Influence of Whey, Whey Component and Malt on the Growth and Acids Production of Lactobacilli in Milk

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Abstract

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The effect of whey powder, whey protein concentrate, caseinomacropeptide, and malt addition into milk on the growth and acid production of lactobacilli (*Lactobacillus casei* Lafti L-26, *Lactobacillus acidophilus* CCDM 151, and *Lactobacillus casei* CCDM 198) was evaluated. The ability of these strains to use different types of saccharides from milk and plant sources was also tested. Glucose, galactose, fructose and maltose were utilised by all tested strains. The results showed that the addition of malt positively affected the growth of lactobacilli strains compared to the growth in milk enriched by whey ingredients. The addition of malt increased significantly the production of D(–) isomer of lactic acid by *Lactobacillus acidophilus* CCDM 151 and *Lactobacillus casei* CCDM 198 and the production of acetic acid by *Lactobacillus casei* CCDM 198.

Keywords: *Lactobacillus*; caseinomacropeptide; whey protein; malt; acetic acid; lactic acid

One of the most attractive alternatives of whey usage for human consumption is the manufacture of fermented or non-fermented whey or combined whey-milk products, namely when also some probiotic bacteria are used as further health-promoting component of these products. These kinds of beverages could be considered as a good carrier for the probiotics (Almeida *et al*. 2009) as the whey proteins improve the viability of lactic acid bacteria (LAB) due to enhancing the buffering capacity of culture medium (Kailasapathy & Supriadi 1996). Whey proteins and peptides derived from these proteins were confirmed to possess many health benefits. Apart from being a balanced source of valuable and essential amino acids, whey proteins and their hydrolysates are associated with antihypertensive, antimicrobial, anticarcinogenic, and immunomodulatory activities (Korhonen 2009).

Cereals are other useful fermented milk additional components. Some of them can act as prebiotics due to the content of non-digestible saccharides and also can enhance the survival of LAB during the passage through the gastrointestinal tract (Patel *et al*. 2004). The aim of the present paper was to investigate the growth of selected probiotic lactobacilli in milk with the addition of whey components and malt and also to test the influence of these substances on the production of lactic and acetic acids which possess the impact on the sensory quality of milk based products. As the vitality and viability of LAB are not only influenced by food matrix parameters but also are strain dependent, one commercial probiotic strain of *Lactobacillus casei* Lafti L-26 and two strains with certain probiotic properties formerly proved (Horáčková *et al.* 2011) were used.

MATERIAL AND METHODS

Microorganisms. *Lactobacillus casei* Lafti L-26 (DSM Food Specialties, Heerlen, Netherlands); *Lactobacillus acidophilus* CCDM 151; *L. casei* CCDM 198

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(Culture Collection of Dairy Microorganisms, Laktoflora®, Milcom, Prague, Czech Republic).

Cultivation and determination of lactobacilli count. For the inoculation of tested media, the overnight lactobacilli cultures cultivated in 5% (v/v) CO_2 atmosphere at 37°C in MRS broth (Merck, Darmstadt, Germany), pH 5.6, were used.

For the count determination, lactobacilli were cultivated at 37°C in a CO_2 atmosphere (5% v/v) for 48 h and counted as colony forming units (CFU) per ml on MRS agar (Merck, Darmstadt, Germany), pH 5.6.

Lactobacilli growth in fermentation media with different saccharides. The fermentation medium (5 g yeast extract, 5 g peptone, 2 g sodium acetate, 1 ml Tween 80, 0.4 g $MgSO_4 \cdot 6H_2O$, 0.2 g $MnSO_4 \cdot 4H_2O$, 0.2 g $FeSO_4 \cdot 6H_2O$, 0.2 g NaCl in 1 l of distilled water, pH adjusted to 5.8, sterilised at 121°C 15 min) enriched with different types of saccharides (lactose, glucose, galactose, maltose, arabinose, xylose, cellobiose, and fructose) in a concentration of 20 g/l was used. Cultivations were carried out in microtitre plates in Biotek (Bio Tek U.S., Seattle, USA). The wells were filled with 200 µl of fermentation media with different saccharides and 2 µl of overnight lactobacilli culture. The microtitre plates were incubated at 37°C in an aerobic atmosphere for 26 hours. The cell growth was monitored by the absorbance measurement at 600 nm. All samples were tested in triplicate.

