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DISTINGUISHING BETWEEN METABOLICALLY ACTIVE AND DORMANTBACTERIA ON PAPER

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ABSTRACT

Switching between metabolically active and dormant states provides bacteria with protection from environ-mental stresses and allows rapid growth under favorable conditions. This rapid growth can be detrimental to the environment, e.g., pathogens in recreational lakes, or to industrial processes, e.g., fermentation, making it useful to quickly determine when the ratio of dormant to metabolically active bacteria changes. While a rapid increase in metabolically active bacteria can cause complications, a high number of dormant bacteria can also be problematic, since they can be more virulent and antibiotic-resistant. To determine the metabolic state of Escherichia coli and Salmonella Typhimurium, we developed two paper-based colorimetric assays. The color changes were based on oxidoreductases reducing tetrazolium salts to formazans, and alkaline phosphatases cleaving phosphates from nitro phenyl phosphate salt.

Keywords: - Bacteria, salt, Phage, Dormant, Metabolically.

I. INTRODUCTION

Over 30% of bacteria in sludge, 83% of bacteria in soil, and 50% or more of bacteria in lakes are in a dormant state (Lennon & Jones, 2011). A bacterial bloom occurs when normally dormant bacteria suddenly switch to a metabolically active condition and begin reproducing at an alarming rate. A bloom may reduce the amount of oxygen in the water, leading to a fish mortality; in an industrial context, it can affect the yield and product qualities during fermentation. Contagious diseases may spread rapidly due to the ability of certain bacteria to remain dormant for long periods of time we need rapid ways to assess changes in the ratio of dormant and metabolically active bacteria, ideally strategies that may be used promptly in the field, to avoid or at least mitigate these impacts.

II. REVIEW OF LITERATURE

FRÖDER, HANS, ET AL, (2020) Culture and physiological/biochemical approaches were the first to be used in phenotypic characterization of microorganisms. The second generation of polymerase chain reaction (PCR), known as real-time PCR (qPCR), gradually supplanted the first during the 1990s. Both negative screening and isolating confirmation are greatly aided by this technique. The susceptibility of amplification responses to inhibitors and the inability to discriminate between live and dead cells are two of qPCR's drawbacks. Criteria used to choose an identification method for bacteria include its sensitivity, specificity, robustness, time of response, and cost per assay. As a result, MALDI-TOF MS is rapidly replacing culture-based methods for identifying bacterial and fungal isolates in food systems, down to the genus and species level. Using MALDI-TOF MS, bacterial and yeast colonies grown in petri dishes can be identified in just a few minutes. However, if the spectral database has fingerprints of peptide mass from the strain type of specific microorganism, then new isolates can be identified. Since no single existing technique offers the complete set of benefits sought by the laboratory, current research projects often pair several techniques with different forms of technology.

LIN, DEHUI ET AL., (2020) When compared to cellulose from plants, bacterial cellulose, which is a pure exocellular polysaccharide produced by microorganisms, has many superior properties, such as a high water-holding capacity, high surface area, rheological properties, and biocompatibility (BC has been shown to be a promising low calorie bulking ingredient for the development of novel rich functional foods of different forms, such as powder gelatinous or shred foams, due to its suspending, thickening, water holding, stabilizing, bulking, and fluid properties. This article summarizes and discusses current research on the biosynthesis, structure, and widespread use of bacterial cellulose in the food business. Raw materials, additive components, packaging materials, delivery system, enzyme and cell immobilizers are some of the most common uses for bacterial cellulose in today's food business. We also discuss potential difficulties and investigate possible approaches to implementing BC in other areas.

CHAUHAN, ABHISHESK, AND TANU, JINDAL (2020) since the growth of microbes in food contaminated with pathogenic bacteria and fungi significantly impacts the quality of food and farm products, microbiological analysis of food and food products is crucial in the year 2020. The presence of these potentially harmful microorganisms in food items poses serious risks to human health. Microbiological characteristics have been identified as the causes of foodborne illness; hence, microbiological evaluations of food products have become more important. The main goals of microbiological analysis of food are to determine the nature of the bacteria present, including their pathogenicity and toxicity, and to determine whether or not they are present at the levels indicated for analysis. Understanding the gradations of microbial contamination in food is also helpful. Depending on the extent of contamination, the chosen technique of analysis may change from product to product. Not only should cooked food be subjected to microbiological testing, but also the ingredients and water used in its preparation. The safety and quality of processed foods

depend on the quality of the water and other substances used in their production. This chapter describes a variety of microbiological techniques for assessing the safety of food and food products, including total bacterial count, total coliform count, total yeast and mold count, and enumeration of E. coli, S. aureus, and Bacillus cereus. Discussed are methods for spotting and naming food-borne pathogens such E. coli, Salmonella, S. aureus, Shigella, Vibrio cholerae, Vibrio parahaemolyticus, and Clostridium perfringens. E. coli O157: H7, osmophilic bacteria, Enterobactersakazakii, and thermophilic acidophilic bacteria (TAB) are all discussed in this chapter in relation to their detection techniques in food and food preparations.

