Relationship between milk microbiota, dairy cow mammary health, and calf gastrointestinal development: a postprint

Authors: Wang Yue, Wang Bing, Tong Jinjin, Linshu Jiang, Xiong Benhai

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Abstract

Significant advances in culture-independent microbial technologies and the development of bioinformatics have provided new insights into the complex community of milk microbiota, revealing that milk microbial communities exhibit rich diversity and multifaceted biological functions, can directly reflect the metabolic status of dairy cows, and influence the nutritional metabolism and health of newborn calves. Therefore, this review summarizes the latest research advances on dairy cow milk microbiota, with emphasis on the endogenous pathway hypothesis of milk microorganisms, discusses their crucial roles in bovine mammary health and calf gastrointestinal development, and outlines the application of techniques including 16S genomics and metagenomics in milk microbiome research.

Full Text

Relationship Between Milk Microbial Community and Mammary Gland Health of Dairy Cows as Well as Gastrointestinal Development of Calves

WANG Yue[1], WANG Bing[1], TONG Jinjin[1], JIANG Linshu[1]*, XIONG Benhai[2]* [1]Key Laboratory of Dairy Nutrition in Beijing, College of Animal Science and Technology, Beijing Agricultural College, Beijing 102206, China [2]Beijing Institute of Animal Science and Veterinary Medicine, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Abstract: Recent advances in culture-independent techniques and bioinformatics technologies have provided new insights into milk microbiota, revealing it as a complex community with rich diversity and multifaceted biological functions that can directly reflect the metabolic status of dairy cows and influence the nutritional metabolism and health of newborn calves. This review summarizes current research findings on milk microbial communities, focusing on the endogenous pathway hypothesis of milk microorganisms, discussing their important roles in mammary gland health and calf gastrointestinal development, and outlining the applications of 16S genomics and metagenomics in milk microbiota research.

Keywords: dairy cow mammary gland health; microbial community; milk; calf; gastrointestinal tract

Milk contains a complex microbial flora that has attracted increasing attention in recent years. The development of advanced omics technologies enables more detailed understanding of milk microbiota, triggering extensive research interest. Existing studies have revealed that due to the complexity and interactivity of microorganisms within dairy cows, diverse microbial communities exist and play important roles in bovine biology, significantly impacting cow health status [1]. Research has demonstrated complex and close associations within the gastrointestinal microbiota of dairy cows [2]. In recent years, milk microbial communities have been isolated and identified from various anatomical sites, from obvious locations such as skin and urogenital tract to less obvious ones like the respiratory tract, and even from previously considered sterile regions such as the placenta [3].

Early studies considered the mammary gland and its milk sterile, with milk microorganisms viewed as external contaminants. However, this perspective has been challenged by culture-independent techniques and more precise molecular methods, leading to the proposal of an endogenous pathway hypothesis for milk microorganisms [4]. This review aims to summarize the sources, composition, and detection methods of milk microbiota, as well as their important effects on mammary gland health and calf growth and development, providing a theoretical foundation for understanding the relationship between milk microorganisms and dairy cow health.

1. Sources of Milk Microbial Community

Milk microorganisms have traditionally been considered external contaminants, primarily introduced through environmental microbes accessing the mammary gland via teat skin or oral cavity [5]. Recent research has confirmed an endogenous pathway, demonstrating that microorganisms from various physiological sites can interact and potentially access the mammary gland through specific routes [6].

1.1 Exogenous Pathogen Invasion

Exogenous pathogen invasion represents a major factor inducing bovine mastitis. Mor et al. [7] identified *Corynebacterium bovis*, *Streptococcus agalactiae*, and coagulase-negative *Staphylococcus* as primary exogenous pathogens causing subclinical mastitis. Mastitis-causing pathogens can be categorized into two groups based on transmission characteristics: contagious pathogens that colonize the mammary gland and spread through milking, including *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Staphylococcus aureus*, and *Mycoplasma*; and environmental pathogens including *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Serratia*, *Proteus*, and *Pseudomonas* [8].

Bacteriocins and antimicrobial molecules released by specific milk components may suppress potential pathogen outbreaks, preventing intramammary infections [9]. Quigley et al. [10] demonstrated that bovine microbial communities exhibit host dependency, suggesting their composition may influence mastitis susceptibility. Studies on mammary gland infections and microbe-induced mastitis revealed that gut microbiota may serve as a potential microbial reservoir for mastitis [11]. Human milk oligosaccharides (HMO) can modulate gut microbiota, and bovine milk contains structurally similar oligosaccharides called bovine milk oligosaccharides (BMO). Therefore, BMO may also influence mammary microbial communities [12]. Interestingly, BMO reflects blood type characteristics and is controlled by genetic factors [13]. Research confirmed that *Staphylococcus*, a primary mastitis pathogen, binds only to specific BMO types, indicating that mastitis susceptibility can be determined not only by milk microbial composition or specific pathogen exposure but also by genetic makeup and corresponding BMO types [14].

