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In vitro antioxidant activity and antimicrobial activity against biofilm forming bacteria by the pigment from Desert soil *Streptomyces sp* D25

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ABSTRACT

Streptomyces from rare ecosystems is the promising source for secondary metabolites exhibiting diverse biological activities. The present study reports the antioxidant activity and antimicrobial activity against biofilm forming bacteria by the pigment produced from Desert soil *Streptomyces sp*. D25. Crude pigment from the *Streptomyces sp* D25 was produced by agar surface fermentation using yeast extract malt extract agar and extracted using ethyl acetate. Antioxidant activity of pigment was tested at 100-500µg/ml concentration by DPPH and nitric acid scavenging assay. Antimicrobial activity against the biofilm forming bacteria was tested by disc diffusion method. The Streptomyces pigment showed 35.63% and 96.19% free radical scavenging activity in DPPH assay and nitric oxide assay, respectively. The results of radical scavenging activity of pigment in DPPH and nitric oxide assay showed its antioxidant potential. In antimicrobial assay, the pigment showed 10-20 mm inhibition against biofilm forming bacteria at 25µg/ml. Further *in vivo* studies on this *Streptomyces* pigment pave the way for its biomedical applications.

INTRODUCTION

Reactive oxygen species (ROS) production occurs during normal cell metabolism. Excessive amount of ROS increases oxidative stress and it can cause deleterious effects such as atherosclerosis, reperfusion injury, cataractogenesis, rheumatoid arthritis, inflammatory disorders and cancer. Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to humans against various infections and degenerative diseases. In order to retard the oxidation process, many synthetic antioxidants such as butylatedhydroxycanisole (BHA), butylatedhydroxytoluene (BHT), and propyl gallate (PG) are being used in clinics. However, these synthetic antioxidants have potential health hazards, so it have been attempted to screen alternative antioxidants from natural sources (Lee *et al.*, 2014). Dietary antioxidants have already been shown to be protective against chronic diseases. Modern research is now directed towards natural antioxidants from plants and microorganisms and serves as safe therapeutics (Thenmozhi *et al.*, 2010). Biofilms or microbial communities formed by adherent and cohesive cells on cellular or inert substrata (like medical devices), are involved in 60% of all infections. These infections tend to be chronic as they resist innate and adaptive immune defence mechanisms as well as antibiotics, and the treatment of biofilm infections presents a considerable unmet clinical need. To date, there are no drugs that specifically target bacteria in biofilms; however, several approaches are in early-stage development. For this reason, innovative anti-biofilm agents with novel targets and modes of action are needed (Oja *et al.*, 2015). Actinobacteria are gram positive bacteria that constitute one of the largest bacterial phyla and they are ubiquitously distributed in both aquatic and terrestrial ecosystems.

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Actinobacteria are of great importance in the field of biotechnology, as producers of plethora of bioactive secondary metabolites with extensive clinical, veterinary, aquaculture and agricultural applications. They have extensive secondary metabolism and produce two-thirds of all naturally derived antibiotics in current clinical use as well as many anticancer, antifungal and antiviral compounds (Barka *et al.,* 2016). With this view the present work is focused to test the antioxidant activity and antimicrobial activity of pigment from the Desert soil *Streptomyces sp.*D25 against biofilm forming bacteria.

MATERIALS AND METHODS

Description of Streptomyces sp D25

The soluble yellow-orange pigment producing *Streptomyces sp* D25 (Figure 1) was isolated from the soil collected from Thar Desert, Rajasthan. The yellow-orange pigment showed promising antimicrobial activity against Gram positive bacteria and anti TB activity in vitro (Radhakrishnan *et al.,* 2014). Viability of this strain was maintained on yeast extract malt extract (YEME) agar slants as well as in glycerol broth.

Production and extraction of pigment

Pigment from the *Streptomyces sp* D25 was produced by agar surface fermentation using YEME agar (Radhakrishnan *et al.,* 2015). After 7 days of incubation, the soluble pigment from the agar medium was extraction by solid liquid extraction method using ethyl acetate at 1:2 ratio. The ethyl acetate extract was concentrated using rotary evaporator under reduced pressure and used for testing antioxidant and antimicrobial activity against biofilm forming bacteria.

Screening for antioxidant activity

DPPH radical scavenging assay

The DPPH free radical scavenging assay was conducted based on the method of Rajesh and Natvar (2011). DPPH (1,1diphenyl -2- picrylhydrazyl (H-A) was dissolved in 3.3 ml methanol and it was protected from light by covering the test tubes with aluminum foil. Then about 150µl DPPH solutions were added to 3ml methanol and absorbance was taken immediately at 517 nm for control reading. About 100, 200, 300, 400 and 500µg concentration of crude pigment as well as standard compound (ascorbic acid) was added into each 3 ml of methanol. Then each 150 µl of DPPH was added to all the reaction tubes. Absorbance was taken after 15 minutes at 517 nm on UV-Visible spectrometer, Cyberlab Model using methanol as blank. The DPPH free each radical scavenging activity was calculated using the following formula:

$$\% \text{ scavenging } = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \text{x } 100$$

Nitric oxide free radical scavenging activity

About 100, 200, 300, 400 and 500 µg concentration of crude pigment previously dissolved in DMSO, as well as ascorbic acid (standard compound) was taken in separate tubes. About 2.0 ml of sodium nitroprusside in phosphate buffer saline was added into each tube. The reaction mixture was incubated at room temperature for 150 minutes. After incubation, 5 ml of Griess reagent was added in the each tube including control. The absorbance was measured at 546 nm on UV- Visible spectrometer using methanol as blank (Rajesh and Natvar, 2011).

$$\% \text{ scavenging/reduction} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \text{x } 100$$

Screening for antimicrobial activity

Antimicrobial activity of pigment against the biofilm forming bacteria was tested by disc diffusion method against biofilm forming bacteria previously isolated from marine fouling samples (Gopikrishnan *et al.,* 2013). Five mm diameter filter paper disc impregnated with 25 µg of pigment was placed over nutrient agar plates inoculated with biofilm forming bacteria. Activity was tested in triplicate and the mean value was expressed as zone of inhibition in millimetre in diameter.

