

Antimicrobial Properties of Insect Gut Associated Bacteria

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Abstract: In general microorganisms produce secondary metabolites which are not essential for the survival of the organism. The organism produces an inhibitory product may be an antibiotic which doesn't allow the growth of other organisms. In this present study the bacteria are isolated from the gut of short horned grasshoppers and screened their antagonistic efficacy. A mixed population of bacteria was observed in the respective agar plates and was identified as *Pseudomonas* sp, *Bacillus* sp and *Micrococcus* sp and *Escherichia coli*. To test for antagonistic effect the gut isolated bacteria were tested against five selected human pathogens, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Vibrio parahemolyticus* and *Candida albicans*. It was observed that gut associated *Pseudomonas* sp had broad antagonistic activity against fungus *Vibrio parahemolyticus*, *Candida albicans* and *Escherichia coli*. Although the preliminary screening is in the first stage but in the near future it can pay the way for isolation of newer microbial secondary metabolites in the form of antibiotics from different strains which is isolated from short horned grasshoppers.

Key words: Antagonistic Bacteria • Gut Micro Flora • Bacterial and Fungal Pathogens

INTRODUCTION

Microorganisms are a rich source of new metabolites with a wide variety of biological activities and some of them display significant practical applications [1]. Earth is the planet of insects, as they are found in almost every corner of the earth. Insects belong to the class of insecta, a major group of arthropods. There exists more than 1 million insect species than any other animal species comprising 72.8% of all animals [2]. One of the major features of insects is their extraordinary diversity in terms of numbers and morphological forms. In addition to their ability to survive in different ecological conditions, insect gut is a reservoir of complex microbial communities. These contribute to the host nutrition, growth, development and physiology. Apart from that they also play a key role for the stimulation of the immune system and resistance against the invading pathogens [3]. The protective effect of the gut bacteria are termed as bacterial antagonism is a significant component of host defense against pathogens. Gut bacteria has been documented to show antagonistic activity against pathogenic bacteria and fungi.

Although, a limited numbers of study have been conducted to show the microbial diversity in the insect gut. Less than 1% of described insect species has been studied for microorganism [4]. However, it is rarely possible to identify the bacterial species responsible for antagonistic activity.

Insects harbors a wide variety of antagonistic bacteria producing bioactive substances which are being isolated and characterized with great promise for the treatment of human diseases. However, humans are still far from winning the battle against infectious diseases. This is because the infectious microorganism has acquired resistant against antibiotics for which they were susceptible earlier [5]. The excessive use of antibiotics has promoted the development of resistant strains. Moreover, the traditional screening of soil and marine microorganisms as the source of antimicrobials products provided no results, which became responsible for the current lack of effective antibiotics. This has led to an urge to discover new antimicrobial agents from alternative sources to find new pharmaceutical products. The search for bioactive compounds from insect gut microorganisms has been intensively done during last decade [6].

The aim of the present work is to isolate bacteria from the gut of short horned grasshoppers by using six different types of media, to find out total Number of colonies in each media. All the strains are subjected to Gram staining to find out the microbial community of the gut of short horned grasshoppers. Morphologically different colonies were selected and screened for antagonistic bacteria against the human pathogens, *Klebsiella pneumonia*, *Pseudomonas aerogenosa*, *Vibrio parahameolyticus*, *Escherchia coli* and *Candida albicans*

MATERIALS AND METHODS

Study Area: Short horned grasshoppers (Phylum: *Arthropoda*) were collected from various places in and around VelTech Multi Tech Dr.Rangarajan Dr.Sakunthala Engineering College (Lat. 13°15 N; Long. 80°08 E) Chennai during September-December 2011.

Isolation of Gut Bacteria: There are six different media were used for the bacterial isolation. Surface sterilization has been carried out by using sterile distilled water. Where the isolated samples are rinsed in distilled water followed by a quick rinse in ethanol for few minutes and finally the samples are washed with sterile water in order to remove the traces of ethanol.

Grasshoppers are then transferred aseptically into a mortar and pestle. The gut of grasshoppers is removed by using sterile blades and forceps. The gut part is completely crushed with sterile distilled water in the mortar by using pestle. 50 -100 μ l of sample is inoculated into each medium. The inoculated samples are incubated at room temperature for 24 – 48 hrs. Morphologically different colonies were selected randomly. The number of colonies, pigmented and Non-Pigments colonies was noted. The collected samples are further sub cultured on appropriate medium, where it is again incubated at room temperature for 24 hrs. Likewise 122 strains have been isolated from four different types of grasshoppers. All the samples are then subsequently stored as Nutrient agar stab culture at 4° c. Staining and biochemical test was carried out for the potent strains. From the mixed agar plate cultures, the colonies were specified and were subjected to subculture onto respective fresh agar plates. Again the plates were incubated at room temperature for 24 hrs (bacteria)

The bacterial strains are identified by microbiological diagnostic methods. For this purpose, the colonies on respective medium are streaked on to fresh plates to

isolate individual colonies; the isolated colonies were subjected to macroscopic observation which includes color, consistency, surface texture, appearance and opaqueness. Then the microscopic observation was made by gram staining technique. Four human pathogenic bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Vibrio parahameolyticus* and *Candida albicans* were taken for antagonistic study; the strains were collected from National Culture collection center Pune.