1.2 Endogenous Pathway Hypothesis

The presence of microorganisms in milk has long been attributed to external contamination from teat skin or offspring’ s oral cavity. However, recent studies challenge this view, showing that bacteria in milk differ genetically from the same species found on skin of the same host, indicating that teat skin and streak canal are not the sole sources of milk microbiota [15]. Additionally, strictly anaerobic bacteria such as bifidobacteria make skin an unlikely transmission vector, supporting the endogenous pathway hypothesis [16].

Host microbial communities do not constitute independent environments but are interconnected and continuously interacting. Therefore, microorganisms from other anatomical sites could access the mammary gland through various pathways [17]. The “gut-mammary pathway” has been described based on the ability of microbes to leave the intestinal lumen, traverse mesenteric lymph nodes, and reach the mammary gland [Figure 1: see original paper] [18].

The endogenous origin hypothesis has been validated through mouse studies [19]. While mechanisms for microbial translocation across intestinal barriers remain unclear, research suggests involvement of immune cells, particularly dendritic cells (DCs) [20]. DCs can sample intestinal contents by opening tight junctions between enterocytes and extending dendrites into the lumen without compromising epithelial barrier integrity. These cells can carry live commensal bacteria to mesenteric lymph nodes, where bacteria can survive for days and disseminate to distant mucosal surfaces, including the mammary gland, via mucosal-associated lymphoid system. During lactation, gut-associated lymphoid tissue cells can be transported to the mammary gland through lymphatic vessels and peripheral blood circulation [21]. Yozwiak et al. [22] demonstrated that human peripheral blood mononuclear cells and milk cells contain bacteria and their genetic material during lactation. Reports of viable lactic acid bacteria in human blood further suggest that some gut microbiota members can travel to extraintestinal sites in viable form [23]. Studies also found increased bacterial translocation frequency in mouse intestines during pregnancy and lactation, with DCs serving as carriers when bacteria are present in lactating mammary tissue [23].

Beyond transporting gut microbiota, this mechanism guides offspring immune system recognition and initiation of appropriate responses to symbiotic microbial molecular patterns [24]. This translocation may occur selectively—certain bacteria may be recognized and transported into milk, while others may not, or immune cells may uptake all microbes but only those escaping death can reach the mammary gland [24-25]. Garcia-Garcera et al. [26] reported research supporting endogenous gut-mammary pathway in ruminants by investigating microbial composition and diversity in feces, milk, and blood leukocytes of healthy lactating cows using pyrosequencing of barcoded 16S rDNA amplicons. The study found *Rhodococcus*, *Bifidobacterium*, and *Peptostreptococcaceae* coexisting in all three sample types from the same cow. To avoid external contamination and prevent teat canal stretching or damage, milk was collected via catheter using gravity into sterile containers. This coexistence strongly supports microbial migration between mammary gland and gut via leukocyte circulation [27]. However, the specific cell types responsible for transporting microbiota from mesenteric lymph nodes to milk remain to be determined, requiring further research to dissect mechanisms of gut-to-mammary microbial transport in ruminants, which could have important implications for ruminant, offspring, and human consumer health.

2. Composition of Milk Microbial Community

Milk contains complex and diverse microbial communities, reaching several colony-forming units per milliliter [28]. Abundant microbial communities exist even in milk from healthy cows and mammary glands [29]. Recent research has focused on milk microbiota to understand its physiological and health roles in cows and offspring, and how microbial communities change when milk is consumed directly or processed into dairy products [30].

TABLE:1 Composition of healthy milk microbial community [30]

Types	Genus of microbial community
Colostrum	*Streptococcus lactis*, *Lactobacillus*, etc.

Types	Genus of microbial community
Fresh milk	Hemolytic streptococcus, *Streptococcus pyogenes*, *Brucella*, *Streptococcus mutans*, *Salmonella*, *Shigella*, proteolytic bacteria, fat decomposition bacteria, acid bacteria, *Escherichia coli*, Rhodes bacteria, *Pseudomonas*, *Sphingomonas sp.*, *Oligospora*, aerobic bacteria, slow rhizobia, *Aeromonas*, *Staphylococcus*, etc.
Pasteurized milk	Thermophilic bacteria, *Bacillus*, heat-resistant bacteria, etc.

