

Raw Milk Quality: Forage Type-Dependent Variation in Lactic Acid Bacteria Prevalence and Abundance

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Abstract

The presence of lactic acid bacteria (LAB) in the milk supply has the potential to taint cheese with off flavors and aromas. We analyzed the incidence and quantity of LAB in milk based on the kind of fodder used in dairy cow feeding to discover causes of LAB that may arise throughout cheese manufacturing. Twenty-four farms were randomly assigned to receive a silage diet, a grass diet, and a grass-and-legume diet. The silage was either left uninoculated with marketable products containing a minimum of a *Streptococcus buchneri* strain and *Saccharomyces casei*, the *plantarum* strain enteric bacteria *faecium*. We discovered no correlation between the level of grass used and the number of LAB count of viable cells in the milk. Despite a 35% rise in lactic bacteria in grass fodder after immunization, there was little variation in the LAB profile of the milk. In addition, three out of 481 isolates belonged to the *L. buchneri* group, making up a negligible percentage. Analysis of 406 LAB isolates using random amplified polymorphic DNA typing suggested that a small portion of the organisms had likely been transferred from silage to milk (6%). Therefore, fodder has a negligible role in the spread of LAB in milk.

Keywords: Silage, non-starter lactic acid bacteria, random amplification of polymorphic DNA, bacteriocin

Introduction

Probiotics lactic acid bacteria (LAB) are a widespread microbial family that plays an important role in many types of food fermentation. However, the environmental conditions and ecological niches inhabited by LAB may greatly alter their frequency and dispersion. Dairy products, including yogurt, cheese, and buttermilk, often include LAB. The predominance of bacteria like *Lactobacillus* and *Streptococcus* in these settings is important for fermentation and taste formation (1). Kimchi, pickles, and sauerkraut are only few examples of fermented vegetables that have high levels of LAB. These items often include the probiotic bacteria *Lactobacillus plantarum* and *Lactobacillus brevis*, which aid in fermentation. Meat products, particularly those that have been fermented or cured, may include LAB. Fermented sausages often include the bacteria *Lactobacillus sakei* and *Lactobacillus curvatus*, which aid in the maturation of taste and preservation (2). Beer, wine, and cider are just a few of the many drinks that benefit from adding LAB during fermentation. It depends on the beverage and the fermentation conditions, but species like

Lactobacillus and Pediococcus may be present. Products made from grains, such as sourdough bread, include LAB. A key player in sourdough fermentation, *Lactobacillus sanfranciscensis* helps give sourdough bread its signature taste and chewy texture (3). The human gut microbiome is incomplete without LAB. Species of the probiotic bacteria *Lactobacillus* and *Bifid* bacterium are ubiquitous in the gut and aid in digestion and the fermentation of dietary fibers. LAB is also an important member of the intestinal microbiota of mammals. Animals' intestines may include a variety of LAB species, including *Lactobacillus acidophilus* and *Lactobacillus reuteri*, which are beneficial to the animals' digestive systems and general health (4). LAB several places in the natural world, including plants, soil, and water. Natural fermentation processes and microbial diversity in ecosystems may benefit from LAB's presence even when their numbers are lower there than in more particular niches. Humans may get LAB colonies on their skin and in their mouths. Oral cavity bacteria include *Streptococcus salivarius* and *Lactobacillus salivarius*, whereas skin bacteria include *Staphylococcus epidermidis*. These LAB strains help keep the microbiome in check and the ecosystem functioning normally (5). Probiotics supplements commonly contain LAB strains as an ingredient. Consuming probiotic products, including strains like *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *bifid bacterium bifidum*, have improved both intestinal and general health. Variation in the frequency and distribution of microorganisms according to the kind of habitat or niche they occupy is known as type-dependent variation. The study (6) discussed the microbial communities and the effects they have on different environments makes this idea very pertinent. Various environmental conditions heavily influence the microbial makeup of an ecosystem. These include but are not limited to temperature, pH, nutrient availability, and oxygen levels. For instance, certain microbes may do better in acidic circumstances, while others may do better in alkaline ones. Because of these differences in environmental conditions, various microbial communities have adapted to and come to dominate in various ecosystems (7). Because different types of microbes have different dietary needs, environmental conditions may significantly impact the predominance of particular microbial communities. For instance, organisms that develop quickly and efficiently in nutrient-rich settings may dominate. However, microorganisms with a slower growth rate better suited to restricted resources may thrive in nutrient-poor settings (8). There is a wide variety of microbial interactions, from competition to cooperation to predation. The prevalence of microbes may be dramatically affected by these interactions. For instance, certain bacteria may have evolved the ability to create antimicrobial chemicals, providing them an edge over rivals in particular habitats. Syntrophic partnerships are one kind of cooperative contact that may alter microbial populations (9). The types of microbes present in a given environment might differ from one host to the next. The resources and conditions available to microorganisms vary widely across hosts. For instance, herbivorous and carnivorous animals have different gut microbiota because they consume different foods. Like the microbiota in the soil, the microbiota in the human gut may differ from person to person based on things like food, genetics, and general health (10). Milk that hasn't been pasteurized or treated in any other way thermally is said to have "aw milk quality," which describes its attributes and safety. The safety of customers and the viability of

