International Journal of Food Microbiology 215 (2015) 71–78

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

The fungal community structure of barley malts from diverse geographical regions correlates with malt quality parameters

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ARTICLE INFO

Article history:
Received 12 March 2015
Received in revised form 3 August 2015
Accepted 24 August 2015
Available online 28 August 2015

Keywords:
Barley malt
TRFLP
Microbial community structure
Geographical location

ABSTRACT

Malt is a preferred base for fermentations that produce beer or whisky. Barley for malt is grown under diverse environments in different geographical locations. Malt provides an ecological niche for a varied range of microorganisms with both positive and negative effects on its quality for brewing. Little information exists in the literature on the microbial community structure of Australian malt as well as broader global geographical differences in the associated fungal and bacterial communities. The aims of the present study were to compare the bacterial and fungal community structures of Australian commercial malt with its international counterparts originating from different geographical regions using terminal restriction fragment length polymorphism (TRFLP) fingerprinting and clone library analyses of ribosomal RNA genes. Further, the relationship between malt associated microbial communities and conventional malt quality parameters was also compared. Results showed that differences in fungal communities of malts from different geographical location were more pronounced than bacterial communities. TRFLP analysis discriminated high quality commercial malts with low fungal loads from malts deliberately infected with fungal inocula (*Fusarium*/*Penicillium*). Malt moisture, *beta*-amylase, α-amylase and limit dextrinase contents showed significant correlations with fungal community structure. This investigation concluded that fungal community structure was more important to subsequent malt quality outcomes than bacteria.

1. Introduction

Barley malt is to beer as grapes are to wine (Goldammer, 2008). The most extensive use for barley malt worldwide is as a source of fermentable sugars for alcoholic fermentations, primarily beer but also for whiskey production. Approximately 10% of the world barley crop is used, after malting, for the production of beer. Malt forms the base material for making wort, the liquid extract that is fermented into beer. Different malt types are used to generate different characteristics in beer products including flavor, color and mouthfeel (Bamforth and Barclay, 1993).

Barley for malt is grown in a diverse range of environments and geographic locations. These include sub arctic Scandinavia to near the equator, in the mountains of Ethiopia and in South America, from below sea level near the Dead Sea to high altitudes in the Andes and the Himalayas, from humid, temperate regions, such as western Europe to dry land areas in parts of North America (N. America), Africa, and Australia (Briggs, 1978; Hunter, 1962; Rasmusson, 1985). In Australia Spring, two-rowed, barley is grown as a "winter" crop in semi arid, temperate and intermediate climatic regions resulting in dry maturation and harvest conditions. These conditions usually result in dry barley (<13% moisture) for storage and for subsequent malting. Such conditions maintain the germinative vigor of the barley that is a prerequisite for malting, and inhibit the growth of microbes during storage. Such advantages contribute to Australia's supply of around 32% of the world export malting barley trade (http://www.e-malt.com/). Australian grown barley has a reputation for being "bright and clean", which is suggestive of a low microbial load and rarity of mycotoxins such as deoxynivalenol and ochratoxin A (Kaur et al., 2009).

Barley grains, covered by a fibrous husk, are normally colonized by a wide variety of bacteria, yeasts and filamentous fungi (Flannigan, 2003). These mixed populations are difficult to control and elimination is neither possible nor desirable (Laitila, 2008) in a practical sense. Grain associated microbes have both positive and negative effects on grain quality in the field, in storage, at various stages during the malting process, and on the quality of the resulting malt and beer (Flannigan, 2003; Justé et al., 2011; Noots et al., 1999).

According to Flannigan (2003), barley provides an ecological niche for a diverse range of microorganisms, but the microbiota of different barleys is remarkably similar to each other, and to other cereals. Barley microbiota at harvest has been found to comprise the same limited number of species. Studies on fungi associated with South African (S. African) barley malt reported that predominant species in

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http://dx.doi.org/10.1016/j.ijfoodmicro.2015.08.019
0168-1605/

S. African malt were the same as those found elsewhere in the world; however the counts of these fungal taxa especially *Fusarium* and *Penicillium* species were significantly lower than those reported in the Northern Hemisphere (Rabie and Lübben, 1993). This is not surprising, as the S. African barley growing environment is in many ways similar to that of Australia, in that the grain maturation and harvest conditions are generally dry. The microbial community structure of barley malt can be influenced by other factors including growing location (Birgitte et al., 1996), climatic conditions (Backhouse and Burgess, 2002; Doohan et al., 2003; Krstanović et al., 2005), malting techniques (Flannigan et al., 1982), and storage and handling environments (Hill and Lacey, 1983; Laitila et al., 2003). In addition, the detection and enumeration techniques used for analysis may yield results that lead to different conclusions (Jarvis and Williams, 1987; Rabie et al., 1997). That is the microbial culture conditions may bias the composition determined because some microbes are unculturable or require very specific culture conditions.

A survey of the literature showed that there is a growing body of data for malt associated microbiota derived from N. American, European and S. African locations. There is no little information in this aspect for Australian malt or the broader global geographical differences in malt associated fungal and bacterial communities.