Masoud et al. [31] studied milk microbial composition in healthy dairy cows, finding *Streptococcus* and *Staphylococcus* as the most abundant genera. Other studies showed higher numbers of lactic acid bacteria, followed by streptococci and staphylococci [32]. Montel et al. [33] identified higher levels of bacterial sequences from phyla Firmicutes, Proteobacteria, and Bacteroidetes in milk macrophages. The same study found that the core microbiota of healthy milk includes staphylococci and streptococci, with high inter-individual variability observed among healthy cows. Another study on milk microbial mutants showed Proteobacteria (65%), Firmicutes (34%), *Pseudomonas* (61.1%), and *Staphylococcus* (33.4%) as predominant groups [34]. All these differences correlate with geography, genetics, or diet and warrant further investigation. Valles-Colomer et al. [35] used pyrosequencing of bacterial 16S rDNA genes to assess bacterial DNA diversity in milk samples from 10 different cows, comparing microbial populations from milk samples obtained from healthy udders of the same cow. Results showed significant differences in microbial species among different healthy milk samples, with the most abundant genera being *Pseudomonas syringae*, *Pseudomonas*, *Sphingomonas sp.*, *Stenotrophomonas maltophilia*, *Bradyrhizobium*, *Corynebacterium*, *Pelomonas*, and *Staphylococcus*. In milk samples from a single healthy udder, *Pseudomonas* and *Pseudomonas sp.* abundance was significantly higher than *Lactobacillus* [35]. In a recent study, Tveit et al. [36] described microbial diversity in milk samples with low somatic cell counts (SCC) and uninfected mammary glands. Uncultured Lachnospiraceae and propionibacteria were present in all samples from healthy quarters and could be considered core microbiota of healthy milk. Other commonly found microbial genera in most very low SCC milk samples included *Bacteroides*, *Staphylococcus*, *Streptococcus*, anaerobic bacteria, *Lactobacillus*, *Porphyromonas*, *Komo genus*, *Freundella spp.*, and *Enterococcus*. Certain bacterial genera such as *Lactobacillus* and *Paenibacillus* are associated with mammary gland health.

3. Detection Methods for Milk Microbial Community

Traditional bacterial classification and identification primarily relied on morphological characteristics, physiological and biochemical reactions, ecological features, and serological reactions, such as plate colony counting and Gram staining. However, these conventional methods suffer from long detection periods, cumbersome operations, and difficulty distinguishing closely related bacteria [37]. With molecular biology advances, characterization of entire microbial genomes and metagenomes can be accomplished through targeted 16S rDNA sequences or extensive metagenomic sequencing. The former method relies on PCR amplification and sequencing of 16S rDNA gene fragments (16S genomics), enabling characterization of bacterial components within microbial communities [38].

In 16S rDNA sequencing studies, a pair of "universal" primers is designed to bind conserved regions and amplify variable regions containing taxonomic information [39]. Sequencing the amplified 16S rDNA fragment library allows accurate assignment of each read to its specific taxonomic unit, enabling estimation of relative abundance for each taxonomic group [40]. Hettich et al. [41] used 16S rDNA sequencing to study 20 healthy milk samples with low SCC, finding almost no clonal bacterial sequences. Although *Porphyromonas* sequences were detected, their prevalence was lower compared to mastitic milk samples, suggesting some anaerobes may only act as opportunistic pathogens. However, amplicon-based metagenomics has several limitations, including diversity loss due to PCR bias and variability in diversity estimation [42].

Shotgun metagenomics extracts entire metagenomic DNA without amplifying specific target sites, fragments it, and sequences it, generating large numbers of genomic sequences that can be aligned to genomic positions across all DNA genomes in the community [43]. Muth et al. [44] analyzed hybrid dairy cow milk microbial structure using shotgun metagenomics, generating 63 genes, 11 loci, and 780,000 base pairs (Mb) across 79,856,114 sequences. Results revealed dominance of Enterobacteriaceae members in milk microbiota, followed by *Pseudomonas*, *Bacillales*, and *Lactobacillales*. Metabolic analysis indicated multiple antibiotic resistance potential in microorganisms after antibiotic administration, including sequences for *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Staphylococcus epidermidis*.

