Food Control 67 (2016) 96–113

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Review

# Relevance and analysis of butyric acid producing clostridia in milk and cheese

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ARTICLE INFO

*Article history:*
Received 14 January 2016
Accepted 22 February 2016
Available online 27 February 2016

*Keywords:*
Late blowing
*Clostridium tyrobutyricum*
Hard cheese
Raw milk
Quality
Clostridia

ABSTRACT

Via butyric acid fermentation, clostridia – mainly *Clostridium tyrobutyricum* – are able to transform lactic acid into butyric acid, acetic acid and gas ($H_2$ and $CO_2$). The presence of clostridial endospores in milk may lead to severe quality defects in semi-hard and hard cheeses. As a consequence of butyric acid fermentation during ripening, cheeses tend to swell and develop undesired slits, irregular eyes and a rancid taste, thus resulting in high economic losses for producers. Several measures regarding stable, milking and feed hygiene have already been partly implemented to minimise the risk of raw milk contamination with clostridial endospores. Contamination, nevertheless, cannot be avoided completely. Moreover, some of the existing procedures to reduce the bacterial and endospore count in milk (e.g. bactofugation, addition of bacteriocins) are not always applicable or even prohibited for the production of certain cheese types. Therefore, cheese producers may benefit from integrating the determination of the initial count of clostridial endospores in milk into their quality control system of primary materials. This review discusses the role of butyric acid clostridia in the cheese processing environment and methods for the detection and enumeration of cheese-damaging clostridia in milk and cheese.



**Contents**



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http://dx.doi.org/10.1016/j.foodcont.2016.02.038
0956-7135/

## 1. Introduction

### 1.1. Biology and habitats of butyrate producing clostridia

Butyric acid producing clostridia belong to the large and extremely heterogeneous genus *Clostridium* (Blaschek, 1999). Due to its physiologic and genetic diversity, taxonomically this genus has been modified and reorganised many times over the years, and not all of the currently described 208 species (List of Bacterial Names with Standing in Nomenclature, bacterio.net, 08.09.2015) feature the same characteristics as the type species *Clostridium butyricum* (Rainey, Hollen, & Small, 2009; Wiegel, Tanner, & Rainey, 2006). Already in 1994, Collins et al. have proposed a subdivision of the genus into different clusters, in which butyric acid bacteria (BAB) form part of Cluster I, also called *Clostridium sensu stricto* (Rainey et al., 2009; Wiegel et al., 2006).

The species of the genus *C. sensu stricto* are Gram-positive, rod-shaped bacteria that grow under strictly anaerobic conditions. Their G + C-content varies between 22 and 35% and the 16S rRNA gene sequence similarities range from 92 to 99% (Rainey et al., 2009; Wiegel et al., 2006). Their role as a cause of food spoilage is primarily explained by their capacity to form heat-resistant endospores when they are exposed to unfavourable conditions such as nutrient depletion. Endospores are dormant structures with high resistance to heat or cold, pressure, radiation, drought, chemicals and pH extremes (Postollec et al., 2012; Setlow, 2003). When they regain access to nutrients, endospores can become metabolically active again and develop a vegetative cell via germination and subsequent outgrowth (Paredes-Sabja, Setlow, & Sarker, 2011).

Owing to their capability to form endospores, clostridial habitats are extremely versatile. Their spores have been isolated from desert sand as well as from Antarctic permafrost. They are found in water, soil and the mammal intestinal tract and thus are considered as ubiquitous (Brüggemann & Gottschalk, 2008; Drouin & Lafrenière, 2012; Wanwan, 2010; Wiegel et al., 2006).

Given the high versatility of the genus, it is not surprising that clostridia include pathogenic species (for instance *Clostridium perfringens*, *Clostridium botulinum*, *Clostridium difficile* and *Clostridium tetani*) as well as spoilage organisms like butyrate producing representatives (Brüggemann & Gottschalk, 2008; Doyle et al., 2015).

BAB are found in the stable environment and enter milk as contaminants during the milking process (Leisen, 2011). These bacteria require an anaerobic atmosphere with plenty of organic nutrients and high water content (minimal water activity ~0,96) (Jakob, 2011). Hence, soil usually offers perfect growth conditions for anaerobic spore-formers and is considered as the initial source of clostridial contamination (Driehuis, 2013; Jakob, 2005). Fig. 1 provides an overview of the potential contamination pathway.

Green crops are contaminated already with soil either during growth or during harvesting. The extent of contamination depends on weather conditions and the adequate adjustments of the harvesting machineries, whereas rainy weather and lower cutting height of the grass lead to elevated spore counts in silage, which in turn results in high spore counts in milk (Drouin & Lafrenière, 2012; Leisen, 2011). Dasgupta & Hull (1989) observed the highest spore counts in milk during the autumn–winter period in Tasmania, whereas Feligini et al. (2014) detected highly significant species-dependent seasonal effects. In their study, which was conducted in Northern Italy, *Clostridium beijerinckii* was more abundant in milk during summer-autumn, whereas *Clostridium tyrobutyricum* and *Clostridium tertium* were observed in higher numbers during winter–spring.

For preservation purposes, crops are fermented to silage. As the contamination level of silage is thought to be the factor of highest influence on the final spore count of cheese milk, a well-executed fermentation process, which suppresses clostridial growth, is of particular importance (Vissers, Driehuis, Te Giffel, De Jong, & Lankveld, 2006). The ensiling process is based upon the production of organic acids by lactic acid bacteria in an anaerobic environment (Dunière, Sindou, Chaucheyras-Durand, Chevallier, & Thévenot-Sergentet, 2013).

During the ensiling process, the crop is harvested at the stage of optimum nutritional value, chopped, transferred into a silo, compacted and sealed to create an anaerobic atmosphere. In the beginning, the remaining oxygen enables the continuation of plant respiration and aerobic metabolism by enterobacteria, yeasts and moulds. Afterwards, during the actual fermentation, lactic acid bacteria release organic acids and the pH decreases rapidly (Weinberg & Ashbell, 2003). However, if too much soil is present as a vector for contaminants, the buffering capacity of the silage is increased and the acidification process is delayed. These conditions may promote secondary fermentation by BAB. They transform lactic acid into butyric acid of weaker acidity, which leads to even stronger spoilage due to an increase in pH (Dunière et al., 2013). Especially in the course of corn silage production, the situation is further aggravated by the instability of the silage when the silo is opened after storage and exposed to air again. In this case, yeasts, acetic acid bacteria and moulds are able to metabolise the previously formed organic acids and residual sugars, which causes a rise in pH and temperature (Borreani & Tabacco, 2010; Pahlow, Muck, Driehuis, Elferink, & Spoelstra, 2003; Wilkinson & Davies, 2013). These circumstances paradoxically favour the growth of clostridia in an apparently aerobic environment. The reason lies in the formation of anaerobic niches close to spoiled layers in the silo. Aerobic microorganisms exhaust the oxygen that therefore cannot penetrate into the deeper layers of silage. Subsequently, right under the spoiled surface area, an anaerobic niche with a higher pH permits the growth of spore-forming clostridia (Muck, 2013; Vissers, Driehuis, Te Giffel, De Jong, & Lankveld, 2007a). Several efforts have been made to prevent spoilage by the use of special lactic acid cultures or chemical additives (Stadhouders and Spoelstra, 1990; Te Giffel, Wagendorp, Herrewegh, & Driehuis, 2002; Vissers, Driehuis, Te Giffel, De Jong, & Lankveld, 2007b). In conclusion, careful control of every step during the silage