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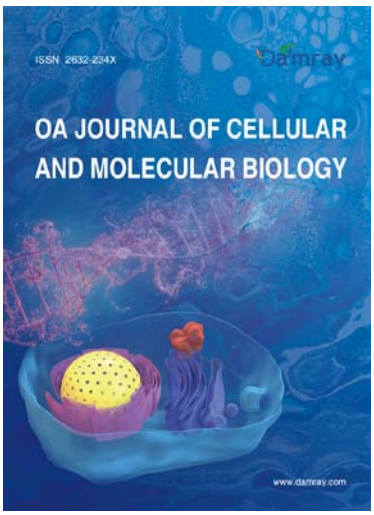
Application of Molecular Blotting Techniques in the Detection of Food Spoilage Bacteria and Pathogenic Microorganisms



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Abstract

In recent years, people have become more health conscious and are paying more and more attention to food safety issues, and food contamination and spoilage have always been a key concern in food supply assurance and food quality and safety management. Only rapid and accurate monitoring of food microorganisms and assessment of food contamination can prevent foodborne illness and food waste. The molecularly imprinted polymer detection technique has the advantages of stability, specificity and ease of preparation. It has a wide range of applications in the detection of food spoilage bacteria and pathogenic microorganisms.

Keywords

Food spoilage bacteria, Pathogenic microorganisms, Molecular blotting technique, Detection

In order to control food spoilage and reduce food-borne diseases, food safety detection techniques such as surface plasmon resonance, ELISA, colony counting and cell culture are commonly used to detect microbial contamination in food, but these detection methods are less sensitive, less specific, and have long detection times and complex operations. In recent years, with the continuous development of modern detection technology, molecular imprinting technology is widely used for the detection of food spoilage bacteria and pathogenic microorganisms. This detection technology ensures food safety through the use of harmful microorganisms by identifying intermolecular forces such as hydrophobic forces, electrostatic forces, Van der Waals forces, hydrogen bonding forces and three-dimensional spatial structures

specific to template molecules and their structural analogues by molecularly imprinting polymers.

1. Analysis of microbial molecularly imprinted polymer synthesis

1.1 Emulsion polymerisation

Emulsion polymerisation, in which the template molecules and stabilisers in the aqueous phase are stirred to evolve into an emulsion, is a way of preparing biomolecularly imprinted polymers, which are more conformationally stable when prepared by emulsion polymerisation, such as proteins, and more specific in terms of target recognition. It was found [1] that polysaccharides from the cell wall of Pseudomonas aeruginosa were used as template molecules, and functional monomers and crosslinkers were selected as acrylamide and N,N-methylenebisacrylamide, respectively, in the principle of emulsion polymerisation, all three of which can be fused in distilled water to form an aqueous dispersion phase and an oil phase with sodium dioctyl sulfosuccinate n-hexane as the continuous phase, polyoxyethylene lauryl ether, N,N,N,N-tetramethylethylenediamine and peroxynitrite. Ammonium sulfate can be prepared in a way that will selectively identify Pseudomonas aeruginosa as the molecularly imprinted polymer.

Emulsions in which the stabiliser is a colloidal particle, also known as Pickering emulsions, are prepared without surfactants and are now widely used by UAN in the preparation of macromolecularly imprinted polymers. Studies have shown [2] that bacteria modified as particle stabilisers for vinyl N-acrylic acid chitosan, and that for oil phase emulsions in the aqueous phase to enhance their stability, the oil phase polymerisation of the crosslinker monomer must be initiated by free radicals, thus allowing the bacteria to imprint on the surface of the polymer microspheres during polymerisation. When the bacteria are eluted, the template bacteria leave adsorption sites on the surface of the polymer microspheres.

1.2 Electrochemical polymerisation

The electrochemical polymerisation method is mainly based on the preparation of macromolecularly imprinted polymers by placing electrodes in an electrolyte solution and inducing the polymerisation of crosslinkers, functional monomers and template molecules on the surface of the electrodes by means of electrochemical reduction and oxidation reactions. This method is simpler and allows better control of the polymer film thickness.

