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Lactobacillus adjuncts in cheese: Their influence on the degradation of citrate and serine during ripening of a washed curd cheese

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Abstract

Lactobacillus adjuncts, differing in metabolic properties, were evaluated in a washed curd, brine salted Dutch-type cheese during long term ripening. Two different adjuncts were used; *Lb. plantarum* INF15D degrading serine (Ser) and citrate and possessing glutamate dehydrogenase (GDH) activity and *Lb. paracasei* ssp. *paracasei* INF448 with only a weak ability to degrade citrate. *Lb. plantarum* INF15D degraded residual citrate in cheese to aspartate (Asp). Early during ripening, Asp was degraded to acetoin most probably when the cells were in a growing state. Degradation of Ser by *Lb. plantarum* INF15D contributed to the production of formate and succinic acid in the early stage of ripening, while acetate was produced during later ripening. *Lb. paracasei* ssp. *paracasei* INF448 produced lactate from residual citrate and did not degrade Ser or Asp in the cheese.

Keywords: Cheese; *Lactobacillus*; Adjunct; Citrate; Serine; Aspartate; Glutamate dehydrogenase (GDH)

1. Introduction

The degradation of lactose, citrate and the catabolism of amino acids by lactic acid bacteria (LAB) play a major role in the aroma formation of Dutch type cheeses. Citrate may be metabolised by many strains of LAB (Hugenholtz, 1993). In cheese made with aromatic mesophilic starters such as Dutch type varieties, citrate is degraded by *Leuconostoc* ssp. and the Cit^+ *Lactococcus* (*Lc.*) *lactis* subsp. *lactis* biovar *diacetylactis* to acetoin and diacetyl in the presence of lactose rapidly after cheese manufacture (Walstra, Noomen, & Geurts, 1993). Decarboxylation to diacetyl requires O_2, and acetoin is therefore most likely to be formed in cheese (Hugenholtz, 1993; McSweeney & Sousa, 2000).

The non-starter lactic acid bacteria (NSLAB) flora in cheese is dominated by mesophilic lactobacilli (Beresford, Fitzsimons, Brennan, & Cogan, 2001; Peterson & Marshall, 1990). A number of studies demonstrated that certain species of NSLAB isolated from milk and cheese such as *Lactobacillus* (*Lb.*) *plantarum*, *Lb. viridensces*, *Lb. casei*, *Lb. paracasei*, *Lb. zeae* and *Lb. rhamnosus* have the capacity to metabolise citrate (Drinan, Tobin, & Cogan, 1976; Dudley & Steele, 2004; Freitas, Pintado, Pintado, & Malcata, 1999; Hickey, Hillier, & Jago, 1983; Palles, Beresford, Condon, & Cogan, 1998; Thomas, 1987). In Cheddar cheese, which normally contains residual citrate, Thomas (1987) found that various *Lb. plantarum* strains degraded citrate under anaerobic conditions. Later a study of Palles et al. (1998) confirmed that both growing and non-growing cells of *Lb. casei* and *Lb. plantarum* metabolised citrate with acetate and acetoin being the major products with optimum at pH 4.5, also in the absence of sugar as an energy source. De Figueroa, De Cardenas, Sesma, and Alvarez (1996) and De Figueroa, Alvarez, De Ruiz Holgado, Oliver, and Sesma (2000) demonstrated that in fact several strains of *Lb. rhamnosus* and *Lb. plantarum* species were capable of using citrate as a sole energy source when grown in complex media. This was confirmed in the study by Jyoti, Suresh, and Venkatesh (2003), which showed that *Lb. rhamnosus* could grow on citrate as the only carbon source producing lactate, acetoin, acetate and diacetyl; once citrate exhausted, it

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continued further growth on lactate. In a study of citrate degradation by NSLAB isolated from Cheddar cheese, Dudley and Steele (2004) showed that *Lb. casei*, *Lb. zeae* and *Lb. rhamnosus* produced acetate, lactate and ethanol from citrate, while the *Lb. plantarum* strains produced aspartate (Asp), acetate, lactate and succinate from citrate. They concluded that *Lb. plantarum* strains produce succinate from citrate via the reductive TCA cycle, while the other lactobacilli strains lacked the enzymes to take the citrate further from oxaloacetate to succinate.

