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In situ enrichment of folate by microorganisms in beta-glucan rich oat and barley matrices

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ABSTRACT

The objective was to study folate production of yeast strains, bacteria isolated from oat bran, and selected lactic acid bacteria as well as one propionibacterium in oat and barley based models. Simultaneously, we aimed at sustaining the stability of viscosity, representing the physicochemical state of beta-glucan. Total folate contents were determined microbiologically and vitamers for selected samples by UHPLC.

Folate in yeast cells comprised mainly 5-methyltetrahydrofolate and tetrahydrofolate. Folate production by microbes in YPD medium was different to that in cereal fermentations where vitamers included 5-methyltetrahydrofolate, 5,10-methenyltetrahydrofolate and formylated derivatives. Microbes producing significant amounts of folate without affecting viscosity were *Saccharomyces cerevisiae* ALKO743 and *Candida milleri* ABM4949 among yeasts and *Pseudomonas* sp. ON8 and *Janthinobacterium* sp. RB4 among bacteria. Net folate production was up to 120 ng/g after 24 h fermentation and could increase during 2-week storage. Glucose addition increased the proportion of 5-methyltetrahydrofolate. *Streptococcus thermophilus* ABM5097, *Lactobacillus reuteri*, and *Propionibacterium* sp. ABM5378 produced folate but in lower concentrations.

Both endogenous and added microbes contribute to folate enhancement. Selection of microbes with folate producing capability and limited hydrolytic activity will enable the development of products rich in folate and beta-glucan.

1. Introduction

Oat (*Avena sativa* L.) and barley (*Hordeum vulgare* L.) are special among cereal grains due to their high content of soluble dietary fibre, mostly (1 → 3)(1 → 4)-beta-D-glucan. They are also valuable sources of many bioactive compounds, including several vitamins. The consumption of oat has been increasing due to its well-documented health benefits. Barley is cultivated mainly for feed and brewing industry. The use of barley for breadmaking is limited but still, with its high dietary fibre content it has gained good reputation as a healthy dietary choice.

Several health promoting effects of oat and barley have been attributed to beta-glucan. Beta-glucan has been reported to act as a prebiotic promoting the growth of beneficial intestinal bacteria (Mitsou et al., 2010; Shen et al., 2012). It has also been postulated to help in reducing and maintaining body weight as well as to improve immune functions (Daou and Zhang, 2012). The most convincing evidence of the beneficial role of oat beta-glucan is related to the regulation of glucose absorption and serum cholesterol reduction. Based on the solid scientific evidence, European Food Safety Authority has permitted the use of cholesterol-lowering health claim in oat and barley foods providing at least 3 g/d of beta-glucan (EFSA, 2009, 2011b). Another health claim involves the reduction of post-prandial glycaemic response, and in order to obtain the claimed effect, 4 g of beta-glucans from oats or barley for each 30 g of available carbohydrates should be consumed per meal (EFSA, 2011a). These beneficial effects of beta-glucan are thought to be related to its viscous properties in the small intestine. Therefore, maintaining high beta-glucan viscosity during food processing is of prime importance in sustaining the health promoting function.

Folate, belonging to the B-vitamin group, functions in providing one-carbon groups for several essential reactions related to amino acid metabolism, nucleotide biosynthesis, and methylation cycle. Folate deficiency causes megaloblastic anaemia and neural tube defects (NTD) in foetus, and there is solid scientific evidence to conclude that periconceptional folic acid supplementation prevents NTDs (MRC Vitamin Study Research Group, 1991). In addition, folate is intensively investigated for other health impacts, many of them dealing with important future challenges of public health. Suboptimal intake of folate has been associated with increased risk for cardiovascular diseases, stroke, certain cancers, and decline in cognitive functions, as reviewed by Iyer and Tomar (2009). Folate intake often falls below recommendations, which typically vary from 300 to 400 μg/d, and optimal health effects of folate are probably not achieved with the current recommended intakes (Dhonukshe-Rutten et al., 2009).

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Table 1
Microbial strains and their origins used in the experiments.

Microorganism		Origin	Reference
Yeasts			
ALKO743	*Saccharomyces cerevisiae*	Baker's yeast; control strain	Mäntynen et al. (1999)
CBS7764	*Saccharomyces cerevisiae*	Rainbow trout intestine	Andlid et al. (1999)
ABM5131	*Saccharomyces cerevisiae*	Kefir grains	Korhola et al. (under review)
ABM4949	*Candida milleri*	Rye sourdough starter	Mäntynen et al. (1999)
ABM5130	*Kluyveromyces marxianus*	Kefir grains	Korhola et al. (under review)
ABM5147	*Clavispora lusitaniae*	Fermented oat product	Korhola et al. (under review)
Bacteria			
ON1	*Pantoea ananatis*	Oat bran	Herranen et al. (2010)
ON8	*Pseudomonas* sp.	Oat bran	Herranen et al. (2010)
RB4	*Janthinobacterium* sp.	Oat bran	Herranen et al. (2010)
ABM5119	*Bacillus* sp.	Oat bran; control strain	Herranen et al. (2010)
ABM5097	*Streptococcus thermophilus*	Commercial youghurt	Kariluoto et al. (2006)
	Lactobacillus reuteri	Fruit juice	
LC-705	*Lactobacillus rhamnosus*	Dairy starter	
ABM5378	*Propionibacterium* sp.	Cheese starter	

Several countries throughout the world attempt to ensure adequate folate intake and prevent NTDs by mandatory folic acid fortification of cereal products. However, also non-fortified cereal products are good folate sources — for instance, in Finland where fortification is not practised cereal products contribute to one third of the daily folate intake. Thus, even a moderate increase in folate content could substantially improve folate status.