1.3 Microcontact blotting method

Bacterial microcontact blotting is a whole cell blotting method in which the blotting film and bacterial template preparation is generally divided into the following steps: (1) Preparation of bacterial template. Microscope glass slides were cleaned using deionized water and anhydrous ethanol, then soaked in acidic piranha solution for 1 h. Vibrio parahaemolyticus suspension was inactivated by formalin and then evenly coated on the glass slides, placed in a 4°C environment for half an hour to allow the bacteria on the surface of the slides to settle, then the slides were placed in a rotary coater for centrifugation at 1500 r per minute to remove excess solvent and prepare bacterial template [3]. (2) Preparation of blotting film. Under vacuum, polydimethylsiloxane was mixed in cyclohexane, an appropriate amount of the mixture was placed on clean glass slides and placed on a hot plate at 80°C for 2 minutes for pre-curing, thus raising the viscosity of the pre-polymer, pressing the bacterial template glass slides into the pre-polymer, maintaining normal room temperature, overnight and then placing them on a hot plate at 80°C for 1 hour for curing, washing the imprinted film using deionised water ultrasonication and drying. To achieve non-specific adsorption of the imprinted film, the evaporation-deposition method can be chosen to complete the preparation of Vibrio parahaemolyticus fluorinated polydimethylsiloxane imprinted films, thereby enhancing selective adsorption properties and capture rates.

2. Testing methods

2.1 Fluorescence detection method

The fluorescence detection method is mainly used to complete qualitative and quantitative analysis with the help of fluorescence signal strength and colour shade. This detection method is easy to operate, highly sensitive, fast response time, and is more frequently used in food testing. It was found that [4], when the emulsion stabiliser was chosen from a complex of Listeria monocytogenes dispersed in phosphate solution and CdTe quantum dots, and the initiator was chosen from N,N-dimethylaniline, benzoyl peroxide, divinylbenzene as functional monomer and trimethylolpropane trimethacrylate, the preparation of bacterial fluorescent probes was completed by Pickering emulsion polymerisation, which could effectively enhance the specificity of the probes. The fluorescence intensity decreased when the adsorption amount of the target bacteria became larger. The detection limit of the probe reached 103 CFU/mL in Listeria monocy-

togenes, which can effectively detect milk contamination.

2.2 Electrochemical sensor detection method

Due to environmental influences, Bacillus spores may appear dormant for long periods of time, germinate and then re-grow in the nutrient, leading to food spoilage and food-borne illness. The electrochemical polymerisation method allows for the appearance of Bacillus cereus bacteriophage macromolecular imprinted polymers on the surface of a carbon paste working electrode, allowing for rapid detection of Bacillus cereus contamination after 5 minutes incubation of the electrode, spores and during cyclic voltammetry electrochemical sensor detection [5]. If the Gram-negative non-bacterium E. coli, for example, this pathogenic bacteria are more common in food and drinking water, is a common factor in inducing gastroenteritis, the template is E. coli O157: H7, the functional monomer is dopamine, after electrochemical methods can be directly synthesized on the surface of the glass electrode polymer film of macromolecular imprinting, in the target bacterial imprinting film adsorption specificity is relatively high, and E. coli multi When combined with E. coli multi-clonal antibodies, the H7-pAb-N-GQDs complex is formed, and under K2S2O8 conditions, the target bacterial adsorption is increased with the help of electrochemiluminescence, and the luminescence intensity is increased, and the range of E. coli O157:H7 electrochemiluminescence detection can even reach 101~107 CFU/mL [6].

2.3 Resonant light scattering detection method

Resonant light scattering detection method is mainly with the help of scattering frequency, electron absorption electromagnetic wave frequency, to promote electronic resonance, thereby enhancing the absorption of light energy, resulting in scattering, this detection method is easy to operate, fast detection speed, high sensitivity, can effectively detect hepatitis A virus and other food-borne viruses and hazards residue detection, through the resonant light scattering detection method can effectively stop the occurrence of large-scale disease [7].

2.4 Surface isochronous resonance detection

Surface isochronous resonance detection is a detection technique formed by plasma-free, non-destructive formation on the surface of a metal dielectric waveguide. This detection technique requires only a small number of samples to complete the detection, which is not only easy and fast to operate, highly sensitive, and also enables real-time monitoring [8]. For example, Enterococcus faecalis belongs to the genus Enterococcus, which is a common faecal contaminant in food and the main pathogen causing zoonotic bacteria, and the surface plasmon resonance detection can reach a detection limit of 0.57 CFU/mL in E. coli, with effective detection of Enterococcus faecalis.

Conclusion

The global food industry is faced with the problem of food contamination and spoilage, which is also a key concern for food supply assurance and food quality and safety management in all countries. According to relevant statistics, the number of people suffering from food-borne diseases reached 600 million each year worldwide, and the number of deaths from food-borne diseases reached 42 people each year. 25% of food losses and microbial spoilage are closely related. Bacterial contamination of food not only causes huge economic losses to producers, but also has a negative impact on the social environment. Molecular blotting is a synthetic material technology with specific identification properties that can efficiently identify microorganisms and biomolecules and detect microbial contamination in food in a timely manner, which can prevent food waste and foodborne disease outbreaks, and is of great value in safeguarding human health.

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