Chapter 4

HOW CAN THE TYPE OF DAIRY FARMING INFLUENCE THE BACTERIAL FLORA IN MILK?

A. Coorevits 1,2, V. De Jonghe 3, J. Vandroemme 4,
A. Van Landschoot 1,2, M. Heyndrickx 3 and P. De Vos 2

1 Laboratory of Biochemistry and Brewing, Faculty of Applied Engineering Sciences, University College Ghent, member of Ghent University Association, Voskenslaan 270, 9000 Ghent, Belgium
2 Laboratory of Microbiology, Department of Biochemistry and Microbiology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium
3 Institute for Agricultural and Fisheries Research, Technology and Food Unit, Brusselsesteenweg 370, 9090 Melle, Belgium
4 Institute for Agricultural and Fisheries Research, Plant Unit, Burgemeester Van Gansberghelaan 96/2, 9820 Merelbeke-Lemberge, Belgium

ABSTRACT

The dairy industry encounters important economical losses and health risks due to harmful spore-forming bacteria. Contamination occurs mainly at the milking stage and microbial numbers grow during conservation before pasteurisation or sterilisation. Some of the contaminants produce degrading enzymes such as lipases and proteases and render the milk unsuitable for human consumption. Others produce toxins and cause severe food illnesses and/or food poisoning. Spore-forming bacteria can survive heat treatments and jeopardize further production processes. Sources of the bacteria are diverse: feed and faeces are paramount, but air, grass, straw, milking equipment and the farmer's skin have been mentioned as well. Logically, the bacterial diversity of a contamination source will influence the resulting bacterial diversity of raw milk. Evidence suggests that differences in season, housing strategy and feed formulation contribute to the bacterial population of milk. Seeing that the operational management on dairy farms determines many of these factors, it seems obvious that this will also determine the bacterial diversity in the produced milk. To test this hypothesis, the diversity of aerobic endospore-forming bacteria in milk obtained from five conventional and five organic dairy farms in Flanders (Belgium) was compared. Although overall diversity was highly similar, some
differences, possibly correlated to operational management, were observed. The greater number of thermotolerant bacteria in milk from conventional dairy farms for example, could be correlated with dietary differences. Cows from organic farms are fed with locally cultivated crops, while cows from conventional dairy farms consume commercially available feed concentrate, which is partially composed of pelleted (and hence heat-treated) and/or imported tropical ingredients. The observed higher occurrence of *Bacillus cereus* - a soil contaminant - in milk from organic dairy farms could be due to differences in housing strategy. Current evidence indicates that differences in operational management might influence the microbial diversity of raw milk, and as a consequence might have an impact on related economical losses and health risks. Further research in this field is needed and should include other bacterial contaminants and other countries.

**INTRODUCTION**

Organic farming is actually the oldest way of farming, i.e. without the use of chemicals (fertilizers) to enhance the production of meat, vegetables, milk and derivatives, and without the use of antibiotics and pesticides to decrease economical losses due to animal sickness and plant disease. Since the introduction of pesticides and fertilizers (mid 20th century), conventional agriculture established all over the world. However, despite the enormous economical advantages seen in the beginning of conventional farming, disadvantages such as soil pollution and insect pest resistance became apparent. This urged some environmentally aware farmers to go ‘back to the roots’ and prefer organic farming instead of conventional farming. For the dairy industry in particular, this implies animal’s welfare and minimization of environmental impact being the main issues.