Cultivation of strains in skim milk and in skim milk with the addition of whey or malt. The effect of the addition of whey powder (Spray dry whey; Moravia Lacto, Jihlava, Czech Republic), whey protein concentrate (WPC) (Whey Protein Concentrate 70; PML Protein Mléko Laktóza, Nový Bydžov, Czech Republic), caseinomacropeptide (CMP) (Ekomilk, Frýdek Místek, Czech Republic), Pilsner malt (Research Institute of Brewing and Malting, Prague, Czech Republic) and dry malt extract Sladovit (Sladovna Bruntál, Czech Republic) on the growth of selected lactobacilli in skim milk was tested during 24 h cultivation. The addition of 5 g into 95 g of skim milk was used for all ingredients. Malt grains were first ground in a KM4 laboratory mill (Labora, Prague, Czech Republic) with a sieve mesh size of 0.5 mm. Media were twice sterilised at 100°C for 15 min in the interval of 24 hours. Lactobacilli inoculum (1 ml) was used for subsequent cultivation at 37°C in CO_2 atmosphere. The samples were taken in 0, 3, 6, 18, and 24 h and the number of cells expressed as CFU/ml was counted by the plate method on MRS agar after the appropriate dilution. pH of samples was measured by digital pH meter 3020 (Jenway, Staffordshire, UK). Titratable acidity was measured by titrating 25 ml of the sample with 0.25 mol/l NaOH and expressed in °SH units.

The results are the means from two independent repetitions, both parallel samples were analysed twice (n = 4).

Determination of lactic and acetic acids. The amount of lactic and acetic acids was determined by Megazyme enzyme kits (Megazyme International, Bray, Ireland) and the results are expressed as a mean from two samples.

Statistical analyses. The significance of differences between the pH values was tested by Student's t-test (α = 0.01).

RESULTS AND DISCUSSION

First, the lactobacilli were cultivated in a medium with different saccharides of milk and cereal origin as carbon sources (data not shown). It was found out that glucose, galactose, fructose, and maltose were utilised by all tested strains well. Fructose, glucose, and maltose represent the main saccharides in malt hydrolysates (Rozada-Sanchéz *et al.* 2008); glucose and galactose are utilised after hydrolysis of lactose, present both in milk and whey, by β-galactosidase, enzyme present in all LAB. *L. casei* Lafti L-26 did not utilise xylose and cellobiose, *L. acidophilus* CCDM 151 did not utilise xylose and arabinose, and *L. casei* CCDM 198 did not utilise cellobiose. There were differences observed between strains, the best growth was proved generally for *L. casei* Lafti L-26.

Table 1 demonstrate the effect of addition of 5 g of whey, WPC, CMP, malt, and malt extract into 95 g of skim milk on the growth of lactobacilli during the 24 h cultivation at 37°C. When cultivated in milk and milk with the addition of whey, WPC and CMP, the final increase in the cell number by 1.5–2 log cycles was found out which was similar to the cultivation in pure milk. Results obtained for supplementation of milk by whey proteins, WPC and CMP are contradictory (Bury *et al.* 1998; Dave & Shah 1998; Martín-Diana *et al.* 2003; Antunes *et al.* 2005). Some authors did not prove a difference in the growth of probiotic lactobacilli in milk with added whey protein supplements (Martín-Diana *et al.* 2003), others proved a stimulation even by the application of 2% WPC into milk (Božanič *et al.* 2004). The growth promoting activity of WPC could be due to CMP and whey protein content (Janer *et al.* 2004). CMP contains not only available nitrogen for the bacterial growth but also amino-saccharides,

Table 1. Number of cells of *L. casei* Lafti L-26, *L. acidophilus* CCDM 151, and *L. casei* CCDM 198 (log CFU/ml) during 24 h cultivation at 37°C in different tested media

