DEVELOPMENT OF MICROARRAY BASED DETECTION OF SUGAR BEET COLONIZING MICROORGANISMS

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ABSTRACT

Growers and sugar factories have to face new challenges because the sugar beet campaign is steadily increasing resulting in expanded storage duration. During storage, sugar beets are subjected to various degrading microorganisms. The resulting root rot is not only responsible for direct sugar losses due to microbial respiration but also negatively interferes with factory processing by accumulating impurities. Juice impurities and the accumulation of invert sugars reduce the technical quality of the beets, thus resulting in an economically important reduction of the potential white sugar yield. Up to know, there is not much known about the microorganisms causing root rots during storage. The exact diagnosis of the causing microorganisms is crucial for the development of management strategies like selection assays for sugar beet breeding. However, species identification by classical *in vitro* isolation is time-consuming and error-prone. Moreover, primary pathogens or wound parasites often cannot be isolated because of the vast number of decomposers additionally colonizing the damaged beets as secondary invaders. Therefore, a microarray based detection assay was developed to allow the specific detection of many microorganisms known to cause root rots in sugar beet.

A microarray in a 2 ml reaction tube (ALERE Technologies) with 225 probes was supplied with oligonucleotides specific to 33 microorganism species known to cause sugar beet root rot. Candidate species belong to the group of pathogens (i.a. *Rhizoctonia solani*, *Helicobasidium purpureum, Phoma betae*), wound pathogens (i.a. *Fusarium* spp.), decomposers (i.a. *Aspergillus* spp., *Penicillium* spp.) and bacteria (i.a. *Pectobacterium betavasculorum*, *Rhanella aquatilis*). For species discrimination, three different genes (internal trancribed spacer, *elongation factor 1 alpha*, *16S rRNA gene*) were used to develop species specific probes. Functionality and specificity of each probe was tested by using pure cultures of the target organisms as well as artificially inoculated sugar beet slices. Furthermore, beets from growers showing severe root rot symptoms were investigated by classical detection methods (isolation, PCR) and by hybridization on microarray. Experimental parameters were optimized allowing specific detection of all targets.

All 33 species were reproducibly detected in pure culture and in artificially inoculated sugar beet tissue as well. Furthermore, the detection assay allows the discrimination of single nucleotide polymorphisms which makes it suitable for differentiation within the genus *Fusarium*. Microorganisms detected in field beets by classical methods were also detected by hybridization on microarray. Interestingly, the results indicate that there is large number of different microorganisms species, especially *Fusarium* spp., present in rotten sugar beet tissue. Finally, the detection of pathogens by means of hybridization on microarray has been proven to be highly sensitive and suitable for routine diagnosis of root rot diseases in sugar beets.