1. Introduction

1.1 Storage of sugar beets

1.1.1 General importance

In North-West Europe, harvest of sugar beets usually starts in September and is finished in mid- or late November when environmental conditions become unfavorable for harvest (Legrand et al., 2012). The high yield and the relatively narrow time frame for harvest result in large amounts of sugar beets exceeding the processing capacity of sugar factories. Thus, sugar beets that are not immediately processed must be stored until they are delivered to the factory. The time period from the first harvesting date to the last processing date determines the length of a sugar beet campaign.

Before the reform of the European sugar market in 2006, most factories finished processing before the end of the year; therefore, beets were stored on average for only 30 days (Legrand et al., 2012). Within the framework of the Council Regulation No 318/2006, the European sugar market has been profoundly changed including cutting raw and white sugar prices as well as the intervention price (EU, 2006). Since then, sugar companies need to achieve a higher profitability by decreasing their production costs. This also led to the closure of 83 European factories (between 2005 and 2011) with the effect of longer processing campaigns. In 2011, sugar beet campaigns in Europe on average lasted already 132 days (Legrand et al., 2012). Within these campaigns, sugar beets were stored for 60 days on average, and in some countries even for up to 100 days (United Kingdom). In North-East and West Germany, sugar factories kept up to 23% of harvested beets in storage by the turn of the year in 2014 (Anonymous, 2015).

On the one hand, the extension of the sugar beet campaign helps factories to reduce their fixed costs, but on the other hand growers and sugar factories have to face increasing storage losses. In Germany, 22 million tons of sugar beets are harvested every year (Anonymous, 2011) and approximately 50% of them are subjected to storage before processing in the factory.

Considering an average sucrose concentration of 17.5% and processing losses of 2%, approximately 1.7 million tons of sucrose are stored every year. Assuming a daily reduction of sucrose by 0.1% (Jaggard et al., 1997), 1,550 t sucrose are lost every day with a monetary value of 627,000 € (EU reference price white sugar: 404,4 € t^{-1}). This highlights the economic importance of storage losses for sugar production, particularly in the context of longer storage periods as well as higher quantities of stored beets in the future.

1.1.2 Quality of sugar beets

The recovery of sucrose from the sugar beet root is mainly determined by its quality which is defined through a variety of parameters like sucrose concentration, non-sucrose substances, resilience, resistance to cutting and soil tare (Dutton and Huijbregts, 2006). Although the sum of these parameters makes up root quality, the concentration of sucrose and non-sucrose compounds mainly determine the economy of sugar recovery. The root constitutes a complex chemical matrix containing a large variety of non-sucrose compounds, but only some of them are of particular importance for stored sugar beets. Amino N, potassium and sodium which are also known as melassigenic substances increase the sucrose loss to molasses. These substances mainly determine the processing quality of freshly harvested sugar beets and their concentration is only little affected by storage except for amino N which can increase substantially (Kenter and Hoffmann, 2009). Considering only these substances for quality assessment of stored sugar beets would lead to inaccurate results. Other non-sucrose compounds with a more severe effect on the processing quality accumulate particularly during storage (Kenter and Hoffmann, 2009). Invert sugar promote color formation and interfere with sugar crystallization and filtration. A high concentration must be controlled during the extraction process by the addition of alkalizing agents leading to a higher energy demand and processing costs (Hein et al., 2012). Since the major invert sugar glucose and fructose are products of the enzymatic hydrolysis of sucrose, their accumulation is also associated with a

direct loss of sucrose. Other non-sucrose compounds of minor importance are trisaccharides like raffinose which impair the polarimetric measurement of sucrose and the crystallization process (Haagenson et al., 2008). Furthermore, the accumulation of cell wall components (e.g. pectin) during storage can decrease the filterability of juices when they become soluble (Hein et al., 2012). Additionally, certain polysaccharides (e.g. dextran and levan) which are exclusively of microbial origin can accumulate in frost damaged beets after thawing. These high molecular compounds decrease the filterability of juice, and therefore must be degraded by the application of dextranases (Bowler and Wones, 2011).

1.1.3 Storage conditions

The major aim of sugar beet storage is to provide optimal conditions for minimization of sucrose losses and accumulation of non-sucrose compounds. In contrast to other crops (e.g. apple, potato), sugar beets are mostly stored in large clamps (Figure 1A) next to the field rather than under controlled conditions in storage halls. During this time, sugar beets are exposed to various abiotic factors which can impair the quality.