Most microbial studies of barley or malt samples have been done using conventional culture-dependent methods, comparing quantitative changes in microbial communities. These conventional microbial culture-dependent methods are biased towards the selective enrichment of fast growing microorganisms adapted to high substrate concentrations that could potentially represent only a minor fraction of the resident microbial community. On the other hand, culture-independent methods are now commonly employed to assess microbial community diversity and dynamics in food based ecosystems (Bokulich and Mills, 2012). PCR-DGGE (denaturing gradient gel electrophoresis) was used to monitor bacterial community dynamics during the malting process in Finland (Laitila et al., 2007).

The objectives of the present study were to compare the bacterial and fungal community structures of commercial Australian barley malt with its international counterparts originating from different geographical regions using terminal restriction fragment length polymorphism (TRFLP) fingerprinting and clone library analysis of ribosomal RNA genes. TRFLP analysis is a method developed for rapid profiling of complex microbial populations. Being a high throughput fingerprinting technique, TRFLP analysis has been applied extensively to the analysis of fungal and bacterial communities (Schütte et al., 2008). While TRFLP shares problems inherent to any PCR-based method (Lueders and Friedrich, 2003; Qiu et al., 2001), it has been shown to provide a facile means to observe changes in microbial community structure on temporal and spatial scales by monitoring the gain or loss of specific fragments from the profiles. When coupled with rRNA gene clone library assessment and sequencing, additional specific information on the composition of microbial communities can be obtained. The final objective was to establish a relationship between malt associated microbial communities and routine physicochemical malt quality parameters used in malting and brewing industry.

2. Materials and methods

2.1. Sample collection and preparation

A total of 34 Australian commercial malt samples were collected from different malt houses representing malt produced from barley grown in different cropping zones and included different commercial varieties (Baudin, Gairdner, Grimmett, Schooner and Sloop). International commercial malts were investigated with the sample numbers and source countries being shown in Table 1. The samples from Finland were malted from barley artificially inoculated with either a *Fusarium* sp. or a *Penicillium* sp. to produce standard malt samples having specified concentrations of deoxynivalenol (DON: 12 and 32 mg/kg) and ochratoxin A (OTA: 126 and 1099 μg/kg) mycotoxins for routine laboratory mycotoxin studies. One malt sample (out of 7) from S. Africa had gushing properties and was prepared by artificially inoculating the malt with *Fusarium culmorum*. Additionally, one sample from N. America malts was produced from barley that was known to be infected with *Fusarium* head blight. These samples had been analyzed for these characteristics and information was provided by the sample providers. The reason for including these known fungal infected malt samples in the study was to examine the discriminative ability of TRFLP technique and further statistical analyses applied in this study. Collected samples were stored in airtight bags at room temperature before grinding. Samples (40 g) were ground in a Cyclone Sample Mill using a 0.1 mm screen (UDY Corporation, CO, USA) and stored immediately at −20 °C until used for DNA extraction. Cross contamination between samples was avoided by blowing high pressure dry air through the grinding mill and collection container in between the samples, and taking only the middle portion of the ground sample from the container for analysis.

Table 1
Detail of barley malt samples used in this study.

Country of origin	No. of samples
Argentina	5
Australia	34
Belgium	2
Denmark	3
Finland	4 (two standard DON and two standard OTA samples)
France	6
North America (N. America*)	3 (includes one standard *Fusarium* head blight infected malt)
Russia	7
Slovakia	3
South Africa (S. Africa*)	7 (includes one standard gushing malt)

* Abbreviated and used there on.

2.2. DNA extraction and rRNA gene PCR amplification

DNA was extracted from ground samples (0.1 g) in duplicate using the FastDNA® Spin Kit for Soil (Q Biogene, CA, USA) according to the manufacturer's instructions except that the samples were homogenized with a Retsch MM300 bead beater (Retsch GmbH, Haan, Germany) at 30/s frequency for 4 min. DNA samples were independently amplified using 16S rRNA gene primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) labeled with D3 WellRED fluorescent dye (Sigma-Aldrich) and primer 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) labeled with WellRED™ fluorescent dye D4 for bacteria and 28S rRNA gene primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) labeled with D3 WellRED fluorescent dye and primer NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) labeled with D4 WellRED fluorescent dye for fungi (Beckman Coulter, Australia Pty Ltd., NSW, Australia). Each 60 μl reaction mixture contained 30 μl of 2 × ImmoMix Red™, 22.5 μl of sterile water (Bioline, NSW, Australia), 3 μl of each forward and reverse primers (10 pmol) and 1.5 μl DNA template. The PCR amplification program was as follows; 95 °C, 10 min; 30 cycles (35 cycles for fungi) of 94 °C, 1 min; 55 °C, 1 min; 72 °C, 2 min with a final extension of 72 °C, 10 min in a PTC 200 Peltier Thermal Cycler (MJ Research, Waltham, USA). After checking amplicons using agarose electrophoresis PCR products (55 μl) were purified using the UltraClean™ PCR Clean-Up Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions.

2.3. TRFLP analysis of bacterial and fungal communities

Aliquots of purified PCR products were digested individually with *Hae*III, *Msp*I and *Rsa*I (for bacteria) and *Hae*III, *Hinf*I and *Rsa*I (for fungi) (New England Biolabs Inc., Ipswich, MA, USA) according to the