milk for different dairy products depend on the quality of raw milk (11). Toxic bacteria, including Salmonella, Escherichia coli (E. coli), Campylobacter, and Listeria monocytogenes, may be found in raw milk, leading to food poisoning. Factors such as animal health, milking procedures, and cleanliness during milk collection and storage might affect the prevalence of these pathogens in raw milk. Milk contains natural antibiotic called somatic cells, which are white blood cells. However, many somatic cells in raw milk suggest that the cows may suffer from udder infections or other health problems. Dairy products with high somatic cell numbers may be lower quality and spoil more quickly (12). Raw milk's fat, protein, lactose, and mineral content all contribute to its overall quality. These factors may influence milk's flavor, nutritional value, and usefulness in different dairy products. Raw milk must be refrigerated promptly and properly to preserve its freshness. To prevent spoilage and the spread of germs, milk should be chilled to less than 45 degrees Fahrenheit (7°C) as soon as possible after collection (13). Raw milk must be handled, stored, and milked following the highest standards of cleanliness to avoid contamination. Cleaning and disinfecting milking equipment, washing hands often, and preventing infection from external sources are all part of this. In addition to the cows themselves, the way a farm is run directly impacts the quality of its raw milk. Dairy animals need to be fed properly, given regular veterinary treatment, milked correctly, and housed in a sanitary environment, all of which fall under this category (14). Raw milk quality may be monitored by testing it often for things like bacterial count, somatic cell count, and composition to catch problems early and keep production up to the line with regulations. Employing quality control methods to correct discrepancies and keep quality consistently high is important. Several jurisdictions have instituted varying restrictions and standards to guarantee the purity and safety of raw milk. Raw milk standards might specify allowable levels of bacteria, somatic cells, and chemical residues (15). There is a greater potential for bacterial contamination in raw milk than in pasteurized milk. Pasteurization, in which milk is heated to destroy germs, is often used to make milk safer for consumption. Raw milk has devoted fans, though; swear by its purported health benefits. Raw milk should only be consumed if obtained from a reputable source that employs stringent quality control processes and abides by all local, state, and federal regulations (16). The study (17) discussed 14 LAB strains representing six species from raw milk fermentations that occurred naturally. Two strains of Lactococcus lactis were chosen due to the technological and nutritional characteristics that allowed them to produce large amounts of GABA in milk when supplemented with 5mmol of monosodium glutamate and co-cultured with Lacticaseibacillus rhamnose or Lacticaseibacillus paracasei. The study (18) summarized recent developments about LAB in conventional raw milk cheeses (made from cow, sheep, or goat milk) and their application as probiotics and makers of bioactive substances with health-promoting benefits. The research (19) aimed to test the hypothesis that no significant differences exist between Adobera cheeses made with pasteurized milk inoculated with a mixture of autochthonous lactic acid bacteria and traditional raw milk cheeses and their technological and quality features. Cheese's texture and color are affected by how water is retained in the matrix due to milk pasteurization. Cheeses from raw milk were denser, more cohesive, and less elastic than their pasteurized milk counterparts. Research (20) suggested

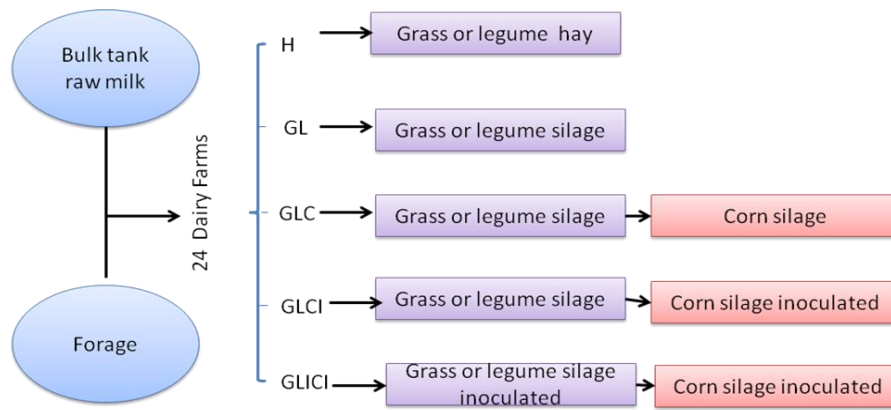
potential for the alpine pasture to directly alter the milk microbiota led researchers to conduct microbiological investigations before, during, and after the cows' summer transhumance. Milk samples at the ALP showed a statistically significant rise in all microbial groupings. The study (21) discussed compiles the relevant information into a coherent whole, outlining the nature of dairy waste and the methods currently used to deal with it. Dairy waste, microbial population, and waste management practices have been analyzed to see how seasonality influences each. Finally, the influence of dairy waste seasonality has been utilized as an estimate to anticipate climate change's impact. The research aims to present the most up-to-date information on how global warming affects dairy waste.

