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Lactobacillus plantarum and *Pediococcus pentosaceus* Starter Cultures as a Tool for Microflora Management in Malting and for Enhancement of Malt Processability

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Lactobacillus plantarum VTT E-78076 (E76) and *Pediococcus pentosaceus* VTT E-90390 (E390) starter cultures were added to the steeping water of normal malting barley in order to balance the microbial community and to enhance malt processability. In this study, we also investigated the effects of lactic acid-acidified MRS-spent medium (MRS-LA) on malting performance. Malting trials with five different two-row barley varieties were carried out in 25 kg pilot scale. The starter cultures promoted yeast growth during malting and restricted the growth of harmful bacteria and *Fusarium* fungi. Furthermore, they had positive effects on malt characteristics. Reduction in wort viscosity and β-glucan content and enhanced xylanase and microbial β-glucanase activities were observed. Starter cultures notably improved lautering performance. Some of the beneficial effects were due to the lactic acid and low pH, as similar effects were obtained with MRS-LA. Starter cultures offer a tool for tailoring of malt properties.

KEYWORDS: Barley (*Hordeum vulgare* L.); malting; lactic acid bacteria; microbial community; malt quality enzyme activity; filtration

INTRODUCTION

Malted barley (malt) is traditionally used in the production of alcoholic malt beverages and distilled spirits. Malt is the key material in brewing, providing all of the nutrients needed for yeast growth during the fermentation process. Moreover, malt has great effects on the brewing efficiency and on the characteristics of the final beer. The production of malt, malting, is a complex biological process in which the germination of barley (*Hordeum vulgare* L.) leads to the synthesis of hydrolytic enzymes and degradation of grain structure (*1*). Barley grains are naturally colonized by a variety of microbes (*2*, *3*). Therefore, the malting process can be considered as an ecosystem consisting of two metabolically active groups: the diverse microbial community and the barley grains. The indigenous microbial community consists of various types of bacteria, yeasts, and filamentous fungi, and it will vary in response to a number of factors including plant species and variety, climate, location, cultivation, storage conditions, and transport (*2*, *4*−*6*). Furthermore, malting conditions favor microbial growth, and each process step can be a source of additional microbes and their metabolites (*7*). Thus, it is evident that the diverse microbial community greatly influences the germination process and thereby the quality of the final product. Depending on the nature and the amount of the microbes, their effects may be either deleterious or beneficial.

Occasionally, microbes can be responsible for reduced grain germination. It is well-known that an excess of aerobic microbes on the wet grain competes with grain tissue for oxygen (*8*, *9*). Microbial activity causes changes in cereal carbohydrates, lipids, and proteins. Uncontrolled degradation of barley components is often observed as discoloration of kernels and as formation of off-odors and off-flavors (*2*, *3*). Certain fungi are known to be active gushing inducers (*10*−*12*). Gushing (spontaneous overfoaming of packaged beer immediately on opening) is a very complex phenomenon, and it can at least partially be explained by the secretion of specific gushing factors by fungi during field conditions, during storage, or during the malting process (*10*, *13*−*15*). In addition, many fungi are capable of producing toxic metabolites, mycotoxins, which are often very stable compounds and can therefore survive throughout processing and enter the final product (*16*). Furthermore, microbes can be involved in poor lautering performance. Some bacteria and yeasts produce extracellular polysaccharides (EPS) during malting, and these slimy compounds may cause problems later during the wort and beer filteration (*17*, *18*). Walker et al. (*19*) reported that dead malt-derived bacteria also had a negative

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effect on the filterability of sweet wort and caused visible haze in the final beer.

Although microbes and their metabolites may have serious negative impacts during malting and later in the brewing process, the positive contribution of the microbial community on the malt characteristics is even more significant. Barley germination is metabolically regulated by a series of plant growth regulators. It is well-known that many microbes take part in the production of metabolites, including plant hormones, which stimulate grain germination (*2*, *3*, *20*). Furthermore, microbes significantly contribute to the malt enzyme potential by being important producers of amylolytic, proteolytic, and cell wall-degrading enzymes (*2*, *3*, *21*). In addition, microbes improve the nutritional value of malted cereals by degrading antinutritive components and also by taking part in the production of health-promoting compounds such as vitamins (*22*, *23*).

Malt-derived microbes, especially lactic acid bacteria (LAB) and also certain fungi, offer a potential alternative as natural, food-grade biocontrol agents. Microbes with antimicrobial potential can be applied as starter cultures in bioprocesses in which the use of chemical antimicrobials is considered undesirable. The success of LAB is due to their ability to improve the safety, flavor, nutritional value, and structure of the products (*24*). Starter technology, in which the barley is inoculated with well-characterized microbes, has also been introduced in the malting industry (*25*−*27*). Our earlier studies revealed two potential LAB strains for malting purposes (*27*, *28*): *Lactobacillus plantarum* VTT E-78076 (E76) *Pediococcus pentosaceus* VTT E-90390 (E390). These two strains added into the steeping waters of barley restricted the growth of harmful bacteria causing wort filtration problems and of *Fusarium* fungi during the malting process (*28*−*32*). The starter cultures have also shown their antimicrobial potential in other cereal-based bioprocesses. Katina et al. (*33*) successfully utilized *L. plantarum* E76 and *P. pentosaceus* E390 in wheat sourdough breads, in which they notably inhibited rope spoilage caused by *Bacillus* species. Furthermore, a combined culture of *L. plantarum* E76 and *P. pentosaceus* E390 effectively suppressed the growth of clostridia during the storage of brewers spent grain (*34*). Several investigations have been conducted to study the antimicrobial properties of LAB isolates from barley and malt and their potential against microbial contaminants in malting (*32*, *36*−*40*). The antagonistic action of LAB is based both on competition for nutrients and space and on the production of various antimicrobial compounds such as organic acids, hydrogen peroxide, bacteriocins, and low molecular weight antimicrobial compounds (*41*). Recently, Lowe and Arendt (*42*) reviewed the potential of LAB in malting and brewing applications. Along with LAB, certain fungal cultures with antagonistic properties can be used as biocontrol agents in malting. *Geotrichum candidum*, a yeastlike fungus, inhibited fungal growth and prevented mycotoxin production during malting (*25*, *43*).

In addition to their antimicrobial potential, the use of starter cultures in malting has led to improvements in the physical and chemical quality of malt. Malt-derived thermophilic LAB have traditionally been used in the production of biologically acidified malt, mash, or wort (*44*, *45*). In addition to improved microbiological stability, biological acidification has contributed to the technological and organoleptic properties of malt, wort, and beer (*45*−*48*). Our previous studies showed that LAB starter cultures added into the steeping contributed to malt enzyme potential and to lautering performance (*28*, *29*). Bol et al. (*49*) reported increased β-glucanase activities when they inoculated unspecified microbes into laboratory scale maltings. Later, starter culture technology based on the use of *Rhizopus oligosporus* was developed with the specific aim of compensating for deficiencies in malt cell wall modification (*26*, *50*, *51*). *Rhizopus* starter culture produced cell wall-degrading enzymes, mainly β-glucanase, xylanase, and proteases with beneficial effects on malt characteristics.

The inoculation stage and composition of starter preparation are critical with respect to the functions of microbial cultures in bioprosesses. The indigenous microbial community is rapidly activated during the first step of malting, steeping. Therefore, starter cultures are often applied at this stage (*28*, *43*, *51*). It has been shown that whole LAB cultures (cells and spent medium) are needed for the maximal restriction of harmful microbes, because the antimicrobial effect of LAB is to a large extent based on the antimicrobial compounds present in the culture broth, and growth medium also provides beneficial nutrients for starter strains (*28*, *32*, *37*). Therefore, starter cultures are usually activated in the broth before use (*28*, *52*). However, very little is known about the effects of cell-free spent medium of starter cultures on the overall microbial community and malt quality.