4. Milk Microbial Quantity, Composition, and Mammary Gland Health

According to national standard GB 19301-2010, raw milk quality and safety are compromised when total bacterial count reaches 2×10 CFU/g (or CFU/mL). Raw milk samples exceeding this limit cannot be used as raw material for dairy processing. Moreover, extremely high bacterial loads in raw milk indicate extensive microbial colonization within the mammary gland, potentially including pathogenic microorganisms threatening mammary health [45]. Beyond quantity, microbial composition significantly affects mammary health. Invasion by exogenous pathogens (e.g., *Staphylococcus aureus*, *Streptococcus uberis*) disrupts normal microbial development, triggers mammary inflammation, and leads to elevated milk SCC and decreased milk production. Conversely, probiotic colonization (e.g., lactic acid bacteria, bifidobacteria) benefits mammary health and milk quality.

Liu Yanyan [46] used fluorescent quantitative PCR to determine total microbial counts in 100 raw milk samples, finding 37 samples with cycle threshold (CT) values below 29.81, indicating bacterial loads exceeding 2×10 CFU/mL. Health examination of cows producing these 37 samples revealed 16 with clinical mastitis and 21 with subclinical mastitis symptoms detected by California Mastitis Test (CMT). Hettich et al. [47] found that inoculating cows with *Lactobacillus lactis* subspecies isolated from milk increased immunoglobulin isotype levels and memory cell proliferation in blood and milk. Tanca et al. [48] showed that stimulating bovine mammary gland lymphocytes with lactic acid bacteria and bifidobacteria in vitro prevented reaction with *Staphylococcus aureus* surface antigens, suggesting probiotics could serve as natural antibiotic alternatives during dry periods and act as immunomodulators stimulating local and systemic defense mechanisms. Winter et al. [49] detected approximately 56 different species with varying abundances in milk from cows with subclinical mastitis using metagenomics, with *E. coli* being most predominant, followed by *Pseudomonas aeruginosa*, *Pseudomonas pseudomonas*, and *Bacillus cereus*. Interestingly, staphylococcal phages were also present. Tang [50] investigated effects of dietary selenium-enriched probiotics (Se-Pro) on bovine mastitis and milk SCC. Compared to the control group, Se-Pro group (supplemented with 108.2 g/head/day) showed significantly increased blood selenium levels and 35% reduction in mastitis-positive quarter rates. Increased blood selenium helps reduce SCC, decrease mastitis incidence, and enhance neutrophil function. These findings demonstrate that milk microbial quantity and composition are key indicators of mammary health, with certain probiotics promoting mammary gland health.

5. Impact of Milk Microbiota on Calf Gastrointestinal Microbial Colonization

Milk contains immune cells and various bioactive molecules, including sugars, nucleotides, lipids, immunoglobulins, antimicrobial proteins, cytokines, and other immunomodulatory factors [51]. Maternal milk significantly impacts offspring development, particularly by transmitting microorganisms to the developing gastrointestinal tract [52].

5.1 Maternal Delivery

Morrow et al. [53] found that delivery mode significantly affects milk microbiota. Using 16S rDNA gene amplicon pyrosequencing, they compared microbial diversity in milk from vaginally delivered versus cesarean-delivered cows. Milk from vaginally delivered cows showed higher microbial diversity, while cesarean-delivered cows' milk had higher relative abundances of staphylococci and bifidobacteria but lower streptococci content. Delivery mode also affects newborn calf gut microbiota composition [54]. Compared to vaginally delivered calves, cesarean-delivered calves showed significantly reduced similarity to maternal microbiota and lower microbial diversity [54-55].

Microbial colonization in birthing mammals provides crucial stimuli for immune maturation in newborn offspring. Offspring microbial community formation is influenced by prenatal and postnatal factors, including genetic and environmental variables (gestational age, delivery mode, nursing, and nutrition) [56]. Based on these findings, we hypothesize that perinatal factors can affect newborn calf health through microbial transfer in milk, suggesting that microbial transfer during perinatal periods could provide new targets for dietary strategies to reduce non-infectious diseases [57]. These data may help guide improvements in reproductive management practices with significant impacts on dairy cow health.

Milk microbiota affects other health factors, including *Lactobacillus*, *Bacteroides*, and *Clostridium*, which influence mucin production, enhance mucosal permeability, maintain T-cell balance, and reduce mucosal inflammation [58]. Studies in germ-free mice demonstrate that early microbial colonization is essential for complete immune system development [59]. Milk microorganisms are crucial for bovine immune functions against pathogens and symbiotic bacteria. Offspring gut microbiota and immunity evolve through movement and colonization of milk microorganisms from maternal mammary channels, with synchronized development during nursing through interconnection between maternal mammary gland and calf oral microbiota [60].