Methodology

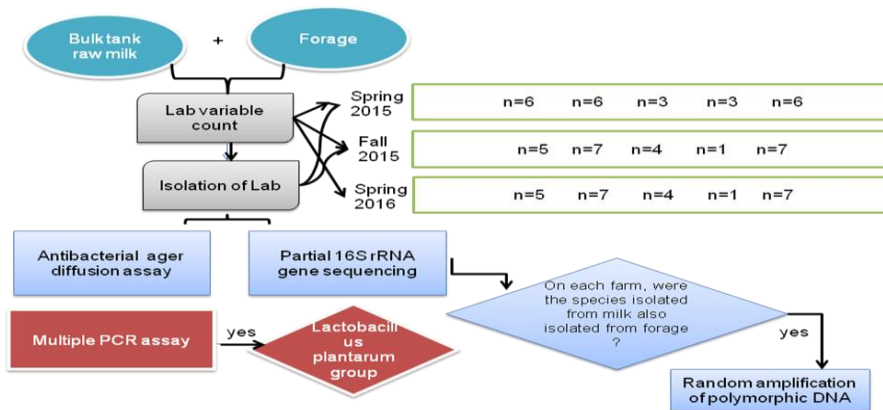
Farm sampling

Collecting, Isolating, and Measuring LAB

There were three samples taken from the dairy farms (Figure 1A). Three farms switched to a different feeding method in the time between our first and second samples. Three farms were converted: 2H to 7GL, 1GLCI to 7GLICI, and 2GLCI to 4GLC. Raw milk and forage were gathered from each location. It took at least 45 days of ensiling to ferment the forages. All samples were transported to the laboratory within 24 hours and stored in a chiller at 4 degrees Celsius until analysis. About 500 grams of fodder was taken from silage storage areas. A sterile scoop or probe was used to collect samples from bundled substances, and the specimens were subsequently moved to a bag devoid of any germs or other live microbes. The DM and pH of the initial subsample sent to the Lactanet lab were measured by infrared and absorbance spectroscopy. Since distinguishing homofermentative from heterofermentative colonies based on color alone proved to be challenging, it was found that total LAB corresponded to the number of viable colonies on ABEV agar. We restored both forms of fermentative LAB, as shown by the bacteria we isolated and their subsequent identification. Milk viability testing was carried out in the same manner as forages. The first group's fodder and milk samples were cultured on Petri plates with a disk, and many of the isolates survived for at least 24 hours. For future use the stock cultures were stored in a solid state at 80 degrees Celsius and 20% glycerol. Isolates were identified as RKG and numbered 1 or 2 depending on the sampling time and the sequence in which they were isolated. The MRS-BPB agar medium was inoculated with commercial inoculants and rehydrated in peptone-buffered saline to mimic the conditions seen in commercial farms. Extracting DNA for RAPD required selecting, purifying, and freezing colonies in the same manner as forage and milk samples.



[A]



[B]

Figure (1) [A]: Schematic depicting the steps involved in collecting agricultural samples, **[B]:** isolating LAB and characterizing their effects

Herd Selection

Farmers were randomly chosen from a list of profitable dairy farms ($n = 24$) in Quebec provided by Canadian DHIA. To better understand the general feeding techniques in eastern Canada, farms were classified into one of five feeding categories based on the forages they employed (Figure 1B). The first group, the "control," consisted of farms that relied only on silage (H; non-fermented). The second group consists of GL farms, which feed grass or legume silage. To round out their diet, the third group supplemented their animals' grain with grass or legume silage (GLS). Four groups also fed their animal's grass or legume silage that had been infected at harvest. Providing a mixture of inoculated corn silage and grass or legume silage (GLI; GLICI) was common among the fifth group. (Table 1) shows the inoculation agents utilized by GLCI and GLICI dairy producers. These included the available on-the-market inoculants Biotal Buchneri 500, Biotal Supersile, 11CFT, 11C33, and 11G22. There was no grass available for the animals at the dates of sampling.

