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Characterization of Bacteria Isolated from Decayed *Vitis Vinifera* and *Musa Paradisiaca* Fruits and Assessment of their Susceptibility to Antimicrobial Animal Derivatives

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Abstract: ***Bacteria are the major and important factor for fruit spoilage. A study was carried out to examine the presence of bacterial species in decayed grapes (Vitis vinifera) and banana (Musa paradisiaca). Two types of bacteria namely (AHM1-Grapes) and (AHM 2-Banana) were successfully isolated by Serial Dilution –agar plating method (Spread plate method). Gram stain results demonstrated that one sample (AHM1) is Gram negative rod-shaped bacteria and other sample (AHM2) is Gram positive rod-shaped bacteria. Antimicrobial Susceptibility testing was carried out using three different antimicrobial agents against the two types of bacteria that were successfully isolated. The different antimicrobial agents used were Ant peptide extracted from Tetramorium sp. Citric acid solution and chitosan extracted from shell of prawn. The two colonies were identified by 16SrRNA sequencing. Molecular characterization revealed that the Bacterial species were (AHM1-grapes-Sequencing Results awaited for this isolate)) and Bacillus siamensis (AHM2-Banana). Phylogenetic analysis were also carried out for Bacillus siamensis. The antimicrobial susceptibility profile of the bacterial isolates obtained from spoilt grape and banana fruits samples was determined using the Disc-diffusion method in nutrient agar medium. All two bacterial isolates were susceptible to the three antimicrobial agents that were used. These antimicrobial agents can be used as coatings in fruits and vegetables as food packaging agents as these agents inhibits the growth of microorganisms.***

Keywords: Bacteria, Decayed fruits, Antimicrobial Susceptibility, Antimicrobial Agents, Molecular Characterization and Phylogenetic Analysis

1. Introduction

Introduction about the research area:

The Research area that I have chosen for my Project is Food Microbiology or Microbiology of Food. Organisms depend on the food for their growth and survival. It is the basic need of life. But sometimes, the food we eat for a good health may cause illness or discomfort. This may be because of infection or spoilage of food by the microbes. Uncontrolled growth of microbes in food causes the spoilage of food. These microbes can alter the taste, odor, color, and texture of the food. Food microbiology deals with the study of microbial infection of the food, food spoilage, food-borne diseases and preservation of foods from microbial infection. The study is very important to prevent the contamination of food and to control food-borne diseases. Fruits are more susceptible to microbial growth. The nutrient content in fruits support the growth of bacteria, yeast and moulds. They are likely to be infected by microbes during different stages of growing, harvesting, transport and storage.

Though all foods are prone to be contaminated by microbes, the degree of susceptibility of food to microbial spoilage depends on Physical or chemical characteristics of the food. Some foods such as meat, fish , poultry , eggs, milk and most fruits are readily spoiled by microbes , such foods are called **Perishable Food.** Those foods which remain unspoiled for prolonged period of time is called as **Semi perishable Foods**. Foods like pulses, cereals, rice and sugars do not spoil normally when kept under normal conditions. Such foods are called as **Non-perishable foods.** These foods, too, are contaminated by Bacteria and fungi.

Antimicrobial agents have different activities on different pathogenic microorganisms due to their various diverse physiologies. Antimicrobial agent is integrated either directly into food particle or to the packaging material where it is released over a period of time to maintain the products quality, as well as its safety leading to its extended shelf life. Characterization of microorganisms can be very helpful for the choice of an antimicrobial agent.

Microbiology is important to food safety, production, processing, preservation, and storage. Microbes such as bacteria, moulds, and yeasts are employed for the foods production and food ingredients such as production of wine, beer, bakery, and dairy products. On the other hand, the growth and contamination of spoilage and pathogenic microorganisms is considered as one of the main causes to loss of foodstuff nowadays. Although technology, hygienic strategies, and traceability are important factors to prevent and delay microbial growth and contamination, food remains susceptible to spoilage and activity of pathogen microorganisms. Food loss by either spoilage or contaminated food affects food industry and consumers' leading to economic losses and increased hospitalisation costs Food Microbiology is important to make sure our

foods are safe to consume. The food is a potential source for microorganisms to grow and multiply. Food microbiology is important because microbes and food interact in both positive and negative ways. They can make or be the food, enhance it, spoil it, or make it unsafe to eat. Knowing how to bring out the good and prevent the bad is very important since people need to eat. Microbial activity on the food can observed in order to determine the quality through the microbial population on the food.

