlear, and breast implants, dentures, and ventricular assist and ocular devices) surfaces (Magana et al., 2018). The unremitting frequency of antibiotic resistance in *S. aureus* (eg. methicillin resistance) due to recalcitrance by biofilms is a serious threat and highlights an urgent call for novel drug discovery that inflict least selection pressure on the pathogens. Coherent and cumulative study for suppression of *Staphylococcal* virulence and pathogenesis could be a better perspective for the development of new antibiotics.

In this study we have determined the potential of *Streptomyces* sp. ADR1 metabolite extract against *S. aureus* and Methicillin-resistant *S. aureus*. The extract has also been checked for the inhibition of biofilm produced by these pathogens.

Materials and Methods

Metabolite extract of endophytic actinobacteria, *Streptomyces* sp. ADR1 was obtained by solvent-solvent extraction (Srivastava and Dubey, 2016). This extract was tested against *Staphylococcus aureus* strains: ATCC 29213, ATCC 25923 and methicillin resistant *S. aureus* 562 and *S. aureus* ATCC 43300 by well diffusion assay as per CLSI guidelines. The minimum inhibitory concentration (MIC) of ADR1 extract was determined using various concentrations in Mueller Hinton broth by micro-dilution method with 0.5 McFarland cell suspension of the pathogens prepared from overnight grown culture. (Weigend et al., 2008; Bussmann et al., 2015). Biofilm formation inhibition protocol was adapted from Frank et al. (2006) with slight modifications. It was tested against inhibition of biofilm formation as well as preformed biofilms of pathogenic *S. aureus*.

Results and Discussion

In well diffusion assay the zone of inhibition by ADR1 was comparable to standard drugs tetracycline and ampicillin. The MIC value of the crude extract against *Staphylococcus* spp. was

found to be in the range of 15.625 µg/ml - 0.49 µg/ml. Further studies confirmed MIC values against MRSA strains, which were between 00.4 µg/ml - 0.2 µg/ml. The minimum Biofilm inhibitory concentration (MBIC) was less than 15.625 µg/ml and against preformed biofilm it was <500µg/ml. These results indicate a good effect of the ADR1 extract against various *Staphylococcus sp.* and MRSA.

Conclusions

The work reported in this study shows the potential of crude extract against a common but significant pathogen, *S. aureus* and its methicillin resistant strains. It showed very potent activity to inhibit biofilm formation as well as to disintegrate preformed biofilms. The extract after purification could lead to the isolation of very effective anti-staphylococcal molecules that could be evaluated for their suitability as drug candidate.

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