Table (1): The dairy industry's reliance on commercial inoculants is discussed

Feed type	Farm number	Lactobacillus casei	Inoculant	Lactobacillus buchneri	Species in inoculants		
					Pediococcus Pentosaceus	Lactobacillus Plantarum	Enterococcus faecium
GLICI	1		1 and 2	X	X	x	
	2		1	X	X		
	3	x	1 and 3	X	x	x	
	4		1	X	x		
	5		1	X	x		
	6	X	3 and 5	X		X	X
	7		4 and 5	X		x	x
GLCI	1		4	X			x
	2	x	3	X	x	x	
	3		1	X	x	X	

Bacterial Identification and Typing

Partial sequencing of the 16S ribosomal RNA gene was used to identify all the sampled isolates. A customized Geneaid Presto Mini gDNA Bacteria Kit was used first to extract genomic DNA. The lysozyme concentration in the gram-positive buffer was raised to 20mg/mL, and mutanolysin was added. All sample preparation incubation periods were, at long last, doubled. The LAB strains that were typed might have come from a variety of sources, including silage. We chose raw milk and forage-collected isolates for each farm from the same species. For instance, if *L. plantarum* were found in milk on a particular farm, we would type all milk, fodder, and inoculant isolates. Clustering was carried out using the unweighted pair group approach with an arithmetic mean, and similarities between isolates were evaluated using Pearson's correlation. Genotype definition was set at a cutoff of 80% similarity.

Diffusion Plates for Bacteria Growth Inhibition

The agar well dissemination strategy was utilized to test all disconnects for their antibacterial movement against decay pointer microscopic organisms and cheddar starter microorganisms. The strains of *Listeria ivanovii* HPB28, *Clostridium tyrobutyricum* ATCC 25755, and *Lactococcus lactis* spp. were all grown in their respective optimal broths (tryptic soybean broth with 0.6% fermented yeast extract, enhanced clostridia medium, and Elliker material, respectively) in a single day. The identical scenario medium, this time with 0.75 percent agar, was infected with a 0.6 percent quantity of the bacteria. Wells were drilled onto agar plates before being inoculated with aliquots of isolate supernatant (80 L each). The next day, I took

an MRS culture incubated overnight and centrifuged it (10,000 g for 10 minutes at 4°C) to obtain the residue. The plates were perused after 24 hours, brooding at 37 degrees Celsius (in anaerobic circumstances for *C. tyrobutyricum*).

Statistical Analysis

We used a the pupil's t-test, one-way analysis of variance, Tukey's various comparisons, or a difference of statistical significance test for valid count data that fulfilled these requirements. The remainder of the information was analyzed using the Kruskal-Wallis-Wilcoxon test, a parametric alternative to the ANOVA. When it came to crunching the numbers, we turned to JMP 14. In the R programming environment, we converted data on the relative abundances of different taxonomic groups. The STAMP 2.1.3 program was then used to evaluate the abundance ratios. Multigrain analysis of variance (ANOVA) was used to determine statistical significance, and P-values were corrected using Storey's FDR method.

Results

Forages varied in their qualities depending on the kind. For instance, inoculation did not change the fact that C silage had a lower pH than GL silage. Since MRS-BPB agar was not a LAB-specific medium, we only reported viable counts from the ABEV medium. Both inoculation and uninoculated silages had higher LAB viable counts than H (Figure 2). Only during the second sample period did inoculated grass have a higher LAB viable count than non-inoculated silage. Seven hundred and fifty-eight LABS were found in forages during the first two sample times. There were a total of 42 taxa representing six different genera.

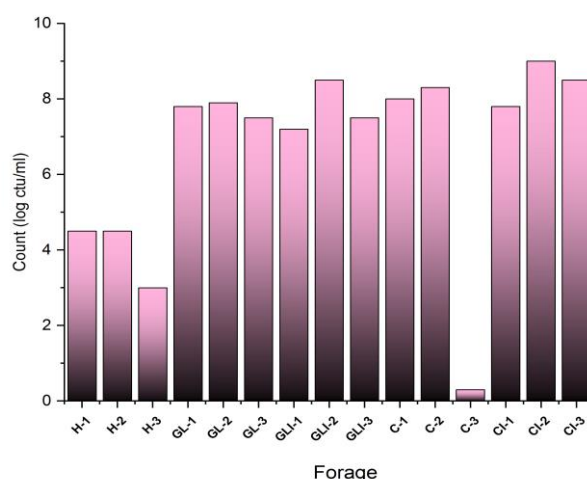


Figure (2): Cultured lactic acid bacteria with high viability on arginine-rich agar

The STAMP statistical analysis revealed that the LAB profiles of H and silages were distinct. Mean rates differed by 20% among H and silages and 15% among *Enterococcus* and *Leuconostoc* at the family level (Figure 3) [A] *Enterococcus*, [B] *Lactobacillus*, [C]

Leuconostoc, and (Table 2). Lactobacillus spp. was found in higher concentrations in the silages than in the H.