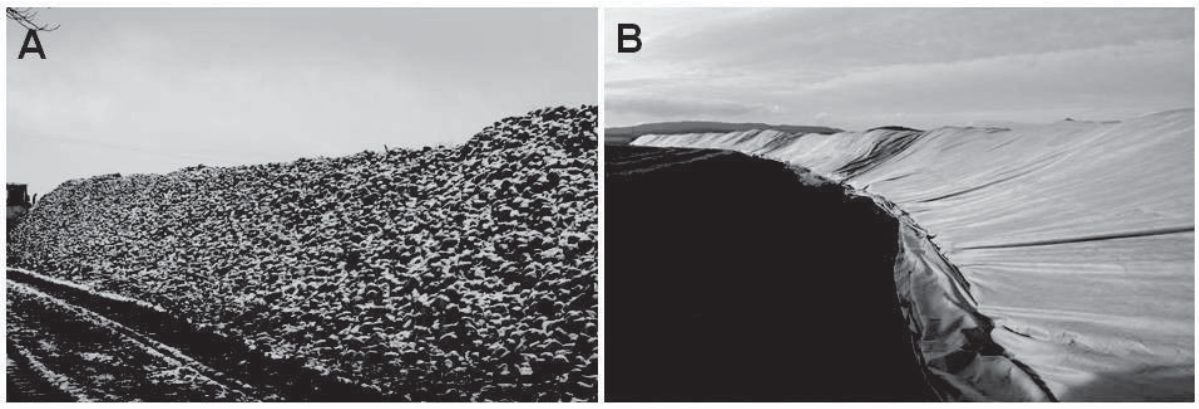

Fig. 1: Typical sugar beet clamps in Germany before (A) and after covering (B) with fleece material (Photograph: Institute of Sugar Beet Research).

Intensive sun and wind exposure increase sucrose and weight loss due to elevated dehydration (Lafta and Fugate, 2009). According to Campbell and Klotz (2006b), longest storability can be expected when sugar beets are stored just above the freezing point within the range of 1.5 to 5°C. Since beets are produced worldwide comprising different climatic areas (e.g. China,

France, Italy, Russia, United States), storage conditions are extremely variable with strong implications for post-harvest handling. For example, storage in Mediterranean countries like Italy with high temperatures after harvest is not feasible and restricted to a few days (Vaccari et al., 1991). Similar conditions also predominate in California where sugar beets are immediately processed after harvest without any storage period. In contrast, sugar beet producers in North-West European countries have to face temperatures below the freezing point which must be avoided as it leads to cell rupture accompanied by the accumulation of microbial polysaccharides (Bowler and Wones, 2011). This constitutes a serious problem as the extension of storage periods in Europe till the end of February has dramatically raised the risk of frost damage. Statistically, sugar beets stored in North-West European countries are exposed to frost events on 25 to 30 days during December (Legrand et al., 2012). Additionally, large fluctuation in day and night temperature can cause freezing and thawing of beets which accelerates microbial degradation. Therefore, clamps are usually covered in December with fleece material (Figure 1B) to avoid freezing of sugar beets. In field trials, covering reduced daily losses to 56 g sucrose per ton beet compared to uncovered clamps with 275 g sucrose (Huijbregts et al., 2013). Additionally, covering also protects beets from rain, sun and wind exposure. In North Dakota (North America), the very low ambient air temperature (up to -25°C) is utilized in complex ventilation systems to keep beets frozen (-20°C) throughout the whole storage period (Backer et al., 1979).

Despite all covering strategies, the microclimate within a clamp remains highly variable and difficult to control. For example, Jaggard et al. (1997) reported that in some clamps the temperature nearest the surface were coolest and similar to the air temperature, whereas in other clamps the lowest temperature was measured at the deepest point in the clamp. The authors also observed 1% greater sucrose concentration at the bottom than near the top of the clamp. Furthermore, the daily reduction of sucrose concentration was shown to be highly variable depending on the clamp (ranging between 0 to 0.03%). Therefore, tools for real time

assessment of storage conditions within a clamp would be helpful in order to determine the time point when conditions become unfavorable and sugar beets should be processed immediately.

