Lactate racemization in cheese by *Clostridium tyrobutyricum*

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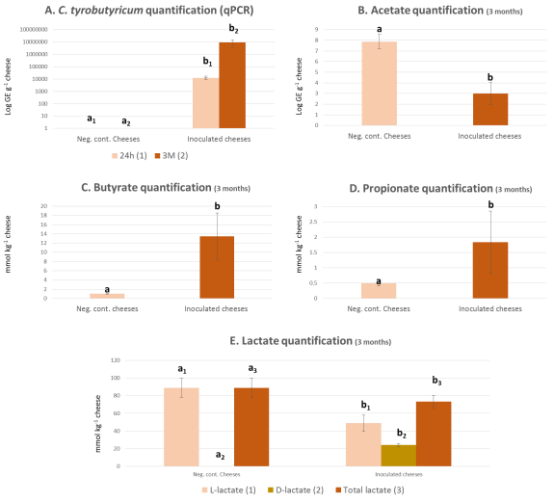
Background and objectives

Late blowing is one of the most relevant fermentation defects observed in hard and semi-hard cheeses in Switzerland. Signs of late blowing appear during cheese maturation and include high concentration of butyrate as well as the presence of eyes caused by the formation of gases. *Clostridium tyrobutyricum*, a spore-forming obligate anaerobic bacterium, is considered the main cause of late blowing defect. Other late-blowing causing Clostridia are *C. butyricum*, *C. sporogenes* and *C. beijerinckii*.

Within the general objective of better understanding the metabolism of late blowing-causing Clostridia in cheese, we conducted an *in situ* study in semi-hard red-smear cheeses produced with mesophilic starter culture producing only L-lactate. A novel qPCR-based method was first developed to facilitate the quantification of *C. tyrobutyricum* in cheese matrix. Surprisingly, the decrease of L-lactate in cheeses inoculated with *C. tyrobutyricum* was accompanied by the formation of D-lactate. Lactate racemization has been well characterized in Lactic Acid Bacteria (LAB). However, information about the role of lactate racemization by lactate-consuming microorganisms are scarce. We present here a deep *in silico* and *in vitro* study aiming to understand the racemization of lactate in cheese by *C. tyrobutyricum* and other relevant late blowing causing Clostridia.

Fig. 1. Growth of *C. tyrobutyricum* in cheese



Growth of *C. tyrobutyricum* in cheese

To characterize the growth of *C. tyrobutyricum* in cheese and its effects on cheese three cheeses inoculated with a mixture of five strains were analyzed after 3 months of ripening and compared to negative controls (Fig. 1). Quantification of *C. tyrobutyricum* DNA with qPCR showed concentrations of genome equivalents (GE) in three months old cheeses up to a thousand times higher than those of samples harvested at 24 h. Butyrate and propionate concentrations were significantly higher in inoculated cheeses, whereas acetate and lactate concentration were lower. L-lactate was the only lactate enantiomer detected in negative controls, whereas inoculated cheeses showed also the presence of D-lactate. The ratio of both entantiomers was approximatively of 2:1 (L:D).

Table 1. Lactate racemization by late-blowing Clostridia *in vitro*

Species	Strain	L-lactate medium		D-lactate medium	
		L-lactate (mmol kg^{-1})	D-lactate (mmol kg^{-1})	L-lactate (mmol kg^{-1})	D-lactate (mmol kg^{-1})
C. tyrobutyricum	FAM22547	37.5	30	32.5	39
	FAM22549	37	32	nt	nt
	FAM22551	36	33	nt	nt
	FAM22552	36.5	34	nt	nt
	FAM22553	36	33	35	36
C. beijerinckii	FAM1743	37.5	35.5	37	38.5
	FAM21718	42.5	35	nt	nt

nt = not tested

Lactate racemization by late-blowing Clostridia *in vitro*

The ability of several *Clostridium* strains isolated from cheese to racemize lactate was evaluated by growing them in media containing L- or D-lactate and acetate for four days (Table 1). All *C. tyrobutyricum* and *C. beijerinckii* strains tested could racemize L-lactate to D-lactate almost up to a 1:1 ratio. The same was also true for the racemization of D-lactate in L-lactate. *C. butyricum* and *C. sporogenes* could not be grown in media containing only lactate and acetate.

Organization and expression of *lar* genes

Putative orthologs of *lar* genes described in *L. plantarum* were identified in genomes of *C. tyrobutyricum* as well as in single genomes of *C. beijerinckii* and *C. butyricum*. Differently from *L. plantarum*, *lar* genes were not found to be arranged in a single cluster (Fig. 2). Expression of putative lactate racemase *larA* as well as those of accessory genes *larB*, *larC* and *larE* were assessed in *C. tyrobutyricum* FAM22553 grown for 32 h in MRS (no lactate) and L-lactate medium by rt-qPCR (Fig. 3). The results were normalized with the house-keeping gene *rpoA*. Lar genes showed a higher expression in medium with lactate.

Fig. 2. Organization of *lar* genes in late-blowing causing Clostridia

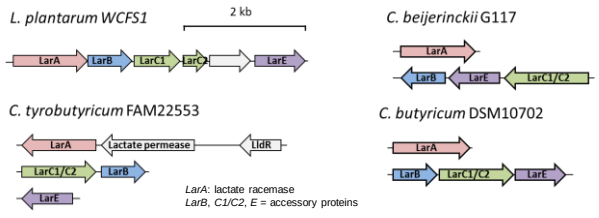
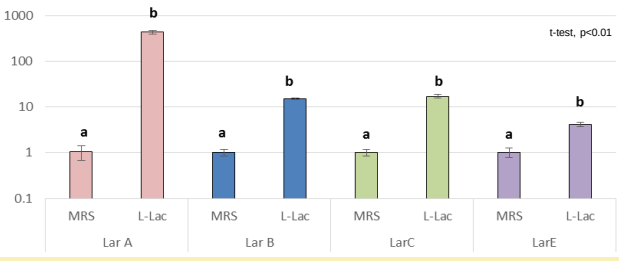


Fig. 3. Relative gene expression measured with rt-qPCR



Conclusions and outlook

This work reports for the first time the racemization of lactate by lactate-consuming microorganisms in a complex matrix such as cheese, providing a deeper understanding of the development of late blowing. Lactate is one of the few biological molecules synthetized in two enantiomeric forms, L- and D-lactate. It has been shown that the relative concentrations of these enantiomers can affect the growth rate of lactate-consuming microorganisms, playing a role in shaping bacterial populations. The characterization of their ability to racemize lactate is therefore important to understand the dynamics of microbial communities in important ecosystems such as fermented food or animal and human gut.

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