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SCREENING AND IDENTIFICATION OF MICROBES IN PACKED AND PROCESSED FOODS: A STUDY ON MICROBIAL CONTAMINATION

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Abstract:

This study focuses on the crucial task of identifying and counting microorganisms found in pack or processed foods to ensure their safety and quality. The project also utilizes molecular and biochemical techniques to characterize these microorganisms. Various techniques such as serial dilution, enrichment culture, filtration, and direct plating methods are utilized to screen and isolate the microorganisms, and they undergo PCR amplification, sequencing and biochemical tests for characterization. The research discovered various microorganisms such as bacteria, yeasts, and fungi present in different food products with varying contamination levels. An analysis of the microorganisms showed that a few of them could cause food spoilage, including Pseudomonas, Bacillus, and Aspergillus species. Also, the study identified useful microorganisms such as lactic acid bacteria, which can be used as probiotics or for food fermentation. This research provides valuable insights into the microbiological quality of packed or processed foods and contributes to the development of effective strategies to improve food safety and quality.

1. Introduction:

In the food industry, food quality and safety are important concerns, and the presence of microbes in packaged or processed meals can have a big impact on both.[1] Food rotting brought on by microbial contamination can result in financial losses and public health issues. A few microbes can also result in foodborne infections, which can be serious and even fatal.[2] Hence, it is crucial to screen and isolate microorganisms from packaged or processed meals in order to ensure the safety and quality of the food. Identification and counting of the microorganisms in a sample are necessary for screening and isolating germs from food products.[3] In order to accomplish its goal, this project will screen and isolate bacteria from packaged or processed foods using a variety of microbiological techniques, and then identify and describe the isolated microorganisms using molecular and biochemical methods.[4] The

outcomes of this project may offer insightful information about the microbiological consistency of packaged or processed foods, as well as aid in the creation of novel approaches to enhancing food quality and safety[5].

2. Materials and Methods:

2.1 Sample Collection:

Packed and Processed foods were purchased around the areas of Madurai district. A portion of each sample was aseptically moved to sterile tubes to reduce the risk of contamination. A total of 10 samples were purchased namely,

SNO	SAMPLES	COLLECTED AREA
1	Almond Milk	Madurai
2	Aloevera Drink	Madurai
3	Dried Ginger Powder	Madurai
4	Ginger Garlic Paste	Madurai
5	Grape Juice	Madurai
6	Idly Chilli Powder	Madurai
7	Paruppu Saadha Podi	Madurai
8	Pomegranate Juice	Madurai
9	Tomato Puree	Madurai
10	Water	Madurai

2.2 Serial Dilution:

During the sequential re-suspension of an original solution into predetermined amounts of a liquid diluent, a known or unknown substance is systematically reduced through serial dilution.[6] 1 gm or 1 mL of the sample is added to 1 mL of the double-distilled water to create the stock solution. The first tube (T1), which has the greatest dilution factor label and contains 900 µL, receives a 1 mL aliquot of the stock solution.[7] Repeat the procedure by aliquoting 1 mL of the fresh solution1 and putting it in tube 2. The stock concentration is diluted by a factor of 10 with each stage of aliquoting and resuspension until the last tube is reached.[8]

2.3 Preparation of the substrates:

Nutrient agar and Luria-Bertani medium were used for the majority of the samples as they promote bacterial growth.[9]

2.3.1 Nutrient agar medium: Medium was prepared by dissolving 2.8 g of commercially available Nutrient Agar Medium (HiMedia) in 100 ml of distilled water.[10] The dissolved medium was autoclaved at 15 pounds of pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured into 100 mm petri dishes (25-30 ml/plate) while still molten.[11]

2.3.2 Nutrient Broth: It was prepared by dissolving 2.8 g of commercially available medium (HiMedia) in 100 ml of distilled water and boiling until the medium was completely dissolved.[12] Media was dispensed as needed and autoclaved at 15 lbs (121°C) for 15 minutes.[13]

2.3.3 LB Agar: Medium was prepared by dissolving 2.5 g of commercially available LB medium (HiMedia) in 100 ml of distilled water.[14] The dissolved medium was autoclaved at 15 pounds of pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured into 100 mm Petri dishes (25-30 ml/plate) while still molten.[15]

2.4 Spread Plating:

Plate the bacteria: Pour the agar medium into a sterile Petri dish and allow it to solidify. Take a small volume of the diluted sample suspension (usually 0.1 mL) and place it on the centre of the agar plate.[16] Using the sterilized spreader, spread the bacterial suspension evenly over the surface of the agar plate or LB plate.[17]

Sterilize the spreader: Sterilize the spreader by dipping it in 70% ethanol and flaming it until it turns red hot. Allow it to cool for a few seconds before use. In a clockwise/counter clockwise motion, glide the horizontal portion of the spreading rod to equally distribute the sample within the petri dish.[18]

Incubate the plate: Invert the agar plate and incubate it at the temperature of 37°C for 24 hours and conditions for the bacterial strain being tested.[19]

2.5 Subculture Plating:

Streak the mixed culture: Using a sterile loop, streak a small amount of the mixed culture onto the surface of the agar plate in a zigzag pattern.[20] The streaking should be done in such a way that the bacterial cells are diluted as the streaks progress.