1.2 Variability and sources of post-harvest storage losses

1.2.1 Variability

As already indicated in the previous chapter, the storage temperature is one key factor determining the long-term storability of sugar beets. Wyse (1978b) demonstrated large variation in sucrose losses under controlled conditions (ranging between 43 and 91 kg per ton) depending on the temperature regime (-1 to 10°C). Besides storage conditions, different authors described genotypic effects on storability (Akeson and Widner, 1981; Cole, 1977; Van Swaaij and Huijbregts, 2010). Large differences in sucrose losses (30% and 60%) between two modern sugar beet hybrids were also observed by Kenter and Hoffman (2009) after storage for 110 days at 20°C. The invert sugar content displayed a similar response with a range between 500 and 1500 mmol kg^{-1} DM. The genotype effect was further studied by Campbell and Klotz (2007) including different environments of cultivation. Significant variations in the extractable sucrose concentration were observed after storage for 120 days at 5°C depending on the environment (ranging between 99 and 122 kg t^{-1} root). Within the same environment, the extractable sucrose content of five different genotypes ranged between 92 and 135 kg t^{-1} root. The author stated that relatively small genotype effects were superimposed by large environmental effects and interactions between both factors. Understanding environmental effects on sugar beet storability is crucial but rather difficult as a large number of agronomical factors need to be considered. However, mechanical damage during harvest was identified very early as one important factor impairing the storability of sugar beets (Cole, 1977; Wyse, 1978a). During storage for 21 days at 20°C, the daily sucrose reduction of mechanically damaged beets amounted 0.5%, whereas untreated beets lost only

0.1% (Kenter et al., 2006). Another study showed that the topping method affects storability as well with highest sucrose losses for badly topped beets followed by defoliated and topped ones (Hoffmann, 2012). Furthermore, sugar beets which were exposed to drought stress during the growing season displayed a poor quality after storage for 25 days at 10°C and 25°C (Kenter and Hoffmann, 2008).

Considering the aforementioned studies, the storability of sugar beets is characterized by a strong variability at least attributed to effects of environment, genotype, mechanical damage and storage temperature. A clear understanding of these effects is crucial for the improvement of sugar beet storability. In this context, it is necessary to deal with the metabolic processes responsible for the degradation of sucrose and the accumulation of non-sucrose substances.

1.2.2 Plant endogenous processes

The sugar beet is a biennial species with vegetative growth in the first year and generative growth in the second year. The root developed during the vegetative stage is a storage organ and as such constitutes an energy reservoir for the plant to initiate the reproductive phase after winter. Consequently, the sugar beet root has to maintain its physiological activity to prevent destruction during the resting phase. Thus, post-harvest losses occur from the first day after harvest and cannot be completely prevented. Energy is not only required for the maintenance of cellular processes but also for wound healing. Topping and mechanical harvesting creates severe injuries which need to be repaired to prevent leaching of sucrose and water as well as microbial colonization. Wound healing is a complex process comprising the formation of brown deposits (probably melanin), lignin-like substances and suberin (Ibrahiml et al., 2001). Furthermore, Rotthues et al. (2008) demonstrated that storage is also associated with transcriptional changes including the up-regulation of genes involved in wound response, pathogen defense, dehydration stress, and detoxification of reactive oxygen species. Another

reason for elevated energy demand occurs when defoliated or badly topped beets start sprouting during storage (Kenter et al., 2006).

Since stored beets are not photosynthetically active, energy and substrates required for plant endogenous processes must be mobilized from the storage organ. In all living plants, at the cellular level, sucrose is the key carbon source for growth, development, and defense (Ruan, 2014 and references therein). As the primary substrate for cellular respiration in sugar beet (Barbour and Wang, 1961), sucrose must be cleaved into glucose and fructose prior to glycolysis. This step is catalyzed by two different sucrolytic enzymes, namely sucrose synthases and invertases. SBSS1 (Hesse and Wilmitzer, 1996) and SBSS2 (Haagenson et al., 2006) belong to sucrose synthases which catalyze the reversible cleavage of sucrose into UDP-glucose and D-fructose-6-phosphate. In contrast, the hydrolytic cleavage of sucrose by invertases is an irreversible conversion. Depending on their localization, invertases found in sugar beets are divided into the three classes: vacuolar (vacuole), extracellular (apoplast) and neutral invertases (cytoplasm) (González and Cejudo, 2007; Rosenkranz et al., 2001). Generally, invertases are key enzymes in all plants and involved in various processes including control of sugar composition and partitioning as well as response to drought stress, pathogen infection and wounding (Roitsch and González, 2004). In sugar beet, invertases have been shown to be associated with wound-induced sucrose losses as they provide hexoses for wound-induced cellular metabolism (Rosenkranz et al., 2001). Furthermore, several studies demonstrated the activity of both sucrose synthases and invertases during storage (Klotz and Finger, 2004; Lafta and Fugate, 2009). Sucrose can also be utilized directly in other metabolic pathways like the synthesis of raffinose, particularly during cold storage temperatures (Haagenson et al., 2008). Besides reduction of sucrose, the activity of the aforementioned enzymes is also associated with the accumulation of invert sugar. It is supposed that endogenous sugar beet enzymes are the main source for invert sugar