Time (h)	Milk	M+whey	M+WPC	M+CPM	M+malt	M+extract
L. casei Lafti L-26						
0	6.77	6.72	7.19	6.69	6.66	6.78
3	6.89	6.96	7.25	6.80	6.67	6.75
6	7.54	7.59	7.65	7.44	7.67	7.83
18	8.57	8.53	8.67	8.93	9.12	8.70
24	8.60^{a}	8.76^{a}	8.89^{a}	8.97^{b}	9.49^{b}	8.91^{b}
L. acidophilus CCDM 151						
0	6.47	6.42	6.75	6.34	6.53	6.54
3	6.59	6.79	6.84	6.46	6.64	6.75
6	6.61	7.01	7.27	6.75	6.97	7.08
18	8.63	8.84	8.89	8.27	8.84	8.83
24	8.64^{a}	8.88^{a}	9.01^{b}	8.42^{a}	9.84^{b}	8.93^{b}
L. casei CCDM 198						
0	6.47	6.42	6.75	6.34	6.53	6.54
3	6.59	6.79	6.84	6.46	6.64	6.75
6	6.61	7.01	7.27	6.75	6.97	7.08
18	8.63	8.84	8.89	8.27	8.84	8.83
24	8.64^{a}	8.88^{a}	9.01^{b}	8.42^{a}	9.84^{b}	8.93^{b}

M = milk; WPC = whey protein concentrate; CPM = caseinomacropeptide; extract = malt extract; values in the same row followed by different lowercase letters are significantly different (α = 0.01)

such as sialic acid and *N*-acetylgalactosamine, which could be utilised as prebiotics by some probiotics, namely bifidobacteria (Azuma *et al*. 1984). It is evident from Table 1 that a significant increase in the number of viable lactobacilli cells was detected after the supplementation of 95 g milk by 5 g malt or malt extract. After 24 h cultivation the number of cells was significantly different for all three tested lactobacilli.

When using milk with malt, the number of viable cells increased by 3 log cycles in the case of *L. casei* Lafti L-26 and by 3.3 log cycles in the case of *L. acidophilus* CCDM 151 after 24 h cultivation. Also, the specific growth rates of *L. casei* Lafti L-26 and *L. casei* CCDM 198 were significantly higher compared to those in milk (data not shown). There are not many papers documenting the effect of malt addition to milk on the growth of probiotic lactobacilli, but Ranadheera *et al.* (2010) found also the stimulating effect of malt on the growth of several lactobacilli including *Lactobacillus acidophilus* due to the presence of fermentable saccharides and free

Table 2. pH values of different media at the beginning and after 24 h cultivation of tested lactobacilli at 37°C

Media	*L. casei* Lafti L-26		*L. acidophilus* CCDM 151		*L. casei* CCDM 198	
	0 h	24 h	0 h	24 h	0 h	24 h
Skim milk	6.57^{a}	4.81^{a}	6.67^{a}	4.68^{a}	6.58^{a}	4.68^{a}
Milk + whey powder	6.43^{b}	4.86^{a}	$6.35^{b,c,d}$	4.35^{b}	6.43^{b}	$4.89^{a,c}$
Milk + WPC	6.47^{b}	4.94^{a}	$6.45^{b,c}$	4.80^{a}	6.44^{b}	5.51^{b}
Milk + CMP	6.29^{c}	5.13^{b}	$6.30^{b,d}$	5.16^{c}	6.27^{c}	5.37^{c}
Milk + malt extract	6.49^{b}	3.85^{c}	$6.39^{b,c}$	3.72^{d}	$6.52^{a,b}$	3.72^{d}
Milk + malt	$6.54^{a,b}$	3.96^{c}	$6.44^{b,c}$	3.67^{d}	$6.52^{a,b}$	3.65^{d}

WPC = whey protein concentrate, CPM = caseinomacropeptide; values in the same column followed by different lowercase letters are significantly different (α = 0.01)

Table 3. Values of titratable acidity of different media at the beginning and after 24h cultivation of tested lactobacilli at 37°C and lactic/acetic acid ratio at 24 h

