BIOCHEMICAL TESTS FOR CHECKING PRESENCE OF PATHOGENS IN FOOD SAMPLES

A Food Process Technology Report

submitted by

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BACHELOR OF TECHNOLOGY

in

BIOTECHNOLOGY

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(Deemed to be University under section 3 of UGC Act, 1956)

SCHOOL OF BIOSCIENCES AND TECHNOLOGY

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DECLARATION BY THE CANDIDATES

We hereby declare that the Food Process Technology report entitled "**BIOCHEMICAL TESTS FOR CHECKING PRESENCE OF PATHOGENS IN FOOD SAMPLES**" submitted by us to Vellore Institute of Technology, Vellore in partial fulfillment of the requirement for the award of the degree of **BACHELOR OF TECHNOLOGY In BIOTECHNOLOGY** is a record of bonafide work undertaken by us under the supervision of **Mr. Ramalingam C**, **SBST - VIT UNIVERSITY.** We further declare that the work reported in this report has not been submitted and will not be submitted, either in part or in full, for the award of any other degree or diploma in this institute or any other institute or university.

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1. INTRODUCTION

Nowadays, biochemical detection and quantification methods are necessary to study many aspects of food sciences, agricultural, and applied environmental microbiology. These areas are diverse in scope, with a focus on understanding the functional interactions between microorganisms and their environment, at levels ranging from molecules to biomes. Setting up and improving new biochemical tools in order to study these major research areas will significantly contribute to the progress of genetics and molecular biology, enzymology and protein engineering, biotechnology, public health microbiology, environmental, and genomic microbiology, among others.

Selective media are used for the growth of only selected microorganisms. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or tetracycline, then that antibiotic can be added to the medium to prevent other cells, which do not possess the resistance, from growing. Media lacking an amino acid such as proline in conjunction with E. coli unable to synthesize it were commonly used by geneticists before the emergence of genomics to map bacterial chromosomes. Selective growth media are also used in cell culture to ensure the survival or proliferation of cells with certain properties, such as antibiotic resistance or the ability to synthesize a certain metabolite. Normally, the presence of a specific gene or an allele of a gene confers upon the cell the ability to grow in the selective medium. In such cases, the gene is termed a marker.

Selective growth media for eukaryotic cells commonly contain neomycin to select cells that have been successfully transfected with a plasmid carrying the neomycin resistance gene as a marker. Assurance of food safety moves more and more from

end- or finished product testing to proactive food safety management. A food safety management system in a food processing company includes both control and assurance activities. Control activities are aiming at prevention or reduction of a food safety hazard and are typically related to product and process controls.

Preventive measures are prerequisite programs such as cleaning and sanitation, temperature control of the production environment, hygiene of the workers etc. elaborated in order to avoid contamination or outgrowth of microbial contaminants. Interventions in a production process are more focussed on reduction or even elimination of a certain contamination for instance by heat treatments. On the opposite, assurance activities in a food safety management system have the objective to provide evidence that products and processes are within set specifications.

Despite the recent advances in food pathogen detection, there still exist many challenges and opportunities to improve the current technology. Culture based methods have been the oldest methods in detecting the microorganisms, even the pathogenic strains. This method gives a confirmed result regarding the presence of a pathogen. The success rate is found to be high, and these methods are cost-effective. However, the biggest drawback in the culture-based method is the slow growth due to which excess time is lapsed to get the final result, which can turn out to be fatal.

2. MATERIALS

The selective media used is MacConkey agar which is a selective media for E.coli and Mannitol salt agar which is a selective media for Staphylococcus. The food sources used

