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Wound healing property of bioactive compound from actinomycetes

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ABSTRACT

The main objective of this work was to find out the angiogenic or wound healing property of the bioactive compound from actinomycetes isolated from mangrove soil. Five potential actinomycetes strains were isolated from mangrove soil and screened for bioactive compounds. Bioactive compounds from actinomycetes exhibited an effective antibacterial activity against the both Gram positive and Gram negative bacterial strains, Bacillus, Pseudomonas, Staphylococcus and E.coli. The wound healing property of bioactive compounds was carried out by Chorio Allontoic Membrane assay in embryonated egg. The bioactive compounds extracted from Actinomycetes strain (GN2) showed an excellent wound healing property in embryonated egg.

Key words: Actinomycetes,bioactive compound, Antibacterial activity, Wound healing property

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INTRODUCTION

Angiogenesis is the physiological process involving the growth of new blood vessels from pre-existing vessels. Angiogenesis is a normal and vital process in growth and development, as well as in wound healing. The identification of an angiogenic diffusible factor derived from tumors was made initially by Greenblatt and Shubik in 1968 [1]. The study of the angiogenic process and the search for novel therapeutic agents to inhibit, or stimulate, angiogenesis has employed in vivo 'angiogenesis' assays by embryonated eggs [2,3,4]. The Chorio Allontoic Membrane (CAM) assay is the most widely used in vivo model for the study of both angiogenesis and ant angiogenesis [2,5] There are two stages of tumor progression regarding blood vessel formation [6,7]. In first stage of tumor growth, it receives nutrition and oxygen from blood by diffusion. When tumor mass grows larger than the initial stage nutrition through diffusion is not sufficient for further growth leads to the formation of new blood vessels (angiogenesis) is necessary to draw nutrition directly from the systemic circulation [8,9]. The tumor remains in a dormant state until it can stimulate blood vessel growth from nearby pre-existing capillaries [10, 11,12]. Inhibiting angiogenesis has been considered as an important anticancer strategy to suppress tumor growth and metastasis. Antiangiogenic therapy designed to block neovascularization is an evolving anticancer strategy [13, 14]. Antiangiogenic tumor therapies are recently attracted because of their broad spectrum of action, low toxicity, and absence of drug resistance [13]. A number of therapeutic agents having antiangiogenic property have been developed and used in clinical trials [15,16,17,18, 19]. In the present investigation the ability of extracellular metabolites of Actinomycetes from mangrove soil was tested against different pathogenic bacteria and their wound healing property has been reported.

MATERIALS AND METHODS

Isolation of Actinomycetes

Mangrove soil samples were collected from rhizosphere region of the mangrove plants in Pennar, Nellore District, and Andhra Pradesh, India. The collected sample was transferred to laboratory under sterile conditions. The sample was air dried for 15 days and pretreated at 50^0C for 10 minutes to eliminate the unwanted bacterial population in the soil. The pretreated sample was used for isolation of actinomycetes by using Starch Casein Agar medium [20]. After isolation the Actinomycetes. The strains screened for potent strains for production of bioactive compound.

Screening of actinomycetes for bioactive compound production

The actinomycetes colonies in the medium were cultured by streaking on isolation medium to obtain pure cultures. Well grown cultures were inoculated in 50ml of optimized medium with 60% distilled water and 40% Sea water, pH 7.2 and incubated for 5days in rotary shaker at 120 rpm maintained at 28^0C temperature. After incubation the broth was filtered with Whattmann No.1 filter paper, and the filtrates were used as source of bioactive compound to test the antibacterial and antifungal activity by agar diffusion method [21]. The following pathogens are used in this study *Bacillus, Pseudomonas, Staphylococcus* and *E.coli*.

Production and extraction of angiogenic compounds

The fermentation broth was inoculated with 72h old culture of inoculum at 120rpm for 7days and the temperature was maintained at 28^0C. After incubation the broth was filtered followed by centrifugation at 5000 rpm to remove the cells and debris from the fermentation broth. Then broth was mixed with equal amount of ethyl acetate and kept for stirring by using magnetic stirrer. The organic phase was separated and submitted to TLC analysis on silica gel plate with dichloromethane, ethylacetate and methanol as mobile phase. UV light with range 200 to 350nm was used for detection.

Angiogenic activity of bioactive compound

The fluroscent compounds in the UV light was scrapped from the TLC plate and mixed with the mobile phase and centrifuged at 5000rpm to remove the silica gel. Then the supernatant was collected and the organic solvents was evaporated at 50^0C and the thick solvent remained was crystallized by using lyophillizer. These crystals were dissolved in distilled water and used to check the angiogenic activity. The angiogenic activity of the isolated compound was conducted on CAM region of fertile eggs collected from the Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India. These eggs were incubated for 3days at 37^0C and on 4^{th} day the eggs were surface sterilized by wiping with 70% alcohol and different concentrations of the compound was injected into the CAM region. Celecoxib was used as positive control and a negative control was also maintained. After 48hours the eggs were opened and the formation of new blood vessel was observed by comparing the control eggs without compound.