Media	*L. casei* Lafti L-26			*L. acidophilus* CCDM 151			*L. casei* CCDM 198		
	titratable acidity (°SH)		acid ratio*	titratable acidity (°SH)		acid ratio*	titratable acidity (°SH)		acid ratio*
	0 h	24 h		0 h	24 h		0 h	24 h	
Skim milk	6.8	27.6	0.5	7.4	28.6	10.3	7.4	28.2	8.9
Milk + whey powder	9.8	37.8	1.3	10.0	26.6	7.9	9.2	23.8	12.0
Milk + WPC	9.8	25.6	2.9	9.2	57.6	15.6	9.4	27.8	11.8
Milk + CMP	15.2	29.2	1.2	15.6	37.2	14.2	12.4	28.8	15.1
Milk + malt extract	8.4	51.8	1.9	10.8	66.6	19.0	8.2	66.4	3.3
Milk + malt	7.8	50.4	4.0	8.8	68.6	19.8	8.0	71.2	1.0

WPC = whey protein concentrate, CPM = caseinomacropeptide; *lactic/acetic acid molar ratio after 24-h cultivation

nitrogen. Attention was also paid to the effect of malt on the viability of probiotic lactobacilli in culture media. The positive effect of malt on the viability of several lactobacilli strains of different species including *L. acidophilus*, *L. plantarum*, and *L. reuteri* was proved (Charalampopoulos *et al.* 2002; Charalampopoulos & Pandiella 2010).

Significant differences in the final pH value between milk containing whey component and milk supplemented by malt or malt extract were found after 24 h cultivation (Table 2). At the beginning of cultivation, the pH values were influenced by different supplements and also by the strain inoculated into the media. While differences in pH value were from 0.9 to 2.0 in the case of milk with whey components, in malt supplemented milk they reached 2.6 to 2.9 after 24 hours. In general, all lactobacilli decreased pH in milk supplemented by whey components to a lesser extent and in the malt supplemented milk to a higher extent compared with the skim milk. Oliveira *et al.* (2001), who compared the effect of milk supplementation and culture composition on milk acidification, concluded that the acidification activity was greatly improved with casein hydrolysate but not by the addition of whey. The positive effect of malt on the acidification activity of lactobacilli was also confirmed by Charalampopoulos *et al.* (2002). The final pH of fermented products is influenced by the buffering capacity of the medium which is in relation to the amount and type of proteins present.

Lactic and acetic acids are the main products of carbohydrate fermentation in *Lactobacillus* genus. The content of lactic and acetic acids has an impact on sensory properties of fermented products. Furthermore, lactate and acetate as representatives of non-branched short chain fatty acids can play a positive role in human nutrition. The presence of short chain fatty acids in the intestine lowers pH, increases bioavailability of calcium and magnesium and inhibits the growth of potentially harmful bacteria (Craeyveld *et al.* 2008). The content of both acids was analysed in all tested media after 24 h fermentation and the results are presented in Figure 1. All