From the beginning of the re-introduction of organic farming, research has been focused on differences between this ‘new’ and the ‘old’ - conventional - type of dairy farming. Most papers report on differences in milk quality as outlined later on in this chapter. However, few research has dealt with the possible impact of these two types of dairy farming on the microbial aspect of dairy farming. Bacteria intrude raw milk via diverse contamination sources and can have a negative impact on milk quality and safety due to enzyme production causing milk spoilage on one hand and toxin production causing severe food illnesses on the other hand. These contaminants mainly are psychrotrophic pseudomonads or aerobic and anaerobic endospore-forming bacteria. To our knowledge, aerobic spore-formers are the only micro-organisms included in research comparing the microbial quality of milk from conventional and organic dairy farming thus far. Consequently, focus in this chapter will be on these aerobic spore-formers. Firstly, all problems the dairy industry encounters with this type of contaminants will be discussed. Secondly, differences between organic and conventional farming will be considered, with special attention to those differences possibly affecting the microbial flora of raw milk. In a third part, results on research conducted thus far will be disclosed. Finally, conclusions will be drawn on the microbial quality of milk from conventional and organic dairy farming.
AERobic Endospore-Forming Bacteria in the Diary Industry

Aerobic endospore-forming bacteria are the cause of many problems in the dairy industry concerning food safety and product quality. These problems arise due to the production of spoilage enzymes, toxins or through interference with cheese preparation due to gas formation or nitrate reduction.

Aerobic spore-formers contaminate raw milk through both the vegetative form and the heat resistant spore structure, and it is specifically this spore configuration that poses problems. A lot of studies have been performed on the contamination sources of these aerobic spore-formers (most research focussed on Bacillus cereus), but there is no consensus as to their origin in raw milk [64]. Very diverse contamination sources are indicated: soil [5, 8, 11, 29, 58], bedding [11, 13], feed [13, 29], hay [5], dust [5, 13], air [43], faeces [29, 58], dirty teats [11] or teat cups [2] and milking equipment [28, 29, 41]. Once these spores have intruded the milk, they cannot be destroyed by conventional heating processes - which are applied to destroy bacteria and thus assure safety of the product - such as pasteurization (e.g. 15 s 72°C) [1]. Some spores are even known to survive Ultra High Temperature (UHT)-treatment (e.g. 2-5 s 140-145°C) [44, 50, 51]. After the sterilisation step, spores can germinate into vegetative cells, a process which is even activated by heat; and subsequently grow in the milk or derived products, possibly causing problems mainly due to enzyme or toxin production.

It is well-known that aerobic spore-formers belonging to the genus Bacillus and related genera are involved in milk spoilage, hampering the quality of raw milk and derived products. Spoilage enzymes such as proteases, lipases and lecithinases are responsible for off-flavours and structural defects in pasteurized milk [37]. The most important spoilage organism in the dairy industry is undoubtedly B. cereus, causing ‘bitty cream’ (floating clumps of fat) due to lecithinase activity and ‘sweet curdling’ (curdling of the milk without acidification) due to protease activity [23]. The latter also causes bitter and rotten off-flavours, whereas fruity and rancid off-flavours are caused by lipolytic activity [37]. Furthermore, these enzymes have also been found in Bacillus species other than B. cereus, and appear to have heat resistant characteristics [7]. Another important trait of B. cereus, and some other bacilli too, is their ability to grow at the storage temperature of milk (4-7°C), which mainly determines the shelf life of pasteurised milk and derived milk products [37, 58].

Spoilage caused by aerobic spore-formers is mostly limited to pasteurized milk. Spoilage of UHT and sterilized milk only occurs occasionally and can mostly be attributed to recontamination with proteolytic Bacillus species or Gram-negative spoilage organisms during the filling step [14, 52, 66]. Despite this fact, there are problems with aerobic spore-formers in UHT-milk, not necessarily related to spoilage but more to a non-achievement of sterility standards. EG-regulation 92/46 requires that the number of colonies counted from incubated (30°C during 15 days) unopened UHT-cartons, does not exceed 10 colony forming units (cfu) per 0.1 mL. This requirement could not be met in Italy, Austria (1985) and Germany (1990) and later-on in other European (France, Benelux, Spain) and non-European (Mexico, USA) countries [20, 50]. Hammer et al. [20] linked this problem to highly heat resistant (HHR) spores of a mesophilic micro-organism described later as Bacillus sporothermodurans [44]. Since this organism does not have any pathogenic or toxic activity
[20, 21], and also only rarely causes minor spoilage defects in the form of a slight pink discoloration [25, 32], this is not so much an issue from a hygienic point of view, but all the more from a technological perspective, being a mere sterility issue.