The aim of the proposed research is to investigate about the microorganisms associated with the spoilage of fruits and testing for antimicrobial activity using different antimicrobial agents. The microbial contamination of the fruits and vegetables leads to various health effects in human population. Spoiled fruits and vegetables are not good for human consumption as this may lead to health effects or illness. Food may also carry pathogenic microorganisms which when ingested can cause disease. When food with microorganisms that produce toxic substances is ingested, it results in food poisoning. Some microorganisms are used in the preparation and preservation of food products. This proposed study will be useful to farmers, fruit juice industries and consumers of the fruits .The outcome of this research will guide the users of different fruits on the best method to avoid the spoilage.

Some traditional food preservation techniques like drying, freezing, heating, fermentation, salting can extend the shelf-life of food products. Antimicrobial packaging system is a novel development which incorporates antimicrobial agent into a polymer film to suppress the activities of targeted microorganisms that are contaminating foods Food pathogens endangering consumers' health can be dealt with the multifunctional bio-based antimicrobial packaging agents that sought to provide enhanced food safety. The antimicrobial activity can be achieved by adding antimicrobial agents in the packaging system to prevent the growth of microbes by extending the lag period and decreasing live counts of microorganisms by reducing growth rate. Conventional food packaging aims at shelf life extension, maintenance of quality, and assurance of safety of the food product. Naturally occurring antimicrobials have gained attention among researchers and food manufacturer due to their safety and nontoxic status. Natural preservatives are easy to obtain from plants, animals and microbes. These naturally occurring antimicrobial agents can be isolated from indigenous sources using various advanced techniques. Natural preservatives such as nosing, essential oils, and natamycin have effective potential against spoilage and pathogenic microorganisms.It is important that sellers should be properly educated and sensitised on the need to improve their own personal hygiene. It is suggested that proper handling would ensure a better quality of fruits and vegetables being sold in the local markets.

2. Literature Survey

Fruits play a vital role in human nutrition by supplying necessary growth factors such as vitamins and essential minerals in daily diet which help to live a healthy life (AlHindi *et al.*, 2011).India is the fourth largest producer of fruits in the world, yet due to losses in the field, during storage, transit or trans-shipment, during handling processes of the crop from the grower to the whole sale dealer and to retailer and finally to consumers (Chukwuka *et al.*, 2010 and Zubbair, 2009), they become inadequate (Barth *et al.*, 2013). The succulent nature of fruits makes them to be easily invaded by microbes. The high concentration of various sugars, minerals, vitamins and amino acids also provide a good platform for the successful growth and survival of various microorganisms (Bhale,2011). Most microorganisms that are initially observed on whole fruit surfaces are soil inhabitants **(**Andrews and Harris, 2000**;** Janisiewicz and Korsten, 2002).

Several novel mechanisms have been reported by researchers that either inhibit the pathogen spore germination and vegetative growth or directly kill the vegetative cells by producing active antimicrobial diffusible compounds. A report by Sipiczki (2006) demonstrates the antagnostic activity of red-maroon pigment-producing Metschnikowia strains against filamentous fungi, yeasts, and bacteria which has been hypothesized to be based on inhibition of growth of sensitive microorganisms by depletion of free iron in the medium due to iron binding pigment formation by the yeast strain or other than the siderphore. "Bacteriocins," peptides having antimicrobial activity of bacterial origin, are best-suited candidates for food bio preservation as their use would help in retaining the organoleptic and nutritional properties of particularly the fresh produce or the minimally processed fruits and also would help to reduce the practice of use of chemical preservatives and intense heat treatments for preservation (Leverentz *et al.*, 2003; Galvez *et al.*, 2007).