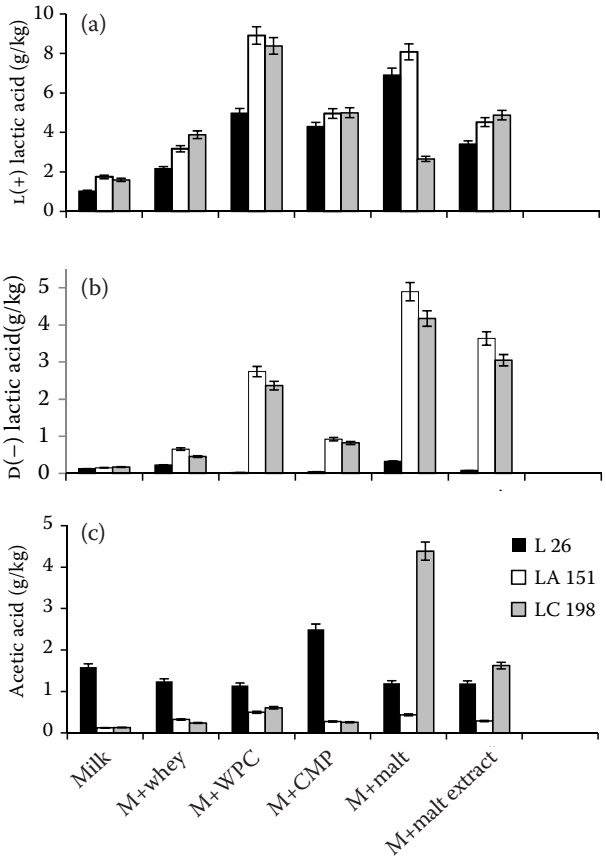

Figure 1. The content of (a) L(+) actic acid, (b) D(–) lactic acid, and (c) acetic acid after 24 h cultivation of tested lactobacilli at 37°C

L 26 = *Lactobacillus casei* Lafti L-26; LA 151 = *Lactobacillus acidophilus* CCDM 151; LC 198 = *Lactobacillus casei* CCDM 198

added substances increased the amount of lactic acid. The highest content of L(+) lactic acid was detected in the case of *L. acidophilus* CCDM 151 and *L. casei* CCDM 198 in milk with whey protein concentrate (8.9 and 8.3 g/kg, respectively) and in the case of *L. casei* Lafti L-26 in milk with malt (6.9 g/kg). After cultivation of all three tested strains in milk, only traces of D(–)lactic acid were detected. The addition of malt and malt extract increased significantly the production of D(–) isomer of lactic acid at two out of the three tested lactobacilli strains (*L. acidophilus* CCDM 151 and *L. casei* CCDM 198; 4.9 and 4.2 g/kg, respectively). Malt and malt extract addition also increased the production of acetic acid by *L. casei* CCDM 198. Charalampopoulos and Pandiella (2010) found a lower level of lactic acid production in malt-containing media after the cultivation of *L. plantarum* (2.1 g/l) similarly like Helland *et al.* (2004), who reported only 1.36–4.0 g/kg of lactic acid in maize porridge with added malted barley. However, some authors determined more than 15.0 g/kg of lactic acid in a malt medium after the cultivation of *Bifidobacterium breve* (Rozada-Sanchez *et al*. 2009). Furthermore, the values of titratable acidity and lactic/acetic acid molar ratio were compared (Table 3). It is evident that titratable acidity is influenced not only by the production of lactic and acetic acids but also by acid reactive residues of amino acids contained in the proteins of culture media. Different lactic/acetic acid molar ratios were obtained between different species and also between two strains of *L. casei*. These results are in accordance with statements of other authors (Zalán *et al*. 2010) who proved that the real fermentation profile of LAB is intensively influenced by the composition of media, cultivation conditions and strain and that it can differ from the theoretical end products of metabolic pathways.

CONCLUSION

It was proved that the addition of 5 g of malt-based supplements into 95 g of milk increased the final number of probiotic lactobacilli used for fermentation. At the same time, it influenced more the amount and composition of produced metabolites compared with the supplementation of milk by whey products. Furthermore, the differences in growth and biochemical activity between the tested strains were proved in the same media. Careful selection of probiotic LAB strains and cultivation conditions for the new milk fermented products supplemented with whey or cereal-based additives is necessary.

References

Almeida K.E., Tamine A.Y., Oliviera M.N. (2009): Influence of total solids content of milk whey on the acidifying profile and viability of various lactic acid bacteria. Food Science and Technology, **42**: 672–678.

Antunes A., Cazetto T., Bolini H. (2005): Viability of probiotic micro-organisms during storage, postacidification and sensory analysis of fat-free yogurts with added whey protein concentrate. International Journal of Dairy Technology, **58**: 169–173.

Azuma N., Yamauchi K., Mistuoka T. (1984): Bifidus growth-promoting activity of a glycomacropeptide derived from human κ-casein. Agricultural and Biological Chemistry, **48**: 2159–2162.