Incubate the agar plate: Invert the agar plate and incubate it at 37°C for 24 hours and conditions for the bacterial strain being tested.[21]

Observe the colonies: After incubation, observe the agar plate for bacterial colonies. There may be several different types of colonies present on the agar plate.

Pick a single colony: Using a sterile loop, pick a single colony of the bacteria of interest. The colony should be well-separated from other colonies.[22]

Subculture the colony: Streak the single colony onto a fresh agar plate using the streaking technique. The goal is to produce isolated colonies of the bacteria of interest.[23]

Incubate the agar plate: Invert the agar plate and incubate it at 37°C for 24 hours and conditions for the bacterial strain being tested.[24]

Observe the colonies: After incubation, observe the agar plate for bacterial colonies. There should be isolated colonies of the bacteria of interest.[25]

2.6 Bacterial Suspension

Sterilize equipment: Sterilize all equipment that will come into contact with the bacterial culture, including the test tubes, pipettes, and loop or swab.[26]

Prepare the nutrient broth: The Nutrient broth were prepared using commercially available nutrient medium (HiMedia) and autoclaved for free of contamination.

Obtain the bacterial culture: Obtain a bacterial culture that has been grown on an agar plate or stored in a culture collection.[27,28]

Inoculate the nutrient broth: Using a sterile loop or swab, transfer a small amount of the bacterial culture to the nutrient broth. The amount of bacterial culture added will depend on the desired cell density for the experiment.[29]

Incubate the nutrient broth: Incubate the nutrient broth at the appropriate temperature and conditions for the bacterial strain being tested. Ensure that the broth is well mixed by gently swirling the test tube.[30]

2.8 Biochemical Tests

Out of the 10 sub culture plates, 5 cultures which are unique in morphology was chosen for biochemical tests.

Nutrient broth was prepared and the cultures were grown in suspension.

The major biochemical tests used in this research are Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test.[31, 32].

Indole Test:

It is used to test whether bacteria can break down the amino acid. Tryptophan.[33]

Procedure:

1. Inoculate a 24 hours culture in Tryptophan Broth
2. Incubate at 37^0 C for 24 hours
3. Add 0.5ml of Kovac's Reagent to the broth

Methyl Red Test:

It is used to check the ability of bacteria to perform Mixed Acid Fermentation of glucose into Acetic acid, Lactic acid and Succinic acid.[34]

Procedure:

1. Inoculate a 24 hours culture in MR VP broth
2. Incubate at 370 C for 48 hours
3. Add 5 drops of methyl red to the broth

Voges Proskauer Test:

It is used to check the ability of bacteria to produce acetoin – a non-acidic neutral end product of glucose fermentation.[35]

Procedure:

1. Inoculate a 24 hours culture in MR VP broth
2. Incubate at 370 C for 48 hours
3. Add 0.3ml of Barritt's reagent A then add 0.2ml of Barritt's reagent B
4. Shake the tube and expose to atm. O2 and keep the tube for 10 – 15 mins undisturbed.

Citrate Utilization Test:

It is used to determine the ability of bacteria to utilize Sodium Citrate as its only carbon source inorganic Ammonium dihydrogen phosphate as the sole nitrogen source.[36]

Procedure:

1. Inoculate a 24 hrs culture in Simon Citrate Agar slant
2. Incubate aerobically at 37 C for 24 hrs
3. Some organism may require up to 7 days of incubation due to their limited rate of growth on citrate medium

2.9 Isolation of Genomic DNA:

1) Take 1.5 ml of O/N culture. Centrifuge at 10,000 rpm for 5 mins.

2) Discard the supernatant. To the pellet add 560ul of TE buffer and mix well.

3) Add 60ul of 10% of SDS and mix well. Incubate for 1hr at 37C.

4) Add 100ul of 5M NaCl and mix well. Then add 80ul of CTAB/NaCl solution. Mix and incubate at 65C for 10mins.[37]

5) Then add equal volume of phenol:chloroform:isoamyl alcohol(25:24:1).Mix well and centrifuge at 12,000rpm for 5mins.

6) Transfer the aqueous supernatant to a fresh tube and leave the interface behind.

7) Add equal volume of chloroform:isoamyl alcohol(24:1).Mix well and centrifuge at 10,000rpm for 5mins.[38, 39]

8) Transfer the supernatant to a fresh tube and add double the volume of absolute ethanol to precipitate DNA, shake the tube back and forth till the strongly white DNA precipitate becomes clearly visible.

9) Centrifuge at 10,000rpm for 5mins.Discard the supernatant and add 200ul of 70% ethanol.

10) Wash it and air dry it. Add 40ul Milli Q Water.[40]

Solution preparation:

TE buffer: 1ml of 1M Tris,0.2ml of 0.5M EDTA and make up to 100ml using distilled water.

10%SDS: 10g SDS in 100ml of distilled water.