Screening of Antagonistic Activity: The gut associated isolates were tested for antagonistic effect by double agar overlay method [7,8] against five human pathogens. Five numbers of human pathogens were used for antagonistic effect study. The human pathogens were *Candida albicans*, *Vibrio parahameolytics*, *klebsiella pneumonia*, *Pseudomonas aerogenosa*, *Escherichia coli*. All the human pathogens are obtained from NCC Culture Collection Center; Pune. The isolates were grown on nutrient agar medium. All the isolates were tested for the production of antimicrobial metabolites using Double agar overlay method. The 24 hours of old isolates were spotted on the nutrient agar medium and incubated at room temperature for 16 hours. About 1000 μ l of the test cultures were suspended in 100 ml of soft agar (0.75%) mixed vigorously and were pored immediately over the colonies of the antagonistic bacteria on the nutrient agar plates. The plates were then incubated at room temperature for 24 hours. The cleared zone around the macro-colonies of the antagonistic bacteria was measured.

RESULTS AND DISCUSSION

The study of different environments throughout the world has yielded different types of antimicrobial agents which has a great value for the treatment of various infections. Animals are of considerable interest since they are capable of defending themselves against pathogenic organisms by producing quick and powerful antimicrobial compounds. Totally 122 strains has been isolated from four different types of Short horned grasshoppers with various types of medium. All the strains are screened for antagonistic activity against human pathogens *Vibrio parahameolytics*, *klebsiella pneumonia*, *Pseudomonas aerogenosa*, *Escherichia coli* and *Candida albicans*. Out of 122 strains isolated 26 strains showed antagonistic activity against the human pathogens. By performing gram staining identified that most of the organisms which are present in the gut of grasshoppers are gram negative

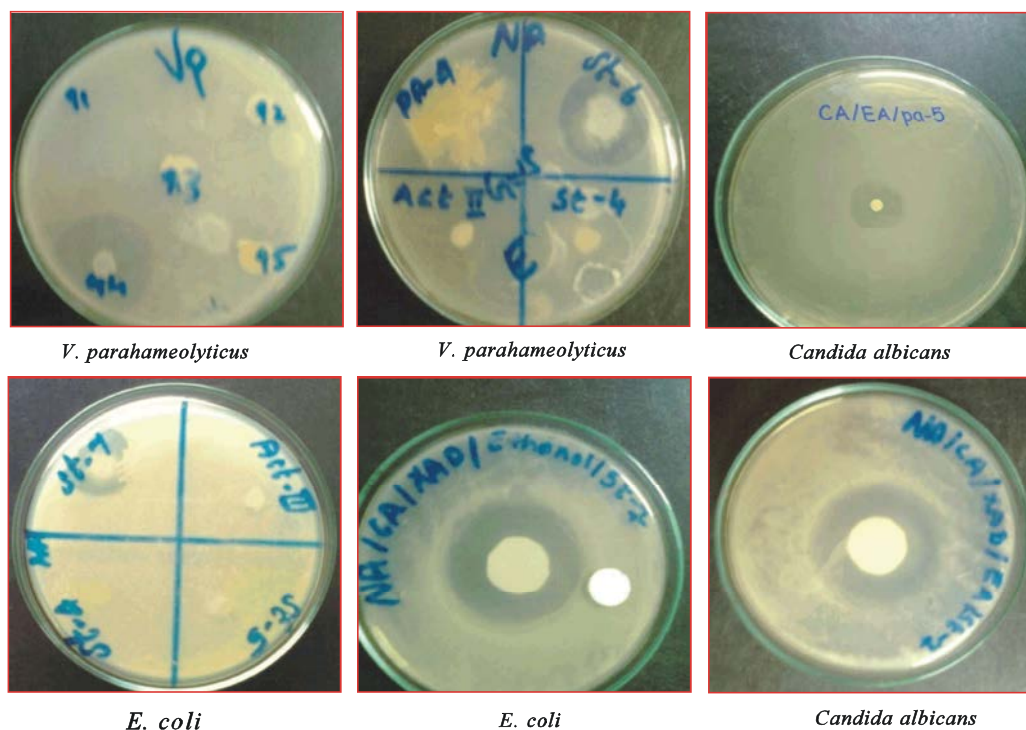


Fig. 1: Antagonistic effects of gut bacteria against *V. parahameolyticus*, *E. coli* and *C. albicans*

which constitutes around 65% and gram positive which constitutes around 35%. The organisms which are present predominantly in the gut regions are *Pseudomonas* sp., *Bacillus* sp. and *Escherichia coli* apart from that *Micrococussp* and *staphylococussp* are also present but low in number. The present study shown that bacteria are present predominantly in the gut of short horned grasshoppers, it has associations with microorganisms and thus it possesses an open system that is suitable for different kind of organisms (Fig.1). Small significant differences were observed in the total microbial counts of the four different types of bacteria. Maximum number of colonies is seen in Nutrient agar medium in all the four cases. Where, minimum number of colonies was observed in starch casein agar medium.

Results of the microbial load of the gut indicated that more bacteria than molds and yeasts were found in the gut [9]. This is likely due to the characteristics of the organisms. Bacteria are known to be ubiquitous, living in nearly all environments, while fungi and yeast are more selective in their hosts. This concept aggress with the findings of Chapman's [10] that the most commonly occurring microorganisms in insects are bacteria. The roles played by microorganisms in insect digestion are highly significant. In some insects, microorganisms ferment the wood and without them the insect is unable to

utilize the cellulose content of the wood. Microorganisms supply essential vitamins and other substances, hence change a poor diet into adequate one, also microorganisms play a subtle roles in the life of insects, means being involved in the digestion of food and detoxification of secondary plant compounds [9]. The present study reports about the preliminary observation of antagonistic bacteria from the gut of short horned grasshoppers against human pathogenic bacteria and fungi, but this study will pay the way for identification of new antibiotics from the gut microbes of grasshoppers.

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