accumulation in healthy roots being induced when sucrolytic activity exceeds the demand for respiration and other substrates (Campbell and Klotz, 2006b).

The respiration rate of sugar beets, measured as carbon dioxide emission, is regarded as an indicator for the physiological activity and was therefore determined in several studies (Akeson and Stout, 1978; Campbell and Klotz, 2007; Cole, 1977; Hoffmann, 2012; Kenter and Hoffmann, 2009; Lafta and Fugate, 2009; Wyse, 1978a). Typically, highest respiration rates occur after harvest due to wound healing processes followed by a continuously decrease during long-term storage. Furthermore, the level of respiration losses is affected by environment, genotype, mechanical damage, storage conditions and topping method. Wyse and Dexter (1971) estimated that approximately 80% of sucrose losses during storage is attributed to plant respiration whereas the other 20% account for sucrose transformation into invert sugar and raffinose. However, a pitfall of respiration measurement is the contribution of microbial derived carbon dioxide, making a clear separation from plant induced sugar losses and invert sugar accumulation impossible. Thus, it is not a reliable indicator for the physioloigical activity of sugar beets during storage.

1.2.3 Storage rots

Post-harvest decay by storage pathogens is a well-known issue in many stored harvest products like apple (Konstantinou et al., 2011), banana (Lassois et al., 2010), blueberry (Mehra et al., 2013), cranberry (Oudemans et al., 1998; Stiles and Oudemans, 1999) and potato (Estrada et al., 2010; Peters et al., 2008). The high sugar and water content of sugar beets as well as the presence of severe injuries after harvest provide ideal conditions for post-harvest microbial growth. Thus, apparently healthy harvested sugar beets can become deteriorated after storage (Campbell and Klotz, 2007; Christ et al., 2011b). Post-harvest microbial colonization can be recognized visually on the root surface by the presence of fungal mycelia (Figure 2). An extensive review on the current knowledge about storage rots

and their impact on the storability of sugar beets is provided in manuscript I (Liebe and Varrelmann, 2014). Therefore, this section focuses only on issues relevant to the research questions of the present thesis.