Folate contents in oat and barley are relatively high. According to an extensive diversity screen organised in the European Healthgrain project, the average total folate contents in oat and barley cultivars (566 and 657 ng/g dm, respectively) were at the same level or even higher than in winter wheat cultivars (561 ng/g dm) (Andersson et al., 2008; Piironen et al., 2008; Shewry et al., 2008). Edelmann et al. (2012, 2013) reported somewhat higher concentrations – on average 685 ng/g for oat and for 773 ng/g for barley – for cultivars analysed soon after harvesting. Folate is known to be concentrated in the outer layers of the kernel (Edelmann et al., 2012, 2013).

Oat and barley are consumed largely as whole grain products. Although a few novel applications, such as snacks and milk substitutes, have been developed, the potential health benefits of these two cereals still remain underutilized. In order to increase the human consumption of oat and barley, new processes and applications are needed. Aqueous processes could be tailored to combine the enhancement of bioactive compounds with the beneficial effects of dietary fibre, especially beta-glucan. For instance, Angelov et al. (2005) found heat-treated oat mash a suitable medium for fermentation by both pure and mixed cultures of probiotic lactic acid bacteria and yeast strains, and the resulting ferments had high and stable beta-glucan contents. However, since the physiological effects of beta-glucan are related to its rheological properties, factors affecting its viscosity have to be controlled.

Biofortification and particularly fermentation fortification have been intensively studied in recent years. Folate overproduction by metabolic engineering has been successfully carried out among several lactic acid bacteria (Burgess et al., 2009). It was proved in a depletion-repletion bioassay that folate produced by metabolic engineering of *Lactococcus lactis* is indeed bioavailable and alleviates folate deficiency (LeBlanc et al., 2010). However, due to legislative limitations and negative perception of genetic modification by consumers, selection of natural overproducers has gained favour.

Fermentation of cereal substrates offers an economical way of improving nutritional value, sensory and functional properties, and shelf life. Traditional fermented, cereal-based foods cover a wide variety of products and exploit not only lactic acid fermentation but also other endogenous bacteria, yeasts and moulds (Blandino et al., 2003). We have shown that endogenous bacteria may produce folate, and that traditional sourdough fermentation increases the folate content in rye bread (Kariluoto et al., 2004, 2006, 2010). Hjortmo et al. (2008b) demonstrated that by selection of yeast strain and optimization of cultivation procedure the folate enhancement can be transferred to baking and can result in white wheat bread with 3–5-fold higher folate contents.

In our previous studies we have investigated bacteria isolated from oat bran products, screened them for their hydrolytic activities and studied their folate production capability as well as vitamer distribution in rich medium (Herranen et al., 2010; Kariluoto et al., 2010). The objective of the current study was to examine the folate production potential of endogenous bacteria, selected lactic acid bacteria, and yeasts in different oat and barley based cereal models while sustaining the stability of viscosity, i.e. the physicochemical state of beta-glucan. In addition, yeasts were characterised for their folate contents and compositions in YPD medium.

2. Material and methods

2.1. Microbes and cereal fermentations

Based on previous screening of yeasts and bacterial strains isolated from oat and rye products (Herranen et al., 2010; Kariluoto et al.,

Table 2
Raw materials and processes in oat and barley fermentations.

Matrix	Process	Microbes
3.5% oat bran OatWell® 14.0% beta-glucan (Swedish Oat Fiber AB, Bua, Sweden)	Heat treatment at 70 °C overnight Mixing with sterile water (3.5% flour in water, w/v) Cooking for 10 min	*w/ and w/o 1% glucose*: ALKO743, ABM4949, ABM5130, ABM5147, ON1, ON8, RB4, ABM5119
10% oat flour Elovena oat flake 4.6% beta-glucan (Raisio Plc, Raisio, Finland), milled in a laboratory mill to 0.5 mm particle size	Mixing with sterile water (10% flour in water, w/v) Cooking for 10 min Autoclaving at 121 °C for 20 min	*w/o 1% glucose*: ALKO743, ABM5131, ON8, ABM5097 *w/ 1% glucose*: ALKO743, ABM5131, ABM4949, ON8, ABM5097, *L. reuteri*, *L. rhamnosus*, ABM5378
3.5% barley bran Bonafibre barley bran 16.3% beta-glucan (Bonafiber Oy, Lahti, Finland)	Mixing with sterile water (3.5% flour in water, w/v) Cooking for 10 min Autoclaving at 121 °C for 20 min	*w/o 1% glucose*: ABM5130, ON8 *w/ 1% glucose*: ALKO743, ABM4949, ABM5130, ABM5147, ON1, ON8, RB4, ABM5119, ABM5097, *L. reuteri*, *L. rhamnosus*, ABM5378
20% barley flour Barley groats, wholegrain 4.5% beta-glucan (Myllyn Paras Ltd, Hyvinkää, Finland), milled in a laboratory mill to 0.5 mm particle size	Mixing with sterile water (20% flour in water, w/v) Cooking with Termamyl for 20 min (15 ml Termamyl/3 l flour–water mixture) Autoclaving at 121 °C for 20 min	*w/ and w/o 1% glucose*: ALKO743, ABM4949, ABM5130, ABM5147, ON1, ON8, RB4, ABM5119