CTAB/NaCl solution: Dissolve 2.5g of NaCl in 4oml distilled water. Add 5g CTAB and heat it at 65C.Adjust the volume to 50ml.[41, 42]

2.10 16s rDNA Amplification

Three microliters of the extracted DNA was amplified with primers 16sFp1(5'-GTGCCAGCAGCCGCGGTAA-3') and 16sRp1 (5'-TACGGYTACCTTGTTACGACTT-3') generating a PCR product corresponding to nucleotide positions 8 to 926 of the Escherichia coli 16S rDNA sequence. All reactions were performed in 25 µL volumes containing 12.5 µL of master mix, 1 µL of each forward and reverse primer, 2 µL of template DNA, 8.5 µL of PCR-H2O.[39]

PCR was performed in the pattern of the following thermocycling program: Denaturation at 95°C for 5 minutes, followed by 1 minute denaturation at 95°C up to 30 cycles, annealing at 55°C for a minute, extension at 72°C for a minute, and a final extension step at 72°C for 5 minutes. Visualization of PCR products was done in electrophoresis in 1% (wt/vol) agarose gels and the used staining was ethidium bromide (0.5 µg/ml). The amount of PCR product used for visualization is 10 microliters. [43]

Sterile water was used to prepare all solutions which was double distilled and treated with UV for 30 mins to avoid contamination. 3 separate rooms were used for the process of master mix preparation, template addition, and the gel electrophoresis of PCR products.[44, 45] To reduce the probability of false positive PCR results which occurs due to cross contamination, 2 negative controls were used for each master mix.[46]

2.11 16s rDNA Sequencing

16S rDNA sequencing is a molecular biology technique used to identify and classify bacteria based on their DNA sequences. The 16S rDNA gene is a small subunit of the bacterial ribosomal RNA gene, and it is present in all bacteria.[47] The PCR products can be sequenced using a next-generation sequencing platform, such as Illumina or PacBio. The generated sequence data can be analysed using bioinformatics tools, such as BLAST or QIIME, to identify and classify the bacterial species.[48]

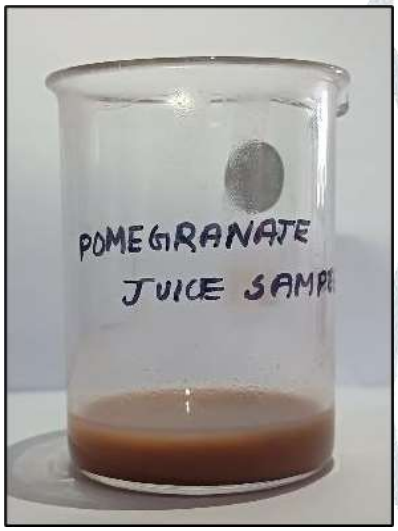
3. RESULTS AND DISCUSSION:

3.1 SAMPLE COLLECTION:

GINGER GARLIC PASTE

TOMATO PUREE

ALMOND MILK

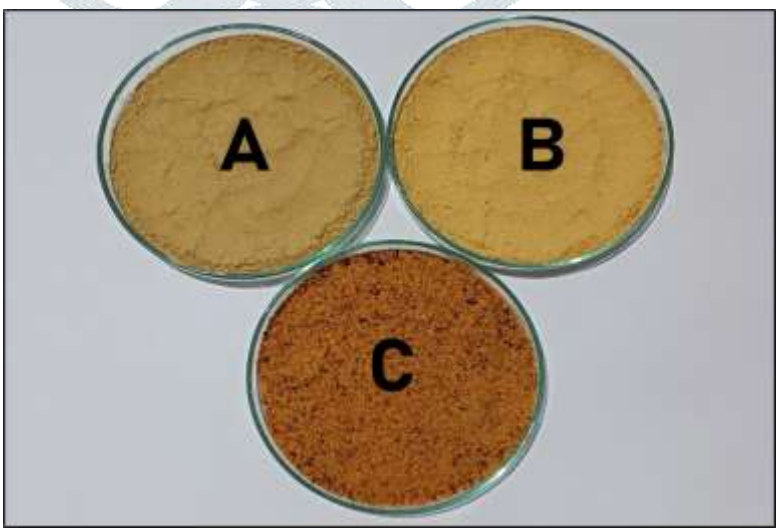


POMEGRANATE JUICE

GRAPE JUICE

WATER

ALOEVERA DRINK



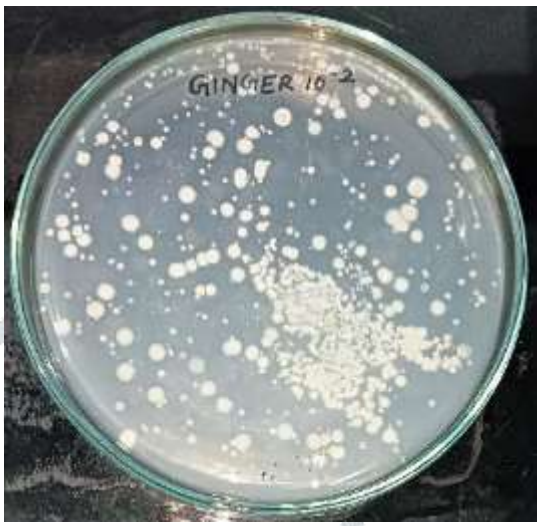
A – DRIED GINGER POWDER
B – PARUPPU SADHA PODI
C – IDLY POWDER

All 10 samples were collected around the areas of Madurai and immediately transferred into sterile containers to avoid cross-contamination.

