

Original Research Article

Bacteriostatic effect of *Lampito mauritii* (Kinberg) coelomic fluid and cell extract on pathogens

J. Marlin Cynthia^{1*}, K. Uma¹ and R. Uma Devi²

¹Department of Zoology, National College Trichirappalli, Tamil Nadu, India

²Department of Zoology, Bishop Heber College, Trichirappalli, Tamil Nadu, India

*Corresponding author

A B S T R A C T

Keywords

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The earthworm is one of the typical saprozoic organisms, living in the environment replete with microorganisms some of which may be a threat to their existence. To survive in such an environment, they have developed efficient immuno-defense mechanisms against invading microorganisms. In the present study antibacterial properties of earthworm coelomic fluid and cell extract was investigated for bacteriostatic effect against five different pathogens (*Aeromonas hydrophilia*, *Bacillus megaterium*, *Staphylococcus aureus*, *Salmonella typhi*, and *Klebsiella pneumoniae*). The bacterial growth inhibition was observed after 16 hrs as well as 24 hrs. In our investigations both extracts showed bacterial inhibition. *Bacillus megaterium* and *Staphylococcus aureus* are inhibited the most. This study indicates that earthworm extract can be used for antimicrobial agent against pathogenic microorganisms causing serious disorders

Introduction

Invertebrates have innate immune mechanisms that recognize conserved molecular patterns for detecting of pathogens. Immune mechanisms of earthworm include both cellular and humoral components which comprises nonspecific and specific responses. The cellular defense carried out by two immune cells namely amoebocytes and oeleocytes which are responsible for phagocytosis, agglutination and cytotoxicity (Hong, 2007). Earthworm coelomocytes respond to the presence of

pathogens by phagocytosis, encapsulation /brown body formation, and NK-cells activity (Cooper *et al.*, 2001). It is claimed that earthworm's coelomic fluid possess some bactericidal capacities. They secrete the humoral molecules for the humoral defense, release molecules having the capacity to lyse erythrocytes and agglutinate them (Stein *et al.*, 1982; Wojdani *et al.*, 1982), or protect earthworms against bacterial infection (Lassegues *et al.*, 1989; Stein *et al.*, 1986). Also the earthworm's fibrinolytic enzyme

show antitumor activities on human hepatoma cells. It is also reported that extract isolated from earthworm, (*Lampito mauritii*) show anti-inflammatory and antipyretic activities.

The Annelid coelomic fluid inhibits the growth of both Gram-positive and Gram-negative bacteria previously shown to be pathogenic for the worms (Valembois *et al.*, 1982). It is supposed that earthworms living in the pathogen-abundant environment must have peptides against bacteria (Balamurugan *et al.* 2008). Bacteria pathogenic to earthworms include *B. megaterium*, *B. thuringiensis*, *Aeromonas hydrophila* and a species of *Breibuterium* and *B. diphtzerie* (Roch 1980). Antimicrobial agents of earthworms are formed in the earthworm body but not by the soil microorganism entering their digestive tract (Khomyakov *et al.*, 2007). We report here results of tests on the effect of the coelomic fluid, of coelomocytes and extracts of coelomocytes on different bacteria. In an attempt to determine the bacterial specificity of this humoral antibacterial activity, we focused on five sensitive bacterial strains.

Materials and Methods

Isolation of Coelomic fluid (CF) and Cell extract (CX)

Twelve healthy adult earthworms with grown clitellum were selected and kept in clean water for 3 hours to clear the gut contents. Worms were taken and washed several times in sterile saline water. Worms were given a shock of 5mV electric current for 10 seconds. Extruded coelomic fluid was collected and centrifuged for 5 min at 1000 rpm. The cell pellet were homogenized by adding

0.5 ml of saline and labelled as Cell extract (CX) and cell free coelomic fluid as CF.

Bacteriostatic Activity

Stock cultures of 5 strains of bacteria (*Aeromonas hydrophilia*, *Bacillus megaterium*, *Stephylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*) were obtained and maintained in sterile chambers on nutrient solid agar and was serially diluted to get standard density of 4×10^6 . One ml of Log phase growing bacteria were centrifuged and re-suspended in nutrient broth and labeled as controls. The experimental strains were incubated in nutrient broth with 200 ul of coelomic fluid and cell extract were for 24 hours under orbital shaking. The optical density was recorded after 16 hrs and 24 hrs, and percentage of inhibition was obtained using formula

$$\frac{\text{Test absorbance} - \text{control absorbance}}{\text{Control absorbance}} \times 100$$

Statistical analysis of student t test was carried out using SPSS. There was statistically significant difference ($p < 0.005$) observed in control and treated samples.

