Total bacterial count as an attribute for raw milk quality

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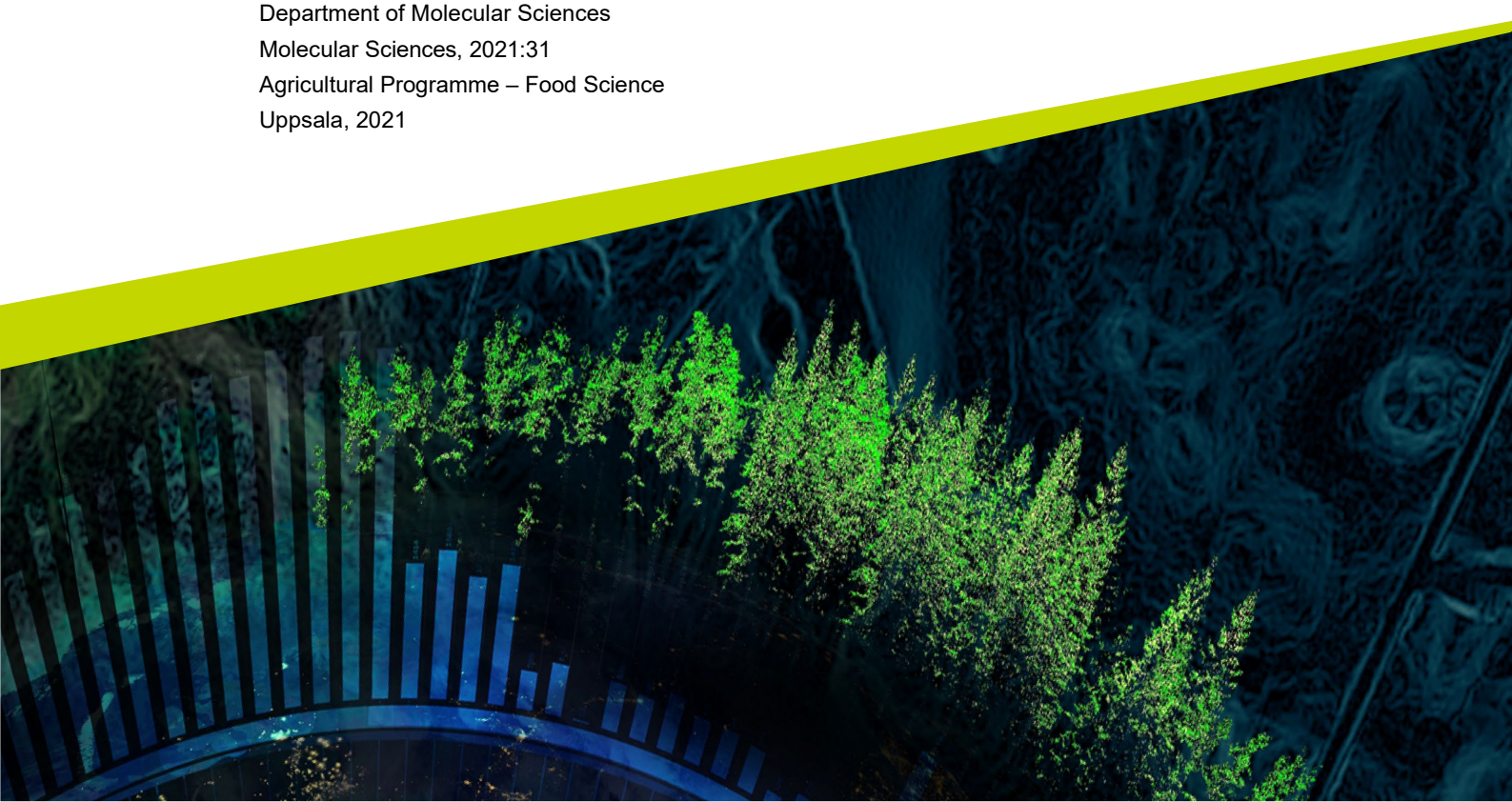