Another issue regarding aerobic spore-formers in milk is the possible production of toxins. *Bacillus cereus* is a well known food pathogen that can cause two types of food poisoning syndromes: an emetic and a diarrhoeal type. The former is caused by a small ring-formed heat-stable dodecadepsipeptide named cereulide. The diarrhoeal syndrome is caused by vegetative cells that are thought to produce a variety of heat-labile enterotoxins in the small intestine [18]. Three pore-forming cytotoxins have been associated with diarrhoeal disease: haemolysin BL (Hbl) and nonhaemolytic enterotoxin (Nhe), which are both two homologous three-component toxins, and cytotoxin K (CytK), a single component cytotoxin.

Two different forms for the latter have been described, the highly cytotoxic CytK-1 and the moderate cytotoxic CytK-2 variant, encoded by cytK-1 and cytK-2 genes [13]. The cytK-1 variant has thus far only been detected in a limited number of *B. cereus* strains, that have been proposed to form a novel bacterial species, for which the name “*Bacillus cytotoxicus*” or “*Bacillus cytotoxicus*” is suggested [31].

Although *Bacillus* species other than *B. cereus* have been incriminated as food poisoning agents, the link between toxin production and foodborne illness has not been fully established. Increasing evidence for the production of both heat-stable and heat-labile toxins is becoming apparent through cellular assays that confirm both production and functionality of the toxins. In these assays, strains identified as *Brevibacillus brevis*, *Bacillus circulans*, *Bacillus lentes*, *Bacillus subtilis* [3], *Bacillus licheniformis* [3, 33], *Bacillus pumilus* [33] and *Bacillus amyloliquefaciens* [45] are shown to produce heat-labile enterotoxins. Strains identified as *B. licheniformis* [3, 17, 38, 49, 57], *B. pumilus* [16, 17, 55], *Bacillus mojavensis* [15], *B. subtilis* [3, 17], *Bacillus simplex* [57], *Bacillus firmus* [57], *Bacillus megaterium* [57], *B. brevis* [3], *B. circulans* [3] and *B. lentes* [3] can produce novel heat-stable toxins, resembling the physico-chemical characteristics of cereulide [17, 49, 55, 57]. Some of these toxins, heat-labile as well as heat-stable toxins, have been characterised as surfactin isoforms, namely lichenysin (described in *B. licheniformis* [38, 49]) and pumilacidin (described in *B. pumilus* [55]). The surfactin superfamily is a family of structurally diverse, low molecular weight cyclic lactonic lipopeptides, that is well-described in strains of the *B. subtilis* group.

Another concern regarding aerobic spore-forming bacteria is interference with cheese production through nitrate reduction, whether or not in combination with gas production during fermentation. Nitrate is often added to raw milk during the cheese preparation process to prevent growth and germination of anaerobic spore-forming species belonging to the genus *Clostridium*. Specifically *Clostridium tyrobutyricum* can grow fermentatively with gas production, thereby causing ‘late blowing’ defects in Gouda type cheeses [26]. The reduction of nitrate to nitrite or nitrogenous gasses, is a process carried out by many *Bacillus* species [60], thus nullifying the growth inhibitory effect of supplemented nitrate. Recently, it became apparent that also gas producing aerobic spore-formers, namely *Paenibacillus polymyxa* and *Paenibacillus macerans*, can be directly implicated in this type of spoilage in Argentinian Cremos and Mozarella cheeses [47].

Even though the dairy industry is often confronted with severe implications caused by aerobic spore-forming bacteria, their identity is most often either not determined onto the species level, or not reliably determined because identification has been based on phenotypic...
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and biochemical characteristics of the isolates only [36]. Based on these inadequate identifications, the spoilage potential was thought to be restricted to *B. circulans*, *Bacillus coagulans*, *Brevibacillus laterosporus* [53], *P. polymyxa* [60] and *B. cereus* [42]. In view of current taxonomic insights in the aerobic spore-forming microbiota, up to 48% of all aerobic spore-forming isolates from raw milk could not be identified in some previous studies [19, 56]. It is clear that future diversity studies should focus on these yet unknown groups of bacteria, enabling an important improvement of our knowledge on contaminating aerobic spore-formers in the dairy industry.