Several investigations with LAB have been carried out in laboratory scale with artificially and heavily contaminated material or with barley expected to cause problems in the brewing process. To our knowledge, comparatively little has been reported of their impacts on the normal malting process in a larger scale. The present investigation was set up to study in more detail the effects of *L. plantarum* E76 and *P. pentosaceus* E390 starter cultures on the malting process as well as on the malt quality of normal malting grade barley. Malting experiments with five different barley samples of the same harvest were carried out in 25 kg scale in an automated pilot malting equipment. We also monitored development of the microbial community during the pilot scale malting process and the influence of starter cultures on microbial growth during processing. Furthermore, we investigated the effects of chemically acidified, unfermented MRS culture broth on the microbial community and on malt quality.

MATERIALS AND METHODS

Lactic Acid Starter Cultures. *L. plantarum* VTT E-78076 (E76, isolated from beer) and *P. pentosaceus* VTT E-90390 (E390, isolated from split barley kernels) were provided by the VTT Culture Collection. During the experiments, the bacteria were maintained as frozen stock cultures (Protect Bacterial Preserves, TSC Technical Consultants Ltd., Lancashire, United Kingdom) at −70 °C. LAB were routinely propagated twice in MRS broth (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) in anaerobic conditions at 30 °C for 2 days. The final LAB starter preparation was produced using 1% inoculum and 3 days of incubation at 30 °C without shaking. The cultures were stored in a cold room (+4 °C) overnight before adding them into the steeping water.

Pilot Scale Malting Experiments. Five different two-row barley varieties (Kymppi, Kustaa, Saana, Tofta, and Mentor) from the same harvest were malted in pilot scale (**Table 1**). A total of 17 pilot scale malting experiments were carried out. Barley samples were analyzed using the following EBC (*53*) recommended methods: moisture content (EBC 3.2), protein content (EBC 3.3.1), germination capacity (EBC 3.5.1), and germination energy (EBC 3.6.2). Before malting, barley samples were sieved to remove grains <2.5 mm. For moisture content analysis, a 1 kg sample of barley was packed into a netted fabric bag and malted along with the batch. The sample was then weighed daily for the moisture content estimation. Batches of 25 kg barley were steeped in an automated conical steeping vessel (Inssitiimi, Turku, Finland) for 2 days at 12 °C to a moisture level of 45−46% with two alternating wet steep periods (8 h each) and two air rests (16 h each).

Table 1. Basic Characteristics and Microbiological Quality of the Barley Samples Malted in 25 kg Pilot Scale Experiments

	Kymppi	Kustaa	Saana	Tofta	Mentor
barley characteristics					
moisture (%)	13.6	12.7	13.4	12.5	13.6
protein (% d.w)	11.2	11.5	12.6	11.2	12.0
germination capacity (%[a])	98	98	100	96	100
germination energy (4 mL, %[b])	98	98	99	98	100
germination energy (8 mL, %[b])	68	85	85	76	90
bacteria and yeasts (cfu/g)					
aerobic heterotrophic bacteria	3×10^7	2×10^7	2×10^7	5×10^7	2×10^7
Pseudomonas spp.	8×10^6	5×10^6	2×10^6	4×10^7	3×10^6
LAB	1×10^2	<50	<50	5×10^1	2×10^2
yeasts	1×10^4	2×10^3	3×10^3	6×10^2	2×10^2
field fungi (kernels contaminated with fungi, %)					
Fusarium	22	7	10	13	12
Alternaria	53	53	36	68	61
Cephalosporium	43	9	1	30	13
Cladosporium	9	4	13	9	12
Drechslera	1	3	18	0	5

[a] Germination capacity (hydroperoxide method). [b] Germination energy determined using 4 or 8 mL water for 100 grains.

The barley−water ratio was 25 kg:27.5 L. The steeping water was aerated for 5 min every half hour, and during the air rest, carbon dioxide was removed, simulating the industrial steeping procedure. The LAB cultures (120 mL/1 kg barley), including cells and spent medium, were added into both steeping waters. To compare the effects of culture broth components and lactic acid on the malt quality, two malting experiments with Kymppi and Mentor barley were carried out in which a corresponding volume (120 mL/kg) of chemically acidified, unfermented MRS broth (later referred as MRS-LA) was added into the steeping instead of starter culture. Unfermented MRS broth was prepared without glucose and supplemented with 2.5% DL-lactic acid (BDH Laboratory Supplies, Poole, England). The pH of the MRS-LA was adjusted with NaOH to 3.8, the same pH value as in the starter cultures. After the steeping, samples were transferred to an automated pilot germination/kilning unit (designed at VTT Biotechnology) and germinated at 14 °C for 6 days. The percentage of germinated grains was calculated from 150 to 200 kernels daily. The samples were kilned for 21 h with a stepwise temperature increase from 50 to 85 °C. The moisture content after kilning was approximately 4%. After kilning, the rootlets were removed in an ejector-feeded grain precleaner (Kongskilde precleaner KF-12/FRL10, Kongskilde, Denmark).

Microbiological Analyses. Samples for the microbiological analyses were taken from untreated barley and from barley after steeping, germination, and kilning (after rootlet removal). The following microbial groups were analyzed from homogenized barley samples: aerobic heterotrophic bacteria, *Pseudomonas* spp., LAB, and yeasts. A sample of 10 g was homogenized for 10 min with 90 mL of sterile saline in a Stomacher Lab Blender 400 (Seward Medical, London, United Kingdom). Aerobic heterotrophic bacteria were determined on plate count agar (PCA, Difco Laboratories, Detroit, MI) and *Pseudomonas* spp. on C-F-C agar (Oxoid Ltd.). Samples were incubated in aerobic conditions at 30 °C for 2−3 days. The number of LAB was determined on MRS agar (Oxoid), and samples were incubated in anaerobic conditions at 30 °C for 5 days. To prevent fungal overgrowth of bacterial determinations, 0.001% cycloheximide (Sigma Chemical, St. Louis, MO) was added to PCA, C-F-C, and MRS media. Yeast counts were determined on YM agar (Difco Laboratories). Samples were incubated in aerobic conditions at 25 °C for 3−5 days. Chlortetracycline and chloramphenicol (both at 0.01%) were added to YM medium to prevent bacterial growth. In addition, 0.02% of Triton-X 100 (BDH) was used to limit the spreading of fungal colonies on YM agar. The bacteria and yeast results are expressed as colony forming units/gram barley (cfu/g).

For *Fusarium* analyses, 100 randomly selected kernels were placed on a selective Czapek−Dox agar containing Iprodion and Dichloral (CZID agar) (*54*, *55*). The CZID plates were incubated at 25 °C for 7 days. Other filamentous fungi such as *Alternaria* spp., *Cephalosporium* spp., *Cladosporium* spp., *Drechslera* spp., *Epicoccum* spp., *Mucor*, and *Rhizopus* spp. were determined on wet filter paper using direct plating of 100 kernels (*55*). Filter paper plates were incubated at 25 °C for 21 days. Fungi were identified under a stereomicroscope on the basis of typical colony form and color. Identification was confirmed by conidia morphology with a light microscope (magnification 400×). The results are expressed as percent of kernels contaminated with fungi.