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Abstract

Milk is a nutritious food and at the same time a substrate where microorganisms thrive. Their metabolic impact can be useful in dairy fermentation, but the degradation of compounds within its habitat can also cause dairy food spoilage. The amount and type of bacteria directly affect the quality of the milk, in industry mostly evaluated by total bacterial count (TBC). TBC is an indicator of the hygienic conditions at farm level, as well as for the hygienic quality of the raw milk, where premium payment systems use TBC in the prizing of milk to the farmer. However, TBC merely gives the enumeration of bacteria in milk and not its microbial composition. Milk microbiota is today known to mainly consist of a few genera, but with high diversity and variations, thus the gap between TBC and milk microbiota has widened. Therefore, the aim in this literature study was to evaluate TBC as an attribute for milk quality and to relate TBC to alternative methods. Many factors related to farm management influence both bacterial counts and microbiota. Properly maintained hygiene and milk storing is of major importance from farm to dairy plant, and deviations cause bacterial counts to increase and contaminants with spoilage capacity to dominate. Correlations between TBC and bacteria of specific concern have been reported, although not consistently. TBC and psychrotrophic counts show similar dynamics, whereas thermotolerant bacteria are more frequent in milk with low total counts. Spoilage of pasteurized and ultra-high temperature processing (UHT) milk is mainly due to the heat-resistant enzymes produced by *Pseudomonas, Acinetobacter* and *Bacillus.* The numbers of these bacteria are of great importance for the industry in a milk production of high hygienic quality. Proteolytic activity is shown to have a relatively strong correlation with those bacteria and could thus be a more adequate indicator of milk quality, preferably evaluated close in time for processing. A focus on establishing alternative methods is needed to find more adequate indicators that meet dairy industry´s current needs.

Keywords: total bacterial count, milk microbiota, raw milk hygienic quality, spoilage

Sammanfattning

Mjölk är en näringsrik vätska men samtidigt ett substrat där mikroorganismer trivs. Deras metaboliska inverkan kan vara användbar i fermenteringen för olika mejeriprodukter, men omvandlingen av mjölkens komponenter kan också orsaka svinn. Den hygieniska mjölkkvaliteten beror på mängden och typen av bakterier i råmjölk, i mejeribranschen mestadels utvärderad som totala antalet bakterier. Bakterietal är en hygienindikator på gårdsnivå, liksom för den hygieniska kvaliteten på mjölk där betalningssystem använder måttet vid prissättning av råmjölk till mjölkproducenten. Men bakterietal ger bara antalet bakterier och inte någon information om dess mikrobiotiska uppbyggnad. Mjölkens mikrobiota är idag känt för att huvudsakligen bestå av några få släkten, men i hög mångfald och variation. Avståndet mellan bakterietal och mjölkens mikrobiota har således ökat. Syftet med denna litteraturstudie var att utvärdera bakterietal som attribut för hygienisk mjölkkvalitet i relation till andra analysmetoder. Många faktorer på gård påverkar både bakterietal och dess mikrobiotiska sammansättning. God hygien och bibehållen kyllagring från gård till mejeri är avgörande och avvikelser gör att bakterietal och andelen oönskade bakterier ökar. Samband mellan bakterietal och bakterier av särskilt intresse finns rapporterat. Dock finns studier där sambandet saknas. Totala antalet bakterier och psykrotrofa bakterier visar en likartad dynamik medan termotoleranta bakterier är rapporterat att vara vanligare i mjölk med lågt bakterietal. Pastöriserad och UHT-mjölk härsknar främst på grund av de värmetoleranta enzymer som produceras av *Pseudomonas, Acinetobacter* och *Bacillus*. Antalet av dessa bakterier är i och med detta av stor betydelse för industrin i en mjölkproduktion av hög hygienisk kvalitet. Proteolytisk aktivitet har visat sig vara jämförelsevis starkt relaterat med dessa bakterier och kan vara lämpligare som indikator av mjölkkvaliteten direkt före vidare förädling. Fokus på att finna mer lämpliga indikatorer för att möta branschens nuvarande behov är nödvändigt.

Nyckelord: totalantalet bakterier, bakterietal, mjölkens mikrobiota, hygienisk råmjölkskvalitet, svinn

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Abbreviations

AOAC	Association of Official Agricultural Chemists
ATP	adenosine-triphosphate
BTM	bulk tank milk
CFU	colony forming unit
FC	flow cytometry
FTIR	Fourier transform infrared
HTS	high-throughput sequencing
IBC	individual bacterial count
ISO	International Standards Organization
LPC	laboratory pasteurization count
PBC	psychrotrophic bacterial count
PBC-LPC	psychrotrophic-thermoduric counts
qPCR	quantitative polymerase chain reaction
SLB	Swedish Friesian cow
SPC	standard plate count
TBC	total bacterial count
UHT	ultra-high temperature

1. Introduction

The fluid secreted by mammals to nourish their young is what we call milk. Milk mainly contains water, fats, proteins, and lactose (Adams & Moss 2007). Since the beginning of farming, milk from animals has been consumed by humans. Bovine milk is globally one of the most common animal milks obtained, being a highly nutritious food. Milk is also a highly suitable growth media for a heterogeneous number of microorganisms. They come from a large variety of sources, such as the mammary gland and teat canal, udder skin, milking machine, and farm environment (Quigley et al. 2013). Milk microbiota includes bacteria, yeast and mould and their metabolism changes and build up molecules within its habitat. Their presence in milk can be beneficial in the fermentation of dairy products, as well as detrimental due to foodborne illnesses or spoilage (Quigley et al. 2013).