The previous paragraphs indicate that problems with aerobic spore-formers encountered by the dairy industry might be affected by farm management (e.g. various types of feed). Indeed, bacteria intrude raw milk via various contamination sources as feed, hay, bedding, grass, and it can be expected that differences in these contamination sources between individual farms or between farm types might consequently have an impact on the composition of the microbiota of raw milk, thus affecting the quality and safety of milk and derived products [9]. One way to test this hypothesis could be by comparing the microbial load and diversity of milk from two types of farm management (i.e. organic versus conventional dairy farming) which may show differences in the relative importance of the above mentioned contamination sources.

**ORGANIC VERSUS CONVENTIONAL DAIRY FARMING**

Organic farming aims at sustainable farming. It emphasises the animal’s welfare and the use of local natural resources as much as possible, excluding chemical fertilizers and pesticides. The main principle of this type of farming is to minimize negative effects on the environment, preserve natural resources and maintain biological diversity on farms and their neighbourhood. Organic farming is gaining popularity over the last decade, however, in 2005, only 0.5% of all farming was regarded as organic in the U.S. and around 4% in the E.U. [34, 62]. This rather low percentage may be due to high managerial costs, risks of shifting to a new way of farming, limited awareness of organic farming systems and lack of marketing and infrastructure [62]. To obtain the label of organic, farms are obligated to follow some rules (determined by the Council Regulation in Europe and by the United States Department of Agriculture in America), which are created to preserve and protect the organic farming method. These rules imply, specifically for the dairy sector, restrictions on feed and housing strategy of animals, and also on health strategy.

Regarding the feed, organic farmers are compelled to use mainly locally cultivated crops, rather than imported feed concentrate. Even more, since 2007, all feed offered on organic dairy farms must be 100% organic in origin [34], precluding the use of chemical fertilizers and pesticides. The diet of animals from organic farms thus mainly consists of grass, cereals, fresh vegetables, barley and trefoil. On the other hand, cattle from conventional dairy farms are fed conventional crops, usually grain, supplemented with vitamins, minerals and feed concentrate. The latter provides the animals with the necessary proteins and is often composed of imported soy or manioc and tropical waste ingredients from products such as coconut meal and citrus pulp.
The regulations also specify a minimum space allocation per animal. Cattle from organic farms usually have more space, with larger stables and access to the outdoors. These measurements should have a positive impact on the animals’ welfare, and indeed it has already been shown that reducing stocking density - which is a logical consequence of providing more space per head - does have a positive impact on dairy cow welfare [27]. High stocking densities however, affect a cow’s ability to access feeders and lying areas [30].

Regarding health strategy, disease prevention rather than cure is emphasised in organic farming. Animals labelled as organic may not receive hormones nor antibiotics. Off course, organic farmers are allowed (or better obliged) to treat sick or injured animals with a prohibited medication, but these animals are not considered organic anymore. This lower treatment policy of organic herds might raise the concern that udder health is worse compared to conventional cattle. However, comparative studies on somatic cell counts of animals, which are used as an indicator of mastitis, reveal no significant difference between organic and conventional herds [22, 67]. In addition, the occurrence of mastitis, ketosis and milk fever was less abundant in organic herds, indicating an overall better condition of the cows compared to cows of conventional farms [22]. This was confirmed by a Danish study on herd health, stating fewer treatments for retained placenta and ketosis in organic herds [4]. In contradiction, Hörning et al. [24] reported that cows on German organic farms seem to be more susceptible to mastitis, indicated by higher somatic cell counts, but fewer fertility problems, and a longer life span. This discrepancy probably could be due to an underestimation of the occurrence of mastitis in organic herds. After all, the use of alternative herbal medicine and homeopathic treatments for mastitis - mostly applied in organic herds - is not recorded in databases, while antibiotic treatment - mostly applied in conventional herds - is always recorded [61].