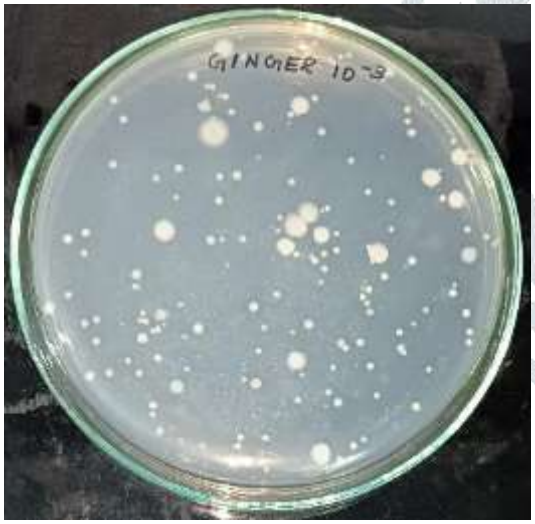
3.2 SPREAD PLATING RESULTS OF SAMPLES

Culture plates of the sample DRIED GINGER POWDER

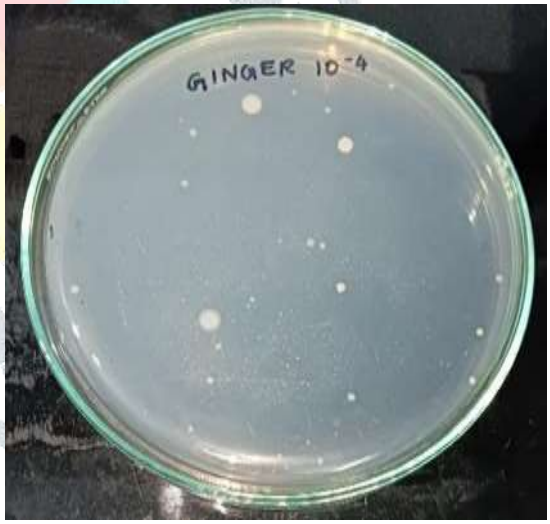
Concentration 10^{-1}



Concentration 10^{-2}



Concentration 10^{-3}

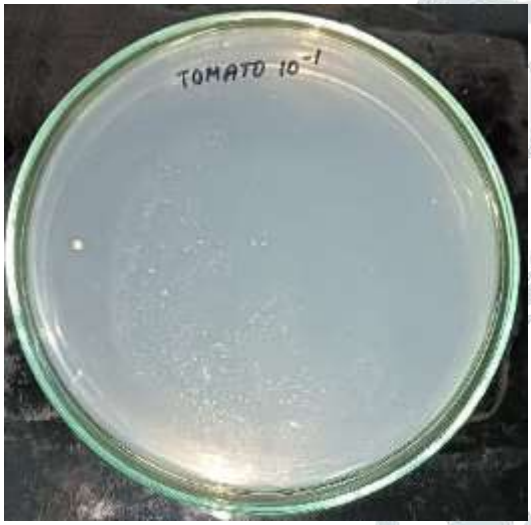


Concentration 10^{-4}

Concentration 10^{-5}

Control plate

Culture plates of the sample TOMATO PUREE



Concentration 10^{-1}

Concentration 10^{-2}

Concentration 10^{-3}

Concentration 10^{-4}

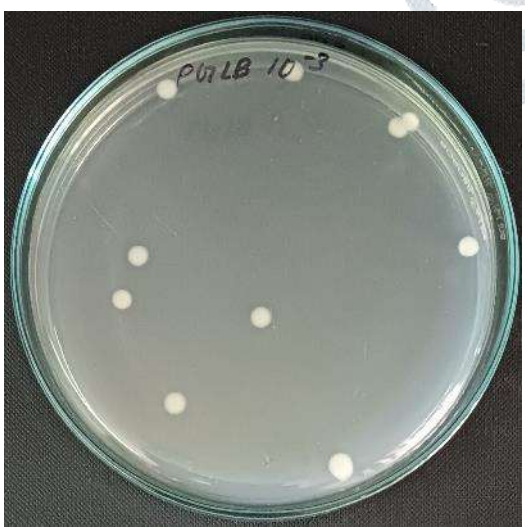
Concentration 10^{-5}

Control plate