The composition of the milk microbiota was for long studied using culture-dependent methods. However, molecular methods and high throughput sequencing (HTS) allowed to realize that milk microbiota is far more complex than earlier believed. Almost 2000 taxa at genus level or above have been identified in bulk tank milk. Most of the genera belong to the phyla *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* where a few make up most of the microbiota, with a high relative abundance. The core microbiota found include psychrotrophs, gut associated bacteria, bacteria found on teat skin, and microorganisms of potentially beneficial importance (Parente et al. 2020).

Microbial spoilage of milk is a great concern for the industry, both environmentally and economically. It is mostly due to a few bacteria, mainly psychrotrophic species of *Pseudomonas*, *Acinetobacter* and *Bacillus* (Fusco et al. 2020). Psychrotrophic bacteria proliferate in refrigerated temperatures and produce extracellular heat stable enzymes, such as lipases and proteases. Their spoilage activity has a considerable effect on the shelf-life of milk and dairy products and degrade milk fat and casein, causing milk to go rancid and develop bitter off-flavours (Quigley et al. 2013). Although most bacteria can be eliminated by pasteurization, heat tolerant enzymes and their enzymatic activity persist. This makes them one of the main problems related to microbial contamination of milk, together with some spore-forming bacteria surviving heat treatment (Murphy et al. 2016).

In the industry, microbial counts are an imperative quality indicator of raw milk and greatly impact the milk price to the dairy farmer. Aerobic mesophilic, psychrotrophic, and coliform microorganisms belong to the groups most used as hygiene indicators in milk quality programs. A high concentration of mesophilic bacteria is considered an indication of inadequate hygiene practises (Nero & De Carvalho 2018). International standard bodies such as International Standards Organization (ISO) design most analytical standard methods used for the hygienic evaluation of milk. If other analytical methods are used, they must be validated by accredited organizations e.g. AOAC International (Association of Official Agricultural Chemists), and any alternative method must be validated against a standard method (Burke et al. 2021). Standard plate count (SPC) is validated as reference method providing total bacterial counts (TBC), according to ISO 4833-1:2013. TBC is determined as colony forming unit (CFU) and criteria for raw milk is 100 000 cfu/ml, according to Regulation (EC) No 853:2004 of the European Parliament and of the Council. Other culturable microbiological tests include the enumeration of preliminary incubation count, coliform bacteria, psychrotolerant bacteria, thermotolerant bacteria, and counts for specific agents causing mastitis (Murphy et al. 2016).

Culturable methods are simple and considered the "gold standard" but come with the disadvantages of having a long time-to-result and requiring much labour. More, only viable cells able to replicate and grow under the provided conditions are counted. Instrumental techniques such as flow cytometry (FC) is another method used for analysing milk quality. Instead of CFU, bacteria are then counted individually and determined as individual bacterial count (IBC). FC give results faster and can enumerate otherwise non-cultivable bacteria, but only bacteria over the detection limit of 10^3-10^4 cells/ml are counted. Both methods give no further information of which bacteria is present in the microbiota (Sohier et al. 2014).

With new knowledge about the variations in milk microbiota, and its compositional change during cold storage, it is possible that total bacterial counts are too general to adequately evaluate milk quality. Psychrotrophic proteolytic bacteria are behind most spoilage of pasteurized and ultra-high-temperature (UHT) process milk, highlighting the need for a more specific count of those bacteria (Fusco et al. 2020). Other bacteria of concern for the industry are thermoduric spore-forming bacteria which can be present in milk of high quality and still cause product defect (Rodrigues et al. 2017).

2. Objective

The objective of this literature study is to evaluate the suitability of total bacterial count as a quality attribute of raw dairy cow´s milk, considering milk´s microbiotic complexity, variation, and effect on pasteurized fluid milk and UHT milk.
Questions of relevance are:

- What variations are seen in total bacterial counts and raw milk microbiota?
- How is total bacterial count correlated with farm hygiene, microbiota, and milk spoilage?
- Are there alternatives to total bacterial count that more accurately describes the quality of raw cow's milk and thereby better predicts the shelf life of liquid pasteurized milk?