Abreu et al. [61] demonstrated that milk microbial communities change throughout lactation, further suggesting their role in shaping offspring gut microbiota. Dairy cows harbor another complex microbial community—the rumen microbiota—which plays a key role in digesting plant material and converting indigestible compounds into useful substances. Rumen microbial colonization occurs continuously, and recent studies have demonstrated mature microbial functions in calves. Using pyrosequencing, cellulolytic bacteria were shown to be present in newborn calf rumen on day 1 after birth, increasing by day 3. Vankerckhoven et al. [62] proved that key rumen bacteria were present from day 1 of life when calves were only fed colostrum, before feed intake. These early gastrointestinal microbial communities in newborn calves likely originate from maternal transmission through skin, birth canal, milk, or saliva.

5.2 Calf Feeding

Current dairy management practices create barriers to interaction between cow and offspring microbial communities. Jost et al. [63] surveyed 149 newborn calves fed colostrum, pooled colostrum, or colostrum replacers, disrupting mother-offspring microbial transmission and potentially losing associated functions in offspring gut microbiota evolution. Dairy farm management should consider the quality of colostrum and milk, recognizing that healthy, nutritionally balanced milk with microorganisms is essential for offspring immune system development. Under stress conditions, such as intensive housing systems, calves likely experience gut microbiota imbalance, including decreased lactobacilli and bifidobacteria and increased pathogenic microorganisms [63]. Maldonado et al. [64] found increased proportions of lactic acid bacteria and *E. coli* in calf intestines when fed whole milk, further demonstrating milk' s complex effects on calf gut microbial populations.

High-quality colostrum should be provided to newborn calves within 6 hours after birth. However, many farms use unsaleable waste milk for calf feeding. Waste milk is milk unfit for human consumption, typically from cows with high SCC or treated with antibiotics [65]. Feeding waste milk to calves is common practice; Collado et al. [66] reported that 87.2% of US dairy farms fed waste milk to calves in 2002. Most farms consider waste milk use economically beneficial and safe, particularly after pasteurization. However, antibiotic residues and potential pathogen contaminants in waste milk can significantly damage healthy gut microbial colonies in calves. Akyol et al. [67] evaluated effects of waste milk feeding on calf fecal microbiota diversity, finding that microbial diversity in calf feces increased with age, including *Salmonella* and *Treponema pallidum*, while important beneficial bacteria like eosinophils decreased.

More dangerous factors in waste milk include antibiotic residues and potential enrichment of antibiotic resistance genes (ARGs) that could transfer to pathogens [68]. Continuous antibiotic pressure may increase ARG transfer opportunities. Liu et al. [69] fed antibiotic-contaminated milk to newborn mice, then administered ampicillin and tetracycline via oral and intravenous routes. Oral administration increased ampicillin resistance gene copy numbers 4-fold, while intravenous administration increased tetracycline resistance gene copy numbers 2-fold, possibly because intravenous ampicillin was cleared through urine without interacting with gut microbiota. Baldassarre et al. [70] assessed effects of antibiotic-contaminated milk on calf fecal microbiota using Illumina MiSeq sequencing of microbial 16S rDNA genes. Results showed that drug residues in milk affected fecal microbiota composition, suggesting even trace antibiotics could exert selective pressure on microbial competition. Antibiotic residues can selectively pressure immature microbiota with low or no ARG content, allowing exogenous microorganisms to colonize, mutate, and gradually evolve into characteristic calf microbiota, disrupting normal gut microbial development and function.

Another practice disrupting milk microbial balance is intramammary antibiotic treatment during dry or lactating periods [71]. This practice is considered more effective than selective treatment for preventing infections during dry periods. Intramammary antibiotic therapy is the most common treatment for mild and moderate mastitis cases, often used without knowing the infecting bacterial type [72]. However, due to concerns about antibiotic resistance selection, this practice has not been implemented in Nordic countries for decades and is increasingly abandoned in the Netherlands. The impact of this practice on milk microbiota and potential selection for ARGs warrants further investigation.