Culture plates of the sample POMEGRANATE JUICE

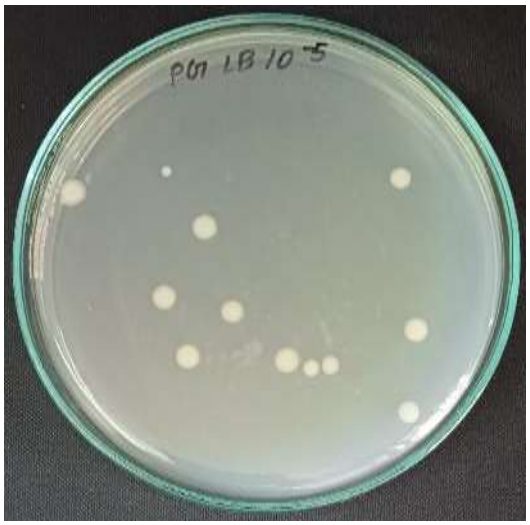
Concentration 10^{-1}

Concentration 10^{-2}



Concentration 10^{-3}

Concentration 10^{-4}



Concentration 10^{-5}

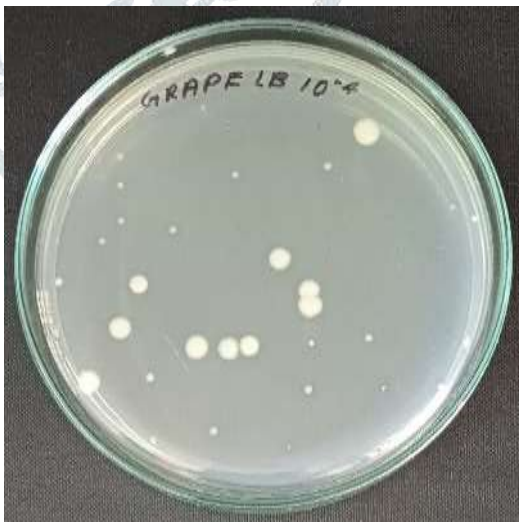
Control plate

Culture plates of the sample GRAPE JUICE

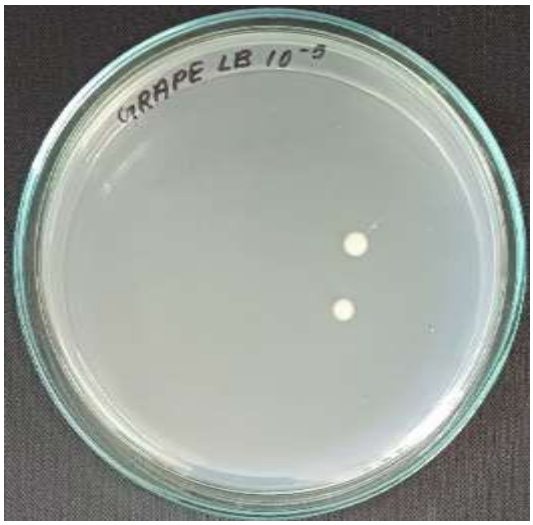
Concentration 10^{-1}

Concentration 10^{-2}

Concentration 10^{-3}



Concentration 10^{-4}



Concentration 10^{-5}

Control plate

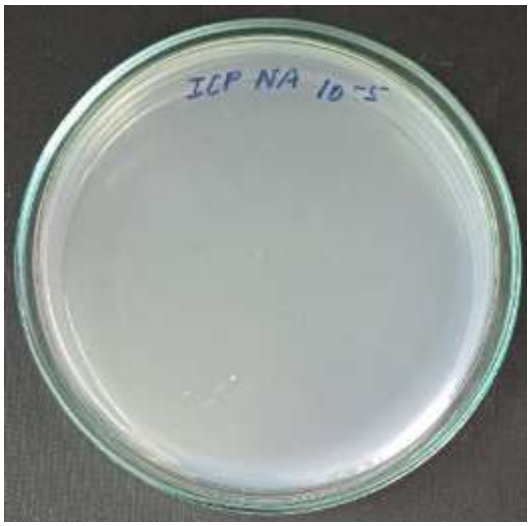
Culture plates of the sample IDLY POWDER

