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Hygiene Monitoring per Chip

Detecting Human- and Phytopathogens during Postharvest Vegetable Processing

Fresh produce contaminated with human- and phytopathogenic microorganisms may result in high losses during storage and pose a health risk. Testing microbiological contamination levels is time consuming and for this reason cannot be routinely done during fresh vegetable processing. The development of an on-chip-system based on molecular and cytometric technologies provides an opportunity to adapt the processing steps effectively to the grade of microbial contamination.

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Keywords

PCR, flow cytometry, food, vegetables, pathogens

After harvest agricultural products like vegetables and fruits are more or less contaminated with attached soil and therefore with a changing number of microorganisms [1]. This microbial contamination of vegetables and salad can result in severe food poisoning [2].

Microbial load of vegetable washing water

Several time and money consuming cleaning steps may be necessary during the processing of the produce. This results in a conflict between the economic input for cleaning and the consumer's wish for less processed food on one side and the optimal microbiological hygiene on the other side. Recent experiments conducted at the Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V. (ATB), have shown a load of 10^6 colony forming units per ml washing water (cfu/ml) during processing of carrots. For the most part Gram-negative bacteria were detected. In addition, high numbers of microorganisms (10^7 cfu/ml) were detected in the washing water of a spinach processing plant.

A molecular genetic analysis of the microbial diversity in the washing water samples showed that approx. 43 % of all detected DNA-sequences can be affiliated with potential pathogens [3], e.g. of the genus *Pectobacterium, Clostridium* and *Pseudomonas*. Some species of *Pectobacterium*, e.g. cause rot of fruits and vegetables, while the genera *Pseudomonas* and *Clostridium* include a variety of human- and phytopathogenic species.

Development of an on-chip system

As part of the research network ProSenso.net 2, supported by the Federal Ministry of Education and Research, solutions for an efficient monitoring of the hygienic status shall be developed, which allows the specific detection of human and phytopathogenic microorganisms. With such a monitoring system, processing steps following the cleaning can be adapted efficiently to the degree of contamination by pathogens.

At the ATB highly specific cellular and molecular techniques are developed and adapted for the rapid identification of pathogens. These methods will be the basis of a miniaturised on-chip system (*Fig. 1*). The on-chip system will be designed in close cooperation with the Anwenderzentrum für Mikrotechnik (AZM) located at the Berliner Elektronenspeicherring-Gesellschaft für Synchrotronstrahlung m.b.H. (BESSY) and the private company "ELBAU GmbH Berlin“ as industrial partner.

Sampling and sample analysis in the automated system

In the processing plant it will be essential to obtain a result before the vegetables reach the packaging area, so that further processing can be adapted if necessary. Essential for

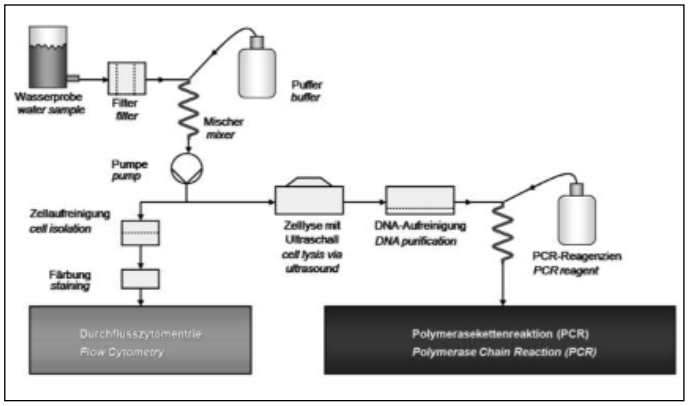

Fig. 1: Scheme of the on-chip system for the detection of pathogens in water used to wash vegetables during postharvest processing