3. Method and material

This literature study included a search of scientific articles in different databases, mostly through the Swedish Agricultural University´s own platform Primo, but also from PubMed and Google Scholar. Also, information regarding industrial guidelines and international regulations have been gathered from European Food Law to Industry Guidelines from the Swedish National Food Agency and International Standard Organization.

In the search of articles regarding raw milk quality and microbiological monitoring, a combination of words was used, including “bulk tank milk” “total bacterial counts”, “milk quality indicator” and “microbiological quality”. In the search regarding milk microbiota, the search included words such as “raw milk microbiota” and “dairy milk”. To delimit the search into finding more recent studies and reviews, year of publications were concentrated to 2010 and newer. This was combined with a focus on studies on cow´s milk.

After reading abstracts of generated findings, around 50 articles were selected. Within those, the most relevant ones correlated to the objective of this study were used in the evaluation of total bacterial counts. Studies focusing on mastitis and udder health was discriminated to aim for the importance bacteria have for hygienic milk quality rather than animal health.

4. Hygienic quality of raw cow´s milk

Hygienic milk quality is related to the amount and type of bacteria present in milk. The quality of pasteurized milk and dairy products depend on a high microbiological quality of the raw milk. To avoid spoilage, the industry works to reduce the microbial load. This is achieved by preventing contamination at farm level, storage of milk at refrigerated temperature to prevent bacteria from multiplying, and by monitoring bacterial levels by microbiological analysis (Fusco et al. 2020).

4.1. Variations in total bacterial counts

Many farms related factors contribute to the variation in milk TBC. The factors presented here show some of the major causes for variations found in this literature study.

Mastitis is a major reason for rising bacterial counts, as reported in numerous studies (Múnera-Bedoya et al. 2017; Skeie et al. 2019). TBC can vary with lactation stage, being higher in late lactation (Paludetti et al. 2019). In a Swedish study, higher TBC were found in milk from larger farms, characterized by loose housing, robotic milking, and The Swedish Friesian (SLB) breed (Bernes et al. 2019). Milk collected in summer months when cows are grazing outdoors revealed modestly higher counts (Kable et al. 2019; Priyashantha et al. 2021). Conversely, the opposite has also been reported when enumerating with quantitative polymerase chain reaction (qPCR) technique (Doyle et al. 2017b).

Several studies show a clear association between teat hygiene and raw milk quality, where disinfection lowers the amount of bacteria (Bava et al. 2017; Bradley et al. 2018; Bernes et al. 2019). In a recent evaluation of associations between TBC and different bedding material, Bradley et al. (2018) pointed out teat preparation as the critical step to prevent bacteria from being transferred to milk. Interestingly, Doyle et al. (2017b) observed higher TBC in milk after teat preparations, especially in milk from grazing cows. It was suggested that bacteria originating from outdoor environment may have difficulties adhering to teat skin, and instead shed down into the milk (Doyle et al. 2017b). Milking system has also been observed to be associated with differences in bacterial counts. Pipeline milking system is

associated with higher counts than milking parlour systems (Cempírková 2012), and automatic milking system has been correlated with higher counts compared to tie-stall milking (Bernes et al. 2019; Skeie et al. 2019). The observations were suggested to be due to the differences in teat cleaning routines.

Numerous studies show correlations with storage temperature, time and the interaction between temperature and time on microbial loads (O'Connell et al. 2016; Doyle et al. 2017a; Vithanage et al. 2017; Paludetti et al. 2018a). TBC levels are positively correlated with both temperature and time of storage. In general, in milk stored at ≤4°C, no considerable increase of TBC is seen, however, significant increases are observed in milk stored at 6°C. Unhygienic milking and improper storage of milk are common in association with higher TBC (Olofsson et al. 2018).

As milk is pasteurized, TBC and psychrotrophic counts are reduced. However, the reduction is seen to be of lower magnitude in samples with initially higher TBC and thermoduric counts have been reported not to be efficiently reduced (Paludetti et al. 2019).

4.2. Compositional variation of raw milk microbiota

Even though the raw milk microbiota mostly consists of a few main genera, it shows for high diversity and variation. Variations in raw milk are seen during the whole process chain from farm level to dairy plant (Parente et al. 2020).

Falardeau et al. (2019) suggested that to the point where milk is stored, farm environment highly affects the microbiota, whereas the subsequent change in bacterial composition is due to the thriving environment for psychrotrophs and environmental contaminants.