Some studies report on the adoption of these ‘rules for organic farming’ into practice by interview questionnaire with the farmers. Zwald et al. [67] reported on the following differences between organic and conventional dairy farms in the US: organic herds are usually smaller and have a lower milk production, use of antibiotics is reduced, and is never used to treat mastitis in organic farms, calves are placed separately from the organic cows with a separate water supply which reduces the risk of disease transfer and conventional farms tend to buy their feed and concentrate, rather than producing it themselves. Coorevits et al. [9] described differences in feeding and housing strategy between organic and conventional dairy farming in Flanders, Belgium. Organic farmers indeed feed their cows locally cultivated crops such as maize, trefoil, barley, wheat, grass, carrots, turnip and chicory, supplemented with minerals, salt and seaweed calcium, while conventional farmers feed their cows with maize, grass and wheat supplemented with feed concentrate containing components including or derived from soy, rape, coconut, citrus and cacao. Differences in housing strategy were most obvious during summer season. During winter season, cows were mostly stabled day and night on both type of farms. In late summer/autumn, cows from organic farms more often grazed outside. Langford et al. [30] found that main differences between organic and conventional farming in the UK were milk yield (which was lower in organic herds), level and composition of used concentrate feed, management of heifers and calving, and use of alternative treatments to prevent and treat mastitis.

It can be concluded that management practices on organic dairy farms are in compliance with the regulations enforced to protect and preserve organic farming, and differ from
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management practices on conventional dairy farms, which is most obvious when comparing animal disease treatment, feed and housing strategy.

Various studies have dealt with the influence of these two different types of management on the animal’s welfare, and on the quality of milk. Butler et al. [6] for instance, showed an influence of the length of the grazing period, and diet composition on sensory and nutritional quality of milk. Vicini et al. [65] reported on a greater protein concentration in milk from organic farms, which could be due to the use of another breed, or to the lower milk production by organic herds, implying a higher protein level. However, in the Netherlands, Nauta et al. [40] mentioned a highly significant decrease in protein percentage and also in fat content due to conversion from conventional to organic dairy farming. Very recently, Molkentin [39] clearly showed a difference in fatty acid composition of milk from organic and conventional dairy farms (e.g. higher levels of α-linolenic acid), which might be used to discriminate between the two types of milk. To our knowledge however, almost no studies report on differences in microbial quality of different types of milk. Only Vicini et al. [65] mention a non-significant lower bacterial count in milk from conventional farms, but no efforts to further characterize this bacterial flora have been made.

The hypothesis of our recent study [9] was that more detailed information (identification and characteristics) about these aerobic spore-formers would allow to trace the possible contamination sources, and find a relationship between these sources and the management practices on conventional and organic dairy farms. After all, a better insight in the factors influencing the introduction of spores into raw milk is crucial to take necessary actions to improve the quality and safety of milk.

Various papers report on correlations between micro-organisms found in raw milk and their contamination sources. For instance, soil has been identified as a major source of contamination of raw milk with B. cereus spores, the contamination being even more severe when cows grazed outside [8, 46]. Seasonal variations in psychrotolerant spore content have been reported in several investigations [5, 19, 46, 58], indicating a possible effect of pasturing, and thus grass and soil, as a contamination source [54]. As cows are more often grazing outside during summer period, the contamination sources soil and pasture can be linked to seasonal variation, and also to the housing strategy of the animals. Indeed, cows from organic farms are more often grazing outside than cows from conventional farms.