Concentration 10^{-1}

Concentration 10^{-2}

Concentration 10^{-3}

Concentration 10^{-4}

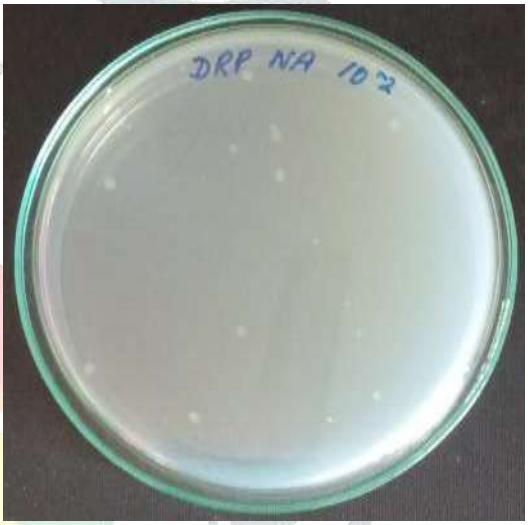


Concentration 10^{-5}

Control plate

Culture plates of the sample PARUPPU SADHA PODI

Concentration 10^{-1}



Concentration 10^{-2}

Concentration 10^{-3}

Concentration 10^{-4}

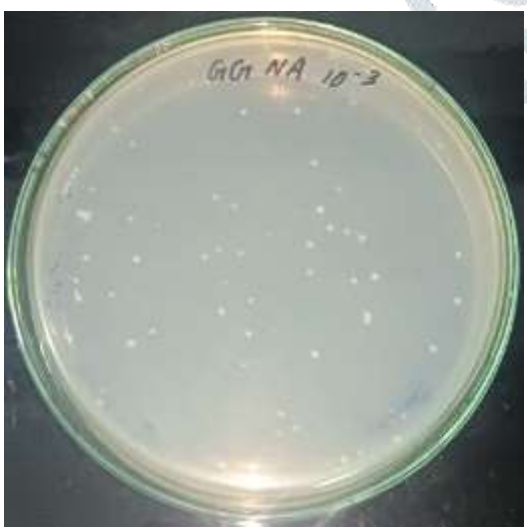
Concentration 10^{-5}

Control plate

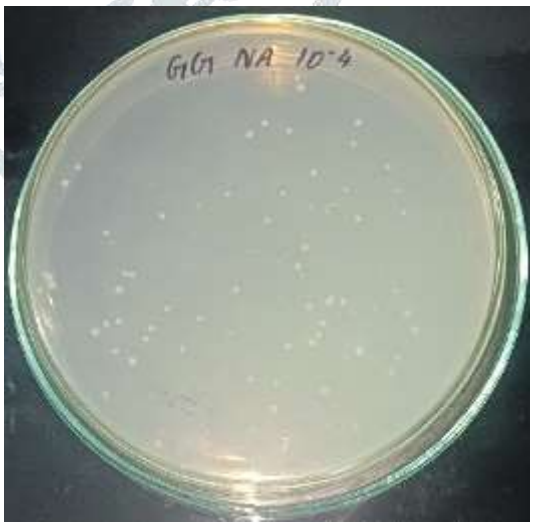
Culture plates of the sample GINGER GARLIC PASTE

Concentration 10^{-1}

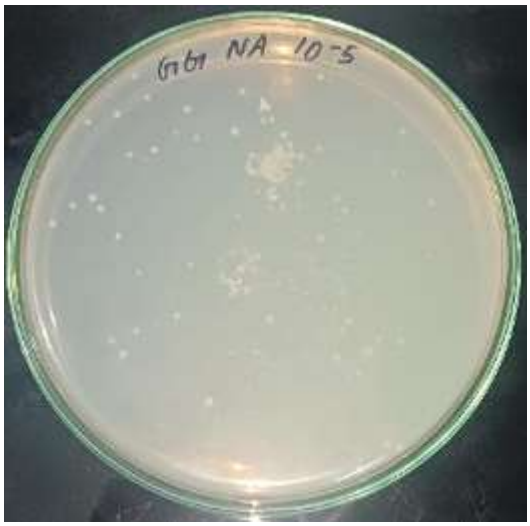
Concentration 10^{-2}



Concentration 10^{-3}



Concentration 10^{-4}



Concentration 10^{-5}

Control plate

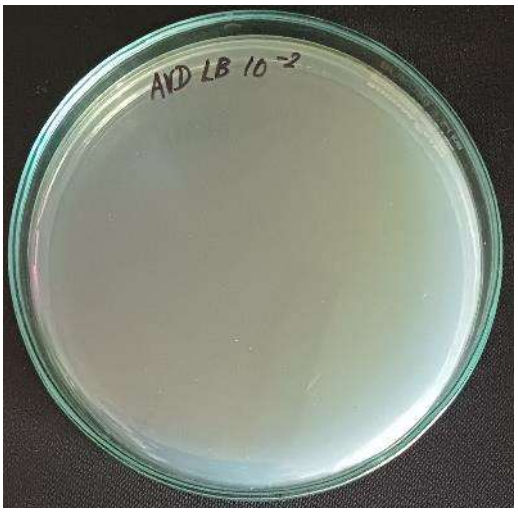
Culture plates of the sample ALMOND MILK

Concentration 10^{-1}

Concentration 10^{-2}

Control plate

Culture plates of the sample ALOEVERA DRINK



Concentration 10^{-1} Concentration 10^{-2}

Control plate

Culture plates of the sample WATER

Concentration 10^{-1}

Concentration 10^{-2}

Concentration 10^{-3}

Control plate

3.3 SUBCULTURE STEAKING:

Among the spread plates, the cultures which are unique in their morphology and colour were chosen for subculture. 16 specific colonies were subcultured by steaking.

POMEGRANATE JUICE

TOMATO PUREE

WATER

PARUPPU SADHA PODI

ALOE VERA DRINK

ALMOND MILK

GINGER GARLIC PASTE

IDLY POWDER

DRIED GINGER POWDER

GRAPE

3.4 BIOCHEMICAL TESTS:

Out of the 10 sub culture plates, 5 cultures which are unique in morphology and color was chosen for biochemical tests. Agar broth was prepared and the cultures were grown in suspension. The major biochemical tests used in this research are

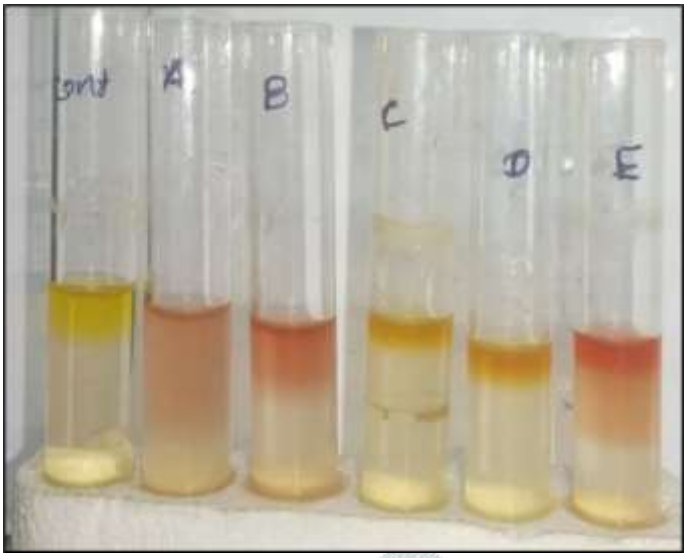
1. Indole test
2. Methyl Red test
3. Voges Proskauer test
4. Citrate utilization test