6. Summary and Outlook

Molecular biology advances have enabled breakthroughs in microbial community research, revealing the diversity and complexity of microorganisms inhabiting organisms and their mutual associations with hosts. Milk microbial communities, like somatic cell counts and biomarkers (expressed proteins, blood factors), serve as important biological indicators reflecting physiological health of cows and offspring and dairy product quality. The existence of the bovine "gut-mammary pathway" demonstrates the importance of maternal microbial communities in influencing calf gastrointestinal development and health, while waste milk use severely disrupts calf gut microbial community structure and function. These issues may cause significant economic impacts on dairy cow health and industry development. Therefore, further in-depth research on milk microbiota will contribute to comprehensive understanding of mammary gland physiology and milk quality safety, including mechanisms of subclinical and hidden mastitis, effects of farm management on mammary and offspring health, the role of gut as a reservoir for mastitis pathogens, and strategies for controlling antibiotic resistance.

References

[1] ENDESFELDER D, ZU C W, ARDISSONE A, et al. Compromised gut microbiota networks in children with anti-islet cell autoimmunity [J]. Diabetes, 2014, 63(6): 2006-2014.

[2] BRANISTE V, AL-ASMAKH M, KOWAL C, et al. The gut microbiota and the blood-brain barrier permeability in mice [J]. Science Translational Medicine, 2014, 6(263): 263ra158.

[3] HAMADY M, KNIGHT R. Microbial community profiling for human microbiome projects: Tools, techniques, and challenges [J]. Genome Research, 2009, 19(7): 1141-1152.

[4] SAVAGE D C. Microbial ecology of gastrointestinal-tract [J]. Annual Review of Microbiology, 31(1): 107-133.

[5] SENDER R, FUCHS S, MILO R. Revised estimates for the number of human and bacteria cells in the body [J]. PLoS Biology, 2016, 14(8): e1002533.

[6] CAO B, STOUT M J, LEE I, et al. Placental microbiome and its role in preterm birth [J]. Neoreviews, 2014, 15(12): e537-e545.

[7] MOR G, KWON J Y. Trophoblast-microbiome interaction: a new paradigm on immune regulation [J]. American Journal of Obstetrics and Gynecology, 2015, 213(4S): S131-S137.

[8] TOLLE A. MASTITIS, the disease in relation to control methods [J]. Doc Int Dairy Fed, 1979: 3-11.

[9] HOOD L. Tackling the microbiome [J]. Science, 2012, 336(6086): 1209.

[10] QUIGLEY L, O' SULLIVAN O, STANTON C, et al. The complex microbiota of raw milk [J]. FEMS Microbiology Reviews, 2013, 37(5): 664-698.

[11] LI J, NASIDZE I, QUINQUE D, et al. The saliva microbiome of Pan, and Homo [J]. BMC Microbiology, 2013, 13(1): 204.

[12] LAMENDELLA R, VERBERKMOES N, JANSSON J K. 'Omics' of the mammalian gut-new insights into function [J]. Current Opinion in Biotechnology, 2012, 23(3): 491-500.

[13] KUCZYNSKI J, LAUBER C L, WALTERS W A, et al. Experimental and analytical tools for studying the human microbiome [J]. Nature Reviews Genetics, 2012, 13(1): 47-58.

[14] JI H, GREENING D W, KAPP E A, et al. Secretome-based proteomics reveals sulindac-modulated proteins released from colon cancer cells [J]. Proteomics Clinical Applications, 2009, 3(4): 433-451.

[15] SHARPTON T J. An introduction to the analysis of shotgun metagenomic data [J]. Frontiers in Plant Science, 2014, 5: 209.

[16] Jumpstart consortium human microbiome project data generation working group. Evaluation of 16S rDNA-based community profiling for human microbiome research [J]. PLoS One, 2012, 7(6): e39315.

[17] LIU Z, DESANTIS T Z, ANDERSEN G L, et al. Accurate taxonomy assignments from 16S rRNA sequences produced by highly parallel pyrosequencers [J]. Nucleic Acids Research, 2008, 36(18): e120.

[18] SCHLOSS P D. The effects of alignment quality, distance calculation method, sequence filtering, and region on the analysis of 16S rRNA gene-based studies [J]. PLoS Computational Biology, 2010, 6(7): e1000844.

[19] ACINAS S G, MARCELINO L A, KLEPAC-CERAJ V, et al. Divergence and redundancy of rDNA sequences in genomes with multiple operons [J]. Journal of Bacteriology, 2004, 186(9): 2629-2635.

[20] FRANZOSA E A, MORGAN X C, SEGATA N, et al. Relating the metatranscriptome and metagenome of the human gut [J]. Proceedings of the National Academy of Sciences of the United States of America, 2014, 111(22): E2329-E2338.