The core microbiota in bulk tank milk (BTM) has been investigated in a few studies and is suggested to consist of *Bacillus, Pseudomonas, Streptococcus, Lactococcus*, and *Acinetobacter.* Many bacteria are found in low abundance (Porcellato et al. 2018; Skeie et al. 2019). Falardeau et al. (2019) found anaerobic bacteria to be the most abundant genera, believed to proliferate in the enclosed compartment where milk is stored.

Significant variation in tank milk microbiota was observed between farms in Norway, suggesting that each farm might have its own niche of dynamic microbiome. Variations were observed between farms and between geographical locations, as well as within farms between sampling occasions (Skeie et al. 2019). A longitudinal study on the same farms did however observe a more persistent microbiota with increased abundances of *Bacillus* and *Pseudomonas*. It was suggested to be mostly related to weather during harvest season, together with selected feed. The reason behind this conclusion was the results of questionaries to the farmers revealing that a change in feed with an increase in concentrates and

imported roughage was the main difference between the two studies (Porcellato et al. 2021).

Li et al. (2018) studied how seasonal temperature and humidity fluctuations can influence milk microbiota. *Acinetobacter* and *Firmicutes* were related to low and high temperature, respectively, and *Bacteroidetes* and *Proteobacteria* with low and high humidity, respectively. Also other studies reported variations in milk microbiota correlated with season (Doyle et al. 2017b; Porcellato et al. 2018). Spring and summer milk samples were associated to a more diverse milk microbiota (Kable et al. 2019). Housing was concluded to be major factor behind the composition of milk microbiota, and teat skin the major contributor of bacteria according to Doyle et al. (2017b). Indoor milk samples had a higher prevalence of host/gut-associated bacteria and Gram-positive bacteria such as *Ruminococcus* and *Eremococcus*, and outdoor samples had a higher proportion of soil-derived and environmental bacteria such as *Pseudomonas* and *Acinetobacter*. *Bacillus* spp. have also been seen in a higher abundance during summer months, and *Pseudomonas* spp. and *Lactococcus* in higher relative abundance in winter months (Porcellato et al. 2018).

Teat preparation has been reported to have a significantly reducing effect on *Pseudomonas, Lactococcus* and *Lactobacillus.* Additionally, it was observed that indoor milk samples had increased proportions of *Pseudomonas* after teat preparation (Doyle et al. 2017b).

The diversity of the milk microbiota reported by Bernes et al. (2019) was seen to be strongly correlated with the milking system in use, where farms with automatic milking had higher proportions of *Streptococcus* in their tank milk. Diversity was also associated with the relative abundance of certain bacteria, e.g., a low diversity was associated with an increasing abundance of *Pseudomonas*.

A compositional change in the microbial community, with psychrotrophic bacteria becoming dominant after storage of milk, was observed in several studies (Kable et al. 2016; Porcellato et al. 2018; Bernes et al. 2019; Falardeau et al. 2019). This corresponds with the knowledge about the profitability of these bacteria, including *Pseudomonas,* but also *Psychrobacter, Lactococcus* and *Staphylococcus* to grow in lower temperatures (Yuan et al. 2019). Doyle et al. (2017a) also reported that the proportions of *Pseudomonas*, *Streptococcus* and *Acinetobacter* increased more after storage at 4°C and 6°C in mid-lactation milk, than in late-lactation milk.

Heat processing changes the milk microbiota to be dominated by a few thermoduric groups of bacteria with spoilage character. The relative abundance of *Bacillus* species in pasteurized milk is seen to be a direct consequence of the initial prevalence in raw milk (Porcellato et al. 2018).

Monitoring microbiological quality in raw milk

Today, the dairy industry monitors the microbiological quality of the supplied bulk tank milk and the milk price to the dairy farmer is affected by the TBC (Murphy et al. 2016). EU legislation sets the maximum criteria for TBC in cow´s milk to ≤100 000 cfu/ml, based on rolling geometric average over a two-month period of at least two randomly collected samples per month. TBC is also performed immediately before any processing of dairy products, where the plate count must not exceed 300 000 cfu/ml for raw cow´s milk, or 100 000 cfu/ml for processed cow´s milk (EC 853:2004).

TBC is often a criterion in "premium" payments, with the goal to encourage milk production of high hygienic standard at farm level. In Sweden, a dairy farmer who produces the premium quality milk is given a Gold Medal by the royal family. Among the criteria, milk supplies are to be consistent with TBC levels below 50 000 cfu/ml over a period of 23 years (LRF Mjölk, 2019).

