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EXPERIMENTAL CONFIRMATION OF INVITRO ANTI-PSEUDOMONAL ACTIVITY OF COELOMIC FLUID OF EARTHWORM OCTOCHAETONA SURENSIS.

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ABSTRACT

Introduction: Earthworms are terrestrial invertebrates belonging to the phylum Annelida. They exhibit a cylindrical structure and contain a tubular cavity within which is called as the coelomic cavity. This cavity is usually fille with a colourless white to opaque or a yellowish translucent fluid which is called as the coelomic fluid. This fluid contains specialised cells called as the coelomocytes which is widely studied to confer anti-microbial activity to the earthworm. They are called as natural recyclers of the earth and hence derives its name as earthworm as they are commonly seen within the soil in different habitats. *Pseudomonas aeruginosa* is a commonly found gram negative anaerobe, which is an encapsulated rod-shaped bacterium that is implicated in dangerous nosocomial infections to the humans viz a viz., plants and animals. In the current era, due to the emergence of resistance to antibiotics as a result of injudicious and irrational usage, the discovery and availability of antibiotic has come to a standstill, in the same context, due to the emergence of resistance among most of the bacterial species, the effectiveness of the conventional antibiotics have decreased and gram negative anaerobes poses major challenge in medical practice as the availability of antibiotic that could treat the infection is scarce. Hence, newer modalities of drug discovery and finding major leads that could become a potential candidate for further antibiotic discovery is the grave need of the hour. In our study we found excellent anti-pseudomonal activity of coelomic fluid of earthworm *Octochaetona surensis* against *pseudomonas aeruginosa* which was much better than the drug used to treat the infection in the present condition. Further investigations and basic in-depth research could help us develop a potential newer antibiotic that could tackle the menace of pseudomonal resistance. **Aims and Objectives:** In this study we aim to demonstrate the antimicrobial activity of varied concentrations of coelomic fluid of earthworm *Octochaetona surensis* against gram negative anaerobe *Pseudomonas aeruginosa*, which is an ATCC (American Type Culture Collection) strain preserved through serial cultures in the microbiology laboratory. The concentrations used were (5 μl, 10 μl, 15 μl, 20 μl/ disc). **Materials and Methods:** A standard antibiotic disc of AMP (Ampicillin) 10 μg /disc was used as positive control for pseudomonas and a plane Whatman's filter paper disc was used as a negative control in a Petri plate. The entire Petri plate with the inoculum was incubated overnight in the temperature of 37°C following which the results were tabulated. **Results and Conclusion:** Coelomic fluid of earthworm has demonstrated a definite and excellent zone of both cidal and inhibitory activity that is visible as zone of clearance at a concentration as less as 5 μl/disc against *P. aeruginosa* when compared to the positive control which is the 10 mg standard antibiotic disc inoculated in the same petri-plate. Since *P. aeruginosa* is implicated in variety of nosocomial infections, identifying the susceptibility pattern of coelomic fluid of different species of earthworm would help us to move forward towards a newer antibiotic discovery route and enable us to generate leads for better antibiotics in the future.

KEYWORDS : Earthworms; Antimicrobial activity; Coelomic fluid; Octochaetona Surensis; P. aeruginosa;Antimicrobial substances; Antimicrobial resistance.

INTRODUCTION

Earthworms have inhabited earth since time immemorial, records says that they existed on this planet for as early as 700 million years ago[1]. These organisms are annelids belonging to the class oligochaete. They are referred by multiple names like farmers friend, rain dew worms, nature's ploughman etc. these are tube like organisms that contain a coelomic cavity filled with coelomic fluid, they are hermaphrodites which have a characteristic segmentation of their body called as the metameric segments, and these segments are present both externally and internally[2]. The coelomic fluid of earthworms contain four distinct types of cells which are biologically active molecules and are of four types namely amoebocytes, mucoytes, circular cells and chlorogogen cells which are together called as the coelomocytes. Apart from these cells the coelomic fluid contains watery matrix and plasma[3]. These coelomocytes are biologically active molecules which exhibit phagocytosis and encapsulation, they are known to secrete effector modulators of innate immunity such as the cytotoxic proteins and antimicrobial molecules[4]. Lysozymes and fetidines of the coelomic fluid are known to exert antimicrobial activity[5].

Superbugs have dominated the planet in the recent times, due to injudicious usage of antibiotics, irrational prescription patterns and erroneous intake of antibiotics without a proper indication or antimicrobial culture and sensitivity patterns have led to the development of resistance to antibiotics and emergence of antimicrobial resistant strains are on the high[6]. This has led to increase in the number of hospital infections and fatality due to acute shortage of antibiotics. The pharmaceutical pipeline has hardly any leads that could become a potential antibiotic to combat the emerging resistant patterns specially the nosocomial infections[7]. In this

context, uncertainty and heavy cost incurred by pharmaceuticals for bringing out new antibiotics in the market has led to a standstill in antimicrobial drug arena[8].