Antimicrobial peptides from animal origin have a broad range of antibacterial activities as well as antiviral**.** Burrowes *et al*. evaluated pleurocidin in food applications using 18 microbial species. Pleurocidin was effective against *E. coli* O157:H7, *L. monocytogenes*, *P. expansum*, *S. cerevisiae*, and *V. parahemolyticus* with MIC of 5.3, 23.0, 20.6, 5.5, and 69 µM, respectively; no haemolytic or cytotoxic effect on intestinal cells were found. Chitosan is produced commercially from chitin, a by-product obtained from exoskeletons of crustaceans and arthropods with capacity to inhibit the growth of moulds and yeasts.

3. Background Information

Food is very important in part of all living organisms. We get our food from various sources. Microbiology is the study of microbes that are known to affect the animals and human beings around us. Some microbes are also useful in nature, and they can be used for the preparation of food products but many of them are harmful in nature and are the main causes of death around the world. The microbes are also the major cause of spoiling the food in our daily lives. So, food microbiology is the mixture of food and microbiology where we learn about the effects of microbes on the food we eat. This is what food microbiology is where we study the microbes and how they cause food spoilage. Antimicrobial packaging which is thought to be a subset of active packaging and controlled release packaging is one such promising technology which effectively impregnates the antimicrobial into the food packaging film material and

subsequently delivers it over the stipulated period to kill the pathogenic microorganisms affecting food products thereby increasing the shelf life to severe folds. Food preservation is another very important thing, many microbes are causing the spoilage of food, these microbes can be killed using different antimicrobial agents. The antimicrobial agents can be Organic acids, Antibiotics, Enzymes, Peptides, Chitosan extract, different plant extracts and so on.

4. Methods/Approach

Sample collection

Fresh grapes and banana were collected from the local markets. Samples were left free of dust, insect etc. and were under room temperature for 5-6 days to undergo natural decay.

Fresh Grape Fruit

Spoiled Grape Fruit

Fresh Banana Fruit

Spoiled Banana Fruit

Isolation of the bacteria from the decayed fruits

All glassware used in the experiment were sterilized in the autoclave at 121°C for 15 min. The nutrient agar medium was prepared by dissolving the required grams in distilled water and was sterilized in the autoclave before use. To maintain, a sterile atmosphere, all procedures were performed under the laminar air flow hood. Samples of the decayed fruit were suspended in sterile distilled water and serially diluted up to 10^{-1} to 10^{-6} times (Aneja, 2009). 100 µl of each serial dilution were transferred for culturing to nutrient agar plates by spread plate method. The nutrient agar plates were then incubated at 37°C for 24 h to observe the bacterial colonies.

Inspection of the colony morphology and preparation of pure cultures

Two morphologically different colonies were identified, one colony each from the plates with the 10^{-6} times serially diluted grape and banana samples, respectively. These colonies were sub cultured on nutrient agar slants and incubated at 37°C for 24 h. The slants with the two sub cultured bacterial isolates were stored at 4°C for further use.

Gram Staining:

The primary stain (crystal violet) is used to stain the heat fixed bacterial smear on clean glass slide. Gram's iodine (Iodide and potassium iodide) is applied as a mordant or fixative. Gram-positive cells form a crystal violet-iodine complex. Alcohol or acetone is used to decolorize the cells. Gram-negative bacteria have much less peptidoglycan in their cell walls, so this step essentially renders them colourless, while only some of the colour is removed from gram-positive cells, which have more peptidoglycan (60-90% of the cell wall). The thick cell wall of gram-positive cells is dehydrated by the decolorizing step, causing them to shrink and trapping the stain-iodine complex inside. After the decolorizing step, a counterstain is applied (safranin) to colour the bacteria pink.

Molecular Characterization of the isolated bacteria (pure culture) by 16SrRNA Gene Sequencing:

Genomic DNA was isolated from the cultured bacterial sample (Pure Cultures) and Polymerase Chain Reaction (PCR) was performed to amplify the 16SrRNA gene. The Amplified fragments were separated by Agarose Gel

Electrophoresis and then Sequenced. Identification of the bacteria was done by BLAST analysis of the 16SrRNA gene sequences.