4.2.1. Enumeration of total bacterial counts

Standard plate count for total bacterial counts and other bacterial counts are considered reference methods, and commonly used by dairy industry (Quigley et al. 2013). Culture-dependent analyses are simple but time-consuming and require lots of labour and material. Another issue with these methods is that viable but non-cultivable bacteria will not be enumerated, and thus the results are discriminating and counts sometimes underestimated (Sohier et al. 2014).

Another globally accepted assay for TBC is a cytometric analysis, which reveals the individual bacterial count (IBC). Flow cytometry (FC) gives an exact number due to a staining of various cellular components with a fluorescent dye (Sohier et al. 2014). FC does however only determine stainable bacteria with a signal above the discriminator level (ISO 21187:2021(E)). Legislation is adapting to this new methodology by introducing new limit values, but in countries where CFU is still set as the current limit, a conversion of the IBC results is necessary (Cassoli et al. 2016). The correlations between culturable methods and FC are shown to be good (Gunasekera et al. 2000). ISO has set up international guidelines explaining how conversion relationships are established and verified, according to ISO 21187:2021(E). A scatter diagram is set up to confirm a conversion relationship where the given average of 10 results from each method in the respective unit is compared and calculated. Many factors need to be considered when establishing these relationships as both environmental and analytical factors can influence the results.

4.2.2. Total bacterial count as a quality attribute for farm management

Frequent audits are performed to assess different criteria at the farms as part of quality assurance and certification programs. Their purpose is to improve the quality and safety of milk where TBC is used as one control point. In the study by Múnera-Bedoya et al. (2017) milk quality was clearly associated with the milker´s effort in hygiene. Higher milk quality was related to the knowledge and attitude towards dairy hygienic routines, such as cleanliness and proper milking practices. In Bosnia and Herzegovina, milk payment models were implemented first 2010, with the goal to improve milk quality (Pašić et al. 2016). The implementation confirmed how payment reassurance can improve milk quality production, with a 10% increase of milk fulfilling standard criteria of 200 000 cfu/ml.

In comparison, other studies show little use of TBC as screening tool in farm evaluations. It has been implied that farmers with higher respect to animal welfare are likely to pay more attention to the environment, udder- and milking hygiene. However, in a recent study with focus on the correlation between different quality data and scores on animal welfare at herd level, this could only be partly confirmed (Ginestreti et al. 2020). The study reported that the correlation between TBC and animal welfare data was extremely weak, and TBC therefore only give limited information regarding animal welfare. Vice versa, Flores-Miyamoto et al. (2014) comparison of farms with approved audits and those with negative audits reported that TBC was only 2-6% lower for farms fulfilling audit criteria. Though the results concluded that there is an association between TBC and the result of audits. It was mentioned that it has not always been the case in previous studies and that audits only give limited information on bulk milk quality.

4.2.3. The association between total bacterial counts and other bacterial counts

Cold storage of milk increases the relative abundance of psychrotrophic counts. In high quality milk, psychrotrophs initially constitute approximately 10% of the total counts but after storage their dominance can make up 90% of the total microbiota (Machado et al. 2017). O'Connell et al. (2016) investigating the effect of storage time, temperature, and the interaction between storage time and temperature on the microbiological quality of milk, found that TBC and psychrotrophic bacterial counts (PBC) were strongly correlated. Both counts increased upon storage of the milk at 4°C and 6°C during 96 h. However, PBC showed a significantly higher increase. Other counts were also evaluated, including proteolytic and lipolytic activity, laboratory pasteurization count (LPC) and psychrotrophic-thermoduric

counts (PBC-LPC). For LPC and PBC-LPC, no increase was observed during storage, independent on temperature.

The study of Paludetti et al. (2018) revealed that although samples from different farms had similar initial counts, TBC differed significantly after 72 h when stored at 4°C. Similar patterns were observed for PBC and proteolytic count, whereas no change was seen in LPC or PBC-LPC.

4.2.4. Can the total bacterial count give a reflection of the microbiome?

No distinct correlation between TBC and higher taxonomic groups were identified in a study by von Neubeck et al. (2015). In their study, the diversity of the milk microbiome was evaluated at the end of cold storage by a culture-dependent approach with a further identification of 150 isolates. In contrast, Rodrigues et al. (2017) found associations between TBC and the identified microbiome. In their study, bacterial DNA was extracted from milk samples and sequenced. A core microbiota was identified, including spoilage, spore-forming and pathogenic bacteria. *Streptococcus* was strongly related with TBC above >3.6 log cfu/ml, disclosing how mastitis bacteria can enhance TBC. Also, spoilage associated bacteria such as *Acinetobacter, Enterobacteriaceae* and *Corynebacterium* were all found to be in significantly higher relative abundance in samples with higher TBC. On the contrary, *Bacillus* and *Thermoanaerobacterium* were found to associate with lower TBC. The diversity was negatively correlated with increasing TBC, suggesting that a few taxa were dominant in milk samples with higher bacterial counts. It was concluded that these results correspond with earlier theories that milk quality parameters and total bacterial level are mainly related to the presence of udder pathogens and spoilage bacteria.

