

# Contamination Control of Agricultural Products by On-Chip PCR and Flow Cytometry

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Food and Agriculture

## Summary:

**A growing demand for quality standards for foodstuffs and the avoidance of commercial damage require innovative concepts for fast, reliable and cost-effective in situ analysis for the detection of pathogenic microorganisms. An approach for an on-line detection of microbial contamination in water using to wash of agricultural products by means of an on-chip PCR and flow cytometry shall be presented.**

## Abstract:

According to the marketing standard for fruit and vegetables the produce must be “clean and practically free of any visible foreign matter”, among other things. Hence, much produce needs to be carefully washed to fulfill these claims. In general, washing helps to reduce the product temperature and to decrease their microbial load. On the other hand, the microorganisms suspended in washing water are a potential source for produce contamination and therefore a fast detection of the relevant bacterial load can be a helpful tool with respect to safety and quality aspects and controlled processing. Here, microtechnology's many advantages for developing high-end technologies and manufacturing low-cost, highly automated, robust and mobile detection systems with a high level of integration for fast and reliable in-situ analysis can be applied.

Essential for the development of marker-based detection systems is the knowledge of the presence and diversity of microbial contaminants within process water. Therefore, samples were taken from representative commercial vegetable-processing. Out of the pooled samples, total microbial DNA was purified and a 16S rDNA library was constructed. 430 clones of this library were analyzed by amplified rDNA restriction analysis (ARDRA). For the 80 individual ARDRA fingerprints indicating distinct groups of micro-organisms, the 16S rDNA nucleotide sequence was determined and assigned to corresponding reference species for taxonomic classification. First results indicate the presence of a broad range of members of Epsilon- and Gammaproteobacteria with close relationship to certain human- and phytopathogens like *Pectobacterium* sp.

Based on the results of the molecular diversity analysis, a group- or species-specific PCR assay shall be developed. Initial tests are conducted to develop PCR assays based on the detection of known pathogen factors of the plant pathogen *Pectobacterium* sp. and to design a lab-on-a-chip-system for a fast and reliable detection by means of real time PCR. In a parallel approach, the quantitative detection of *Pectobacterium* sp. with flow cytometry was established as a reference for the marker-based detection system. Depending on the dyes used, it is possible to differentiate between viable, non-viable and viable-but-not-cultural bacteria in a continuous system.

The combination of both detection systems will give quantitative information about the microbial contamination and allow special produce treatment after washing steps if necessary.