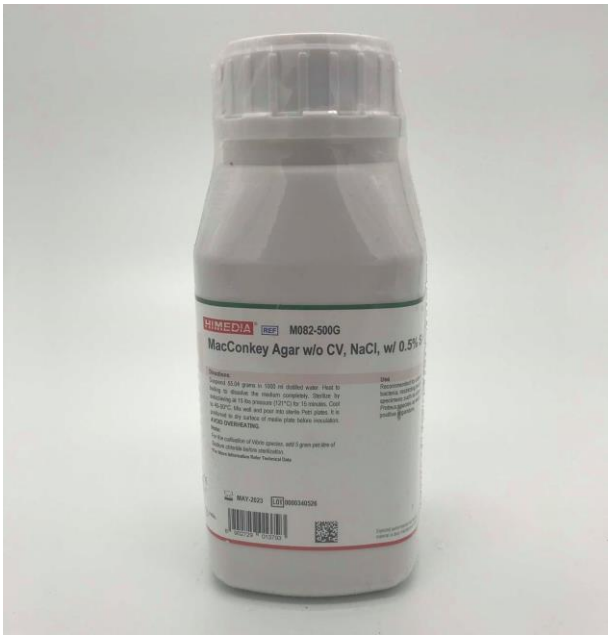

Figure 1: MacConkey Agar HIMEDIA

Figure 2: Mannitol Salt Agar HIMEDIA

The food samples used is Canned Sardine and Gravy from a local restaurant.

Figure 3: Gravy from a local restaurant

Figure 4: Canned Sardine

The diluent used is Monobasic Phosphate buffer and the glassware's are as follows:

1) 6 x Petri plates
2) 1 ml micropipette
3) 6 x 10 ml test tubes
4) L-Rod
5) 3 x 250 ml conical flasks
6) Test tube rack

3. PROCEDURE

- The preparation of agar media is as follows, where for MacConkey Agar, 5 gram of MacConkey agar is added to 100 ml of distilled water in a conical flask and for Mannitol Salt Agar, 12 gram of Mannitol salt agar is added to 100 ml of distilled water in a conical flask. For preparation of the buffer, 9 gram of Monobasic Sodium Phosphate is added to 150 ml of water.

- All materials including glassware's, media and buffers used for the experiment was autoclaved in a cooker for 50 minutes. After autoclave, the materials used for the experiment is kept in the laminar air flow which has been sterilized by both UV and Ethanol cleaning, 10 minutes prior to performing the experiment.

- To all 6 test tubes, 4.5 ml of monobasic phosphate buffer is added, and the test tubes are labelled as t1, t2 and t3 for each sample. Then 0.5 ml of sample (canned sardine/ gravy) is added to t1 of corresponding sample test tubes. The t1 with the sample is diluted by serial dilution method onto t2 and from t2 onto t3.

- The MacConkey agar and Mannitol Salt Agar in the conical flask is poured onto each of the corresponding Petri plates. The petri dishes were labelled, control, sample one (canned sardine) 3rd dilution, sample two (Gravy) 3rd dilution for each petri dish with the agar. The petri dish with the agar is left to solidify in the laminar air flow for 15 - 20 minutes.

- After the agar plates have solidified, the corresponding, samples are streaked, and the petri dishes are incubated based on the media incubation time and temperature.
- Petri dish with MacConkey Agar is incubated for 24 hours at 33 – 37 ºC. The petri dish with Mannitol Salt Agar is incubated for 48 hours at 33 – 37 ºC.

Figure 5: Serial dilution of samples

4. RESULTS

Figure 6: Petri dishes with MacConkey Agar after 24 hours of incubation

The results were observed after the following incubation time, where MacConkey agar which is a selective media for E.Coli even after 24 hours, had no growth of the pathogen due to absence of red/pink non mucoid colonies.
The Mannitol Salt Agar which is a selective media for Staphylococcus Aureus even after 48 hours, had no growth of the pathogens due to absence of yellow colonies with yellow zones.

Figure 7: Petri dishes with Mannitol Salt Agar after 48 hours of incubation

5. CONCLUSION

The biochemical test was a success as this test assures whether, the food sample had the common pathogens. The experiment can be considered a quality control procedure for determining whether the food sample can be consumed without causing any negative effects to the consumer.

Due to absence of Staphylococcus Aureus and E.coli the food is much safer for consumption, however due to unavailability of selective media for Salmonella, the presence of this pathogen couldn't be determined.

6. REFERENCES

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