Food Microbiology 39 (2014) 39–46

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Bacterial community dynamics during industrial malting, with an emphasis on lactic acid bacteria

A. Justé [a,b,c,d], S. Malfliet [c,d,e], M. Waud [a,b,c,d], S. Crauwels [a,b,c,d], L. De Cooman [c,d,e], G. Aerts [c,d,e], T.L. Marsh [f], S. Ruyters [a,b,c,d], K. Willems [a,b,c,d], P. Busschaert [a,b,c,d], B. Lievens [a,b,c,d,*]

[a] Laboratory for Process Microbial Ecology and Bioinspirational Management (PME&BIM), Department of Microbial and Molecular Systems (M^{2}S), KU Leuven, Campus De Nayer, B-2860 Sint-Katelijne-Waver, Belgium
[b] Scientia Terrae Research Institute, B-2860 Sint-Katelijne-Waver, Belgium
[c] Leuven Food Science and Nutrition Research Centre (LFoRCe), KU Leuven, Belgium
[d] Leuven Institute for Beer Research (LIBR), KU Leuven, Belgium
[e] Laboratory of Enzyme, Fermentation, and Brewing Technology (EFBT), M^{2}S, KU Leuven, KAHO Sint-Lieven Technology Campus, B-9000 Gent, Belgium
[f] Center for Microbial Ecology, Michigan State University, East Lansing, MI 48824, USA

A R T I C L E I N F O

Article history:
Received 8 February 2013
Received in revised form
9 October 2013
Accepted 30 October 2013
Available online 7 November 2013

Keywords:
454 pyrosequencing
Lactic acid bacteria (LAB)
Malt microflora
Microbial community analysis
Terminal restriction fragment length polymorphism (T-RFLP)

A B S T R A C T

Characterization of the microflora during malting is an essential step towards process management and optimization. Up till now, however, microbial characterization in the malting process has mostly been done using culture-dependent methods, probably leading to biased estimates of microbial diversity. The aim of this study was to characterize the bacterial communities using two culture-independent methods, including Terminal Restriction Fragment Length Polymorphism (T-RFLP) and 454 pyrosequencing, targeting the 16S rRNA gene. Studied samples originated from two harvest years and two malting houses malting the same batch of barley. Besides targeting the entire bacterial community (T-RFLP), emphasis was put on lactic acid bacteria (LAB) (T-RFLP and 454 pyrosequencing). The overall bacterial community richness was limited, but the community structure changed during the process. Zooming in on the LAB community using 454 pyrosequencing revealed a total of 47 species-level operational taxonomic units (OTUs). LAB diversity appeared relatively limited since 88% of the sequences were covered by the same five OTUs (representing members of *Weissella*, *Lactobacillus* and *Leuconostoc*) present in all samples investigated. Fluctuations in the relative abundances of the dominant LAB were observed with the process conditions. In addition, both the year of harvest and malting house influenced the LAB community structure.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Malting is a complex biological process involving many biochemical and physiological reactions, leading to the synthesis of hydrolytic enzymes and degradation of the grain structure. Technically, three steps are involved in malting: steeping, germination and kilning. After cleaning and calibration of the grain kernels (mainly barley), grains are submerged and aerated until a water content of 42–46% is reached (steeping). In general, water temperatures of 10–15 °C and steeping times of 24–48 h are used. The grains are then allowed to germinate under humid and aerobic conditions at 16–20 °C for 3–6 days, resulting in enzymatic breakdown of endosperm cell walls and proteins. Germination is ended by drying the grains (kilning) for approximately 21 h at temperatures increasing gradually from about 50 to 85 °C or more. Kilning stops the biochemical reactions and ensures stability and storability of the dried product. During this step, several colour and flavour compounds are produced, thereby influencing the characteristics of the final beer (Laitila et al., 2011). In addition to the germinating grains, a diverse microbial community (Flannigan, 2003; Laitila et al., 2006b; Laitila, 2007; Noots et al., 1998) represents a second metabolically active compound in the malting ecosystem. Microorganisms greatly affect malting performance and malt quality, and thus also beer quality. Depending on the nature and extent of the microorganisms present, their effects may be either beneficial or disadvantageous to the process and/or the final