Multiple Sequence Alignment (MSA) and Phylogenetic Analysis of the 16SrRNA gene sequences using Clustal Omega

MSA of the Cultured bacterial 16SrRNA gene sequences was performed. 16SrRNA gene sequencing using Clustal Omega and the phylogenetic tree was obtained.

Extraction and Characterization Of Antimicrobial Peptide from *Tetramorium Sp.* (Pavement Ant) for Determining the Antimicrobial Susceptibility:

Collection of Ant Sample:

The Ant sample for the study was collected from the Loyola College Campus in a Clean empty bottle and it was identified as *Tetramorium sp.* (Pavement Ant).The Ants were homogenized in mortar & pestle, by adding 10ml of Phosphate Buffer Saline (PBS) solution. Then it is centrifuged at 6000rpm for 15mins. Supernatant was collected and pellet was discarded.

Acetone was added in drop wise to obtain extract in previous step till the protein was completely precipitated out. A Protein Suspension then centrifuged at 6000rpm for 20minutes. Supernatant is discarded and phosphate Buffer is added to pellet for solubilisation. This Protein solution was used as the Antimicrobial Peptide to screen against Bacterial pathogens 100 Microliters of the Peptide Sample was diluted to 3ml and scanned spectrophotometrically in both UV – Visible range to obtain the peak peptide.

Extraction of Chitosan from Prawn Shell for Determining the Antimicrobial Susceptibility:

Extraction of Chitosan from Prawn Shells:

Materials: *Penaeus indica* or Indian prawn was obtained from the local market

P. indica inedible parts including head, body shells and tails were removed from the whole body for extraction of chitosan.

METHOD:

To extract Chitosan, 10 grams of prawn shell waste as raw material was collected. After washing it properly, the prawn shells were under sunlight.

- **Demineralization:**

Then we proceeded with the demineralization process by adding 1.5N HCl at room temperature for 1hour. The spent acid was discarded and the shells were repeatedly washed with distilled water until the pH is neutral.

- **Deproteinization:**

The demineralized shells were then deproteinized with 0.5% NaOH at 100ºC for 30 minutes. This method helped to weaken the protein tertiary structure of the shells. Protein solution was removed and washed thoroughly with distilled water and the pH was checked. The deproteinization process was again repeated for the removal of the remaining protein from the shells, for that 3% NaOH was added to the sample at 100ºC for 30 minutes. After draining the residual proteins along with the effluents, the sample once again washed and the pH was observed till it was approximately near to neutral. This step also helped in decolourization of the shells. Hence the chitin slurry was obtained. The excess water was removed and chitin cake was formed.

- **Deacetylation:**

The Chitosan was prepared by deacetylation of chitin by treating with 42% aqueous NaOH at 95°C for 1.5 hour. After deacetylation the alkali was drained off and washed thoroughly with distilled water until the pH is less than 7.5 and then dried at ambient temperature (30 ± 2°C).

Antimicrobial Susceptibility Testing (AST):

- Antimicrobial Susceptibility Testing, also called as AST is a commonly used method for evaluating resistance to antimicrobial agents.
- There are several different ways to conduct AST like the dilution of broth, agar and disc diffusion tests.
- The disc diffusion method, or 'KirbyBauer procedure involves spreading bacteria onto an agar plate, and then placing discs of paper impregnated with antimicrobial agent onto the plate. After incubation the growth of bacteria is monitored.
- The areas around the discs where there is no growth of bacteria can be observed are known as zones of inhibition. These zones prove that the antimicrobial agent is effective in stopping the growth of bacterial or killing bacteria.

Antimicrobial Susceptibility Testing Using Ant Peptide Extract:

Peptide was extracted from Ant (*Tetramorium sp.*) and checked for antimicrobial susceptibility against two strains of Bacteria that was isolated (Pure Cultures). The Testing was carried out by Disc Diffusion method. 25µL of Bacterial sample was inoculated and spread on each Nutrient Agar Medium Plates. Whattmann Number one filter paper was used for disc –diffusion method. Filter paper (Disc) is coated with a specified volume and the right amount of an antimicrobial and then placed on a dish of susceptibility-testing agar that is uniformly inoculated with the organism of test. The Specified volume of Ant peptide is coated (20µL), (25µL) , (30µL) and control disc without any peptide extract was also placed on the nutrient agar medium. The Plates are kept in the incubator for 24-48 hours to check for the antimicrobial susceptibility (Zone Of Inhibition).