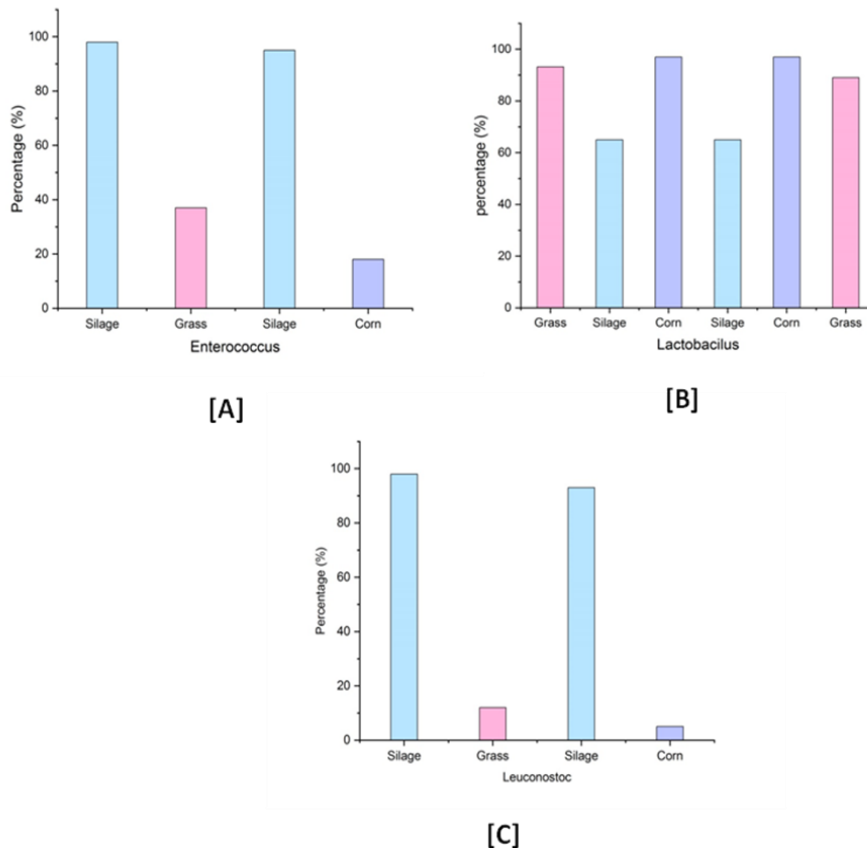


Figure (3): Analyzing the diversity of lactic acid bacteria among various forages genus level [A] Enterococcus, [B] Lactobacillus, [C] Leuconostoc

Table (2): numerical outcomes for forages genus level

Mean proportion (%)	Silage	Grass	Silage	Corn
Enterococcus	98	37	95	18
Lactobacillus	65	93.2	65	97
Leuconostoc	98	12	97	89

Lactobacillus bacteria dominated both corn silage and grass and legume silage. We also discovered important species-level characteristics of the forages (Figure 4) [A] Enterococcus mundtii, [B] Pediococcus pentosaceus, [C] Lactobacillus penthouse [D] Lactococcus lactis and (Table 3). 48% of grass/legume silage and 38% of corn/chicory silage had detectable

levels. Corn silage had a higher concentration of *Lactobacillus brevis/yonginensis/koreensis* than legume silage. Grass and legumes (GL + GLI: 12%) and silage (20%) had higher concentrations of *Pediococcus pentosaceus* than maize (C + CI: 1.5%).