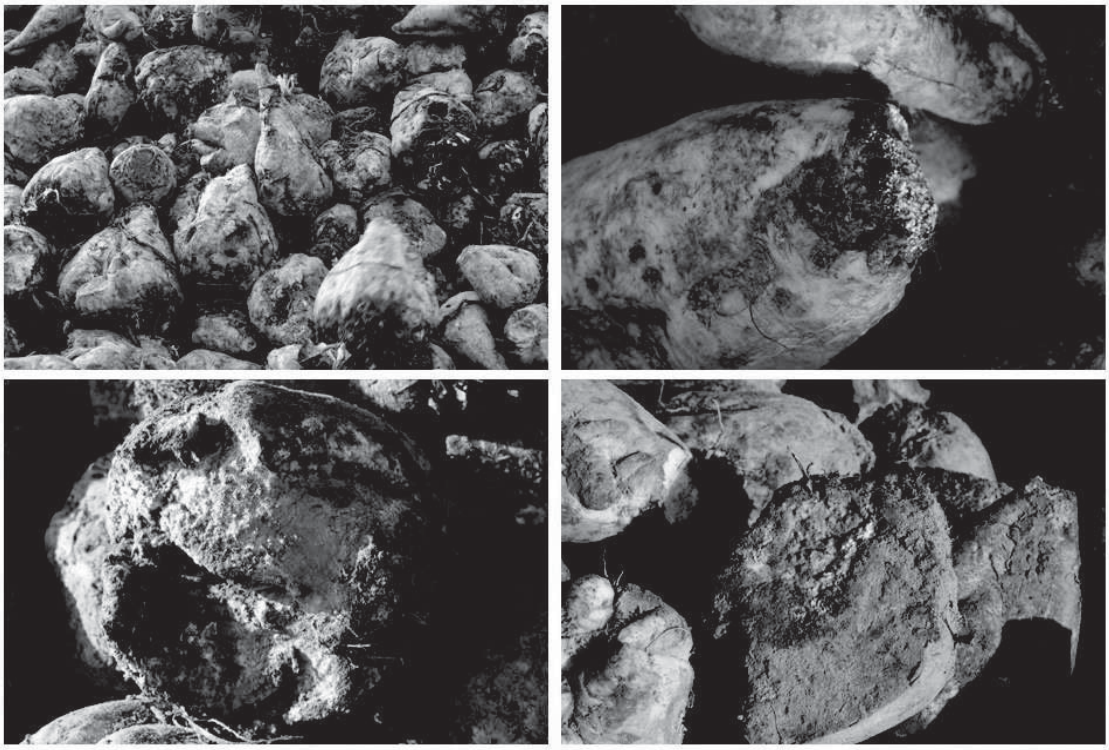

Fig. 2: Post-harvest colonization of sugar beets during clamp storage indicated by the presence of fungal mycelia on the root surface (Photos: Institute of Sugar Beet Research).

The economic impact of post-harvest microbial colonization for sugar factories was demonstrated by Bugbee and Cole (1976) who quantified the amount of storage rots in a survey conducted during a sugar beet campaign. Taking into account both direct sucrose losses during storage and losses due to processing problems in the factory, it was estimated that 1315 tons of sucrose were lost due to storage rots in a single factory. In infection studies, Mumford and Wyse (1976) obtained a correlation of 0.93 between the rotten surface area and the respiration rate of sugar beet roots. Apart from that, a sharp increase in invert sugar content was measured. Invertases cleaving sucrose into glucose and fructose are important sucrolytic enzymes for nutrient acquisition of microorganisms. Parrent et al. (2009) identified invertase genes in genomes of several spoilage fungi like *Aspergillus* spp., *Botrytis cinerea* and *Fusarium* spp.. Moreover, Klotz and Finger (2004) demonstrated the activity of a fungal

invertase from *Botrytis cinerea* in stored sugar beets which caused a decline of sucrose and accumulation of invert sugar. Similar observations have been made for other pathosystems like *Fusarium graminearum*/wheat (Guenther et al., 2009) and *Sclerotinia sclerotiorum*/sunflower (Jobic et al., 2007). Thus, the influence of microbial colonization must be taken into account for understanding post-harvest changes in sugar beet quality.

The microbial colonization of stored sugar beets under controlled storage conditions was studied very early by Karnik (1970) in the US. He reported that *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. were predominant in the early phase of storage but displaced by *Botrytis* sp. and *Fusarium* sp. during long-term storage. Bugbee and Cole (1976) predominantly isolated *Botrytis cinerea*, *Fusarium* spp., *Penicillium vulpinum* (former *P. claviforme*) and *Phoma betae* from different deteriorated root parts including pith, crown, body and tail. However, both studies lacked complete taxonomic classification, particularly for species-rich genera like *Fusarium*. Only the genera *Aspergillus* and *Penicillium* were further studied by identifying the species *A. fumigates*, *P. cyclopium*, *P. funiculosum*, *P. variabile* (Bugbee, 1975; Bugbee and Nielsen, 1978; Halloin and Roberts, 1995). Bosch and Mirocha (1992) were the first authors who focused entirely on *Fusarium* spp. and could identify nine different species in deteriorated sugar beets collected from clamps. The composition and dynamics of the *Fusarium* mycoflora was further addressed in a more comprehensive study conducted under controlled conditions. By testing a total of 4280 roots for infections with *Fusarium* spp., Christ et al. (2011b) elucidated a clear species shift from *F. redolens* to *F. culmorum*, *F. cerealis*, and *F. graminearum* during storage. The investigations did not only include deteriorated but also healthy roots and thus highlighted for the first time that all sugar beets are exposed to an extensive post-harvest microbial colonization. However, the microbial community was only partially addressed and thus has yet to be completely investigated. Moreover, the results raise further questions concerning the association between species

composition and storage rot symptoms as well as the effect of environment, genotype and storage temperature on microbial colonization.