PRIYANKA, B ET AL, (2016) Many illnesses, especially in poorer regions, may be traced back to contaminated food. The monetary effect of this is substantial. They must be contained, and early discovery is of paramount importance in doing so. Early methods of detection and diagnosis depended on culture-based techniques; as immunological techniques, such as enzyme-linked immunosorbent assays (ELISA), and molecular biology-based techniques, such as polymerase chain reaction (PCR), have advanced, so, too, have culture-based techniques. Finding a procedure that is quick, sensitive, specific, and cheap has long been the goal. This objective has united techniques as diverse as microbial culture and future biosensor technologies. This article provides a concise overview of current tendencies and synthesizes many approaches explored throughout time.

III. MATERIALSANDMETHODS

Bacteria and Phage Cultures

Environmental E. coli (E. coli LLV) were isolated from lake water (Lake LaVerne, Ames, IA) by plating the water on modified mTEC agar, while Salmonella enterica subsp. enterica ser. Typhimurium (ATCC 19585) and Escherichia coli K12 (ATCC 25404) were purchased from the American Type Culture Collection (Manassas, VA). Bacteria were cultured in Luria Bertani (LB) broth and counted on agar plates using conventional methods. For the preparation of dormant bacteria, filtered autoclaved lake water (FALW) was inoculated with 109 CFU/ml and then stored at 4 oC for 30 to 40 days (Ozkanca& Flint, 1997). Plate counts were used to determine how many dormant bacteria were present before and after being processed in FALW. About 10% of the bacteria were found to be dormant after being stored. Lambda buffer (100 mMNaCl, 16.6 mM MgSO4, 50 mMTris-HCl buffer pH 7.5, and 0.01% w/v gelatin) was used for the cultivation and counting of T4 (ATCC 11303-B4) and P22 (ATCC 19585-B1) bacteriophages on overlays.

ColorimetricAssaysforBacterialEnzymesonPaper

The reduction of tetrazolium salts or the hydrolysis of para- nitro- phenyl- phosphate formed the basis of the colorimetric experiments performed on Whatman® grade 3 filter sheets. Wax rings of 5 mm diameter were printed on a Xerox Color Qube 8570DNTM (Norwalk, CT) and melted

through the paper (5-8 minutes at 85 °C) to create "reaction zones" (Carrilho, Martinez, &Whitesides, 2009). To conduct the tests, 10 l of the bacteria in PBS at pH 7 and 5 l of the assay solution were applied to the paper, respectively. For metabolically active bacteria, an assay solution was prepared using PBS at pH 7 and varying concentrations of 2-(4-iodophenyl)- 3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) and 5-methylphenazin-5-ium methyl sulfate (PMS) (see Table S1 for specific ratios). Freshly made 0.5 mMTris buffer at pH 10 (details in Table S2) containing 0–3 mM INT and 1–45 mMnitrophenyl phosphate (PNPP) was used to incubate the bacteria in their resting state.

ImmobilizationofBacteriophagesandCaptureofBoundBacteria

Adsorption was used to attach bacteriophages to paper. 200 l of bacteriophage (1010 PFU ml-1) was poured to thoroughly coat the paper, and single circles of Whatman® grade 3 filter sheets were put vertically within a 96-well plate. After 48 hours in the dark at room temperature, the bacteriophage solution was withdrawn, and the sheets were submerged in 200 µl of 1 w/v% bovine serum albumin (BSA) for one hour to block any unreacted sites on the paper. Before using, the blocked sheets were given a wash in a PBS buffer solution (100 µl). Five minutes were spent at 37 degrees Celsius with 300 revolutions per minute of shaking to allow for interaction between E. coli (103 to 107 CFU) and Salmonella (102 to 106 CFU). Four washes with 100 µl of PBS buffer were used to eliminate any free microorganisms. The bacteria samples were tested in the same way as before. Each experiment was repeated eight times.

IV. RESULTSANDDISCUSSION

Development of Colorimetric Assays

There was a separate paper test for each metabolic condition. Bacteria with an active metabolism interacted with a tetrazolium salt at neutral pH, whereas quiescent bacteria reacted with a combination of tetrazolium salt and nitrophenyl phosphate at an alkaline pH.

Iodonitrotetrazolium Chloride for Metabolically Active E. coli on Paper: In In the presence of bacteria, the color of a number of tetrazolium salts in solution shifts (Berridge et al., 2005). While methyl-thiazolyl-diphenyl-tetrazolium bromide and neotetrazolium chloride did not change color when exposed to E. coli, iodophenyl-nitrophenyl-phenyl tetrazolium chloride (INT) did. Methylphenazinium methyl sulfate (PMS) is an electron transporter that accelerated INT's color development. No discernible tendencies in the produced color were seen when the INT and PMS concentrations and ratios were varied while the E. coli concentration was held constant at 5*103 CFU/mm2.