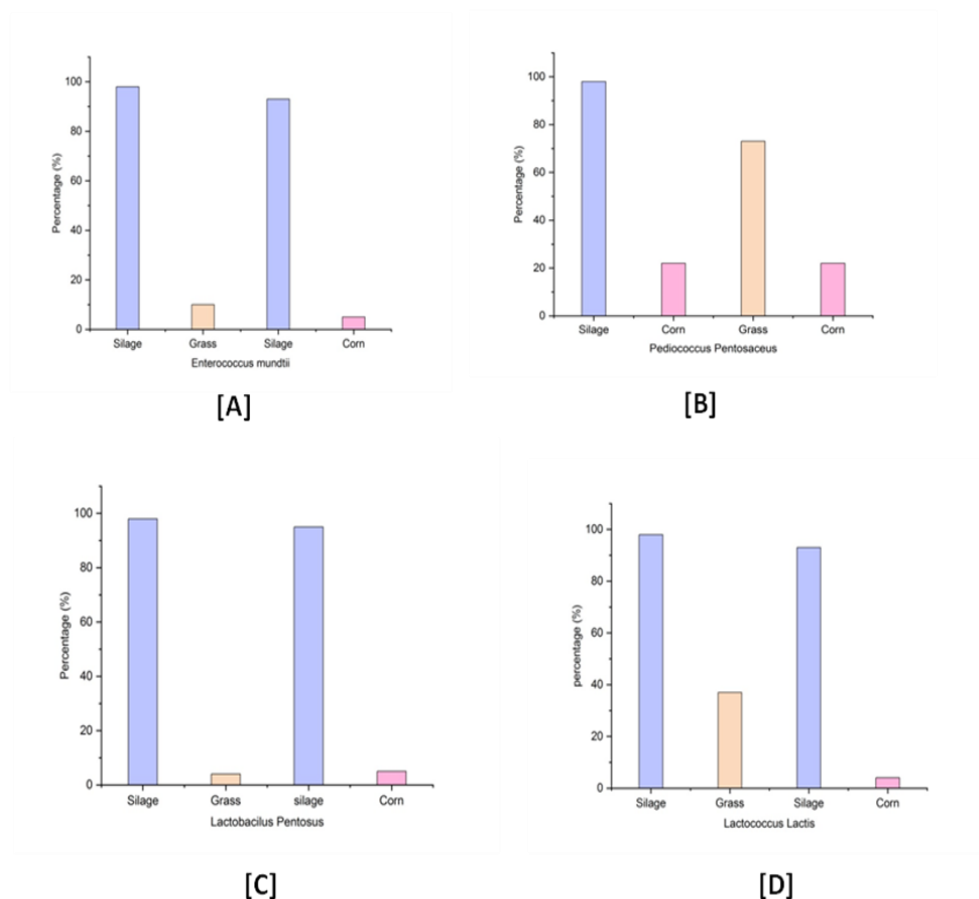


Figure (4): Analyzing the diversity of lactic acid bacteria among various forages species level, [A] *Enterococcus mundtii*, [B] *Pediococcus pentosaceus*, [C] *Lactobacillus penthouse* [D] *Lactococcus lactis*

Table (3): numerical outcomes for forages species level

Mean proportion (%)	Silage	Grass	Silage	Corn
<i>Pediococcus Pentosaceus</i>	98	73		22
<i>Enterococcus mundtii</i>	98	10	93	5
<i>LactobacillusPentosus</i>	98	6	95	5
<i>Lactococcus Lactis</i>	98	37	93	3

There was no difference in the prevalence or quantity of LAB between the C and CI silage samples. (Figure 5) shows differences in the relative abundance of some taxa between GL and GLI silages during the first two sampling periods, namely *Lactobacillus*, *Pediococcus*,

and *Weissella*. GLI silage had 35% more *Lactobacillus* spp than control silage. *Weissella* spp. and *Pediococcus* spp. were found in greater numbers in the GL silage. The LAB profiles of the GL silage samples were in the center of the spectrum when compared to H profiles and other silages. *Weissella paramesenteroides/thailandensis* was shown to be the most common bacterium in GL silage, followed by *P. pentosaceus* and enterococci, just as it was in grass. Overrepresented in the GL silage, as in the GLI, C, and CI samples, was the *L. buchneri* group.

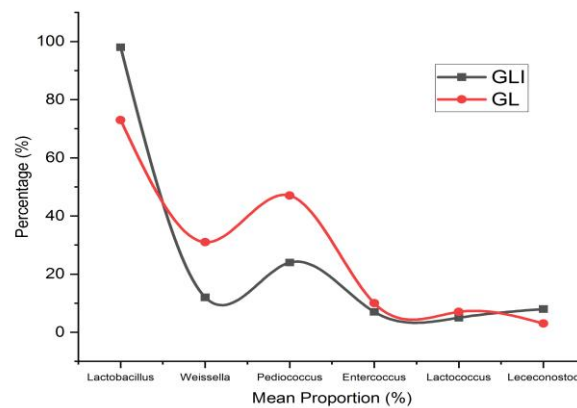


Figure (5): Inoculation's influence on grass and legume silage (GL)

