

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

L Joseph, V Josekumar, P George

## Citation

L Joseph, V Josekumar, P George. *Detection of antimicrobial activity in accessory gland secretions of the virgin male red palm weevil, Rhynchophorus ferrugineus*. The Internet Journal of Microbiology. 2008 Volume 7 Number 1.

## 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, antibacterial 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 (Cruickshank 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 30°C in a BOD incubator for 24 hrs to get fresh bacterial inoculum. Fresh inoculum (0.1 ml) of each culture containing 10<sup>8</sup> 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 µl 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 37°C 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**

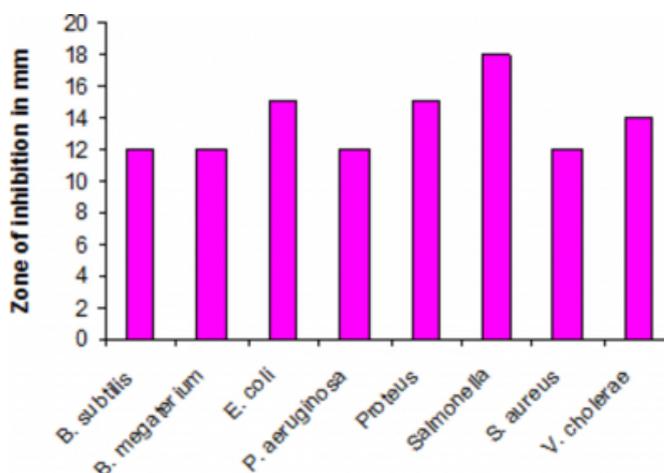
Table – 1: Antibacterial activity of accessory gland (MARG) secretion

Sl no.	Bacterial species	Growth inhibition zone in m.m		
		Accessory gland extract	Ampicillin	Chloramphenicol
1	<i>Bacillus subtilis</i>	12	15	17
2	<i>Bacillus megaterium</i>	12	16	18
3	<i>Escherichia coli</i>	15	17	18
4	<i>Pseudomonas aeruginosa</i>	12	17	20
5	<i>Proteus vulgaris</i>	15	15	18
6	<i>Salmonella</i>	18	18	20
7	<i>Staphylococcus aureus</i>	12	15	18
8	<i>Vibrio cholerae</i>	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 from 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 peptide from the reproductive gland of med fly, *Ceratitis capitata*.

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 (Zaslouff, 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.

## ACKNOWLEDGEMENT

Authors like to thank Prof. M I Georgekutty, former HOD,

Department of Zoology, St. John's college, Anchal to extend the microbiology lab facilities while VSJ was employed there. L J acknowledges the financial support from UGC by the award of FIP during this study. Gift of bacteriological strains from Prof Dhevendaran K, Department of Aquatic Biology, University of Kerala is also gratefully acknowledged.

### References

- r-0. Cruickshank R., Duguid I P., Marmion B P and Swain, R H A. *Microbiologia medica*, vol. 11 4th ed. Fundacao Calouste Gulbenkian Lisboa. 1975. pp. 393-403.
- r-1. Boman H G. Gene-encoded peptide antibiotics and the concept of innate immunity: an update review. *Scand J Immunol.* 1998. 48:15-25.
- r-2. Com E., Bourgeon F., Evrard B., Ganz T., Collet D., Jegon B., Pineau C. Expression of antimicrobial defensins in the male reproductive tract of rat, mice and humans. *Biol Reprod.* 2002. 68: 95-104.
- r-3. Gillot C. Male accessory gland secretions: modulators of female reproductive physiology and behaviour. *Annu. Rev. Entomol.* 2003. 48: 163-184.
- r-4. Ishibashi J., Saido-Sakanaka H., Yang J., Sagisaka A., Kawa M Y. Purification, cDNA cloning and modification of a defensin from the coconut beetle, *Oryctes rhinoceros*. *Eur. J. Biochem.* 1999. 266:616-623.
- r-5. Hancock R E W., and Chappel D S. Peptide antibiotic. *Antimicrobial Agents and Chemotherapy.* 1999. 43(6): 1317-1323.
- r-6. Lung O., and Wolfner M F. Identification and characterization of the major *Drosophila melanogaster* mating plug protein. *Insect Biochem. Mol. Biol.* 2001. 31:543-551.
- r-7. Lung O., Kuo L., Wolfner M F. *Drosophila* males transfer antibacterial proteins from their accessory gland and ejaculatory duct to their mates. *J. insect Physiol.* 2001.47: 617-622.
- r-8. Marchini D., Manetti A G O., Rosetto M., Bernini L F., Telford J L., Baldari C T., Dallai R. cDNA sequence and expression of the ceraotoxin gene encoding an antibacterial sex-specific peptide from the med fly *Ceratitis capitata* (diptera). *J Biol Chem.* 1995. 270 (11): 6199-6204.
- r-9. Samakovlis C., Kylsten P., Kimbrell D A., Engstrom A., Hultmark A. The andropin gene and its product, a male-specific antibacterial peptide in *Drosophila melanogaster*. *EMBO J.* 1991.10: 163-169.
- r-10. Zasloff M. Antimicrobial peptides of multicellular organism. *Nature.* 2002. 415: 389-395.

**Author Information**

**Leenamma Joseph, Ph.D.**

Lecturer Sel.Gr., Department of Zoology Mar Ivanios College Thiruvananthapuram, India

**V.S. Josekumar, Ph.D.**

Reader, Department of Zoology Mar Ivanios College Thiruvananthapuram, India

**P.V. George, Ph.D.**

Research Director, Department of Zoology Mar Ivanios College Thiruvananthapuram, India