RESULTS

Five Actinomycetes strains were isolated from mangrove soil and screened for bioactive compound production The screening resulted in five potential *Actinomycetes (marked as GN!-GN5)*with morphological differences were tested for their antimicrobial property against both Gram-positive and Gram-negative organisms and results were shown in Table 1.

Table 1 Antibacterial activity of bioactive compounds from actinomycetes

Actinomycetes	Bacterial cultures (Zone of inhibition in cm)			
	Bacillus	*Pseudomonas*	*Staphylococcus*	*E.coli*
GN1	1.5	0.8	1.1	1.6
GN2	1.6	1.2	1.5	1.9
GN3	1.2	0.5	1.0	1.4
GN4	1.5	0.9	1.3	1.7
GN5	1.0	0.7	0.8	1.4
Streptomycin (Control)	1.2	0.8	0.9	1.5

**Values represented in the table are mean of two separately conducted experiments*

Among these strains GN2 was more potential strain when compared to other strains.In the chick CAM model, the ethyl acetate extract of GN2 strain showed an increase in density of blood capillaries on the treated membrane

surface of embryo. The neovascularindex (number of blood vessels/cm2) were measured. Whereas untreated (control)(without addition of bioactive compound) egg showed average neovascular index of 4.1/cm2, while the solvent control (ethyl acetate) showed an index of 3.7/ cm2. The commercial drug (positive control), celecoxib at a conc. of 20 µg/egg showed an index of 4.6/cm2. In comparison to these, extract of the culture filtrate at concentration of 20µl/egg showed the indices of 3.9 /cm2. The negative control (commercial drug Doxirubicin) does not show the blood vessel formation. These results clearly indicate that the bioactive compound extracted from Actinomycetes strain (GN2) shows an excellent wound healing property.

1a **1b**

1c **1d**

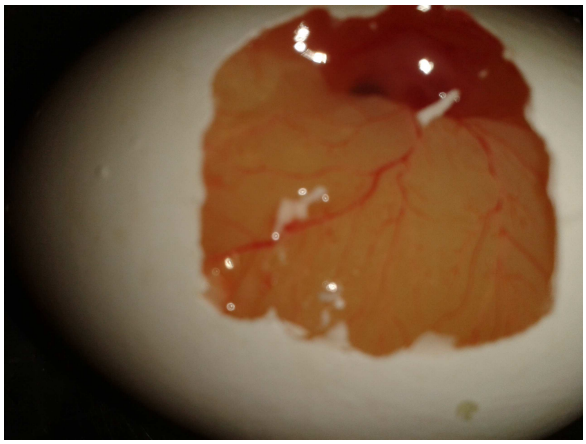

Fig.1a Positive control (celecoxib)- commercial drug for blood vessel formation
Fig.1b. Control without inoculation of bioactive compound/drug
Fig.1c. Negative control (Doxrubicin)- commercial that inhibits the blood vessel formation.
Fig. 1d. Bioactive compound inoculated- formation of blood vessel in embyonated egg.

DISCUSSION

Angiogenesis is important in normal processessuch as the development of the embryo, formationof corpus luteum, and wound healing [22, 23]. Angiogenesis during wound repair serves thedual function of providing the nutrients demanded bythe healing tissues and contributing structural repairthrough the formation of granulation tissue [24]. The streptochlorin, a compound from marine actinomycetes exhibits a potent angiogenic activity and inhibits NF-kB activation. The streptochlorin inhibit the endothelial cell invasion and tube formation with vascular endothelial cell growth factor at low concentration [25]. An unknown compound from *Ziziphusoenoplia* root ethanolic extract was found to have angiogenic compound which was proved in CAM region of fertilized eggs [26]. Sushil [26], used

Mimusops elengi (Sapotaceae) bark along with Yashad Bhasma for treatment of wound in albino rats. The methanolic extract of leaves of Achyranthes aspera was examined for wound healing activity in the form of ointment on albino mice [27].

CONCLUSION

In this study, the bioactive compound which having the wound healing property was isolated from Actinomycetes strain from mangrove soil. Among the Actinomycetes isolates tested in this study, the strain GN2 produces an effective bioactive compound which shows a better wound healing property and it was confirmed by CAM assay method. Further work needs to be identification and characterization of the bioactive compound responsible for wound healing property.

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