Antimicrobial Susceptibility Testing Using Citric Acid Solution:

10% Citric Acid solution was prepared and checked for antimicrobial susceptibility against two strains of Bacteria that was isolated (Pure Cultures). The Testing was carried out by Disc Diffusion Method. 25µL of Bacterial sample was inoculated and spread on each Nutrient Agar Medium Plates . Whattmann Number one filter paper was used for disc – diffusion method. Filter paper (Disc) is coated with a specified volume and the right amount of an antimicrobial and then placed on a dish of susceptibility-testing agar that is uniformly inoculated with the organism of test. The Specified volume of Citric Acid Solution is coated (20µL), (25µL), (30µL) and control disc without any Citric acid solution was

also placed on the nutrient agar medium. The Plates are kept in the incubator for 24-48 hours to check for the antimicrobial susceptibility (Zone Of Inhibition).

Antimicrobial Susceptibility Testing Using Chitosan Solution:

0.4 grams of Chitosan powder is dissolved in 15 ml of Acetic acid solution and solution was made to 100ml with distilled water. 0.4% Chitosan Solution was prepared preparedand checked for antimicrobial susceptibility against two strains of Bacteria that was isolated (Pure Cultures). The Testing was carried out by Disc Diffusion Method. 25µL of Bacterial sample was inoculated and spread on each Nutrient Agar Medium Plates . Whattmann Number one filter paper was used for disc –diffusion method. Filter paper (Disc) is coated with a specified volume and the right amount of an antimicrobial and then placed on a dish of susceptibility-testing agar that is uniformly inoculated with the organism of test. The Specified volume of Chitosan Solution coated is (20µL), (25µL) , (30µL) and control disc without any Chitosan Solution was also placed on the nutrient agar medium. The Plates are kept in the incubator for 24-48 hours to check for the antimicrobial susceptibility (Zone Of Inhibition).

5. Results & Discussion

Isolation of the Bacterial species Present in Decayed Fruits:

After the plating and incubation of the serially diluted Samples (spoiled grapes and spoiled banana), various types of bacterial colonies were observed on the plates. The 10^{-6}plates showed clear separate colonies for both the samples (spoiled grapes and spoiled banana) that were easy to observe and differentiate. The other plates either had overgrowth or a very small number of Colonies. There were some morphologically different bacterial colonies were observed on both the 10^{-6}plates. One bacterial colony from spoiled grape sample and one bacterial colony from spoiled Banana sample were selected for further study.

Isolation of Specific Bacterial Colony from Spoiled Grapes and Banana and Establishment of Pure Culture:

There were some morphologically different bacterial colonies were observed on both the 10^{-6}plates. One bacterial colony from spoiled Grape sample and one bacterial colony from spoiled Banana sample were selected for further study. One bacterial colony (Pure Culture) from Spoiled grape was streaked on Nutrient Agar Slant and one bacterial colony (Pure Culture) from spoiled banana was streaked on Nutrient Agar slant. These two bacterial pure cultures were stored in refrigerator and used for further study.

Pure Culture from Spoiled Grape Sample Streaked on Nutrient Agar Slant

Pure Culture from Spoiled Banana Sample Streaked on Nutrient Agar Slant

Morphological Identification

Morphological identification based on the shape, texture and colour of bacterial isolates were further analysed.