INDOLE TEST:

Sample A, C – Positive

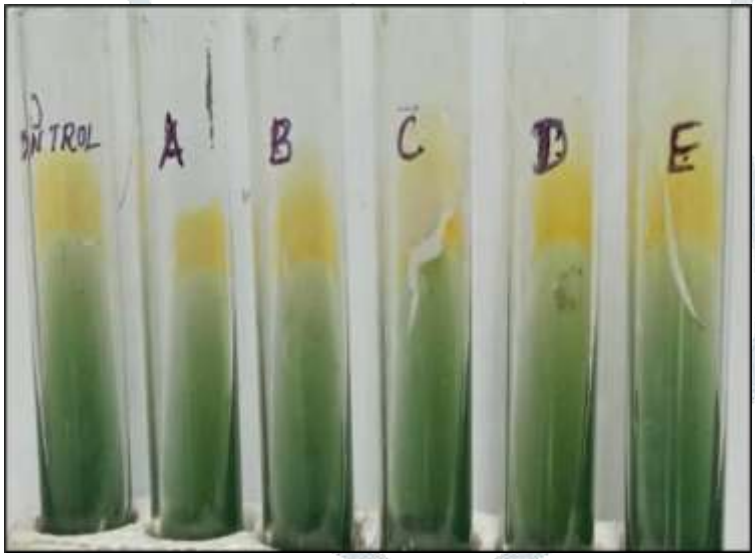
Sample B, D – Negative, Sample E – Either Positive or Negative

METHYL RED TEST:



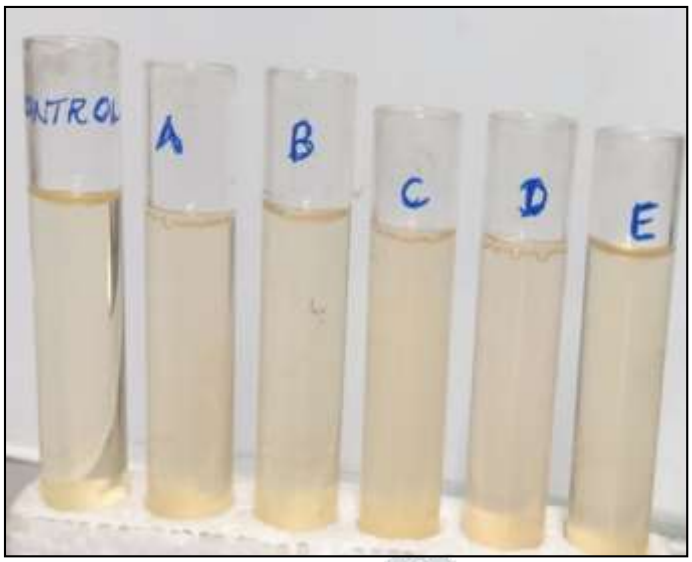
Sample A, B, E – Positive, Sample C, D – Negative

VOGES PROSKAUER TEST;



Sample A, B, C, D, E – Negative

CITRATE UTILIZATION TEST:



Sample A, B, C, D, E - Negative

SNO	SAMPLE	INDOLE TEST	METHYL RED TEST	VOGES PROSKAUER TEST	CITRATE UTILIZATION TEST	NAME OF THE BACTERIAL STRAINS
1	GRAPE - 1	+	+	-	-	*E.Coli*
2	GRAPE - 2	-	+	-	-	*E.Blatte*
3	ALOE VERA DRINK	+	-	-	-	Unpredictable
4	IDLY CHILLI POWDER	-	-	-	-	Unpredictable
5	ALMOND MILK	**v**	+	-	-	*E.coli* (Inactive)

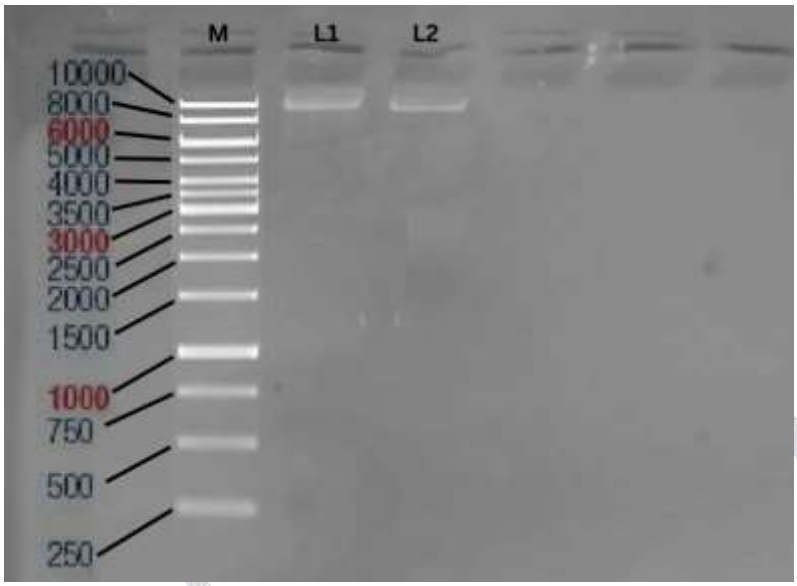
From the table, Sample-A(Grape-1) has been identified as ***Escherchia coli***