Malt and Wort Analyses. Congress mashings were carried out according to Analytica EBC (*53*). Both fine and coarse grinding were used. Extract content (fine grind, congress mash, EBC 4.5.1−4.5.2), color (visual method, EBC 4.7.2), free amino nitrogen (FAN, EBC 4.10), soluble nitrogen (Kjeldahl method, EBC 4.9.1), wort β-glucan content (fluorimetric method, EBC 4.16.2), viscosity (EBC 4.8), and malt modification (Calcofluor method, EBC 4.14) were carried out according to Analytica EBC (*53*). The filtration rate of the congress wort made of coarsely ground malt was determined by measuring the time needed to collect 300 mL of the filtrate. In addition to conventional congress mashing, high gravity mashing and the Büchner filtration test for evaluation of lautering performance were performed as described by Sjöholm et al. (*56*).

Enzyme Activities in Malts. The assay for α-amylase was a modification of the Megazyme Ceralpha method (Megazyme CER 6/93) (*57*). β-Glucanase activity was analyzed using the Megazyme Azo-barley glucan method (Megazyme MBG 6/93) (*58*, *59*). An estimate for β-glucanase activity of microbial origin was obtained by analyzing β-glucanase activity at 60 °C. The malt β-glucanases are inactive at 60 °C and thus contribute only to a minor extent to the measured activity. The xylanase assay was developed on the basis of the Megazyme Xylazyme AX method (Megazyme XYL 8/94) using an extraction time of 15 min. Sodium acetate buffer (25 mM, pH 4.5) was used to extract the enzyme. All of the enzyme analyses were carried out in duplicate.

Statistical Analysis. The overall effects of *L. plantarum* E76 and *P. pentosaceus* E390 starter culture treatment were studied with a paired samples two-tailed *t*-test. Therefore, the data were paired as samples (Kymppi, Kustaa, Saana, Tofta, and Mentor) before and after starter treatment. We compared the magnitude of difference between two means in relation to the amount of inherent variability in the data. The differences were considered to be significant when $P < 0.05$.

RESULTS

Barley Characteristics. Pilot scale malting experiments were carried out with five different, randomly selected barley varieties, Kymppi, Kustaa, Saana, Tofta, and Mentor, from the same harvest. **Table 1** shows the basic characteristics and microbiological quality of the barley samples prior to malting. Samples were considered as normal malting grade barley, with germination energies of 98−100%. The indigenous microbial communities of the barley samples consisted of various types of microbes. The initial aerobic bacterial count in the native barley was (2−5) × 10^7 cfu/g. A significant proportion of this aerobic heterotrophic bacterial population was composed of pseudomonads (2×10^6−4×10^7 cfu/g). Only low numbers, <50−200 cfu/g, of LAB were found in the indigenous community. In addition to bacteria, various types of fungi (yeasts and filamentous fungi) were detected in the barley samples. The number of yeasts ranged from 2×10^2 to 1×10^4 cfu/g. The most frequently detected filamentous fungi belonged to the genera *Fusarium* (7−22%), *Alternaria* (36−68%), *Cephalosporium* (1−43%), *Cladosporium* (4−13%), and *Drechslera* (0−18%). The strorage fungi, *Aspergillus* and *Penicillium*, were not detected in these samples.

Table 2. pH Range in the Steeping Water during the First and Last Hour of the Steeping Phase[a]

steeping phase	untreated samples ($n = 5$)	*L. plantarum* E76 ($n = 5$)	*P. pentosaceus* E390 ($n = 5$)	MRS-LA ($n = 2$)
I steep, beginning	7.2–7.4	3.9–4.3	4.2–4.5	3.9–4.4
I steep, end	7.1–7.2	4.6–4.7	4.6–4.7	4.4–4.5
II steep, beginning	5.6–6.2	3.9–4.2	4.1–4.3	3.9–4.1
II steep, end	5.8–6.5	4.1–4.3	4.2–4.3	4.1–4.3

[a] Starter cultures and MRS-LA were added into both steeping waters.

Effects of Starter Cultures on the Malting Process. *L. plantarum* E76 or *P. pentosaceus* E390 starter cultures were propagated in MRS broth, and the whole cultures (including cells and spent medium) were added into both steeping waters at a level of 120 mL/kg of barley. To study the effects of chemically acidified MRS broth (MRS-LA), unfermented broth without glucose was supplemented with lactic acid. The lactic acid concentration in the steeping water after MRS-LA addition was 29 mM, corresponding to the amount of lactic acid added with the starter cultures. The pH of the steeping water was automatically recorded during the immersion periods. As seen in **Table 2**, the addition of starter cultures or MRS-LA notably altered the acidity during steeping. The pH of the water was reduced to 4.5−4.7 during the first steep after starter or MRS-LA addition, and the pH values ranged from 4.1 to 4.3 at the end of the second steep. In the malting experiments with untreated barley, the pH of the water remained constant during the first steeping. pH values of 7.2−7.4 were measured at the beginning and 7.1−7.2 at the end of this first period. Because of the activation of the indigenous microbial community, lower pH values were recorded in the untreated samples during the second steeping, 5.6−6.2 at the beginning and 5.8−6.5 at the end of the second steep. Both starter cultures and MRS-LA addition decreased the water uptake by the kernels and delayed grain germination. After the second steep, the moisture content of the barley was approximately 1% lower in the treated samples than in the untreated samples. Therefore, it was necessary to spray extra water on the starter and MRS-LA samples at the beginning of germination in order to obtain a moisture level of 46%. Delayed grain germination was recorded as decreased carbon dioxide production during the air rest period and as reduced rootlet growth (data not shown). The number of germinated kernels after steeping was approximately 15−20% lower in starter and MRS-LA-treated samples as compared to the untreated barley samples. However, after 1 day of germination, 92−98% of kernels were germinated in all of the samples, although low steeping and germination temperatures (12−14 °C) were used in this study.

Effects of Starter Cultures on the Microbial Community during Pilot Scale Malting. Figure 1 shows the growth of LAB (**A**), yeasts (**B**), aerobic heterotrophic bacteria (**C**), and *Pseudomonas* species (**D**) during the pilot scale malting. *L. plantarum* E76 and *P. pentosaceus* E390 starter cultures were added into the steeping waters at the beginning of both steeping phases at a level 10^8 cells/g of barley. The determination of LAB counts showed that $(2-5) \times 10^8$ cfu/g were colonized to the steeped grains after 2 days of malting (**Figure 1A**). The number of *P. pentosaceus* E390 adhered to the kernels remained constant throughout the malting process, and as a thermophilic organism, it survived better than *L. plantarum* E76 during the kilning and even multiplied during the first hours of kilning (**Figure 1A**). The viable cell count of *P. pentosaceus* E390 in the final malt samples was still $>10^8$ cfu/g. The number of *L. plantarum* E76 was 2×10^8 cfu/g after germination and decreased by 2 log units during the kilning.

As seen in **Figure 1A**, the indigenous LAB population in the barley samples was low (80 cfu/g) but was activated during the steeping phase. It reached a maximum level of 10^6 cfu/g at the end of germination. Kilning reduced the indigenous LAB population by 2 log units. The addition of MRS-LA into the steeping water had little effect on the growth of the indigenous LAB population. A slight increase, from 9×10^2 to 3×10^3 cfu/g, was noticed after the steeping phase. However, 10-fold less LAB were observed in the MRS-LA samples after germination than in the untreated samples. MRS-LA treatment probably reduced the number of acid-sensitive *Leuconostoc* species in the LAB community. Furthermore, the growth of indigenous LAB appeared to be interrelated with the indigenous yeast population. MRS-LA addition into the steeping water promoted the yeast growth during germination, and 10-fold higher yeast counts were determined in the final malt samples after MRS-LA addition than in the untreated samples (**Figure 1B**). The yeast growth was also accelerated by the starter cultures (**Figure 1B**). Significantly higher yeast counts, over 1 log unit, were detected after the germination ($P < 0.01$) and also in the final malt ($P < 0.001$) when starter cultures were applied. The yeast community was probably composed of species resistant to LAB antimicrobials. Furthermore, the acidic environment appeared to promote the growth of certain yeast populations such as lactate-utilizing yeasts present in the malting process. In addition, the decline of competitive microflora created more opportunities for yeast growth.

