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Antagnostic effect of raze bacteria from the squat horned grasshoppers using (p-dimethylaminobenzaldehyde) and indole test

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ABSTRACT

Bacterial antagonism is a phenomenon in which the growth and activity of other bacteria is inhibited. In general microorganisms produce secondary metabolites which are not as 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 for antagonistic activity. A mixed population of bacteria was observed in the respective agar plates and was identified as Pseudomonas sp, Bacillus sp, Micrococcus and Escherichia coli. To test for antagonistic effect the gut isolated bacteria were tested against four human pathogenic bacteria *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Vibrio parahameolyticus* and one yeast like fungus *Candida albicans*. It was observed that gut associated Pseudomonas sp had antagonistic activity against yeast like fungus *Candida albicans* and *Escherichia coli.* The most potent strain was subjected for 16srRNA gene sequencing in order to identify the antagonistic bacteria. The 16srRNA partial sequences have been run in the Bioinformatics tool, (BLAST) Basic Local Alignment search tool to find out the most similar sequences which matches with our strain. Phylogenetic tree has been constructed and our strain matches with *Pseudomonas otitids* which has (99.1%) similarity.

Introduction

The potential of natural products has been recognizes since antiquity. They continue to contribute a great deal too modern industries by providing a wide range of chemicals such as antibiotics, cardiac drug and insecticides. The explosive growth in modern biology has created a new awareness of the unlimited biotechnological potential natural products such as plants, microbes and animals (Banerjee., 1992)The diversity of life in the terrestrial environment is extraordinary and it consists of 36 phyla of organisms whereas world oceans are represented by 34 phyla.

Many compounds which are isolated from gut micro flora are diverse and novel. Secondary metabolites which are produced by living organisms have been exploited for a variety of purposes including use as food, fragrances, insecticides and medicines. Microbial natural products remain one of the most important sources of bioactive compounds for the pharmaceutical industry. Even though there is shift towards other sources such as computer based molecular modelling and manipulation of biosynthetic gene clusters, the pharmaceutical pipeline remains filled with traditional, microbial based derived natural products. Microorganisms remain nature's best chemists and one of the best sources of novel, biologically active organic molecules from which to build a drug delivery program. Antibiotics are actually rather easy to discover, but few are of medical or any other commercial value. Some are used commercially other than for treating disease for instance a supplement in animal feed. Many antibiotics are toxic to humans or lack any advantage over antibiotics already in use. More than half of antibiotics which are in use today are produced by Streptomyces species, filamentous bacteria that commonly inhabit soil. Only a few antibiotics are produced by Bacillus Spp., one study shows that 400000 microbial cultures that yielded only three useful drugs.

RELATED WORK AND EXISTING SYSTEM

Humans are now exerting a major evolutionary phase on the natural world and the resulting antibiotic resistance problem discloses that new agents need to be developed in the combination with proper techniques to manage them (Palumbi and Noah et al., 2001) New concepts in natural chemistry are formed in the present 'information age'. Rather than just reaping important compounds from nature, an effort is made to understand the naturally occurring chemical interactions (chemical ecology) and to master the biosynthetic principles (biotechnology) (Caprolae, 1995).In the pharmacology of natural products, a wide range of useful drugs have been isolated from plants and animals. These drugs include analgesics, antibiotics, and anti-inflammatory drugs and so on. But insects host an environment of natural products having potential biomedical applications.

Insect gut provides a huge resource bank to the discovery of Novel compounds. The natural compounds are usually about 1000 Dalton, with existing drug properties (Harvey, 2000). Insects harbor's with 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 the traditional screening of soil and marine microorganisms as the source of all antimicrobials products

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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 and microorganisms has been intensively done during last decade. The aim of the present work is to investigate bioactive metabolites from gut bacteria of short horned grasshoppers which are abundantly seen in terrestrial environment. With a preface based effort, the crude extract and partial purified fractions of the grasshoppers are tested against human pathogens. The system isolation of gut associated bacteria from four different types of short horned grasshoppers was carried out.

To test for antagonistic effect the gut isolated bacteria were screened against four primary human pathogenic bacteria Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Vibrio parahameolyticus and one yeast like fungus Candida albicans The precise antimicrobial compound was partially purified by column with high chromatography and through HPLC(High Performance Liquid Chromatography).

MATERIALS AND METHODS

Sample collection:

Squat horned grasshoppers (Phylum: Arthropoda) are collected by using butterfly nets from various places in and around Alpha College of Engineering Chennai.

Four different types of samples were collected. Based upon the external morphology the samples were differentiated.

