“MICROBIAL PLATE COUNT USING IMAGE PROCESSING”

By

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Overview

• Waterborne diseases include those due to microorganisms in water people drink
• First outbreak of E.coli O157:H7 began in US in late 1992
• E.coli infection is usually transmitted through consumption of contaminated water or food
• An effective method to detect bacteria outburst using MATLAB is proposed
• Also methods to find the type of Bacteria is discussed
Worldwide Outbreak of E.coli
Problem Statement

• Detect type of bacteria in given Water Sample
• Predict level of outburst of bacteria in given sample
• Develop an easy and non-chemical method for detection of Bacteria
• Make a portable system for Environmental activists using MATLAB, capable of processing the algorithm designed for this system
Methods of detection

Gram stains

This microbe appears as gram-negative short rods. Older cultures (more than 24 hours) may appear as cocci.

Nitrate Broth reactions

E. coli can reduce nitrate to nitrite.
Methods of detection

Indole from tryptophan

E. coli is the only positive microbe

Reactions in glucose fermentation broth

E.Coli not only ferments glucose to acid, it also produces gas. Observe the bubble in the Durham tube.
Methods of detection

Reactions in lactose fermentation broth

Note the formation of acid (yellow color) and gas

Catalase reactions

Note the bubble formation. Catalase positive
Methods of detection

Motility of selected strains

This microbe is highly motile and will show turbidity throughout the tube.

Colony morphology of test strains

E. Coli forms medium sized colonies with a regular margin and convex elevation. Hence by using image processing, E.coli can be detected. In this method MATLAB is used.
Colony Morphology

• Previous methods involves use of chemicals and may not predict level of bacteria outburst
• Bacteria grow quickly when they are provided with nutrients.
• A colony is defined as a visible mass of microorganisms all originating from a single mother cell, therefore a colony constitutes a clone of bacteria all genetically alike.
• Every Bacteria produce unique colonies having different colors, shape size etc.
• This is defined as colony morphology
• Microbiologists use these methods to identify type of bacteria
• Colony morphology can be automated by Algorithm developed in MATLAB
Colony counting

Ecoli agar plate

Manually operated Agar plate colony counting device
Colony counting

• In order to count number of colonies, the colony counting device is used.

• It involves a tedious process where colonies are counted manually. This kind of system is prone to error.

• Using MATLAB, circular colonies can be easily detected with the time they were recorded.
Significance of Colony Count

Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of 1/10,000 dilution, then the count is 32 × 10,000 = 320,000/ml in sample.)
Direct Measurement of Microbial Growth

- A standard plate count shows the quantity of feasible microbes and presumes that each bacterium grows into a single colony.
- As it’s not possible to say that each colony actually developed from an individual cell (cells clump, fact of life) plate counts are reported as the number of colony-forming units (CFU) instead of the number of cells.
- Colony count directly shows the scale of outbreak.
- So an image processing algorithm for counting these CFU and hence determine the level of outbreak is designed in MATLAB using Image Processing toolbox.
Results in MATLAB

18 Hours
Results in MATLAB

24 Hours
Results in MATLAB

48 Hours
Results in MATLAB Mobile™

```matlab
>> ecoli
Water Sample taken on 19-Mar-2015
02:38:54
Number of E Coli colonies found in the
sample is: 90.
The E Coli bacterial count in the
sample is 900000/ml of water
```
Unique features in different types of Bacteria colonies

Escherichia coli

Klebsiella pneumoniae

Serratia marcescens

Chromobacterium violaceum
How MATLAB is effective for implementation of this idea

• The unique features of bacteria colonies like color, transparency and size can be used to determine Bacteria culture with respect to time

• MATLAB supports logging time and also have function to detect circular object of determined size.

• Using these features in MATLAB exact Bacteria culture can be detected.

• MATLAB can also differentiate between overlapping colonies.

• Compared with Open source software's that requires large computation time, MATLAB can produce the result in fewer seconds.
Future Scope of work

• Utilize other distinctive features of bacteria colonies like growth rate, transparency, illumination in UV light etc. to detect type of bacteria

• Geographic location logging when bacteria is detected using sensor in MATLAB Mobile

• Automate the system using robots or drones capable of taking samples from remote locations
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Conclusion

• Only highly experienced microbiologist can predict type of bacteria by seeing the agar plate.
• Also counting the number of colonies is a tedious process.
• Using MATLAB anyone can tell the type of bacteria and its count.
• This algorithm if programmed on remote android device and MATLAB Mobile, it can help nature activists or common people to prevent outburst of any waterborne bacteria.

• Hence safe water, safe life using MATLAB
THANK YOU