Bulk Tank Raw Milk Contains High Levels of LAB

The mean convergence of LAB reasonable includes in mass tank milk tests was 2.56 1.04 log cfu/mL, and we found that the examining times did not affect this worth. (Figure 6) shows a significant difference in the number of live LAB in the milk samples across the different farm feeding methods. Compared to the GL and H groups, LAB levels in milk produced by the GLICI group of farms were substantially higher. Two rounds of sampling yielded 481 LAB isolates from bulk tank raw milk samples. LAB profiles did not include 12 *Streptococcus* isolates because these bacteria are only found in mastitis and not in forage. A further 469 LAB isolates represent 39 taxonomic groups. Comparable species as those found in human guts were also present in fodder, including *Enterococcus*, lactic acid bacteria, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Weissella* bacteria. Substantial differences in LAB profiles were discovered across silage types, but were far less noticeable between milk types. After making many comparisons at the genus and species levels, it was found that there was only important variation in bulk tank milk samples when categorized by feed methods. Compared to milk specimens from the other feeding regimens, the bacterium *parvulus* was more prevalent (3.1% more abundant) in GLCI milk. However, *P. parvulus* was only found in a single case (12%) in this and different milk samples.

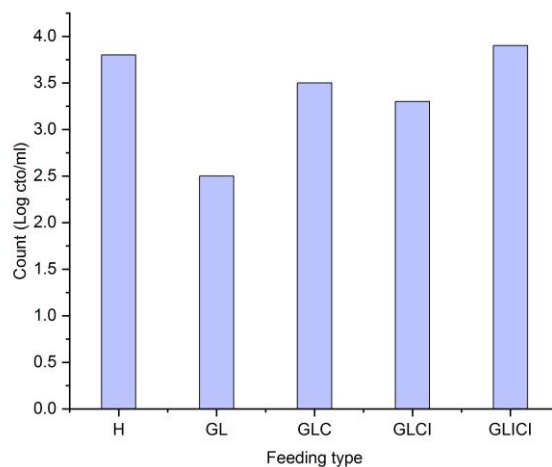


Figure (6): Results of culturing bulk tank milk for lactic acid bacteria on arginine-supplemented agar

Examining Bacteriocin Activity

In this study using *Lis ivanovii* HPB28, only 36 of 1,239 culture supernatants exhibited substantial antibacterial activity, suggesting a bacteriocin effect. Approximately 64% of these supernatants were isolated from *L. plantarum*-related strains. Similar to *Lcc. lactis* sp. *cremoris* SK11, 50% of these inhibited that strain's development. The development of *Lactococcus lactis* subsp. *cremoris* SK11 was suppressed by bacteriocin-like substances produced by 20 different species of *Lactococcus*. The growth of *C. tyrobutyricum* ATCC 25755 was stifled by lactic RKG 2-85. Milk was found to contain 77% of all bacteriocin-active isolates. Nobody of the commercial inoculant isolate showed inhibitory effects comparable to bacteriocin.

Characterization of Forage-Transferred Bacteria in Milk

Similar species isolates were typed from milk and forages from each farm. Only 172 481 milk isolates (representing 18 taxa) were found in at least one pasture or commercial inoculant. Pearson correlation analysis was used to compare the 172 RAPD patterns across farms and species. More than that, five strains showed striking similarities to commercial inoculants. Bacteriocin activity screening suggests that stresses RKG 2-212 and RKG 2-227, which were 94% identical, might be transferred from silage to raw milk due to their antibacterial impact on *Lis. Ivanovii* HPB28. Four of these strains (RKG 1-375, 1-378, 1-380, and 1-500) were shown to have inhibitory activity against *Lis. Ivanovii* HPB28 during bacteriocin screening, proving that they were not isolated from forages or commercial inoculants. The results of the inhibitory activity tests showed that 27 of the bacteria were present in both silage and raw milk from the bulk tank.