Preparation of media:

Six different types of medium are used for isolation of bacteria from the gut of grasshoppers. Since no one medium supports the growth of all types of bacteria. Nutrient agar, Antinomyes agar, Luria-bertani agar, starch casein agar, soya bean casein digest agar, and Peptone agar.

Isolation of the gut associated bacteria from short-horned grasshoppers:

Grasshoppers are transferred aseptically into the lab. Where the gut of grasshoppers are removed by using sterile blade and forceps. The gut part and it's completely crushed with sterile distilled water in the mortar.50-100µl of sample is inoculated into each medium. Control has also been maintained in order to test for contamination.

The inoculated samples are incubated at room temperature for 24 - 48 hrs. Morphologically different colonies were selected randomly. The collected samples are further sub cultured on appropriate medium, where it is again incubated at room temperature for 24 hrs. All the isolated samples are stored at 4° c for future work.

The gut associated isolates were tested for antagonistic effect by double agar overlay method. The human pathogens were Candida albicans, Vibrio parahameolytics, klebisella pneumonia, Pseudomonas aerogenosa, Escherichia coli. All the human pathogens are obtained from NCC Culture Collection centre, Pune.

The isolates were grown on nutrient agar medium. All the isolates were tested for the production of antimicrobial metabolites using double based 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 µl 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. For the bio molecular characterization, one of the bacteria is selected based on the zone of inhibition which is carried out by disk diffusion method. The user selected bacterial strains were grown in appropriate medium and the DNA was extracted. The 16srRNA genes were amplified by PCR using universal primer. Further procedure was then carried out to identify the bacteria. The process of 16s rRNA Sequencing was carried out in Chromas biotech Bangalore.

The supernatant is then subjected in the column chromatography. Where the column is initially packed with the amberlite XAD 16 polymer. The polymer is shipped as water wet product inhibited with sodium chloride and sodium carbonate salts to retard bacterial growth. These salts should be washed from the adsorbent prior to use and it is suggested to wash the adsorbent at a flow rate of 5-10 m/h. The supernatant is passed through the column, after giving a water wash; the adsorbed compound was eluted using Ethyl acetate and Ethanol. Ethyl acetate and Ethanol are used as a solvent system. Each fraction thus obtained was once again evaporated, concentrated & user assayed for antimicrobial activity.

The column fractions of the crude extract of potent strain against candida albicans was eluted as follows: Partial purification of the crude extracts was carried out by the method of Wright (1997). After initial screening, the higher activity was shown by ethanol and ethyl acetate extract and it was fractionated by normal model phase silica gel column chromatography by employing a step-step gradient solvent system from low to high polarity.

Column Purification & Biochemical's:

Sequence of 100% normal hexane; 10% Ethyl acetate: 90% Hexane,25% Ethyl Acetate: 75% Hexane,40% Ethyl Acetate: 60% Hexane, 50% Ethyl Acetate: 50% Hexane,25% Ethyl Acetate: 75% Hexane, 100% Ethyl Acetate,90% Ethyl Acetate: 10% Ethanol, 70% Hexane: 30% Ethanol,50% Hexane: 50% Ethanol, 25% Hexane: 75% Ethanol. Finally 100 % Ethanol and 100% Methanol is used for elution. Each fraction thus obtained was once again evaporated, concentrated and assayed for antimicrobial activity. An aliquot user of the isolated compounds (fractions 5-8,9-10) were subjected to HPLC. Fractions from the separation will be obtained by using a fraction collector and antifungal and antibacterial activity will be carried out for all fractions against human pathogens.

Figure. 1.0 Squat – Horned Grass Hopper

HPLC METHOD AND INDOLE TEST

An aliquot of the isolated compounds (fractions 5-8, 9-10) were subjected to HPLC, hence the fractions were dried. Separation was then performed using c18 reverse phase column (Schizmadzhu). Acetonitrile and water was used as an elutent at

A flow rate of 1.0 ml/minute. The peaks are detected at 252nm in a UV-VIS detector. Fractions from the separator have to be collected by using a normal fraction collector and antimicrobial activity was carried out for all the fractions against Human pathogens.

Twenty of the one twenty two isolated bacteria produced antimicrobial agent. Pseudomonas otitidis was selected for more studying on the basis of most average diameter of the zones of microbial inhibition in disk diffusion method against tested microorganisms. Pseudomonas otitidis was identified by performing 16s rDNA seq Morphological & physiological characteristics of the isolated bacterium were compared with data from Bergey's Manual of systemic bacteriology.