4.2.5. Total bacterial count as attribute for product shelf-life prediction

To investigate the analytical test´s power in predicting the shelf-life of pasteurized milk, Martin et al. (2011) analysed silo tank milk in dairy plants, pre- and post-pasteurization. The milk was stored at 6°C and tested up to 21 days after processing. The correlations of the different microbiological results between pre- and post-pasteurization were weak. Similarly, the correlations between raw milk microbiological test results and sensory tests of the processed milk were weak. It was concluded that test results associated to the raw silo tank milk did not have the power of predicting the quality of the resulting pasteurized milk. According to a review by Murphy et al. (2016), counts in the size of 1 000 000 cfu/ml would be needed to cause product defects. In the study by Martin et al. (2011), the average bacteria count was 18 000 cfu/ml, thus milk of high quality and perhaps the level

of bacteria counts was not of spoilage concern. It was recommended to use the tests when screening milk of poor quality. When milk quality is high and the TBC is low, the results are not giving any useful prediction of the shelf-life of the resulting pasteurized milk.

4.2.6. Alternative milk quality attributes

In studies on the impact of cold storage on bacterial counts, it has been suggested that the count of psychrotolerant bacteria could be important as an indicator of milk quality (O'Connell et al. 2016; Paludetti et al. 2018a). Enzyme activity is a reason for spoilage, therefore, Vithanage et al. (2017) evaluated if any associations could be found with the microbiological quality of the milk. The results showed that protease activity and proteolysis using a protease assay kit were much more capable in predicting spoilage of pasteurized milk, than the use of TBC and PBC.

qPCR is widely used in the detection of pathogens in food (Sohier et al. 2014). qPCR has also been seen as useful for quantitative measurements of bacteria, and Maier et al. (2021) reported the use of multiplex qPCR for rapid and accurate quantification of total *Pseudomonas* count in raw milk. The analysis was also able to distinguish between milk-related species with different proteolytic potential. The results of qPCR were considered more precise than plating, since culturing methods possibly counted bacteria of other genera. Katholm et al. (2012) showed how qPCR could be useful in rapid detection of mastitis. qPCR quantification of spore-formers such as *Bacillus cereus* has also been developed, where qPCR is combined with a sample treatment with propidium monoazide (PMA) to only include viable cells in the quantification (Cattani et al. 2016; Kable et al. 2019). This makes it a good option compared to TBC which cannot give a fast distinction without further analysis. However, all kinds of PCR have disadvantages of being highly sensitive and results are easily influenced by matrix and primers in use (Yuan et al. 2019).

Other technologies available for the analysis of microbes found in the results of this literature study include spectroscopic methods. Fourier transform infrared (FTIR) spectroscopy was e.g., the chosen technique to identify clusters of related bacteria in von Neubeck et al. (2015) study. After culturing and in combination with sequencing of the bacterial 16S rRNA gene, the method was able to identify bacteria down to strain level. According to Ziyaina et al. (2020) review of alterative rapid methods for the dairy industry, FTIR spectroscopy´s ability of distinguishing microbial metabolites could be useful in quantitative and qualitative assessment of microorganisms in raw milk.

Other analytical methods for evaluation of the hygienic quality of milk exist, according to a review of available alternatives (Ziyaina et al. 2020). One method mentioned is the adenosine-triphosphate (ATP) bioluminescence technique. Ziyaina et al. (2020) claim this method is a promising alternative to total counts with high reliability and rapid results. Still, matrix can impact the results and for

milk to be analysed, a pre-treatment is needed to destroy somatic cells and casein micelles which otherwise could affect the results. This necessity for pre-treatment creates a disadvantage in terms of practicality.

5. Discussion

The overall objective of this literature study was to evaluate total bacterial count as an attribute for milk quality. This included to find information regarding variations in TBC and milk microbiota, and their relationship. Information regarding TBC´s feasibility in predicting product shelf-life was also considered, together with alternatives.