Discussion

Consistent with a meta-analysis, the current investigation found LAB in farm-scale silage samples from eastern Canada. Non-inoculated silage had a mean LAB count of 7.04 log cfu/g across all search types (22). The findings are consistent with those of studies conducted on Italian farms. Meta-analysis findings are consistent with our LAB viable count estimates for C silage. Their research revealed that the average LAB in inoculated silage was 7.27, whereas the average LAB in non-inoculated silage was 6.40 log cfu/g. Their research was conducted in synthetic silage created in a lab rather than in a real farm setting. Figures 3, 4, and 5 show how the LAB communities varied depending on the availability of food sources. Indeed, H and GL silage were linked to enterococci (23). They are epiphytic in young alfalfa fields, but their numbers decrease when *Lactobacillus* proliferates in ensiling. In particular, *Enterococcus mundtii* was associated with *H. pylori*. It was discovered that this species thrives as an epiphyte on young grass and legume plants. The ability to persist for extended periods in dry environments may explain why *Enterococcus* spp. is more prevalent than other bacteria in H. Also, the capacity to withstand high osmolarity and dehydration likely contributed to *Lcc. lactic* ubiquity in H. Fermentation of fodder may be started using this species; however, it is less acid-tolerant than *Lactobacillus* spp. Studies have shown that lactobacilli comprise 50 to 98% of corn and grass silage components (24). Whether or not the silage had been infected, species belonging to the *L. buchneri* group predominated. Comparing inoculated and non-inoculated alfalfa silage, the current investigation found that the relative abundance of this organism was similar in both. In mature silage samples, *Lactobacillus buchneri* predominated. Analysis of its genome, transcriptase, and proteome has shown that this species is resistant to rival microbes by many methods (25). These include lactic acid production, acetic acid production, and hydrogen peroxide production. According to the result (26), C silage had a higher concentration of this obligatory heterofermentative group than GL silage. Since *L. brevis* may thrive in acidic environments, it was shown that C silage had a lower pH than GL silage. As a result, the pH of both "C and CI" silage samples may drop readily, rapidly encouraging the growth of acid-tolerant lactobacilli. They conducted a systematic analysis of the inoculation of silage with "homofermentative and facultative heterofermentative LAB." Silage made from grass and legumes fermented better after being inoculated during harvest, while corn silage did not benefit from this method. It's not true that *Pediococcus pentosaceus* can only be found as an epiphyte on grass and legume plants. It has been demonstrated to have a comparable effect on alfalfa as on maize. *P. pentosaceus* was less prevalent after being injected into GL silages. Variations in the composition and abundance of LAB in raw milk from various pasture types were not reflected. Since *L. buchneri* is prevalent in fermented fodder and has been identified in cheese, we reasoned that there would be a higher concentration in milk samples from animals that had been given silage. Raw milk was rarely found to contain this taxon. Although acid sensitivity and the ability to use LAB as an energy source are desirable in raw milk, they are not employed as selection standards. It has been shown that silage is a major contributor to bacterial spore contamination in milk. RAPD typing data, however, revealed

that only a small number of LAB strains, most likely from grass, were found in raw milk. Our research could not determine the source of the pollution. Possible transmission routes include fecal contamination of forages, contact between forages and cows' hair and skin, and transfer inside the barn (27). For instance, silage spores have been detected in cow poop, indicating they are digestion-resistant. Based on the metagenomic data, lactobacilli seem to be highly specialized microorganisms. Genes are acquired by horizontal transfer, and redundant coding sequences are lost when the species adapt to new environments, such as the milk industry. They are less able to make it in vulnerable ecosystems like plants. This may be why so few exploration isolates were found in milk samples. Forage-adapted LAB may also be deficient in genes required for intestine survival. Therefore, there may be fewer possibilities for transmission from feces to milk. One strain's DNA was traced back to its original plant host. *L. plantarum* strains from various environments modify their transcriptase to thrive in MRS media. Raw milk samples from farms that fed their cows *L. buchneri*-inoculated silage showed neither more or lesser prevalence or quantity of LAB than those from other farms. Silage inoculation as a management strategy shouldn't affect cheese production. Additionally, inoculation can be recommended to restrict enterococci in GL silage varieties (28). The chemoresistant bacteria *Enterococcus* spp. is a recognized culprit in cheese manufacturing issues. Typically found as NSLAB in cheese, these facultative heterofermentative species seem better suited to various ecological settings. Additionally, certain silage-isolated *L. plantarum* strains were able to suppress *Lcc. lactis* ssp. *cremoris* SK11. The starter activity and milk acidity during cheese production may be affected by the presence of these NSLAB.

Conclusion

In this study, LAB is found in varying abundances depending on the niche or habitat. LAB may be discovered in a wide variety of places, including foods, the gut microbiota of humans and animals, the environment, the skin, and the mouth. To use LAB for their positive characteristics in food production, human health, and environmental applications, it is vital to understand the type-dependent variation in predominance. In contrast to pasture samples, bulk tank milk did not show variation in LAB prevalence and abundance. This study's results show that silage has a low risk of introducing LAB into raw milk. Only 27 of the 481 milk isolates tested were found to be silage-related. The most common species were those of the *Lactobacillus casei/paracasei*, *Lactobacillus plantarum*, *Penicillium pentosaceum*, and *W. paramesenteroides* families. More research is required on these strains' heat tolerance and possible impacts on cheese production. Only two strains were found to have possible commercial inoculant origins, and neither was found to be *L. buchneri*. Milk contamination by obligate heterofermentative LAB was not impacted by *L. buchneri* inoculation of silage.

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