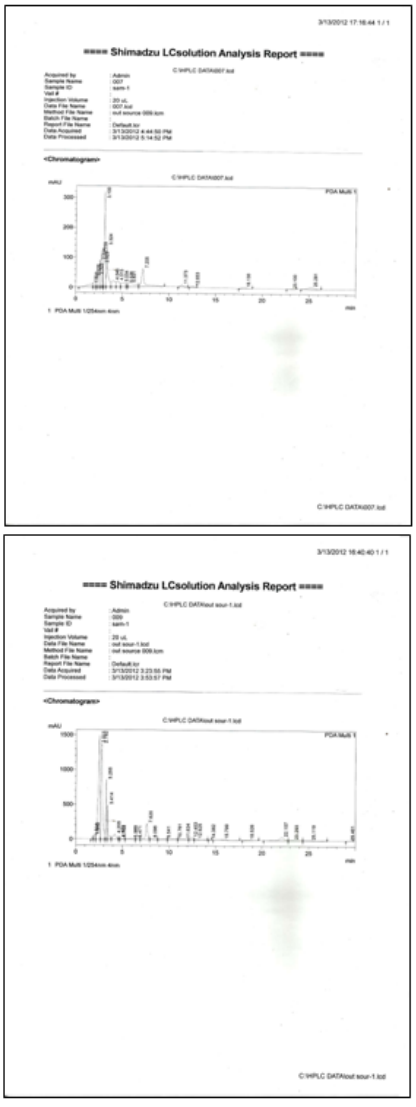

Fig. 2. HPLC Reports for Indole Test

WORK DISCUSSION

So far, antibiotic producing bacterium from the gut of squat-horned grasshoppers has not been reported. Our strain was identified based on phenotypic and genotypic characteristics. Because of these characteristics, this species can be assigned to the species level as P.otitids which is more similar to P.aeruginosa. Antimicrobial agent from this organism shows an interesting spectrum of antimicrobial activity. Differences were observed in the susceptibility to the antimicrobial agent within different analyzed strains. This may be due to presence or absence of receptors for the absorption of normal bioactive compounds or to some mechanism of bacterial resistance.

Some antimicrobial substances such as pyocyanin and pyoverdin have been described for the Pseudomonas spp. Pyoverdin has a good antifungal activity against deleterious fungi and its maximum inhibition zone was against A.niger. Whereas our antimicrobial compound has the ability to inhibit dimorphic fungus Candida albicans. On the other hand, our strain showed a broad inhibitory spectrum against Candida albicans and other bacteria. The activity and stability of this antimicrobial agent in alkaline PH could be vital promising in potential clinical application due to mild alkalinity of human serum.

CONCLUSIONS

Our study showed that the isolated bacterium Pseudomonas otitids has excellent antimicrobial activity against the candida albicans and E.coli. The activity of the compound has to be tested against other human pathogenic bacteria in order to make it as a wide range of antibiotics. It is evident that Pseudomonas otitids constitutively produces a unique and novel compound which may be the substance responsible for antimicrobial activity.

High antimicrobial effects of the bacterium are evident, further structure, functional group of elucidation by using Nuclear Magnetic Resonance (NMR), Infra-red (IR) of the compound is necessary in order to use it for pharmacological purpose.

REFERENCES

Banerji, A.1992.Biotechnological potential of Natural products. Bioelectrochemistry and Bioenergetics, 27; 1057-1065

Berenbaum,M.R. The chemistry of bio defence: theory and practice. In chemical ecology and the chemistry of biotic interaction; eisner, T. & Meinwald,j.,Eds.;National academy press: Washington,D.C.,1995,1-16.

Christophersen. Theory of origin, function and evolution of secondary metabolites. In studies in natural products chemistry; Atta-ur-Rahman,Ed;Elsevier science B.V.:Amsterdam, the Netherlands, 1996,vol. 18, 677-737.

Christophersen. Evolution in molecular structure and adaptive variance in indole based metabolism. Comp. Biochem.Physiology.1991, 98B, 427-432.

Costa R. Rinke. Microbial Diversity in the larval gut of field and laboratory population of the sugarcane weevil sphenophorus levis (coleopteran curculionidae),Genetics and molecular research 10($):2679-2691 (2011).

Gholamhossein Ebrshimipour. (2010) Evaluation of antimicrobial substance produced by a bacterium isolated from parmacella iberica; journal of microbiology. Pg: 7-13.

Julia Kjer, Abdessamad Debbab. Methods for isolation of marine derived entophytic fungi and their bioactive secondary products, Nature protocols: Vol: 5, No: 3 9 (2010).

Meylears, Cerstiaens. antimicrobial compounds of low molecular mass are constitutively present in insects: characterization of β-Alanyl-Tyrosine, journal of current pharmaceutical design, 2002, 8, 99-110