Table Morphological Characteristics of Bacteria Isolated from 2 types of spoiled fruits

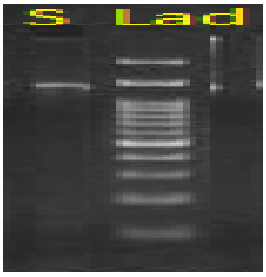
S. No	Fruits	Isolate	Colonial Morphology	Shape and Arrangement
1	Grape	AHM1	Thin, Blue-green, spreading growth	Short rods in scattered arrangement.
2	Banana	AHM2	White, attached spreading with crenate margin, dry	Long rods in scattered arrangement

Gram Staining of Bacterial Isolates:

Gram Staining Procedure Performed on the 2 Bacterial Isolates.

Bacterial Isolates	Name	Gram Staining
1	Sequencing results awaited	Gram Negative(-ve)
2	*Bacillus siamensis*	Gram Positive (+ve)

Molecular Characterization of the isolated bacteria (pure culture) by 16SrRNA Gene Sequencing and BLAST analysis of the 16SrRNA gene sequence.



PCR products of 16SrRNA gene (Lad – 100bp ladder, S - AHM 2)

BLAST Analysis

The amplified sequences belong to 16Sregion were confirmed by similarity index built in the NCBI's BLAST program. Based on the higher percentage similarity against the reference species, the species utilized in this study was assigned as following species. **The Gene Sequencing results for AHM 1(Grape) is awaited as QC got error**

Sample- AHM 2

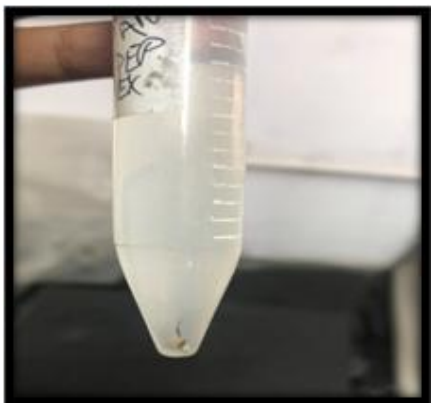
>*Bacillus siamensis*

GATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACG
GGTGAGTAACACGTGGGTAACCTGCCTGTAAGACT
GGGATAACTCCGGGAAACCGGGGCTAATACCGGAT
GGTTGTTTGAACCGCATGGTTCAGACATAAAAGGT
GGCTTCGGCTACCACTTACAGATGGACCCGCGGCG
CATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGG
CGACGATGCGTAGCCGACCTGAGAGGGTGATCGGC
CACACTGGGACTGAGACACGGCCCAGACTCCTACG
GGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGA
AAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAG
GTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAA
CAAGTGCCGTTCAAATAGGGCGGCACCTTGACGGT
ACCTAACCAGAAAGCCACGGCTAACTACGTGCCAG
CAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCC
GGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTT
CTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGG
GGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAG
AAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAT

FASTA Format of the 16SrRNA Gene Sequence of *Bacillus siamensis*

Submitted the Nucleotide Sequences in NCBI GENBANK with Accession number **ON929290.1**

Extraction and Characterization of Antimicrobial peptide from *Tetramorium Sp*. (Pavement Ant).:



Peptide Extract from Ant

UV-Visible Spectrophotometer:

100 Microlitres of the Peptide Sample was diluted to 3ml and scanned Spectrophotometrically in both UV –Visible range to obtain the peak peptide. Spectrophotometer was carried out to confirm the presence of Protein and Peptide in the sample. The **peptide sample showed the peaks in range of 230-280nm, at this wavelength** confirming the presence of protein in the sample

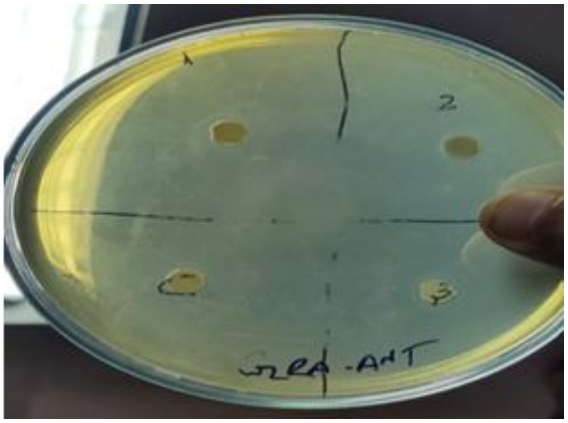
Extraction of Chitosan Powder from Shell of the Prawn by following Chemical Method:

To extract Chitosan, 10 grams of prawn shell waste as raw material was collected. After washing it properly, the prawn shells were under sunlight.