The purpose of the TBC analysis is to give a broad reflection of the hygienic conditions at farm level and to impact the milk prize to the farmer (Fusco et al. 2020). Good hygiene and proper milk storage are major factors for maintaining high milk quality in the results found in this literature study. Undoubtedly, the use of TBC in incentive programs has worked to ensure a milk production of high hygienic quality, both in history and today. Abnormal TBC levels in the everyday production are also effective indicators of deviations, as seen in the studies by Skeie et al. (2019) and Doyle et al(2017b). In their studies, higher TBC was traced back and found to be signs of mastitis or insufficient cleaning of bulk tanks, disclosing its effectiveness as raw milk quality attribute. Also, when investigating the importance of bedding material, it was pointed out, that proper cleanliness was more related to TBC than the material itself (Bradley et al. 2018).

The investigations on TBC´s correlation with numbers of specific bacteria show a relationship between TBC and psychrotrophic bacteria, especially in culture-dependent studies. The results found when studying the relationship between TBC and the microbial composition through sequencing reveal different outcomes. The correlations observed by Rodrigues et al. (2017) could indicate that TBC can give a simplified reflection of mastitis causing bacteria and contaminants, but not thermotolerant bacteria. However, compositional variation may exist without being reflected in TBC. It is possible that the differences in findings can be explained by the analytical methods that were used and their ability to influence the analytical results. Parente et al. (2019) expressed a need for standard operational procedures in research on the milk microbiome to improve comparability between studies. Having the opposing results found in this literature study in mind, further evaluation of relationships between TBC and the microbial structure is needed.

Higher numbers of total bacterial counts are associated with increasing enzyme activity, which has the potential of damaging milk components and cause product defects. When milk production is of high hygienic quality, it can be questioned if

TBC below the criteria 100 000 cfu/ml will satisfactorily predict the shelf-life of pasteurized or UHT milk (Fusco et al. 2020). TBC will not give further information of which bacteria that are present. Nevertheless, one study concluded that due to the strong correlation between TBC and PBC, TBC is a sufficient indicator of milk quality (Cempírková 2012). Yet, in the study by Martin et al. (2011) also PBC was insufficient to predict shelf-life of pasteurized milk. There is a consensus that a more specific analysis is required since *Pseudomonas, Acinetobacter* and *Bacillus* stand for a lot of spoilage in pasteurized and UHT milk. Spoilage is mainly associated with their production of bacterial enzymes with the ability to withstand heat treatment, thus a focus on certain bacteria might be of good value for the industry (Murphy et al. 2016). As seen in the evaluation by Vithanage et al. (2016), the results from the use of a protease assay showed stronger correlation with the level of proteolytic bacteria of psychrotrophic and thermoduric character, than both TBC and PBC. Perhaps enzymatic activity could be a better indicator for the shelf-life of heat-treated milk since lipases and proteases degrade milk components into compounds which give rise to off-flavours. Alternatively, the numbers of the enzyme producing bacteria since their growth is directly related to the amount of enzymes present (Murphy et al. 2016).

Recent reviews of alternative methods for evaluation of milk quality highlight the more comprehensive information given by other analytical methods (Ziyaina et al. 2020). As new methods show promising use for the industry in its work to improve milk quality, the legislation on raw milk criteria might be subjected to change. It is stated by regulation nr (EC) 853:2004 that the criteria for raw milk currently in use "apply pending the establishment of standards in the context of more specific legislation on the quality of milk and dairy products". Although TBC has its limitations, it is still mostly used by the industry, where plating continues to be the referential method (Burke et al. 2021). New alternative methods need to be fully established to be adopted by the industry and food law. Perhaps more focus is needed in establishing new ways of improving the monitoring of hygienic milk quality to live up to the modern standards requested today and in the future (Fusco et al. 2020).

It can be concluded that incentive payments with the use of TBC work to ensure a production of high-quality milk. TBC has some capability of reflecting the microbiome, although, correlations need further evaluation. Proteolytic and lipolytic activities are the major reasons for spoilage of heat-treated milk. Instead of controlling milk quality using TBC, a focus on quantifying bacteria with the highest enzymatic power could perhaps better meet the industries´ needs.

5.1. Future studies

Future research is needed in the field of milk microbiota and milk quality. One example is the correlations between TBC and the composition of the milk microbiome, considering that the microbiotic diversity is a great challenge for the industry. It could be of interest to evaluate how culture-dependent and culture-independent studies differ, as opposing results appear.

Moreover, better methods for prediction of shelf-life are needed. Research on the level of proteolytic bacteria or their proteolytic activity appear to be lacking, and reviews show how given limits for product defects are based on previously culture-dependent studies. As the technologies develop, an updated evaluation of this could possibly reveal new information.

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