Starter cultures, especially *L. plantarum* E76, effectively restricted the growth of aerobic bacteria during steeping (**Figure 1C**,**D**). A statistically significant ($P < 0.001$) 2−3 log unit reduction in the number of aerobic heterotrophic bacteria, comprising mainly Gram-negative bacteria such as enterobacteria, was observed after addition of *L. plantarum* E76 or *P. pentosaceus* E390 starter cultures into the steeping (**Figure 1C**). Furthermore, starter cultures greatly inhibited growth of the *Pseudomonas* population throughout the pilot scale malting experiments (**Figure 1D**). A decrease of 3−4 log units was observed during the steeping. The viable counts of *Pseudomonas* spp. also remained significantly lower (approximately 1 log unit) during the germination and in the kilned malt. The antagonistic action against *Pseudomonas* species was mainly due to organic acids and the reduction of pH, as similar results were obtained with chemically acidified MRS broth (**Figure 1D**).

In addition to bacterial and yeast determinations, malting samples were analyzed for filamentous fungi. **Figure 2** shows the *Fusarium* contamination level in native barley, steeped barley, and final malt. Although the *Fusarium* contamination in the native barley was low (only 7−22% of the kernels were contaminated with fusaria), intensive growth was observed during the steeping. Approximately 30−50% higher counts were measured after the steeping. The addition of *L. plantarum* E76 or *P. pentosaceus* E390 notably restricted the growth of fusaria. The contamination level decreased to approximately 20% after starter addition. Interestingly, the addition of MRS-LA had a similar effect against *Fusarium* fungi present in barley. The steeping water contained only 0.27% of lactic acid, but marked reduction in the *Fusarium* counts was observed. The main population of the two-rowed barley samples consisted of *F. avenaceum* species. The growth of this species was obviously affected by the synergistic effect of lactic acid and sodium diacetate, an ingredient present in the commercial MRS broth. Kilning effectively reduced the viable count of fusaria in all of

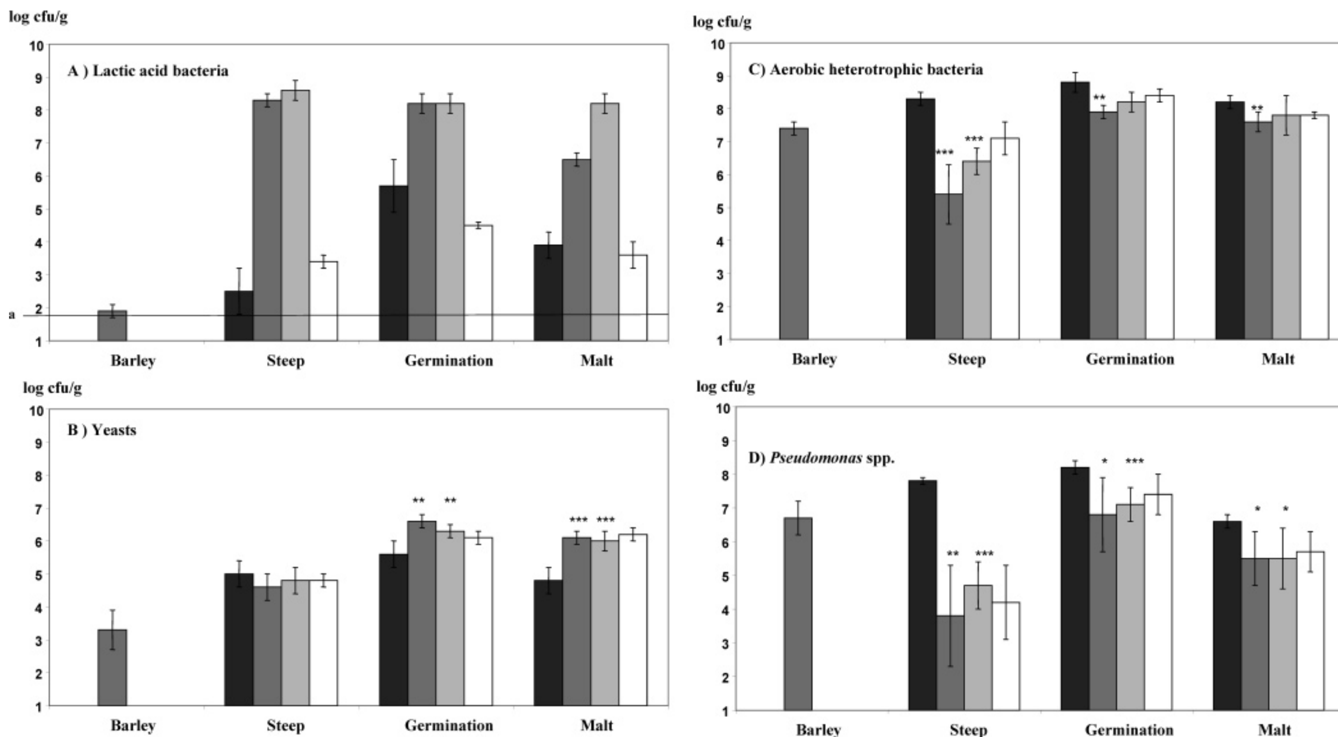

Figure 1. Counts of LAB (**A**), yeasts (**B**), aerobic heterotrophic bacteria (**C**), and *Pseudomonas* spp. (**D**) during the pilot scale malting experiments. Black bars, untreated control samples ($n = 5$); medium gray bars, *L. plantarum* E76 added into the steeping waters ($n = 5$); light gray bars, *P. pentosaceus* E390 added into the steeping waters ($n = 5$); and white bars, MRS-LA solution (pH 3.8) added into the steeping waters ($n = 2$). The significance levels in the paired *t*-test: $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. The detection limit in bacterial and yeast determinations was 50 cfu/g (log 1.7 cfu/g).

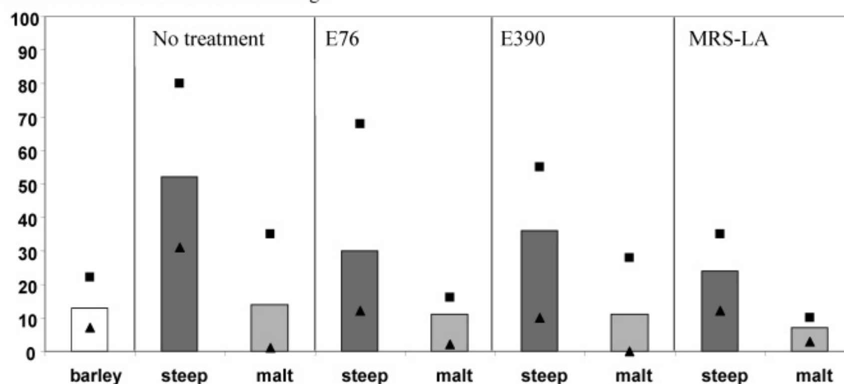

Figure 2. Effects of starter cultures (*L. plantarum* E76 and *P. pentosaceus* E390) and MRS-LA (unfermented MRS broth, containing 2.5% lactic acid) added to steeping water on the occurrence of *Fusarium* fungi during pilot scale maltings. The counts are mean values obtained from five different individual malting experiments. Two malting experiments were also carried out with MRS-LA. In addition to mean values, minimum (▲) and maximum values (■) are presented.

the samples. In the finished malt samples, approximately 7−14% of kernels were contaminated by *Fusarium* fungi.