* Corresponding author. Laboratory for Process Microbial Ecology and Bioinspirational Management, Fortsesteenweg 30A, B-2860 Sint-Katelijne-Waver, Belgium. Tel.: +32 15 305590; fax: +32 15 305099.
E-mail address: bart.lievens@kuleuven.be (B. Lievens).

0740-0020/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fm.2013.10.010

product (Boivin and Malanda, 1997; Flannigan, 2003; Laitila et al., 1997, 2007; Lowe and Arendt, 2004). Consequently, more insight into the microbial communities that are involved in malting may contribute to the improvement of malt characteristics and a safe malting process and beer (Laitila et al., 2011).

In general, the microbial load and composition of barley and malt have been determined using traditional microbiological methods based on plating, counting and identifying colonies (Justé et al., 2011). As these techniques rely on the culturability of the organisms, our view on the total microflora in the malting ecosystem is probably heavily biased and might be different from reality (Rappé and Giovannoni, 2003). Therefore, these classical approaches are increasingly being complemented or replaced by culture-independent, molecular methods (Justé et al., 2008). Fingerprinting techniques like Terminal Restriction Fragment Length Polymorphism (T-RFLP) and Denaturing Gradient Gel Electrophoresis (DGGE), often complemented with sequencing, have been widely used to describe the diversity and dynamics of microbial communities in all kind of ecosystems and habitats (Jany and Barbier, 2008; Justé et al., 2008; Ranjard et al., 2000; Tolvanen and Karp, 2011). More recently, technological advances such as 454 amplicon pyrosequencing have enabled rapid characterization of microbial communities at a greater sequence depth than was deemed possible via cloning and Sanger sequencing, enabling highly efficient in-depth microbial community analysis (Sogin et al., 2006). Surprisingly, with the exception of only a few studies using fingerprinting techniques (Laitila et al., 2007; Kaur, 2009), these modern techniques have not yet been used to investigate microbial communities during malting.

One important group in many food applications is the lactic acid bacteria (LAB), which have as a common metabolic property the production of lactic acid from the fermentation of carbohydrates (Carr et al., 2002). LAB are Gram positive, catalase-negative, non-sporulating, and acid tolerant bacteria that belong to the Firmicutes, including members of for example *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* and *Weissella* (Stiles and Holzapfel, 1997; Axelsson, 1998; Rouse et al., 2007). LAB are commonly exploited for the bio-preservation of various foods, feed and beverages (Rouse et al., 2007). In addition, LAB are used in the production of probiotic foods (Rathore et al., 2012). In the malting and brewing industry, LAB strains have been extensively used for several reasons (Lowe and Arendt, 2004). One example is the development and use of LAB starter cultures as inoculants during the malting process in order to improve the malt quality and safety (Boivin and Malanda, 1997; Haikara and Laitila, 1995). Biological control methods using LAB have shown high promise for the control of spoilage organisms or toxigenic fungi like fusaria, both in malting and in brewing (Dixon, 1959; Haikara et al., 1993; Haikara and Laitila, 1995; Laitila et al., 1997; Lowe and Arendt, 2004). Furthermore, certain LAB produce antimicrobial substances which restrict the growth of harmful bacteria that compete with grain tissue for dissolved oxygen and may also retard mash filtration (Lowe and Arendt, 2004; Van Campenhout, 2000). Also Laitila and co-workers demonstrated an enhanced malt processing potential after LAB addition to the steeping water (Laitila et al., 2006a; Raulio et al., 2009). Although the importance of LAB is highly recognized in the malting and brewing industry, so far most studies have focused on individual isolates (Booysen et al., 2002; Rouse et al., 2007), while complete LAB communities that are associated with barley and the malting process have not yet been studied in detail.