[21] GODZIK A. Metagenomics and the protein universe [J]. Current Opinion in Structural Biology, 2011, 21(3): 398-403.

[22] YOZWIAK N L, SKEWES-COX P, STENGLEIN M D, et al. Virus identification in unknown tropical febrile illness cases using deep sequencing [J]. PLoS Neglected Tropical Diseases, 2012, 6(2): e1485.

[23] DEUSCH S, TILOCCA B, CAMARINHA-SILVA A, et al. News in livestock research - use of Omics-technologies to study the microbiota of the gastrointesti-

nal tract of animals [J]. Computational and Structural Biotechnology Journal, 2015, 13: 55-63.

[24] MENDE D R, WALLER A S, SUNAGAWA S, et al. Assessment of metagenomic assembly using simulated next generation sequencing data [J]. PLoS One, 2012, 7(2): e31386.

[25] SCHMIEDER R, EDWARDS R. Quality control and preprocessing of metagenomic datasets [J]. Bioinformatics, 2011, 27(6): 863-864.

[26] GARCIA-GARCERÀ M, GARCIA-ETXEBARRIA K, COSCOLLÀ M, et al. A new method for extracting skin microbes allows metagenomic analysis of whole-deep skin [J]. PLoS One, 2013, 8(9): e74914.

[27] KIM M, LEE K H, YOON S W, et al. Analytical tools and databases for metagenomics in the next-generation sequencing era [J]. Genomics & Informatics, 2013, 11(3): 102-113.

[28] CUI L, MORRIS A, GHEDIN E. The human mycobiome in health and disease [J]. Genome Medicine, 2013, 5(7): 63.

[29] BARTRAM A K, LYNCH M D J, STEARNS J C, et al. Generation of multimillion-sequence 16S rDNA gene libraries from complex microbial communities by assembling paired-end illumina reads [J]. Applied and Environmental Microbiology, 2011, 77(11): 3846-3852.

[30] WHITELEY A S, JENKINS S, WAITE I, et al. Microbial 16S rDNA ion tag and community metagenome sequencing using the ion torrent (PGM) platform [J]. Journal of Microbiological Methods, 2012, 91(1): 80-88.

[31] MASOUD W, VOGENSEN F K, LILLEVANG S, et al. The fate of indigenous microbiota, starter cultures, *Escherichia coli*, *Listeria innocua* and *Staphylococcus aureus* in Danish raw milk cheeses determined by pyrosequencing and quantitative (qRT)-PCR [J]. International Journal of Food Microbiology, 2012, 153(1/2): 192-202.

[32] QUIGLEY L, O' SULLIVAN O, BERESFORD T P, et al. Molecular approaches to analysing the microbial composition of raw milk and raw milk cheese [J]. International Journal of Food Microbiology, 2011, 150(2/3): 81-94.

[33] MONTEL M C, BUCHIN S, MALLET A, et al. Traditional cheeses: rich and diverse microbiota with associated benefits [J]. International Journal of Microbiology, 2014, 177(54): 136-154.

[34] FRANZOSA E A, HSU T, SIROTA-MADI A, et al. Sequencing and beyond: integrating molecular 'Omics' for microbial community profiling [J]. Nature Reviews Microbiology, 2015, 13(6): 360-372.

[35] VALLES-COLOMER M, DARZI Y, VIEIRA-SILVA S, et al. Meta-omics in inflammatory bowel disease research: applications, challenges and guidelines [J]. Journal of Crohn' s & Colitis, 2016, 10(6): 735-746.

[36] TVEIT A T, URICH T, FRENZEL P, et al. Metabolic and trophic interactions modulate methane production by Arctic peat microbiota in response to warming [J]. Proceedings of the National Academy of Sciences of the United States of America, 2015, 112(19): E2507-E2516.

[37] MCCLURE R, BALASUBRAMANIAN D, SUN Y, et al. Computational analysis of bacterial RNA-SEQ data [J]. Nucleic Acids Research, 2013, 41(14): e140.

[38] GOSALBES M J, ABELLAN J J, DURBÁN A, et al. Metagenomics of human microbiome: beyond 16S rDNA [J]. Clinical Microbiology and Infection, 2012, 18(4S): 47-49.

[39] ABRAM F. Systems-based approaches to unravel multi-species microbial community functioning [J]. Computational and Structural Biotechnology Journal, 2015, 13: 24-32.

[40] AHRENS C H, BRUNNER E, QELI E, et al. Generating and navigating proteome maps using mass spectrometry [J]. Nature Reviews Molecular Cell Biology, 2010, 11: 789-801.