The deepest purple appeared at the greatest INT (2.5 mM) and PMS (1 mM) concentrations, whereas the second-darkest purple appeared at the lowest INT (0.5 mM) and PMS (0.25 mM) values. Weak but discernible coloration also occurred at two additional INT/PMS doses and ratios (1.5 mM INT/0.5 mM PMS and 2 mM INT/1 mM PMS).

Para-nitrophenylphosphate and INT for Dormant E. coli on Paper

Two types of enzymes, oxidoreductases and alkaline phosphatases (ALP), were used to identify bacterial slumber. These enzymes have been found to function normally in bacteria that appear to be dormant. As substrates, we used both inositol pyrophosphate (INT) and para-nitrophenyl phosphate (PNPP). By the action of oxidoreductases, INT is converted to purple formazan, while PNPP is hydrolyzed by ALP to yield yellow para-nitrophenol.

We combined PNPP and INT substrates to detect concurrently ALP and oxidoreductases activity because para-nitrophenol was difficult to notice on white paper and because both types of enzymes are present in quiescent bacteria.

V. CONCLUSION

The physical characteristics of the Ni(II)- and Dy(III)-based MILs were studied, and their potential antibacterial effects were evaluated, in Chapter 4 of this Study. Nine different Salmonella serotypes and eight different E. coli O157:H7 strains were evaluated for this study. Potentially harmful effects of the MILs, such as cell damage, were investigated using non-selective (TSA) and selective (BSA, SMAC) media for 15 minutes. Evaluation of the MIL's potential antimicrobial properties is done by plating MIL-treated cells on both non-selective and selective medium simultaneously. The outer membrane of injured cells is often the first thing to go. 1-ethyl-3-methylimidazolium thiocyanate ([EMIM+]SCN-), which has been shown to promote cellular harm, was used as a comparison agent for MIL-based exposure. Neither non-selective nor selective medium could revive cells exposed to Dy(III) MIL, whereas Ni(II) MIL had no detectable antimicrobial effects on the cell. After dissecting the Dy(III) MIL, researchers concluded that the cytotoxic effects were caused by the ammonium salt ([NH4+][Dyhfacac-]).

The methods for detecting bacterial pathogens that were discussed throughout this study have four main benefits: fast results, cheap costs, mobility, and user-friendliness. Our disclosed quick detection methods have the potential to provide easy and inexpensive sampling of bacterial pathogens, which may be employed in agricultural testing and to enhance industrial efficiency in food processing settings. As a versatile and robust sample preparation technique, MILs have many potential applications, such as the particle-free capture of sequence-specific DNA, and offer a novel method for purifying and preconcentrating bacterial pathogens. Extending MIL-based capture and recovery of critical foodborne pathogens such as Listeria monocytogenes and other members of the Enterobacteriaceae will be a major focus of future research. Using fluorescently-labeled cells may make it easier to see the connection between the MIL and the cells, even if our study reveals that weak electrostatic interactions drive MIL-based capture. Bacteria tied to MIL might be seen under a fluorescent microscope after being labeled with Syto-9, washed, and then spread out in a model media; magnetic focusing of the cell-charged MIL could further enhance visibility. Better purification and enrichment of harmful bacteria will be possible with the help of newly designed MIL structures made possible by a better knowledge of the MIL capture process.

REFERENCES:-

1. Achbergerová, L., Nahálka, J., Lartigue, C., Noskov, V., Chuang, R., Algire, M., … Venter, J.(2011). Polyphosphate - an ancient energy source and active metabolic regulator. MicrobialCellFactories, 10(63). doi:10.1186/1475-2859-10-63

2. Adkins,J.A.,Boehle, K.,Friend,C.,Chamberlain, B.,Bisha,B., &Henry,C.S.(2017).Colorimetric and electrochemical bacteria detection using printed paper- and transparency-based analytic devices. Analytical Chemistry, 89(6), 3613-3621.doi:10.1021/acs.analchem.6b05009

3. Almaas, E., Kovács, B., Vicsek, T., Oltvai, Z. N., &Barabási, A. L. (2004). Global organizationof metabolic fluxes in the bacterium Escherichia coli. Nature, 427(6977), 839-843.doi:10.1038/nature02289

4. Anany, H., Chen, W., Pelton, R., & Griffiths, M. W. (2011). Biocontrol of Listeriamonocytogenes and Escherichia coli O157:H7 in meat by using phages immobilized onmodified cellulose membranes. Applied and Environmental Microbiology, 77(18), 6379-87.doi:10.1128/AEM.05493-11

5. Ayyash, S., Wu, W. I., & Ravi Selvaganapathy, P. (2014). Fast and inexpensive detection ofbacterial viability and drug resistance through metabolic monitoring. In: 2014 IEEEHealthcareInnovation Conference, HIC 2014. (pp.22-25)

6. Becker,D.,Selbach,M.,Rollenhagen,C.,Ballmaier,M.,Meyer,T.F.,…Bumann,D.(2006).

7. Robust Salmonella metabolism limits possibilities for new antimicrobials. Nature,440(7082),303-307. doi:10.1038/nature04616

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