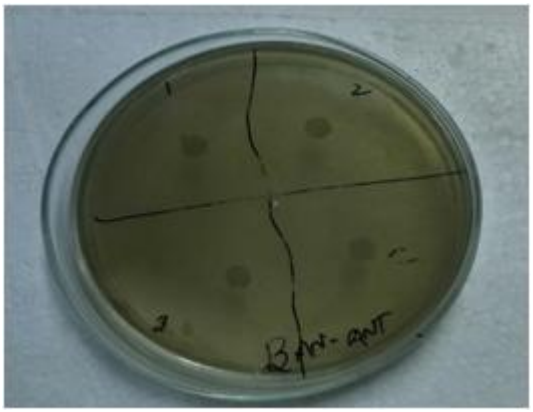
Chitosan Powder

Chitosan solution

Screening of Antimicrobial Peptide against Two Bacterial Isolates:

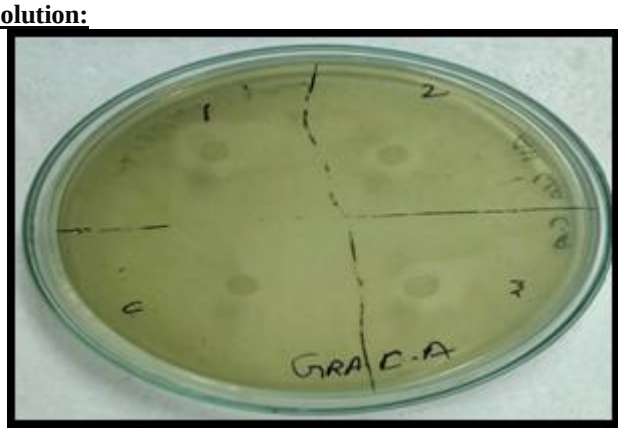


Antimicrobial activity of the peptide extract from ant against AHM-1(Grape)

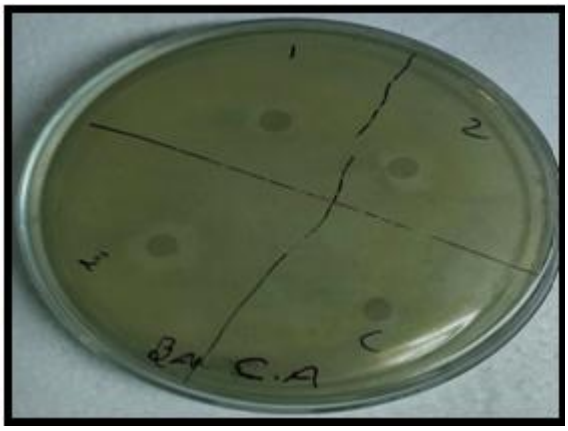


Antimicrobial activity of the peptide extract from ant against *Bacillus siamensis*

Antimicrobial Susceptibility Testing Using Citric Acid Solution:



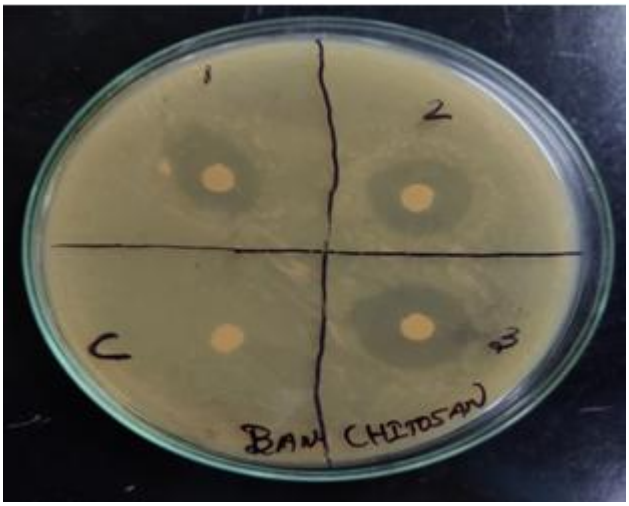
Antimicrobial activity of the Citric acid solution against AHM-1(Grape)