The antifungal effect of starter cultures was selective against *Fusarium* fungi. The growth of other common field fungi such as *Alternaria*, *Cephalosporium*, *Cladosporium*, or *Drechslera* was not inhibited by the addition of LAB (**Figure 3A**). On the contrary, a statistically significant increase of *Alternaria* fungi was recorded during the germination when *L. plantarum* E76 ($P < 0.05$) or *P. pentosaceus* E390 ($P < 0.001$) cultures were added into the steeping waters. The common field fungi were not affected by the addition of unfermented MRS-LA. The *Mucorales* fungi such as *Mucor* and *Rhizopus* did not belong to the normal microbial community of barley (**Figure 3B**). However, they are commonly detected at elevated moisture conditions, especially during the early hours of kilning. The addition of starter culture or MRS-LA into the steeping had no significant effect on the growth of *Mucor* and *Rhizopus* fungi.

Effects of Starter Cultures on Malt Quality. The effects of starter cultures on malt quality were studied in detail using the high gravity mashing procedure in parallel with the conventional congress mashing procedure (**Table 3**). Student's *t*-test was used to evaluate the difference between the malt analyses of paired observations: untreated malt against starter-treated malt. Although starter addition appeared to retard grain germination during the first days of malting, the malt modification was not significantly affected. By contrast, significantly lower β-glucan levels ($P < 0.05$) were detected in congress worts after *L. plantarum* E76 addition. The wort β-glucan concentration was approximately 30% lower in starter-treated samples as compared to untreated samples. Furthermore, starter

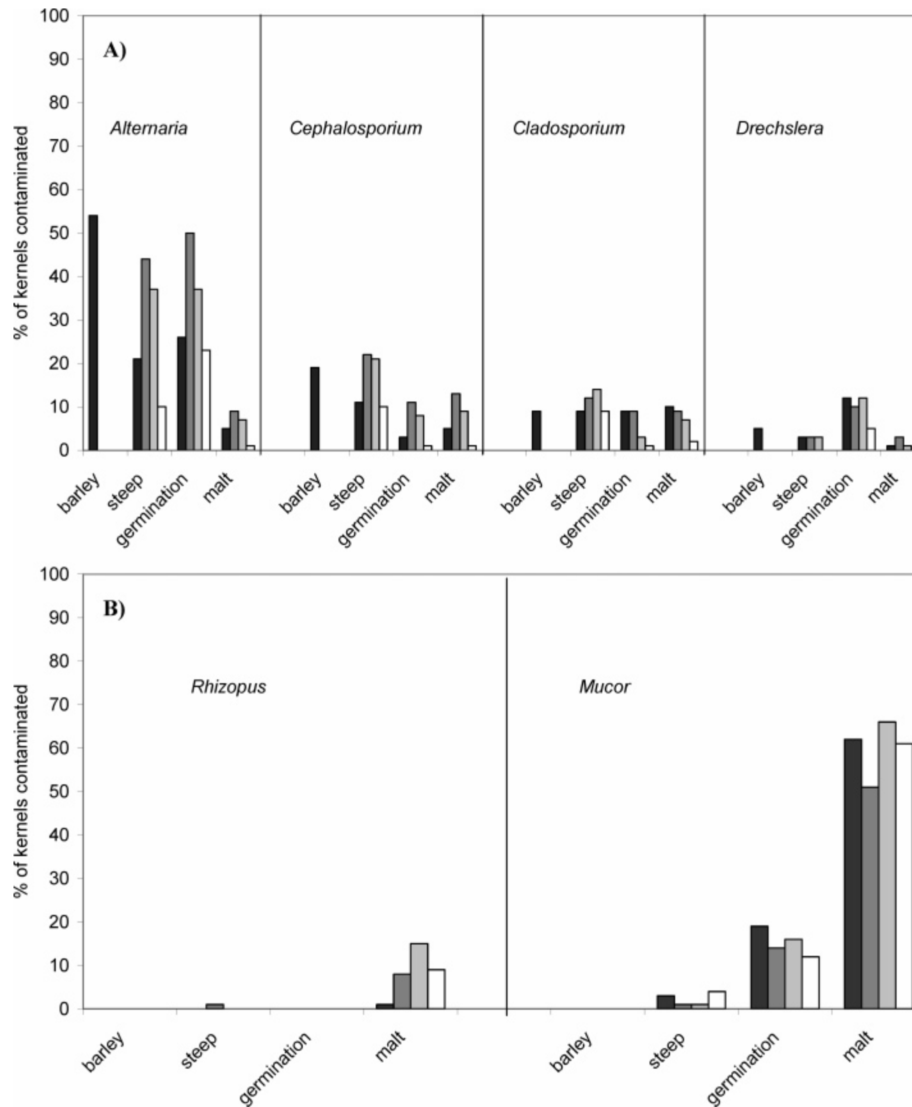

Figure 3. Effects of starter cultures (*L. plantarum* E76 and *P. pentosaceus* E390) and MRS-LA (unfermented MRS broth, containing 2.5% lactic acid) added to steeping water on the occurrence of *Alternaria*, *Cephalosporium*, *Cladosporium*, *Drechslera* (**A**), *Rhizopus*, and *Mucor* (**B**) fungi during pilot scale maltings. Black bars, untreated control samples ($n = 5$); medium gray bars, *L. plantarum* E76 added into the steeping waters ($n = 5$); light gray bars, *P. pentosaceus* E390 added into the steeping waters ($n = 5$); and white bars, MRS-LA solution (pH 3.8) added into the steeping waters ($n = 2$).

malts resulted in notably lower wort viscosity. In the present study, reduced wort β-glucan content and low wort viscosity correlated well with mash filterability (**Table 3** and **Figure 4**). The Büchner filtration test clearly showed that the filterability of starter malts was better than that of untreated malts, although all of the samples were classified as malt with good filterability. The filtration test carried out with coarse grinding confirmed the improved lautering performance with starter malts. Approximately 10 min less time was needed to collect 300 mL of congress wort when starter cultures were applied (**Table 3**).

The starter treatment affected the wort pH. Approximately 0.1 unit reduction in wort pH was recorded in starter-treated samples as compared to untreated samples. **Table 3** also shows the effects of *L. plantarum* E76 and *P. pentosaceus* E390 on α-amylase, β-glucanase, and xylanase activities in the malt samples prior to mashing. No profound differences were observed in α-amylase or endogenous β-glucanase activity between the untreated and the starter samples. However, significantly higher β-glucanase activities were observed in starter malts when the activity measurements were carried out at 60 °C, indicating the presence of microbial β-glucanases. Approximately 60 U/kg higher activities ($P < 0.01$) were detected after *L. plantarum* E76 treatment and approximately 40 U/kg higher activities ($P < 0.04$) after *P. pentosaceus* E390 treatment as compared to untreated samples. Furthermore, malt xylanase activity was greatly influenced by the addition of starter cultures. A paired sample *t*-test showed that untreated and *L. plantarum* E76-treated samples differed significantly ($P < 0.001$). The estimated mean difference was −44 U/kg, which implied a higher xylanase activity after *L. plantarum* E76 treatment as compared to the untreated samples (**Table 3**). Enhanced microbial β-glucanase and xylanase activity detected in starter samples could partly explain the improved wort filtration performance (**Table 3** and **Figure 4**). Furthermore, starter treatment during steeping enhanced proteolytic activity during malting and mashing, which was recorded as higher soluble and free amino nitrogen levels in wort (**Table 3**). In addition, the darker wort color detected in many starter samples was also due to the increased proteolysis.