Catabolism of amino acids is a major contributor for flavour formation in ripened cheeses. By transamination, methionine (Met), aromatic-(ArAA) and branched chain-(BrAA) amino acids are converted to their corresponding α-keto acids, which are further degraded to various aroma compounds (Yvon & Rijnen, 2001). According to Yvon, Berthelot, and Gripon (1998) α-ketoglutarate seems to be the most important amino acceptor. However, the production of α-keto acids by LAB in cheese has been considered to be a limiting step, as only a few natural LAB strains possess glutamate dehydrogenase (GDH) activity (Tanous, Kieronczyk, Helinck, Chambellon, & Yvon, 2002). Rijnen, Courtin, Gripon, and Yvon (2000) obtained a similar amino acid degradation using a strain of *Lc. lactis* overexpressing the gene for GDH, as by addition of α-ketoglutarate directly to the cheese. Interestingly, several *Lactobacillus* ssp. have been shown to possess GDH activity (Tanous et al., 2002). Kieronczyk, Skeie, Langsrud, and Yvon (2003) showed that *Lactobacillus* strains possessing GDH activity could degrade ArAA and BcAA in vitro to precursors of aroma compounds and to aroma compounds when they were associated with *Lc. lactis* ssp. *cremoris*. However, in a cheese paste, Kieronczyk, Skeie, Langsrud, Le Bars, and Yvon (2004) demonstrated that the GDH positive *Lb. paracasei* ssp. *paracasei* INF15D (later reclassified to *Lb. plantarum* INF15D) produced mainly diacetyl and acetoin from catabolism of Asp and not aroma compounds from BcAA. The authors suggested that the nature of the produced aroma compounds depended on the relative aminotransferase (AT) activities due to competition among the ATs for α-ketoglutarate.

It has recently been shown that catabolism of amino acids via non-transaminating reactions could also be of importance for the production of cheese aroma compounds (Liu, Holland, & Crow, 2003). In addition, catabolism of serine (Ser) by LAB could generate potent aroma compounds (Liu, Holland, McJarrow, & Crow, 2003). They showed in vitro that growing cells of *Lb. plantarum* could deaminate Ser to ammonia, acetate, carbon dioxide and formate; while non-growing cells deaminated Ser to acetate, ammonia and carbon dioxide. Although pyruvate was not detected, the production of acetate, formate and carbon dioxide was most probably derived from pyruvate produced from Ser. It has been postulated (Kristoffersen, 1956; Stuart, Chou, & Weimer, 1999) that Ser can serve as an energy source for LAB in cheese, as fermentation of Ser via pyruvate to acetate generates ATP. Kieronczyk, Skeie, Olsen, and Langsrud (2001) found that resting cells of *Lb. plantarum* INF15D utilised Ser when incubated in buffer containing free amino acids when no other energy source was available. In fact, in a previous study (Skeie, Lindberg, & Narvhus, 2001), we found reduced levels of Ser in Norvegia cheese with added *Lb. plantarum* INF15D.

The objective of the present study was to evaluate the influence of two *Lactobacillus* adjuncts, differing in metabolic properties, on the development of metabolites in a washed curd, brine salted Dutch type cheese during long term ripening. Two different adjuncts were used; *Lb. plantarum* INF15D, which degrades Ser and citrate and which possesses GDH activity, and *Lb. paracasei* ssp. *paracasei* INF448, with only a weak ability to degrade citrate.

2. Materials and methods

2.1. Adjunct starter strains

Two adjuncts *Lb. plantarum* INF15D and *Lb. paracasei* ssp. *paracasei* INF448, isolated from Norvegia cheese, were characterised by 16s rDNA sequence analysis, and *Lb. plantarum* INF15D previously described as *Lb. paracasei* ssp. *paracasei* INF15D (Kieronczyk et al., 2001, 2003, 2004; Skeie et al., 2001; Tanous et al., 2002) was reclassified.

2.2. In vitro tests of the adjuncts

The adjunct starters *Lb. plantarum* INF15D and *Lb. paracasei* ssp. *paracasei* INF448 were inoculated (2%) in MRS broth and grown at 30 °C for 22 h.

The GDH activity of the adjuncts was tested according to the method described by Kieronczyk et al. (2003).

Citrate utilisation and α-keto acid production of washed cells was tested in 4 replicates with 200 mM sodium phosphate buffer, pH 5.6, with 10 mM Glu, 10 mM citrate, 10 mM $MgCl_2$ and 1 mM pyridoxalphosphate according to Tanous, Gori, Rijnen, Chambellon, and Yvon (2005) at 30 °C for 10 h.

The Ser catabolism and production of acetate and formate by non-growing cells was tested in 100 mM potassium phosphate buffer (pH 5.8) in 2 replicates according to Liu, Holland, McJarrow et al. (2003) with 4 mM Ser incubated for 8 and 24 h at 30 °C.

The degradation of Asp to volatile compounds as acetoin and diacetyl was tested in potassium phosphate buffer in 2 replicates according to a modified method described by (Liu, Holland, & Crow, 2003). Washed cells were resuspended in 5 mL potassium phosphate buffer (100 mM, pH 5.8), containing 14 mM Asp before incubation at 30 °C for 24 and 48 h.

For all assays, two different controls were made, with buffer substituting either cells or reagents.

Organic acids were measured by high performance liquid chromatography (HPLC) using a modified method of