RESULTS AND DISCUSSION

Streptomyces sp. D25 showed good growth on ISP2 agar with soluble yellow pigment production (Figure 1).

Fig. 1: Growth of streptomyces sp D25 on ISP2 agar.

In DPPH method, the free radical scavenging activity of pigment extracted from the Streptomyces sp D25 was increased with the increasing concentration. From the results, it confirmed that the crude pigment showed maximum of 35.63% of free radical scavenging activity. The result was graphically represented in figure 2. In the previous study also the yellow pigment extracted from the novel *Streptomyces sp* Eri12 from the rhizosphere of *Rhizoma curcumae* Longae showed 28.89 % radical scavenging activity at 500µg/ml in DPPH method (Zhong *et al.,* 2011).

In nitric oxide free radical scavenging assay, the *Streptomyces* pigment showed the dose dependent scavenging/ reductive potential. From the result (Figure 3), when the concentration increases, percentage of scavenging is also increasing linearly for the *Streptomyces* pigment and ascorbic acid standard. The crude pigment showed maximum of 96.19% radical scavenging activity in nitric oxide assay. Nitric oxide (NO) has also been involved in a variety of biological functions, including neurotransmission, vascular homeostasis, antimicrobial, and

antitumor activities. Despite the possible beneficial effects of NO, its contribution to oxidative damage is also reported. This is due to the fact that NO can react with superoxide to form the peroxy nitrite anion, which is a potential oxidant that can decompose to produce OH and NO (Rajesh and Natvar, 2011).

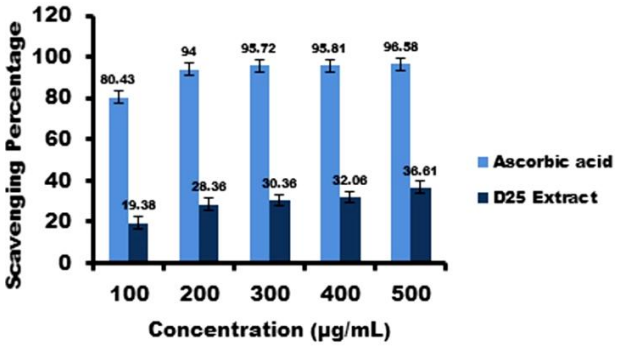

Fig. 2: Antioxidant activity of pigment from streptomyces sp D25 in DPPH method.

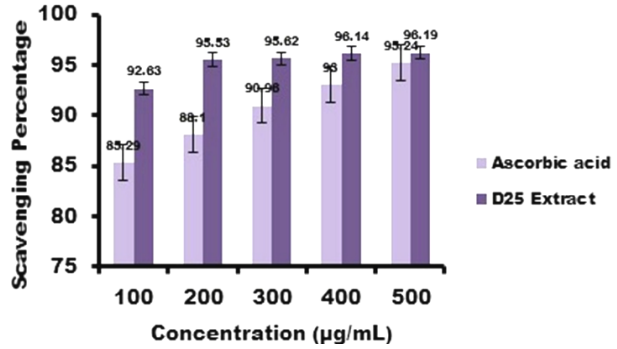

Fig. 3: Antioxidant activity of pigment from streptomyces sp D25 in nitric oxide assay.

Table 1: Antimicrobial activity of Streptomyces pigment D25 against biofilm forming bacteria

Biofilm forming bacteria	Zone of Inhibitionmm in diameter
*Staphylococcus*sp M1	16.3±0.3
*Lactobacillus*sp M6	20.7±0.6
*Alcaligens*sp M28	19.3±0.3
*Bacillus*sp M38	11.7±0.3
*Micrococcus*sp M50	10.7±0.3
*Lactobacillus*sp M51	11.7±0.8
*Pseudomonas*sp P1	15.0±0.5
*Kurthia*sp P3	10.0±0.5
*Lactobacillus*sp P4	16.7±0.3
*Alcaligens*sp P8	16.7±0.3
*Bacillus*sp P13	20.7±0.3

Most bacteria can switch between two life styles and exist either as free-living, planktonic cells or as bacterial biofilms that are surface-associated communities of cells embedded in a self produced matrix. The matrix consists of extracellular polymeric substances (EPS), and it is one of the factors contributing to increased tolerance to antibiotics associated with bacterial biofilms (Oja *et al.,* 2015).In antimicrobial testing, the crude pigment extracted in ethyl acetate showed 10-20 mm inhibition against the biofilm forming bacteria at 25µg/ml in disc diffusion method (Table 1). Studies on the inhibition of biofilm formation by the streptomyces pigment prove its potential further.

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