In this study, we investigated the structure and dynamics of the bacterial communities, and also more specifically the LAB communities, associated with industrial malting, i.e. from barley up till the final malt using T-RFLP. In addition, the endogenous LAB community was deeply characterized using 454 pyrosequencing of 16S ribosomal RNA genes. Study samples were obtained from two harvest years and two different malting houses exhibiting a different germination regime.

2. Material and methods

2.1. Study samples

Both in 2010 and 2011, barley and malt samples were obtained from an industrial malting of the barley variety Sebastian (French harvest). Samples were obtained from two different malting houses exploited by the same malting company, in which grains from the same barley batch were malted. These malting houses represented a system with isolated, closed germination rooms (further referred to as malting house "M1") and a system with open germination rooms in which simultaneously barley from other batches and/or varieties was germinated (further referred to as malting house "M2"). Samples were taken at different steps of the malting process, i.e. from barley, 1 day germinated barley, 5 days germinated barley (also called green malt), and the final kilned malt. For each step, multiple samples were randomly taken, pooled (resulting in a total of about 300 g) and transported to the laboratory for further processing.

2.2. DNA extraction

Ten randomly taken kernels of each pooled sample were soaked in Tris–HCl buffer (pH 8; 10 mM) in a 2 ml screw cap tube for 2 h at 4 °C to improve sample pulverization. Rootlets of germinating kernels were removed. Next, samples were mechanically disrupted by reciprocal shaking for 30 s after addition of 75 µl glass beads (212–300 µm) using a Fast Prep instrument (Thermo Savant, Holbrook, NY, USA). Subsequently, a subsample of 0.1 g from each pulverized sample was subjected to DNA extraction using the MoBio PowerSoil® DNA isolation kits (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's instructions with slight modifications as homogenization was performed with a Fast Prep instrument at maximum speed for four times 30 s. DNA extracts were stored at −20 °C until further use.

2.3. Terminal restriction fragment length polymorphism (T-RFLP)

Two primer sets targeting the 16S ribosomal RNA (rRNA) gene were used for T-RFLP analysis, including the universal bacterial primer set 516F (5′-TGCCAGCAGCCGCGGTA-3′; 5′ FAM-labelled) (Nagashima et al., 2003) and 1541R (5′-AAGGAGGTGATCCAGCC-3′) (McCaig et al., 2001), and the LAB specific primer set 7F (5′-AGAGTTTGATYMTGGCTCAG-3′; 5′ HEX-labelled) and 677R (5′-CACCGCTACACATGGAG-3′) (Heilig et al., 2002). Whereas 7F is non-specific, 677R has been specifically developed to target four important LAB genera, including *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Weissella* (Heilig et al., 2002). PCRs were performed on a Biorad T100 thermal cycler in a 20 µL reaction volume, containing 0.15 mM of each dNTP (Invitrogen, Merelbeke, Belgium), 0.5 µM of each primer, 1 unit Titanium *Taq* DNA polymerase, 1 × Titanium *Taq* PCR buffer (Clontech Laboratories, Inc., Palo Alto, CA, USA) and 1 µL genomic DNA. Samples were denatured at 94 °C for 2 min and then subjected to 30 cycles of 45 s at 94 °C, 45 s at 64 °C (universal primers) or 66 °C (LAB primers), and 45 s at 72 °C, with a final extension at 72 °C for 10 min. Subsequently, labelled PCR products (approximately 200 ng) were digested for 4 h at 37 °C with either *MspI* or *HinfI* (New England Biolabs, Frankfurt am Main, Germany). Restriction fragment analyses were conducted on an Applied Biosystems 373A Automated Sequencer (two technical replicates).

Download English Version:

https://daneshyari.com/en/article/6288731

Download Persian Version:

https://daneshyari.com/article/6288731

Daneshyari.com