We also investigated the effects of chemically acidified steeping on malt quality. MRS-LA added into the steeping water appeared to inhibit grain germination in the beginning of malting more than starter cultures, although the added concentration of LA in steeping water was similar, 0.27%. Despite lower

Table 3. Effects of *L. plantarum* E76 and *P. pentosaceus* E390 Starter Cultures on Malt Quality and Enzyme Potential

analysis		mean ± SD: untreated samples ($n = 5$)	mean ± SD: after E76 treatment ($n = 5$)	d_i[a]	P[b]	mean ± SD: after E390 treatment ($n = 5$)	d_i	P
congress mashing								
extract content, fine grind	%	80.0 ± 0.59	80.5 ± 0.36	−0.5 ± 0.77	0.203	80.5 ± 0.47	−0.5 ± 0.88	0.274
pH		5.97 ± 0.04	5.86 ± 0.06	0.10 ± 0.05	**0.014****	5.87 ± 0.04	0.09 ± 0.04	**0.009****
color	EBC	3.5 ± 0.5	3.8 ± 0.5	−0.26 ± 0.42	0.235	3.7 ± 0.7	−0.14 ± 0.29	0.338
FAN	mg/L	170 ± 28	191 ± 22	−21.2 ± 17.6	**0.054***	191 ± 26	−21.2 ± 19.8	0.075
soluble nitrogen	mg/L	825 ± 91	899 ± 79	−73.6 ± 50.5	**0.031***	898 ± 85	−72.4 ± 49.8	**0.031***
β-glucan	mg/L	188 ± 96	133 ± 78	54.4 ± 41.3	**0.042***	123 ± 41	64.4 ± 71.7	0.115
viscosity	cP	1.53 ± 0.04	1.48 ± 0.01	0.046 ± 0.03	**0.027***	1.49 ± 0.03	0.034 ± 0.06	0.281
filtration time (coarse grinding, approximately 300 mL)	min	87 ± 26	77 ± 30	10.2 ± 14.7	0.195	78 ± 30	9.4 ± 14.10	0.210
modification (Calcofluor)	%	95 ± 3	96 ± 4	−0.6 ± 1.95	0.529	96 ± 3	−0.8 ± 3.03	0.587
high gravity mashing								
extract content (theoretical)	%	81.0 ± 0.66	81.4 ± 0.63	−0.4 ± 0.88	0.390	81.6 ± 1.08	−0.56 ± 1.33	0.386
pH		5.59 ± 0.03	5.48 ± 0.06	0.11 ± 0.07	0.057	5.50 ± 0.04	0.09 ± 0.06	0.058
color	EBC	7.0 ± 0.6	7.6 ± 0.5	−0.6 ± 0.491	0.279	7.8 ± 0.8	−0.8 ± 0.6	**0.000*****
FAN	mg/L	362 ± 66	399 ± 48	−37 ± 38	0.093	375 ± 95	−13 ± 41	0.508
β-glucan	mg/L	277 ± 153	177 ± 126	99 ± 68	0.063	187 ± 91	89 ± 92	0.149
viscosity	cP	2.29 ± 0.06	2.10 ± 0.02	0.18 ± 0.06	**0.002***	2.13 ± 0.02	0.15 ± 0.06	**0.006***
Büchner filtration, 15 min	g	81 ± 10	99 ± 6	−19 ± 5	**0.001*****	92 ± 9	−11 ± 9	**0.047***
Büchner filtration, 30 min	g	103 ± 5	112 ± 4	−8 ± 5	**0.013****	109 ± 6	−6 ± 5	0.073
Büchner filtration, 60 min	g	110 ± 3	116 ± 4	−5 ± 6	0.108	113 ± 5	−3 ± 7	0.382
Büchner filtration, area	gh	91 ± 6	103 ± 4	−11 ± 4	**0.004****	99 ± 6	−8 ± 5	**0.032***
malt enzyme activity								
α-amylase	U/g	246 ± 66	248 ± 62	−1.4 ± 27	0.911	259 ± 61	−12.8 ± 25	0.318
β-glucanase (30 °C) endogenous	U/kg	194 ± 39	181 ± 25	13 ± 26	0.477	189 ± 22	5 ± 39	0.795
β-glucanase (60 °C) microbial	U/kg	51 ± 8	114 ± 39	−63 ± 32	**0.012****	93 ± 32	−43 ± 32	**0.040***
xylanase	U/kg	104 ± 24	148 ± 31	−44 ± 13	**0.001*****	126 ± 44	−22 ± 28	0.154

[a] d_i = mean difference between the pairs of malt samples before and after starter treatment. [b] Two-tailed paired samples *t*-test; *P < 0.05, **P < 0.01, and ***P < 0.001.

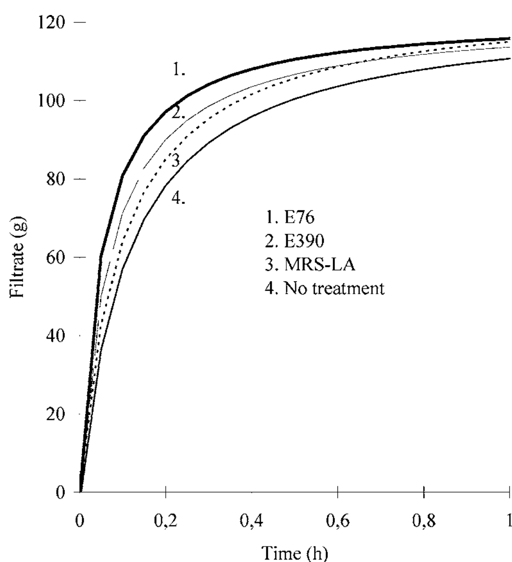

Figure 4. Effect of *L. plantarum* E76, *P. pentosaceus* E390, and MRS-LA (unfermented MRS broth, containing 2.5% lactic acid) on mash filterability measured by the Büchner filtration test. Starter cultures and MRS-LA were added to steeping waters (120 mL/1 kg) of the pilot malting.

endogenous β-glucanase activity and malt modification in MRS-LA samples as compared to the untreated samples, a notably lower β-glucan content and wort viscosity were recorded after MRS-LA treatment (**Table 4**). In addition, MRS-LA treatment increased proteolysis recorded as higher soluble and free amino nitrogen levels. Furthermore, MRS-LA treatment during steeping promoted microbial β-glucanase and xylanase production and led to improved wort filtration (**Table 4** and **Figure 4**). Therefore, part of the beneficial effects obtained with starter treatment can be explained by the lactic acid and the low pH, as the same effects were obtained with MRS-LA treatment. However, the best filtration performance was observed after *L. plantarum* E76 treatment. Starter samples, especially *L. plantarum* E76, exhibited higher xylanase acticity than MRS-LA samples, with a consequent beneficial contribution to lautering performance.