Sample-B(Grape-2) has been identified as ***Escherichia blatte***

Sample-E(Almond milk) has been identified as ***Escherichia coli***, which is in inactive state. The samples C & D are unpredictable and has to be sequenced.

3.5 ISOLATION OF GENOMIC DNA:

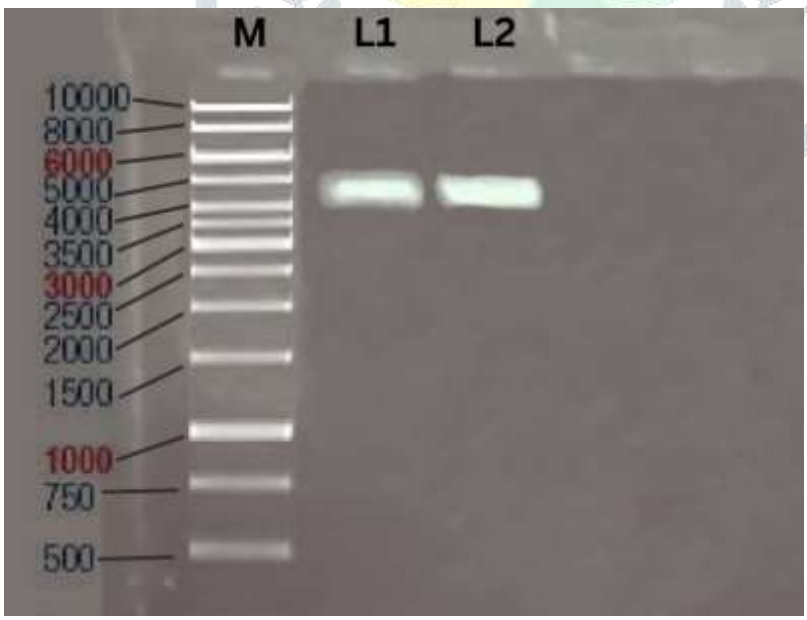
DNA has been isolated for the unpredictable samples C&D and visualization was done through electrophoresis in 1% agarose gel with ethidium bromide staining.



M – 1KB Marker, L1 – Sample 1, L2 – Sample 2

3.6 PCR PRODUCT VISUALIZATION:

After the DNA has been isolated, it is amplified using PCR and visualized in 1% agarose gel with ethidium bromide staining.



M – 1KB Marker, L1 – PCR product of Sample 1, L2 – PCR Product of Sample 2

3.7 16s rDNA SEQUENCING:

Two sequences were obtained by sequencing the samples 1 & 2. Between 88 and 92 percent of the sequences in the NCBI database have similarities to known bacterial sequences. According to the database, the 2 sequences were known to be a strain of

- ***Escherichia coli*** strain NRRI-CPD-COMB29
- ***shigella sp*** strain NCCP-416

Finding ***E. coli***, ***Shigella sp***, and ***E .blatte*** in food samples is a concerning result. These microorganisms are known to cause foodborne illnesses and can pose a significant risk to human health.

E. coli is a common bacterium found in the intestines of humans and animals. Some strains of ***E. coli*** can cause severe food poisoning, leading to symptoms such as abdominal pain, diarrhea, and vomiting.

Shigella sp is another bacterium that can cause foodborne illnesses, specifically shigellosis. Shigellosis can cause diarrhea, fever, stomach cramps, and dehydration.

E. blatte, also known as the oriental cockroach, is not a pathogenic microorganism, but its presence in food samples is a potential indicator of poor sanitation and hygiene.

Based on the results, it is essential to state that even packed foods contain microorganisms which shows that microbes are ubiquitous and unavoidable.[49] Therefore to reduce the risk of getting foodborne illness, consumers must choose the food products correctly and try to avoid the products which are made unhygiene.

Furthermore, it is essential to properly handle and cook all food products to prevent the transmission of these microorganisms to consumers. Food safety is a critical concern, and by taking appropriate measures, we can help ensure that the food we consume is safe and free from harmful microorganisms.[50]

4. CONCLUSION:

To ensure food safety and avoid foodborne illnesses, screening and identification of microorganisms from packaged or processed foods is crucial. Food can get contaminated by microorganisms at any point throughout production, processing, and packing; therefore, it is critical to find and identify these germs before the food is consumed by customers. Strong laboratory procedures, knowledgeable people, and effective quality control procedures are all necessary for the efficient screening and identification of microorganisms from packaged or processed foods. The study has identified both dangerous and non-harmful creatures. Overall, preserving consumer confidence in the food supply chain and safeguarding public health depend on the detection and identification of bacteria in food.

5. REFERENCES:

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