The presence of spore-formers in feed, in particular in silage and food concentrate, has been shown by Slaghuis et al. [54] and Vaerewijck et al. [63], while the correlation between spores in silage and spores in raw milk has been demonstrated by te Giffel et al. [59]. This can also be linked to management practice differences between organic and conventional dairy farming as conventional farmers are allowed to use commercially available feed concentrate, while the diet of organic cows is restricted to locally cultivated crops, without food additives. It thus seems obvious that operational management on dairy farms, which is related to contamination sources, will influence the aerobic spore-forming microbiota of raw milk. To investigate this hypothesis, the aerobic spore-forming flora of milk samples obtained from organic and conventional dairy farms was analysed [9].
DIFFERENCES IN AEROBIC SPORE-FORMING FLORA BETWEEN ORGANIC AND CONVENTIONAL RAW MILK SAMPLES

Before elaborating the results of that study, a short outline of the approach used to isolate and identify the spore-formers in organic and conventional raw milk samples is given, as already described [9].

Five milk samples from each type of dairy farm were collected in the region of Flanders, Belgium, and analysed during a summer and a winter campaign. All samples were heat-treated (10 min at 80°C) to select for spore-formers in particular, and were streaked in parallel on three different media [Brain Heart Infusion Agar (BHI), Nutrient Agar supplemented with egg yolk (NAE) and Skim Milk Powder Agar (SM)], with the latter two being differential media indicating lecithinase (NAE) and protease (SM) activity of isolates, and incubated at three different temperatures to select for psychrotrophic (20°C), mesophilic (37°C) and thermophilic (55°C) bacteria. Bacterial isolates were identified polyphasically. At first, fatty acid profiles of all isolates were generated to obtain a preliminary identification into more manageable groups of isolates. Subsequently, partial 16S rRNA gene sequences of representative isolates of each group were generated. A species allocation was obtained by using the online FASTA tool of EMBL (http://www.ebi.ac.uk/fasta33/), and comparison of the query sequence with 16S rRNA gene sequences of type strains of valid species. Furthermore, all isolates obtained after incubation at 20°C were screened for their potential to grow at refrigeration temperatures, the storage temperature of pasteurised milk (7 days at 6 ± 1°C).

Overall, the isolates turned out to belong to species such as B. licheniformis, B. subtilis, B. pumilus and B. cereus group organisms, as usually cited by papers reporting on microbial diversity in milk and derived products. Additionally, the potential of many isolates to produce lecithinases and/or proteases is shown by characteristic growth patterns on the differential media NAE and SM as well as their potential to grow at refrigeration temperatures (6 ± 1°C) after maximum 7 days. These findings underline again the hazardous implications spore-formers can have on milk quality and safety. Another important conclusion to be stressed out, is that about 7% of all isolates could not be attributed to already described species within Bacillus sensu lato, indicating our knowledge on spore-formers in milk (and to a larger extent in dairy products) is still far from complete, as already mentioned earlier.

It was found that total spore counts were higher in milk of conventional farms compared to organic farms, during summer months, and this difference could be mainly attributed to the higher occurrence of thermotolerant isolates (counts after incubation at 55°C). Previous studies also showed a seasonal variation for winter and summer total spore counts, with Christiansson et al. [8], Ellis et al. [12] and Lukasova et al. [35] reporting higher spore counts in summer due to lower udder hygiene and dietary changes. However, other studies indicate the opposite with higher spore counts in winter season due to housing [10, 56]. The thermotolerant isolates obtained in the comparative study of Coorevits et al. [9] were mainly identified as B. licheniformis and Ureibacillus thermosphaericus. Dietary differences could be a possible explanation for their higher occurrence in milk from conventional dairy farms. Indeed, cows on organic farms are fed locally cultivated crops, while cows on conventional dairy farms are fed maize, grass and wheat, supplemented with commercially available feed concentrate which is often composed of tropical (waste) ingredients. It is this feed concentrate
in particular that is assumed to contribute to the higher thermotolerant spore counts in milk from conventional dairy farms. Indeed, the presence of thermotolerant isolates (e.g. *Bacillus licheniformis*) in feed concentrate has already been demonstrated by Vaerewijck *et al.* [63]. However, the presence of *U. thermosphaericus* in feed concentrate has not been shown. The only isolation source of *U. thermosphaericus* in the dairy industry reported thus far is milking equipment [51]. For that reason, one must be careful drawing conclusions and contamination sources other than feed concentrate should not be excluded. Another finding that possibly also could be attributed to these dietary differences is the higher diversity of isolates in milk from conventional dairy farms and the presence of more unidentified (onto the species level) isolates.