DISCUSSION

The complex microbial community naturally existing in cereal grains has a decisive role in the malting ecosystem. As shown in the present study, the microbial activity during malting has great impacts on grain germination and thereby on the properties of the final malt. Our results were also in agreement with the earlier observations (*2*, *3*, *5*−*7*, *49*, *60*, *61*). Strict control of the incoming barley lots is vitally important in order to reject heavily contaminated material prior to malting. However, malting conditions favor microbial growth and each process step in the barley to beer chain can be a source of additional microbes and their metabolites (*2*, *3*, *7*). Therefore, there is a need for safe ways to control the microbial community during processing. The present study confirmed that steeping activated the microbial community present in barley; therefore, this stage can be considered as the most critical step in the malting process. The number of microbes increased during the germination phase. Although kilning caused a reduction in microbial counts, the number of viable microbes in the finished malt was generally higher than that in the starting barley. Only about 1−2 log reduction in the viable counts of yeasts and bacteria was observed during kilning.

Nowadays, seed−microbial interactions are generally accepted as an integral part of the malting process. Microflora

Table 4. Effects of MRS-LA Treatment (Lactic Acid-Acidified, Unfermented MRS Broth) on Malt Quality and Enzyme Potential[a]

analysis		untreated samples		MRS-LA treatment	
		Kymppi	Mentor	Kymppi	Mentor
congress mashing					
extract content, fine grind	%	81.3	80.0	80.7	80.4
pH		5.86	5.92	5.85	5.83
color	EBC	4.7	4.1	5.0	4.4
FAN	mg/L	200	210	217	228
soluble nitrogen	mg/L	887	974	961	1042
β-glucan	mg/L	410	130	150	94
viscosity	cP	1.55	1.55	1.43	1.45
filtration time (coarse grinding, approximately 300 mL)	min	136	96	85	84
modification (Calcofluor)	%	94	91	90	88
high gravity mashing					
extract content (theoretical)	%	82.5	81.1	82.1	81.9
pH		5.46	ND[b]	5.45	ND
color	EBC	10	ND	13	ND
FAN	mg/L	422	457	441	480
β-glucan	mg/L	490	120	220	120
viscosity	cP	2.35	2.28	2.09	2.1
Büchner filtration, 15 min	g	76	79	100	81
Büchner filtration, 30 min	g	101	104	112	100
Büchner filtration, 60 min	g	108	112	117	113
Büchner filtration, area	gh	75	91	103	93
malt enzyme activity					
α-amylase	U/g	378	354	426	408
β-glucanase (30 °C) endogenous	U/kg	167	249	161	146
β-glucanase (60 °C) microbial[c]	U/kg	45	60	85	116
xylanase	U/kg	92	83	113	115

[a] Four pilot malting experiments (two untreated and two MRS-LA) were carried out with Kymppi and Mentor barley. [b] ND, not determined. [c] Background level, 45 U/kg.

management during malting should be conducted in such a way that the neutral or beneficial microbes are favored with simultaneous restriction of unwanted microbial populations. Several studies have demonstrated that bacteria and fungal cultures originating from barley, malt, or brewery environments can be used as biocontrol agents in malting. The yeastlike fungus *G. candidum*, the filamentous fungus *R. oligosporus*, and several LAB have been applied in malting (*27*−*29*, *43*, *50*, *51*, *62*, *63*). Our earlier studies revealed two strains, *L. plantarum* E76 and *P. pentosaceus* E390 (*28*−*31*, *37*), originating from malting and beer, to be suitable starter cultures for malting. Differences between the activities of the two LAB strains were observed, which makes individual optimization of the malting process necessary for each strain. The different characteristics of the two strains make them applicable to different types of bioprosessess. This study clearly showed that the thermophilic *P. pentosaceus* E390 persisted throughout the processing and survived better than *L. plantarum* E76 during the kilning.

In this study, liquid starter cultures were added into the steeping process of normal malting barley. Both the cells and the spent medium were added into the steeping waters, because not only the extracellular compounds but also the viable cells are needed for the maximum antimicrobial action (*28*, *32*). We also constructed MRS without glucose and supplemented the basal medium with 2.5% lactic acid (MRS-LA) in order to investigate the effects of spent medium and chemical acidification on the malting performance. Biological or chemical acidification notably altered the steeping conditions and thus affected the grain physiology. The present study indicated that low pH during steeping resulted in delayed barley germination and reduced water uptake. However, malt modification and enzyme production were not disturbed. Grain germination as well as starter performance are greatly influenced by the steeping conditions, especially temperature and gas atmosphere. The deficiencies in grain germination caused by starter cultures could be compensated by the temperature and respiratory control during processing. van Campenhout (*21*) reported that *P. pentosaceus* L7230 severely inhibited the acrospire development and slowed germination, which was recorded as reduced carbon dioxide production and heat release. However, she showed that starter performance could be improved by respiration control of barley after the inoculation stage (*21*). This study also indicated that it is extremely important to monitor and control the whole malting ecosystem simultaneously, both microbial activity and grain germination.

The composition of the microbial community was greatly influenced by the biological and chemical acidification of steeped barley. In accordance with our previous results (*28*, *29*, *31*), starter cultures restricted the growth of aerobic bacteria, which were mainly composed of Gram-negative bacteria such as enterobacteria and pseudomonads. A marked reduction in aerobic bacteria was observed throughout the malting process when starter cultures were applied. The strongest antimicrobial potential was obtained with the *L. plantarum* E76 strain. The antimicrobial action was partly due to the organic acids and low pH, as similar effects were obtained using chemically acidified MRS. In addition to organic acids, LAB produced low molecular weight antimicrobial compounds (*37*). Pseudomonads were particularly sensitive to LAB antimicrobials. The restriction of aerobic bacteria during steeping can be regarded as advantageous. Aerobic bacteria are known to be involved in water sensitivity and secondary dormancy, in which the grains do not germinate although they are steeped in optimal conditions (*8*, *9*). Furthermore, EPS produced by aerobic bacteria during malting may cause filtration problems later in the brewing process (*17*, *31*). Kreisz et al. (*18*) reported that even small amounts of bacterial polysaccharides such as gellan, levan, and xanthan released from malt or wort contaminants had negative impacts on wort filtration.

In addition to aerobic bacteria, LAB comprised a significant part of the microbial community in malting, although present in low numbers in the native barley. Our results were in line with an earlier observation that steeping activated the growth of indigenous LAB, although growth in pilot scale was not as intensive as in industrial scale (*7*). O'Sullivan et al. reported an increase from 50−100 to 10^8 cfu/g during the 48 h of steeping (*7*). The present study revealed a 10-fold increase of LAB during the pilot steeping, and the maximum 10^6 cfu/g was reached at the end of germination. The addition of starter cultures obviously changed the composition of the indigenous LAB population, mainly comprised of *Leuconostoc* species. *Leuconostoc* are often present in high numbers in the early stages of fermentations with plant materials (*64*). They are relatively sensitive to acidic conditions, and it has been shown that lactic acid contributes to the early elimination of *Leuconostoc* species in vegetable fermentations (*65*). *Leuconostoc* species are capable of producing slimy microbial polysaccharides, which may cause severe processing and quality problems (*17*, *66*).