Results and Discussion

Earthworms lack real antibodies and efficient innate immune systems to defend themselves against invading foreign materials like vertebrates. Alternatively, it is supposed that earthworms must have some active antibacterial proteins and peptides in the defense mechanism against bacteria. Antibacterial substances in the coelomic fluid of earthworm have been shown to inhibit growth of some bacteria and these substances are enhanced on infection (Lassegueues *et al.*, 1989).

Figure.1 Bacterial Growth inhibition after 16 hr

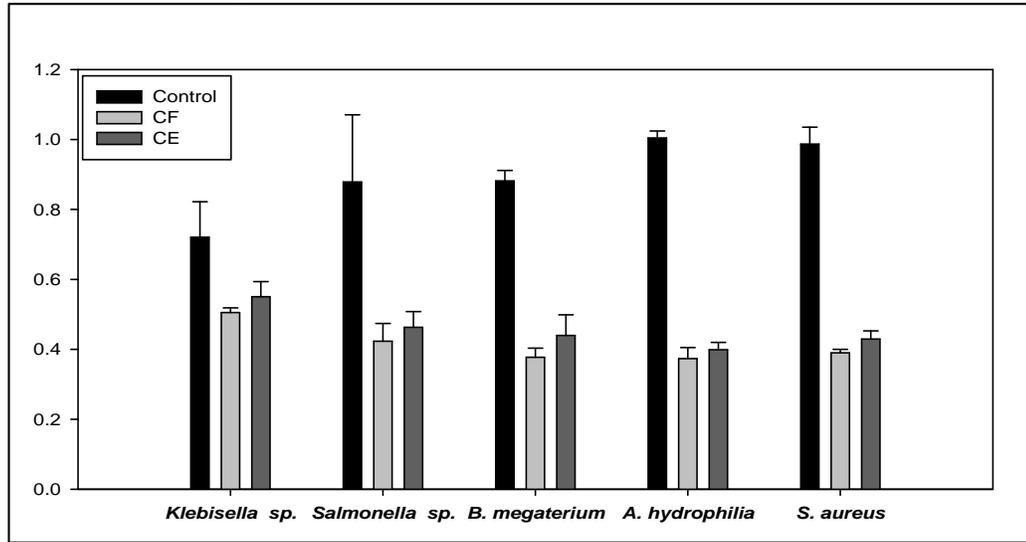


Figure.2 Percentage Bacterial Growth inhibition after 16 hr

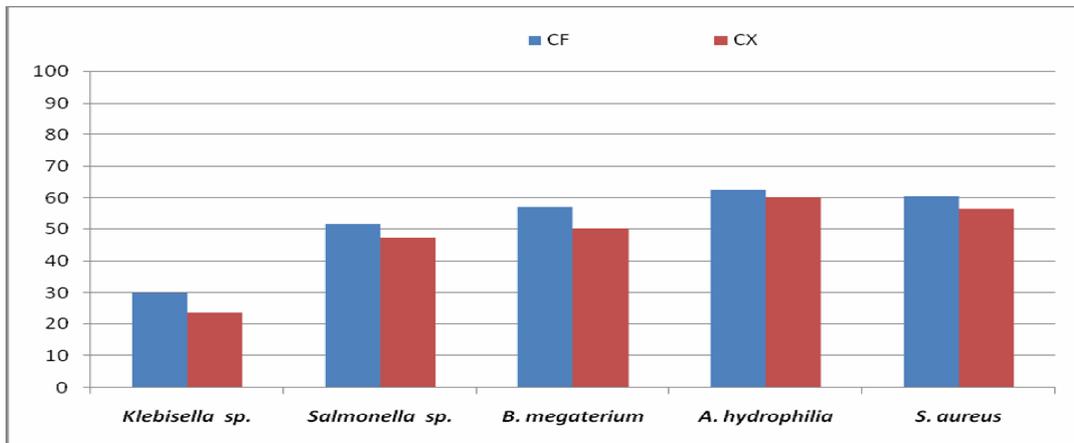


Figure.3 Bacterial Growth inhibition after 24 hr

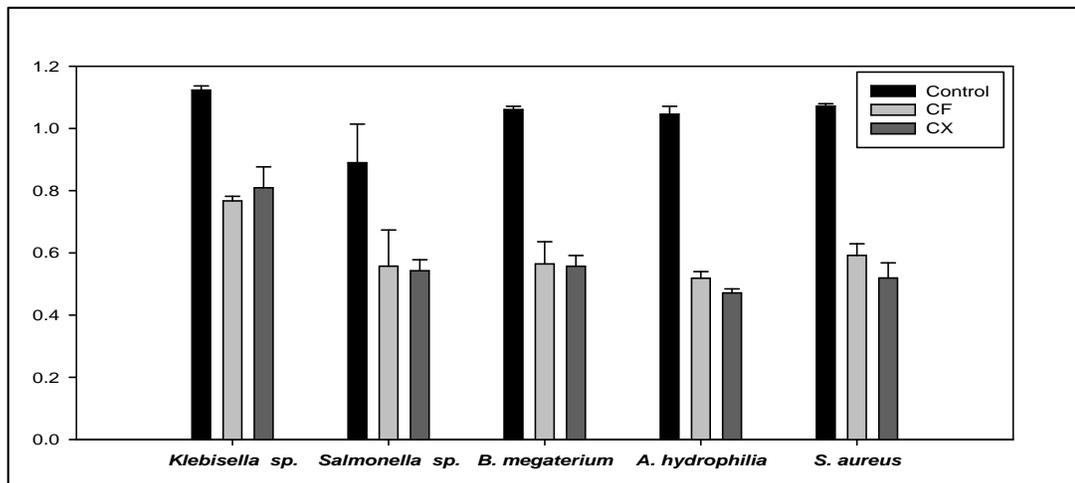
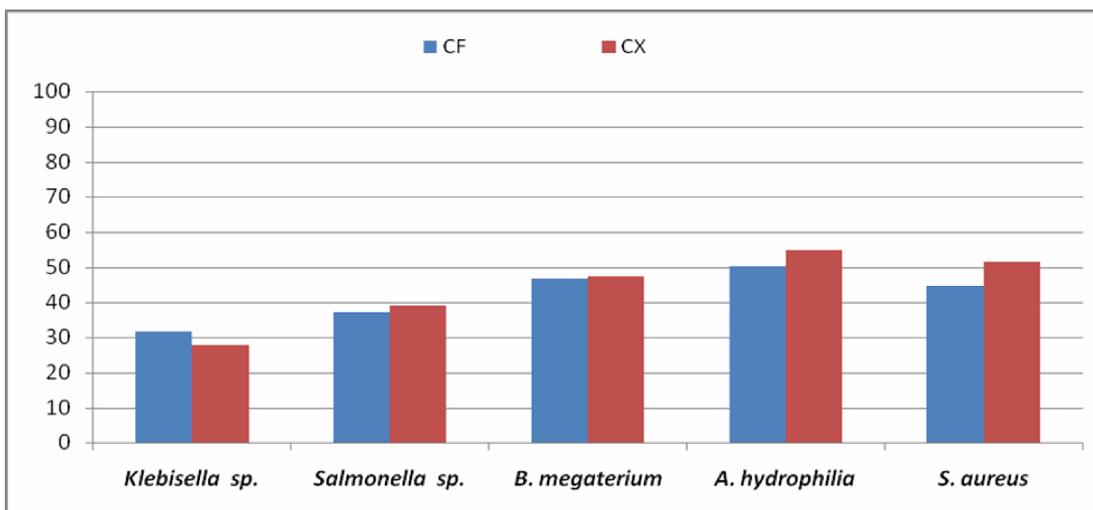


Figure.4 Percentage Bacterial Growth inhibition after 24 hr

In this study both cell extract and coelomic fluid from *Lampito mauritii* was tested for antibacterial activity. The bacterial growth inhibition was observed after 16 hrs (Figure 1 & 2) as well as 24 hrs (Figure 3 & 4). In our investigations both extracts showed bacterial inhibition and the stains of *Bacillus megaterium* and *Staphylococcus aureus* are inhibited the most.

Antibacterial peptides (ABP) are an important defense component. It has been reported that the level of ABP was determined by inducement and gene expression (Shike *et.al.*, 2002). Many antibacterial peptides have been reported in various invertebrates and vertebrates (Jay *et. al.*, 2005, Tomasinsig *et. al.*, 2002). Cell extracts invariably showed greater lysozyme activity than coelomic fluid. Roch *et al.* (1981) have reported that the bacteriolytic activity of the coelomic fluid of another earthworm *Eisenia foetida* Sav. is due to a glycolipid derived from the chloragocytes. Observations on *E. foetida* (Cotuk and Dales, 1984) suggested that there may be at least two bacteriolytic factors in the body fluid and in the coelomocytes, that described by Roch

(1980) and Roch *et al.* (1981), the other resembling lysozyme. This study indicates that earthworm extract can be used for antimicrobial agent against pathogenic microorganisms causing serious disorders.

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