This acute shortage of antibiotics to combat the emerging resistant strains of micro-organisms calls for active screening of crude products and multimodal approach to develop leads which shows promising antimicrobial activity and which can become a potential antibiotic candidate in the coming days is the need of the hour.

Extensive research from the early sixties on the coelomic fluid of earthworm has demonstrated several biological activity ranging from antimicrobial and cytolytic activity[9]. India has many indigenous species of earthworm such as *Eudrilus eugeniae*, *Eisenia fetida*, *Lampito mauritii*, *Dichogaster boluii*, *Eudichogaster prashadii* etc[10]. which have been studied extensively in various regions of Indian subcontinent and other countries.

In the current study we are focussed to evaluate the antimicrobial activity of the earthworm *Octochaetona surensis*, which is an indigenous species of earthworm, whose coelomic fluid is scanty watery and appears white in the day light. This earthworm oozes very scanty amount of coelomic fluid, hence judicious usage is mandated to get effective results.

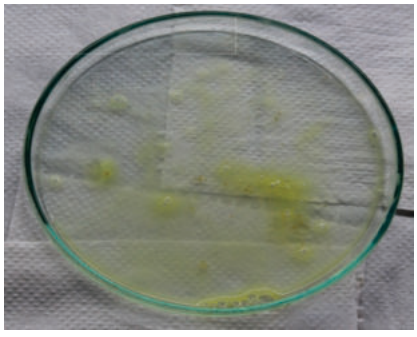

Fig.1 Physical appearance of fresh Coelomic fluid extracted from *Octochaetona surensis*

Fig. 2 Picture of *Octochaetona surensis* in a washing tray

MATERIALS AND METHODS

Collection of Earthworms

Digging and hand sorting method was applied to collect the earthworm *Octochaetona Surensis* for the current study. Worms, were kept in a separate collection and storage basket with proper aeration and their habitat soil, required temperature, moisture was maintained conducive for their viability. This method for collection of earthworms is widely used for sampling. The earthworm in the present study was collected from a field Chittapur Taluk, Kalaburagi, India, Karnataka.

Collection of Coelomic Fluid.

Electric shock method

Octochaetona surensis, the earthworm in the current study were collected and fed with tissue papers for 48 hours to eliminate gastrointestinal metabolites and contaminants. They were then washed under running tap water in a tray and rapidly dried on a filter paper. These worms were then subsequently excited with 5 volt electric stimulations to produce coelomic fluid through their dorsal pores [13]. The collected coelomic fluid was then used for antimicrobial activity.

Collection of pathogenic bacteria for antimicrobial studies

ATCC strains of *Pseudomonas aeruginosa*. With their known pathogenesis and drug resistance profile, were obtained from Department of microbiology, Raja Rajeswari Medical College and Hospital, Bangalore. The pathogenic bacteria Pseudomonas aeruginosa are tested against the collected coelomic fluid of *Octochaetona Surensis*.

Table: 1. Pseudomonas strain against the coelomic fluid of *Octochaetona Surensis*.

SL.NO	MICROBE TESTED (ATCC STRAINS)
4	Pseudomonas aeruginosa (ATCC 27853)

The in-vitro antimicrobial activity was screened against *Pseudomonas aeruginosa* (ATCC 27853) by using Muller Hinton Agar (MHA). To prepare the MHA plates, sterile Petri plate were taken and 15ml of molten media was poured into it, these plates were allowed a resting time of 5 minutes for solidification. Using a sterile swab, 0.1 % of the inoculum was swabbed uniformly over the Petri plate and allowed to dry for five minutes. Subsequently, sterile autoclaved 6mm Whatman's filter paper disc was placed on the surface of the medium. Four concentration of the coelomic fluid (5 μl, 10 μl, 15 μl, 20 μl / disc) of *Octochaetona surensis*, were loaded on 6mm sterile Whatman's filter paper disc. The different concentrations of coelomic fluid loaded in the disc were allowed to diffuse for 5 minutes. The plates were then kept for incubation at 37 degrees Centigrade for 24 hours. At the end of incubation, Inhibition zones formed around the disc were measured with standard zone reader scale in mm (millimeter). The bacterial inoculum was matched with 0.5 McFarland 10^8 CFU/ml adding sterile nutrient broth, before incorporating bacteria (λ=625 nm). A disc of Ampicillin (AMP) (10 μg/disc) was used as a positive control and a plane 6mm Whatman's filter paper disc was used as a negative control.