Božanič R., Tratnik L.J., Herceg Z., Maric O. (2004): The influence of milk powder, whey protein concentrate and inulin on the quality of cow and goat acidophilus milk. Acta Alimentaria, **33**: 337–346.

Bury D., Jelen P., Kimura K. (1998): Whey protein concentrate as a nutrient supplement for lactic acid bacteria. International Dairy Journal, **8**: 149–151.

Charalampopoulos D., Pandiella S.S. (2010): Survival of human derived *Lactobacillus plantarum* in fermented cereal extracts during refrigerated storage. LWT-Food Science and Technology, **43**: 431–435.

Charalampopoulos D., Pandiella S.S., Webb C. (2002): Growth studies of potentially probiotic lactic acid bacteria in cereal-based substrates. Journal of Applied Microbiology, **92**: 851–859.

Craeyveld V., Swennen K., Dornez E., Wiele T., Marzorati M., Verstraete W., Delaedt Y., Onagbesan O., Decuypere E., Buyse J. (2008): Structurally different wheat-derived arabinoxylooligosaccharides have different prebiotic and fermentation properties in rats. Journal of Nutrition, **138**: 2348–2355.

Dave R., Shah N.P. (1998): Ingredient supplementation effects on viability of probiotic bacteria in yogurt. Journal of Dairy Science, **81**: 2804–2816.

Helland M.H., Wicklund T., Narvhus J.A. (2004): Growth and metabolism of selected strains of probiotic bacteria in milk- and water-based cereal puddings. International Dairy Journal, **14**: 957–965.

Horáčková Š., Žaludová K., Plocková M. (2011): Stability of selected lactobacilli in the condition simulating gastrointestinal tract. Czech Journal of Food Sciences, **29**: 30–35.

Janer C., Peláez C., Requena T. (2004): Caseinomacropeptide and whey protein concentrate enhance *Bifidobacterium lactis* growth in milk. Food Chemistry, **86**: 263–267.

Kailasapathy K., Supriadi D. (1996): Effect of whey protein concentrate on the survival of *Lactobacillus aci-*

dophilus in lactose hydrolyzed yogurt during refrigerated storage. Milchwissenschaft, **51**: 566–569.

Korhonen H. (2009): Milk-derived bioactive peptides: from science to applications. Journal of Functional Foods, **1**: 177–187.

Martín-Diana A.B., Janer C., Peláez C., Requena T. (2003): Development of fermented goat's milk containing probiotic bacteria. International Dairy Journal, **13**: 827–833.

Murakami M., Tounouchi H., Takahashi R., Kitazawa H., Kawai Y., Negishi H. (2004): Structural analysis of a new anti-hypertensive peptide (β-lactosin B) isolated from a commercial whey product. Journal of Dairy Science, **87**: 1967–1974.

Oliveira M.N., Sodini I., Remeuf F., Corrieu G. (2001): Effect of milk supplementation and culture composition on acidification, textural properties and microbiological stability of fermented milks containing probiotic bacteria. International Dairy Journal, **11**: 935–942.

Patel H.M., Pandiella S.S., Wang R.H., Webb C. (2004): Influence of malt, wheat and barley extracts on the bile tolerance of selected strains of lactobacilli. Food Microbiology, **21**: 83–89.

Rozada-Sanchéz R., Sattur A.P., Thomas K., Pandiella S.S. (2008): Evaluation of *Bifidobacterium* spp. for the production of a potentially probiotic malt based beverages. Process Biochemistry, **43**: 848–854.

Ranadheera R.D.C.S., Baines S.K., Adams M.C. (2010): Importance of food in probiotic efficacy. Food Research International, **43**: 1–7.

Rozada-Sanchez R., Vázquez J.A., Charalampopoulos T., Pandiella S.S. (2009): Effect of storage temperature and media composition on the survivability of *Bifidobacterium breve* NCIMB 702257 in malt hydrolysate. International Journal of Food Biochemistry, **73**: 14–21.

Zalán Z., Hudáček J., Štětina J., Chumchalová J., Halász A. (2010): Production of organic acids by *Lactobacillus* strains in three different media. European Food Research and Technology, **230**: 395–404.

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