As already indicated in the previous chapters, the storability of sugar beets has been extensively addressed in many studies before. Although different authors mentioned storage rots as another source for sucrose reduction and invert sugar accumulation in particular for heavily damaged beets (Akeson and Stout, 1978; Hoffmann, 2012; Van Swaaij and Huijbregts, 2010), a precise quantification of rot severity is lacking in most studies. A genetic resistance against storage pathogens could be demonstrated in storage trials conducted under controlled conditions (Gaskill, 1950) as well in inoculation studies with different pathogen species (Bugbee, 1979a; Bugbee, 1979b). Furthermore, the development of germplasms with resistance to three major fungal storage pathogens (*P. betae*, *B. cinerea*, *P. claviforme*) has been reported many years ago (Bugbee, 1978). However, further attempts to improve or to understand the storage rot resistance have not been undertaken yet. It is not known whether genetic resistance against storage pathogens is still present in modern sugar beet hybrids since it was not a trait for plant breeders in the past. In addition to genotypic variability, Bugbee and Cole (1986) reported that the amount of storage rots was also influenced by the growing location within a research plot of 4,500 m^2. This evidence for an environmental effect was corroborated by observations of Campbell and Klotz (2007) who reported severe storage rot symptoms for sugar beets from certain sites of cultivation. However, systematic storage trials conducted under controlled conditions providing experimental evidence for an environmental effect on storage rot development are lacking. This is an important aspect as environmental effects on storability would have major implications on storage management as well as on breeding storage rot resistant genotypes.

1.3 Multiplex detection of microorganisms

A major challenge during studying post-harvest microbial colonization of sugar beet is the vast species diversity which needs to be considered. For this purpose, multiplex detection methods, allowing the simultaneous identification of many different microorganism species, are required. A roughly classification of these methods is based on the differentiation between culture-dependent and culture-independent methods. Both approaches comprise techniques which became widely adopted in routine diagnosis of plant diseases as well as in studies on microbial communities. This section summarizes a selection of traditional and newly emerging techniques with an emphasis on molecular assays utilizing nucleic acids for simultaneous species identification.

1.3.1 *In vitro* isolation

In vitro isolation was the first method used to demonstrate that sugar beets are exposed to an intensive post-harvest microbial colonization (Bugbee and Cole 1976; Bosch and Mirocha, 1992; Christ et al., 2011b). As a traditional culture dependent approach, it relies on the isolation of microorganisms using a variety of different culture media, often supplemented with antibiotics or fungicides to prevent the growth of undesirable microorganisms. It is an essential method when it is aimed to characterize isolates, e.g. for their pathogenicity or mycotoxin production (Christ et al., 2011a, 2011b; Strausbaugh and Gillen, 2008). Moreover, it constitutes a key method to fulfill Koch's postulates on plant diseases. By using this approach, it could be shown that isolates of *Fusarium* spp. obtained from stored sugar beets are able to induce rot symptoms after wound inoculation (Christ and Varrelmann, 2011). However, each microbial species has its own ideal growth conditions, and therefore different culture media and incubation conditions are required. In addition, several steps of sub-cultivation and purification are required to obtain single spore/cell isolates for taxonomic classification, either by microscopy or sequencing of marker genes. Thus, *in vitro* isolation is

a labor-intensive and time-consuming procedure with a low throughput. Another problem is unculturability of microorganisms resulting from (i) a lack of knowledge about specific growth requirements (e.g. nutrition, temperature), (ii) fast overgrow by highly competitive species, and (iii) cell death during the stressful extraction and cultivation process (Vaz-Moreira et al., 2011). Nowadays, *in vitro* isolation has hardly any significance in comprehensive studies aiming to describe the whole diversity of a community. Nevertheless, it is still of great importance and often used in conjunction with molecular techniques to study the biological properties of microorganisms *ex situ* (Ellis et al., 2003; He et al., 2008; Kisand and Wikner et al., 2003).