RESULTS

The coelomic fluid from *O. surensis* was tested against standard ATCC strain of bacteria *Pseudomonas. aeruginosa*. The observed zone of inhibition exhibited by the coelomic fluid of *E. prashadi* against the positive and negative control are tabulated and the observed values are furnished in the following Table.

Table.2 Antimicrobial activity of *Octochaetona Surensis* against standard ATCC strain of bacteria *Pseudomonas aeruginosa* at different concentrations.

MICROBES TESTED	CONCENTRATIONS OF COELOMIC FLUID OF O. SURENSIS TESTED IN MILLIMETRES (mm)				POSITIVE CONTROL Ampicillin (AMP) (10μg/disc)	NEGATIVE CONTROL
	5μL	10μL	15μL	20μL		
P. aeruginosa	20	31	35	37	-	-

The activity of different concentrations of coelomic fluid of *Octochaetona surensis* was evaluated against selected ATCC strains of *Pseudomnas aeruginosa*. Four different concentrations (5μl, 10μl, 15 μl and 20 μl) were used. Against standard ATCC strain of *P. aeruginosa*, the coelomic fluid of *Octochaetona surensis* has exhibited maximum zone of inhibition in *P. aeruginosa* at all the concentrations with a maximum value at 20 μl (37mm) followed by minimum zone of inhibition at 5μl (20mm) and no zone of inhibition with the positive control.

Fig.6 Antibacterial activity of Coelomic fluid of *O. surensis* against standard ATCC strains of *P. aeruginosa* with a positive control of Ampicillin (AMP) (10μg/disc) and Negative control as plain 6mm Whatman's filter paper disc.

DISCUSSION

Medzhitov R, carried out extensive research in his article published by the Nature 1997, wherein he has explained in detail the molecular mechanisms of the coelomocytes present in the coelomic fluid of earthworm which exhibits the pathogenic microbe killing[11], the specific determinant in the coelomocyte and the pathway that is responsible for the antimicrobial activity needs to be studied further. It was reported by (Milochau et al 1997) that the coelomic fluid of lumbricid *Eisenia. faetida*e possessed antimicrobial activity.

In the current study attempts were done to evaluate the antimicrobial activity of the earthworm *Octochaetona surensis* coelomic fluid against the ATCC strains of microbe *Pseudomonas aeruginosa*. The results obtained from this experiment shows that, when the antimicrobial activity of the coelomic fluid of earthworm *Octochaetona surensis* was observed against pseudomonas aeruginosa with standard comparator antibiotic Ampicillin 10 μg/disc, it exhibited maximum zone of inhibition

The Petri plate containing lawn culture of *P. aeruginosa* inoculated with coelomic fluid of *O. surensis* as compared with standard antibiotics shows following zones of inhibition at concentrations 20 μl (37mm), 15 μl (35mm), 10 μl (31mm) and minimum zone of inhibition at 5μl (20mm) concentration respectively. The standard antibiotic Ampicillin 10 μg/disc used showed no zone of inhibition. Hence it is evident that coelomic fluid of *O. surensis* has considerable amount of activity and both static (zone of clearance) and cidal (zone of inhibition) potency against *P. aeruginosa* even at least concentrations of 5μl (20mm). Therefore, further studies may lead to discovery of a novel compound responsible for antimicrobial activity in coelomic fluid of *O. surensis*.

CONCLUSION

The results obtained from this In-vitro experiment are quite fascinating, which shows the definite activity of coelomic fluid of *O. surensis* against standard ATCC strains of bacteria *P. aeruginosa* even at a least concentration of 5μl/disc and some visible activity at concentration equivalent to 20μl/disc compared with strain specific antibiotic discs used as positive control. This gives us a lead to carry out further research with clinically relevant ATCC strains of more bacteria and fungi, wherein coelomic fluid of this worm could be used in higher concentrations and antimicrobial activity noted. This study also lays down foundation for carrying out detailed research to understand the molecular mechanism that interplay in inhibiting or killing the microorganisms, it also mandates detail study to find out the active compound, its structure and bond relationships that may help in understanding its biochemical properties. It also gives us hope for discovery of newer agents that are having historical significance in treating human health and diseases and curbing the menace of antibacterial resistance and discovery of antimicrobials.

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Conflict of Interest

The authors do not have any competing conflict of interest.

Ethical Standards

This procedure did not involve any endangered or protected species, and the method of collection of samples were in accordance to the ethical standards and guidelines of the in-house research committee.

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