Antimicrobial activity of the Citric acid solution against *Bacillus siamensis*

Antimicrobial Susceptibility Testing Using Chitosan Solution:

Antimicrobial activity of the Chitosan solution against AHM-1(Grape)



Antimicrobial activity of the Chitosan solution against *Bacillus siamensis*

Antimicrobial Agent	Concentration of Antimicrobial Agent(µL)	Bacterial Isolates	Zone of Inhibition	Interpretation
AMP from Ant	20µL	AHM 1(Grapes)	8 mm	Susceptible
	25µL		16 mm	Susceptible
	30µL		24 mm	Susceptible
AMP from Ant	20µL	*Bacillus siamensis* AHM 2(Banana)	6 mm	Susceptible
	25µL		10 mm	Susceptible
	30µL		20 mm	Susceptible
10%Citric Acid Solution	20µL	*AHM 1(Grapes)*	10 mm	Susceptible
	25µL		16 mm	Susceptible
	30µL		26 mm	Susceptible
10%Citric Acid Solution	20µL	*Bacillus siamensis AHM 2(Banana)*	8 mm	Susceptible
	25µL		18 mm	Susceptible
	30µL		28 mm	Susceptible
0.4% Chitosan solution	20µL	*AHM 1(Grapes)*	18 mm	Susceptible
	25µL		26 mm	Susceptible
	30µL		30 mm	Susceptible
0.4% Chitosan solution	20µL	*Bacillus siamensis AHM 2(Banana)*	16 mm	Susceptible
	25µL		18 mm	Susceptible
	30µL		28 mm	Susceptible

From these three tables, all two bacterial isolates were susceptible to the three antimicrobial agents that were used. The two bacterial isolates were susceptible to Ant peptide extract, citric acid solution and chitosan solution, which means these three antimicrobial agents are effective against the bacteria (kills the growth of microorganisms Isolated Bacteria).

6. Conclusion

Generally fruits are destroyed by different microbes like Bacteria and Fungi and so on, which leads to change in taste and odour of the fruits. Bacteria and Fungi are harmful living microorganisms. The spoilage of most fruits is always associated with Bacteria and Fungi. A study was carried out to isolate and characterize microbial pathogens from spoiled fruit and testing for Antimicrobial Susceptibility. Two Bacterial isolates were successfully isolated from spoiled grape and banana fruits. Through molecular characterization, one of the isolates was identified as *Bacillus siamensis* (from Spoiled banana) and for other isolates gene sequencing results are awaited, as the sequencing got error, I will proceed with the gene sequencing in future.

Antimicrobial Susceptibility testing was carried out using three different antimicrobial agents against the two types of bacteria that were successfully isolated. The different antimicrobial agents used were Ant peptide, 10% Citric acid solution and Chitosan solution. All 2 bacterial isolates were susceptible to three antimicrobial agents. This is just a study to isolate the bacteria from spoiled fruits and testing for antimicrobial susceptibility. These antimicrobial agents are effective against the isolated bacteria. AMPs are important part of innate immune system of different organisms. AMPs have a wide range of inhibitory effects against bacteria, fungi, parasaites and viruses.

7. Future Scope

So these antimicrobial agents can be used in food packaging industry as Biofilm (Edible films). Chitosan coating is one of the promising techniques because of its excellent properties including the property to form the film on the fruit's surface. Chitosan has attracted a growing attention as a food preservative due to its versatility, nontoxicity, biodegradability and biocompatibility. This study can be taken into next level analysis/study, as these antimicrobial agents can be used as coatings in fruits and vegetables as food packaging agents and as preservative. India is a tropical country, so every year we experience the hot climate. So Farmers are affected by spoilage of fruits and vegetables. My work will be very much useful for them as these different antimicrobial agents inhibit the growth of microorganisms and prevent the fruit from getting spoiled.

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