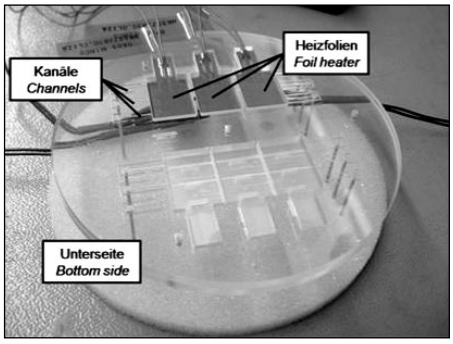

Fig. 2: Preliminary model of the miniaturised PCR unit of the on-chip system

the effective detection of pathogens is that the projected on-chip system is able to fully automatically take samples, and analyse them rapidly and in parallel to the washing process.

After initial treatment of the samples the relevant cell components of the microorganisms will be led to a miniaturised unit (*Fig. 2*) for polymerase chain reaction (PCR), which enables a specific examination of the samples for potential pathogens.

In case of a high bacterial load of the washing water there will be the possibility of adding further washing steps before packaging of the produce. This assures that only hygienically clean products will leave the plants.

Molecular detection of pathogenic microorganisms

Microorganisms often exhibit only a small number of characteristics which help to distinguish pathogenic and non-pathogenic strains. The genetic information of microorganisms is highly species-specific. Its analysis allows the development of species specific detection methods, e.g. by PCR [4, 5]. As essential requirement for any PCR based assay, molecular markers are developed and tested at the ATB for their specificity to *Pectobacterium* and other contaminants (*Fig. 3*).

A sophisticated modification of the PCR is the quantitative real-time PCR (Q-PCR). This method allows both the detection and the quantification of an individual DNA. Q-PCR is based on oligonucleotide primers which are coupled to fluorescent dyes. Thus, the entire PCR process can be monitored by the fluorescence emitted during DNA detection and amplification.

The time it takes until the fluorescence reaches a certain threshold defines the original amount of DNA in the sample [6]. This technique is currently adapted and tested for the detection of several microbial pathogens in washing water.

Characterisation of cells by flow cytometry

By Q-PCR it is not possible to distinguish between living and dead pathogens. Therefore, additionally the usability of flow cytometry for the microbial analysis of washing water is tested.

Flow cytometry or continuous fluorescent microscopy provides an opportunity to measure morphologic and physiologic characteristics of single cells with the help of fluorescent dyes [7]. According to the used fluorescent dyes it is possible to differentiate between living, damaged and dead bacteria. The advantage of the flow cytometry over traditional microbiological methods lies in the saved time and in the detection of unculturable bacteria, at the ATB protocols are currently developed for the optimised detection of pathogens.

Ultimate goal is to provide an analytic facility which enables to verify the success of the industrial hygienic schemes.

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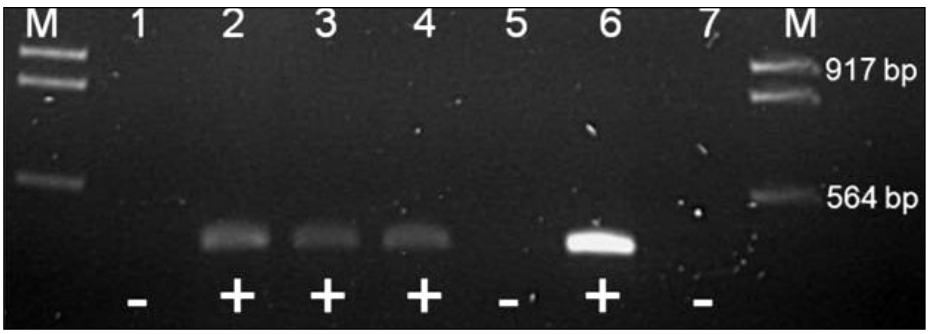

Fig. 3: PCR-based detection of Pectobacterium sp. in spinach washing water. Expected amplicon size was approx. 560 bp. Drinking water (1), washing water 1 (2), washing water 2 (3), washing water 3 (4), blanching water (5), positive control (6), negative control (7)