1.3.2 Polymerase chain reaction (PCR)

Detection assays based on PCR are cost effective and rapidly established molecular tools with great importance for phytopathologists in disease diagnosis (Mumford et al., 2006 and references therein). Classical end-point PCR can be extended for multiplexing through the combination of various primers amplifying polymorphic sites from different species in a single reaction. PCR products can then be separated and differentiated by their size on agarose gel electrophoresis. Using this method, Weiland and Sundsbak. (2000) developed a detection assay for important fungal and oomycetes pathogens on sugar beet, namely *Aphanomyces cochlioides, Cercospora beticola, F. oxysporum, P. betae, Pythium ultimum* and *Rhizoctonia solani*. Over the last two decades, PCR techniques have been constantly developed from end-point to real-time detection of amplified products (real-time PCR). Various chemistries for real time detection are used in disease diagnosis and can be roughly divided into amplicon sequence non-specific (SYBR Green I) and sequence-specific (TaqMan, molecular beacons, and scorpion-PCR) methods (Schena et al., 2004 and references therein). Similar to classical PCR, primers targeting different species can be used in a single reaction and amplified products are then differentiated by using distinct fluorophores. Such multiplex assays are

highly sensitive by detecting less than 100 fg of genomic DNA from their target organisms (Schena et al., 2006). A special feature of real-time PCR is the ability to quantify microbial DNA in complex environmental samples like harvest residues and plant tissue (Burlakoti et al., 2007; Hogg et al., 2010; Köhl et al., 2007). Quantification also enables the differentiation of resistant and susceptible genotypes as shown by De Coninck et al. (2012) for *C. beticola*. However, the establishment of a PCR-based multiplex detection assay is technically challenging (Henegariu et al. 1997) and the number of targets is limited by strong primer interactions (Wei et al. 2008) and available fluorophores. For this reason, PCR-based detection is limited in its applicability on complex microbial communities, but it can be used as a supplemental method for studying single species in more detail by quantification of their biomass.

1.3.3 Clone library preparation and sequencing

Construction of a clone library is a traditional method in microbial community analysis which can be used to analyze heterogeneous PCR products from complex environmental samples. PCR products amplified from genetic markers are ligated into plasmid vectors and transformed into *Escherichia coli*. Each library clone carries an organism-specific PCR amplicon which is sequenced and identified through comparison with known sequences from public databases. A significant advantage of this method lies in the ability to sequence long fragments of marker genes which increase taxonomic identification on species level (Götz et al., 2006). Using this approach, bacterial and fungal endophyte communities from important agricultural crops (e.g. potate and rice) have been described (Götz et al., 2006; Sessitsch et al., 2002; Sun et al., 2008). The microbial community coverage depends heavily on the number of screened clones, and therefore incomplete sampling is a significant problem reported in previous studies. DeSantis et al. (2007) analyzed the bacterial community in air, soil and water by screening 417, 485 and 253 clones, respectively. Despite the great sampling effort,

none of the communities were completely covered. To avoid this problem, clones can be pre-analyzed by amplified ribosomal DNA restriction analysis (ARDRA) and only inserts displaying distinct patterns on agarose gel electrophoresis are subjected to sequencing (Sun et al., 2008). However, this is labor-intensive, time-consuming and becomes overwhelming when multiple replicated samples need to be analyzed. Clone library preparation was the "gold standard" in the past for obtaining the greatest species diversity (DeSantiset al., 2007) but has become less important since the advent of sequencing technologies that allow direct analysis of complex PCR products.

1.3.3 Luminex Technologies

Nucleic acid detection technologies from Luminex are bead-based assays consisting of microspheres (beads) filled with two different dyes. Each bead has a unique spectral signature defined by the mixture ratio of two dyes. Currently, 500 analytes can be analyzed per sample. Color-coded beads can be coated with oligonucleotide probes carrying either target-specific (xMAP technology) or universal tag sequences (xTAG technology). The xMAP technology relies on the hybridization between covalently bound oligonucleotide probes and target specific sequences in biotinylated PCR products (Figure 3). Bound PCR products are labeled with fluorochrome (phycoerythrin) using the high binding affinity of streptavidin to biotin. Finally, the signature of the bead and the fluorochrome present on its surface are analyzed by two separate lasers in a rapidly flowing fluid stream. Up to 100 different signatures can be detected in a single reaction vessel. A slightly different approach is applied by the xTAG technology. Here, PCR products are subjected either to a target-specific primer extension (TSPE) or allele-specific primer extension (ASPE) step coupled with the incorporation of biotin. The 5'-end of each specific primer carries a unique tag sequence complementary to the sequence of oligonucleotide probes present on the surface of the beads. The detection of bound PCR products and the analysis of beads follow the same method as described for the