The fungistatic effect of *L. plantarum* E76 and *P. pediococcus* E390 against *Fusarium* molds has been demonstrated in several laboratory scale experiments (*28*, *30*−*32*). This pilot scale study with five different barley varieties supported our previous findings. The antifungal action of LAB is often due to several mechanisms, and it can be partly explained by the production of organic acids. Many LAB are also known to produce specific antifungal compounds, which are involved in the antimicrobial action (*37*, *67*, *68*). Our results indicated that the unfermented MRS broth containing lactic acid (final concentration in the steeping water, 29 mM) restricted the growth of fusaria in pilot scale experiments. We have shown that MRS broth alone, adjusted with hydrochloric acid to pH 3.8, had no fungistatic effect (*32*). MRS broth contains sodium acetate, which may have a synergistic antifungal effect when combined with lactic acid. Stiles et al. (*69*) reported that this basic component of commercial MRS contributed to the antifungal properties of *L. rhamnosus* culture. We previously reported that *Fusarium* species differed in susceptibility to LAB antimicrobials and that the growth of *F. avenaceum* was restricted by lactic acid. (*32*). However, lactic acid and low pH even when combined with MRS broth could not inhibit the growth of *F. culmorum* or *F. graminearum* fungi (*32*). On the contrary, we have observed that a small amount of lactic acid stimulated the germination of *Fusarium culmorum* macroconidia (unpublished data). Therefore, the similar antifungal effect detected with starter treatment and MRS-LA treatment in the pilot scale malting experiments can be explained by the presence of sensitive *F. avenaceum* species, which have been the most commonly detected *Fusarium* species in Finnish grain samples in recent years (*70*). However, *Fusarium* flora may differ between different crops and locations. Therefore, organic acids alone are not recommended for control of the *Fusarium* community.

The antifungal effect of the studied starter cultures was surprisingly selective against fusaria. Other common field fungi belonging to the genera *Alternaria*, *Cephalosporium*, *Cladosporium*, and *Drechslera* were not inhibited by the addition of starter cultures. In fact, starter cultures actually promoted the growth of *Alternaria* fungi during the germination. Inhibition of fusaria by starter cultures provided more opportunities to *Alternaria*. A corresponding tendency was observed in studies carried out in the laboratory scale. Haikara et al. (*28*) reported that the number of *Alternaria*, *Cephalosporium*, *Cladosporium*, *Drecslera*, and *Mucor* were slightly higher after steeping when starter cultures were applied. *Alternaria* is known to compete on plants with other field fungi such as *Fusarium*, *Cladosporium*, and *Epicoccum*. Several investigators have reported antagonistic interactions between *Alternaria* and other field fungi, especially fusaria (*5*, *71*, *72*). When the number of kernels infected with *Fusarium* increases, a concurrent decrease in *Alternaria* contamination is observed. MRS-LA treatment had no significant effect on growth of filamentous fungi during the malting, although unfermented MRS-LA brought extra nutrients into the malting process.

This study clearly showed that the yeast population was not inhibited by the starter cultures. On the contrary, yeast growth was stimulated by both the starter and the MRS-LA addition. Yeasts are frequently observed in plant-based bioprocesses with low pH and low temperature (*73*). They often occur in plant fermentations together with LAB, and synergistic interactions between these two microbial groups are utilized in many cereal-based fermentation processes (*74*). Many species belonging to thegenera *Candida*, *Hansenula*, and *Pichia* have the capacity to utlilize organic acids as substrates for growth. High numbers of yeasts and yeastlike fungi are frequently observed in the malting process, but the composition and the role of this microbial group are still largely unknown. Yeasts belonging to the genera *Candida*, *Debaromyces*, *Hanseniaspora*, *Rhotorula*, *Saccharomyces*, *Sporobolomyces*, *Torulopsis*, and *Williopsis* have been detected in the malting process (*2*, *3*, *6*). Our preliminary studies revealed that yeasts and yeastlike fungi have a significant contribution to the production of cell wall-degrading enzymes (unpublished data).

The microbial community and especially fungi have a great influence on malt enzyme potential (*5*, *21*, *49*−*51*, *60*, *75*−*77*). This study revealed that by the addition of starter cultures it is possible to enhance the production of microbial β-glucanase and xylanase in the normal malting process. Lowe et al. (*62*) added LAB cultures into the steeping and found that all of the biologically acidified malts exhibited higher β-glucanase activity as compared to that in malt produced from untreated barley. They reported that enhanced enzyme potential obtained by biological acidification can be applied to compensate for reduced enzyme activity when other adjuncts such as unmalted barley are used in processing (*47*, *48*). In our study malt, endogenous β-glucanase activity was not increased by the starter addition. However, significantly higher glucanase activities, 40−60 U/kg, were observed in starter malts when the activity measurements were carried out at 60 °C, indicating the presence of microbial β-glucanases. The endogenous barley β-glucanases are heat labile and are inactivated at 60 °C and thus contributed only to a minor extent to the measured activity (*78*). In addition, MRS-LA treatment promoted the production of microbial β-glucanase. Furthermore, *L. plantarum* E76 treatment during steeping significantly enhanced the production of xylanase. It is well-known that xylanases are mainly produced by microbes, especially filamentous fungi (*79*). van Campenhout (*21*) reported that approximately 75% of malt xylanase activity was derived from the microbial community and 25% from the grain. Increased growth and activity of yeasts could explain part of the increased microbial xylanase and β-glucanase activity detected in starter samples and after MRS-LA treatment.

The enchanced enzyme activities measured in LAB-treated malts led to more intensive degradation of cell wall polysaccharides, which was recorded as decreased β-glucan level and wort viscosity and as improved lautering performance. Thereby, this study confirmed our previous findings (*28*, *29*, *31*). The beneficial effects were more pronounced with *L. plantarum* E76 starter culture. Wort filtration was also positively affected by chemical acidification with MRS-LA. In the pilot malts, the mash filterability was considered as good, and areas under the filtration curve were >85 gh^{-1} indicating good filterability (*56*), but starter cultures could still improve the filtration rates and volumes. Wort separation is often a bottleneck in brewhouse operations, and poor runoffs cause losses in production (*80*). Therefore, good and balanced mash filterability obtained with starter cultures can be regarded as advantageous. Mash filterability is a complex phenomenon, affected by many different factors. The filtration rate is dependent on the complexes formed between proteins and pentosans, β-glucans, residual starch, and lipids (*81*). Furthermore, arabinoxylans contribute to beer viscosity, foam stability, haze formation, and stability (*82*, *83*). Improved filtration observed with starter and MRS-LA-treated samples could also be due to the reduction of slime-forming bacteria. EPS produced by the malt-derived bacteria and also some yeasts may lead to mash filtration problems (*17*, *18*). The effects of starter cultures were more pronounced in the barley samples showing poor lautering performance due to the presence

of EPS-producing microbes in barley-containing split kernels (*31*). Anderson (*84*) reported that barley microflora may secrete flocculents to the surface of the malts, which may influence the filter bed porosity. Malt batches of the same crop but showing great variability in filtration may differ in microbial community; therefore, the amounts of the endogenous flocculants may vary, affecting the porosity of the filter beds. Starter technology offers a potential to equalize the fluctuations observed between different malt batches.

The results of this study revealed that LAB and MRS-LA treatment tended to darken wort color and to increase the amount of soluble and free amino nitrogen in the wort, which were considered to be a consequence of enhanced proteolysis. Lowe et al. (*62*) proposed that treatments such as with starters, which inhibit rootlet growth, cause an increase in soluble nitrogen presumably because nitrogen is not drained away to the roots. Excessive levels of soluble nitrogen are undesirable as they might have an impact on foam and haze properties of beers and as they decrease the microbiological stability of the finished beer (*1*). However, higher proteolysis in malt is preferred when larger proportions of starchy adjuncts are used as raw material.

The present study clearly indicated that lactic acid starter cultures added into the steeping of normal malting grade barley had a great influence on the microbial community and brewing performance of malt. Overall, it can be concluded that starter cultures had many beneficial effects on the malt quality, resulting in improved economy of the malting and brewing processes. Well-characterized microbial cultures offer a new tool for enhancing the antimicrobial potential and other functional activities in germinated cereals. By using a tailored malting process, it is possible to produce novel functional ingredients with the desired combination of valuable properties for the food and beverage industry.

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