Another striking difference between milk from organic and conventional dairy farms was the more frequent occurrence of *B. cereus* group organisms in milk from organic dairy farms, and this was most obvious during summer months. As already mentioned, *B. cereus* is the most important pathogen for the dairy industry due to production of toxins causing food illnesses, production of deteriorative enzymes resulting in decreased milk quality and its ability to grow at storage temperatures (4-7°C) of pasteurised milk and other milk products. It is a common soil organism, and soiling of teats is thus most probably the major contamination source of raw milk with this pathogen. It was already shown that milk is more contaminated with *B. cereus* during summer period, when cows are more often grazing outside [8, 10]. Indeed, keeping cows indoor during summer months does decrease the presence of *B. cereus* [54]. The interview questionnaire performed during the study of Coorevits *et al.* [9] clearly indicated that cows from conventional farms were more often stabled indoor while cows from organic farms were grazing outside. So, differences in housing strategy may match with the observation that *B. cereus* group representatives are more frequently isolated from milk from organic dairy farms.

### CONCLUSION

Quality and safety of milk is affected by the presence of contaminating bacteria, intruding raw milk at the milking stage, and hampering further production processes. Especially spor-formers are of concern, as the spores generated by these bacteria are resistant to the applied heat treatments and subsequently germinate into vegetative cell forms, spoiling milk via the production of deteriorating enzymes, toxins, nitrate reduction or gas production. Introduction of these spores into raw milk occurs via various contamination sources (feed, grass, air, faeces,…), and it can be concluded that differences in these contamination sources do affect the composition of the microbial flora in milk. Comparing the microbial load of milk from organic versus milk from conventional dairy farms clearly revealed a varying microbial constitution, which could most probably be attributed to differences in operational management between the two types of dairy farming.

More thermotolerant isolates (namely *Ureibacillus thermopshaericus* and *Bacillus licheniformis*) were found in milk from conventional dairy farms, which probably could be attributed to differences in feed strategy between conventional and organic farming. It is hypothesised that the use of feed concentrate in conventional dairy farms in particular contributes to this observation, as this feed concentrate is often composed of pelleted (hence
heat-treated) and/or imported tropical waste ingredients. Thermotolerant bacteria are especially an issue in the production of milk powder. They often do not constitute a health risk to the consumer but are used as indicators to monitor good hygienic practices in milk powder production plants [48].

The higher occurrence of *Bacillus cereus* group organisms in milk from organic dairy farms compared to milk from conventional farms is another striking difference. This is most probably due to the housing strategy. For the animal’s welfare, cows from organic farms are more often grazing outside, enabling a higher introduction of *B. cereus* spores into raw milk via grass and soil. As already mentioned *B. cereus* is the most important pathogen in the dairy industry because of its proteolytic and (phospho)lipolytic enzyme production causing structural defects in milk, its toxin production causing severe food illnesses and its ability to grow at refrigeration temperatures. Therefore, the presence of *B. cereus* is determinative for the shelf life of pasteurised milk and derived products.

Although differences in milk quality between milk from organic and conventional dairy farms have often been under study, the microbial diversity differences had not been investigated before to our knowledge. Most research was focused on protein and fat content of milk, as well as on the animal’s welfare (occurrence of diseases). However, the results of our recent study [9] clearly indicate the correlation between operational management and microbial diversity of raw milk when focusing on aerobic spore-formers. In our opinion, an extension of this study to other contaminants and/or other countries is highly mandatory, as this would provide us with the necessary information about contamination sources, to take hygienic or other precautions in order to avoid, or at least reduce contamination risks at the initial phase, i.e. the milking stage.

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