Detection of antimicrobial activity in accessory gland secretions of the virgin male red palm weevil, Rhynchophorus ferrugineus

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Citation

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Abstract

Background: Bioactive molecules have been identified from accessory gland secretions in insects. Antibacterial properties are identified in secretions of insect reproductive system. Study design: Antibacterial screening of male accessory reproductive gland (MARG) secretions in red palm weevil, R. ferrugineus was conducted by disc diffusion sensitivity method against eight bacterial species. Result: Accessory gland secretions showed antibacterial property as growth inhibition zones in all bacteria used in this study. Conclusion: Antimicrobial property of MARG may be evolved to protect the gametes during mating since reproductive tract is also route of microbial invasion in insects.

INTRODUCTION

Male accessory gland secretions in insects contain a variety of bioactive molecules which influences the female reproductive functions (Gillot, 2003). Reproductive ducts are the major route of microbial invasions. The ability of reproductive tracts to defend microbial attack has been explored among animals (Com et al., 2003). Antimicrobial peptides are important in the first time host defense system of many animal species (Boman, 1998). Many antibacterial peptides are clinically important as antibiotic (Hancock and Chappel, 1999). Antimicrobial peptides secreted from male and female reproductive tracts are suggested to maintain the immunity against microbial infection (Lung et al, 2001). An insect defensin, antibacterial peptide was identified and purified from larvae of Coconut beetle, Oryctes rhinoceros immunized with Escherichia coli (Ishibashi et al., 1999). Ceratotoxin, anibacteril 3kDa peptide was isolated from female reproductive glands of Ceratitis capitata (Marchinni et al., 1995).

Lung et al. (2001) identified the male accessory gland derived antibiotic activities in Drosophila. Antimicrobial peptides are transferred from male D. melanogaster to female during mating (Lung & Wolfner, 2001). In this respect the present study was conducted to screen the male accessory gland secretions for the antimicrobial property in the virgin male red palm weevil, Rhynchophorus ferrugineus.

MATERIALS AND METHODS

Insect: Last instar larvae and cocoons of R. ferrugineus were collected from the crown of infected coconut tree. Larvae and cocoons were individually maintained in separate containers in the laboratory and allowed to emerge into adult. Males were identified by examining the presence of rostral hairs. Five day old virgin male insects were selected for this study. Insects were dissected in Ringer bath. Male accessory reproductive gland (MARG) was identified and carefully dissected out into sterile cavity slides. MARG was then homogenized and centrifuged at 3000 rpm in cold centrifuge for 10 minutes. The supernatant fraction was identified containing secretory substance.

Microorganism: Eight bacterial strains including both Gram +ive and Gram –ive were used as test organism and were obtained as a gift from Prof. Dhevendaran K, Department of Aquatic biology, University of Kerala, Thiruvananthapuram. Microbial strains used were Bacillus subtilis, Bacillus megaterium,Escherichia coli,Pseudomonas aeruginosa,Proteus vulgaris,Salmonella,Staphylococcus aureus and Vibrio cholerae

Screening for antibacterial activity: Disc diffusion method was used to screen antibacterial activity (Cruckshank et al., 1975). Strains of bacteria were transferred aseptically with an inoculating loop to a 50 ml sterile nutrient broth in a conical flask and incubated at 300C in a BOD incubator for 24 hrs to get fresh bacterial inoculum. Fresh inoculum (0.1ml) of each culture containing 108 cells was spread over sterile agar plate using sterile cotton buds. Whatman's filter paper No. 1 sterile disc of 5 mm was placed in the middle of each Petri plate seeded with bacterium. Two more paper discs were placed on the agar plate with equal distance apart peripherally. 20 Il of accessory gland sample was loaded in the central paper disc and two antibiotic drugs viz., chloramphenicol and ampicillin (10 mg) were loaded in the peripheral ones as control. Bacterial cultures were incubated at 370C for 18-20 hrs before observing the zone of inhibition. Cultures were examined for the antibacterial activity as zone of growth inhibition and the results were tabulated.

RESULTS

Figure 1

Table – 1: Antibacterial activity of accessory gland (MARG) secretion

no		Growth inhibition zone in m.m		
	Bacterial species	Accessory	Ampicillin	Chloramp
		gland		henicol
		extract		
1	Bacillus subtilis	12	15	17
2	Bacillus megaterium	12	16	18
3	Escherichia coli	15	17	18
4	Pseudomonas aeruginosa	12	17	20
5	Proteus vulgaris	15	15	18
6	Salmonella	18	18	20
7	Staphylococcus aureus	12	15	18
8	Vibrio cholerae	14	17	18

Results were tabulated in table -1. The accessory gland extract showed zones of inhibition towards all the bacterial strains used in this experiment. Maximum activity was shown against Salmonella sps. Both E.coli and Proteus vulgaris were affected considerably and stand as second in growth inhibition. All other species were moderately inhibited.

Figure 2

Fig. 1. Inhibition zones in different bacterial colony



DISCUSSION

Antibacterial property of accessory gland secretions form virgin male of R. ferrugineus is demonstrated in the present study. All the bacterial strains used in this screening were sensitive to the MARG as zone of inhibition was noticeable above 2 mm. The present study can be included in the existing list of the few reports of antibacterial properties of insect reproductive secretions. Antibacterial proteins have been identified in Drosophila melanogaster and found expressed within the male reproductive tract (Samakovlis et al., 1991; Lung et al., 2001). A novel member of insect defensins was purified from the larvae of coconut beetle, Oryctes rhinoceros (Ishibashi et al., 1999). Marchini et al. (1995) identified ceratotoxin, an antibacterial sex peptides from the reproductive gland of med fly, Ceratitis capita.

Microorganism carried by the male genitalia can be introduced into the female during mating (Lung et al., 2001). This could be overcome by antibacterial secretions produced from the male accessory glands which can also protect the sperm from microbial attack. Lung et al. (2001) suggested that proteins of MARG induce systemic protection in female since seminal fluid proteins are reported to enter haemolymph of females. Antimicrobial peptides have served a fundamental role in the successful evolution of complex multicellular organisms (Zasloff, 2002). Thus the antibacterial property of MARG in virgin males of R. ferrugineus can be considered to be evolved to assist reproductive success of this insect by eliciting microbial protection in adverse mating conditions.

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