[41] HETTICH R L, PAN C L, CHOUREY K, et al. Metaproteomics: harnessing the power of high performance mass spectrometry to identify the suite of proteins that control metabolic activities in microbial communities [J]. Analytical Chemistry, 2013, 85(9): 4203-4214.

[42] MAO L, JACQUELINE F. Symbiosis, dysbiosis, and rebiosis—the value of metaproteomics in human microbiome monitoring [J]. Proteomics, 2015, 15(5/6): 1142-1151.

[43] KOLMEDER C A, DE VOS W M. Metaproteomics of our microbiome - developing insight in function and activity in man and model systems [J]. Journal of Proteomics, 2014, 97: 3-16.

[44] MUTH T, BENNDORF D, REICHL U, et al. Searching for a needle in a stack of needles: challenges in metaproteomics data analysis [J]. Molecular Biosystems, 2013, 9(4): 578-585.

[45] 赵玉琴. 关于《食品安全国家标准—生乳》的解读 [C]//江苏省计量测试学术论文集. [S.l.]: 江苏省计量测试学会, 2011.

[46] 刘艳艳. 鲜乳细菌总数荧光定量 PCR 检测方法的建立 [D]. 硕士学位论文. 长春: 吉林大学, 2014.

[47] HETTICH R L, SHARMA R, CHOUREY K, et al. Microbial metaproteomics: identifying the repertoire of proteins that microorganisms use to compete and cooperate in complex environmental communities [J]. Current Opinion in Microbiology, 2012, 15(3): 373-380.

[48] TANCA A, PALOMBA A, PISANU S, et al. A straightforward and efficient analytical pipeline for metaproteome characterization [J]. Microbiome, 2014, 2: 49.

[49] WINTER G, KRÄMER J O. Fluxomics-connecting Omics analysis with phenotypes [J]. Environmental Microbiology, 2013, 15(7): 1901–1916.

[50] TANG J. Microbial metabolomics [J]. Current Genomics, 2011, 12(6): 391–403.

[51] XIONG W L, ABRAHAM P E, LI Z, et al. Microbial metaproteomics for characterizing the range of metabolic functions and activities of human microbiota [J]. Proteomics, 2015, 15(20): 3424–3438.

[52] YOUNG W, HINE B C, WALLACE O A M, et al. Transfer of intestinal bacterial components to mammary secretions in the cow [J]. PeerJ, 2015, 3(6): e888.

[53] MORROW A L, RANGEL J M. Human milk protection against infectious diarrhea: implications for prevention and clinical care [J]. Seminars in Pediatric Infectious Diseases, 2004, 15(4): 221–228.

[54] BOEHM G, MORO G. Structural and functional aspects of prebiotics used in infant nutrition [J]. The Journal of Nutrition, 2008, 138(9): 1818S–1828S.

[55] PENTTILA I A. Milk-derived transforming growth factor-beta and the infant immune response [J]. Journal of Pediatrics, 2010, 156(2S): S21–S25.

[56] WALKER A. Breast milk as the gold standard for protective nutrients [J]. Journal of Pediatrics, 2010, 156(2S): S3–S7.

[57] KUEHN J S, GORDEN P J, MUNRO D, et al. Bacterial community profiling of milk samples as a means to understand culture-negative bovine clinical mastitis [J]. PLoS One, 2013, 8(4): e61959.

[58] ZHANG R Y, HUO W J, ZHU W Y, et al. Characterization of bacterial community of raw milk from dairy cows during subacute ruminal acidosis challenge by high-throughput sequencing [J]. Journal of the Science of Food and Agriculture, 2015, 95(5): 1072–1079.

[59] CABRERA-RUBIO R, COLLADO M C, LAITINEN K, et al. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery [J]. American Journal of Clinical Nutrition, 2012, 96(3): 544–551.

[60] GUEIMONDE M, LAITINEN K, SALMINEN S, et al. Breast milk: a source of bifidobacteria for infant gut development and maturation? [J]. Neonatology, 2007, 92(1): 64–66.

[61] ABREU N A, NAGALINGAM N A, SONG Y L, et al. Sinus microbiome diversity depletion and *Corynebacterium tuberculostearicum* enrichment mediates rhinosinusitis [J]. Science Translational Medicine, 2012, 4(151): 151ra124.

[62] VANKERCKHOVEN V V, VAN AUTGAERDEN T, HUYS G, et al. Establishment of the PROSAFE collection of probiotic and human lactic acid bacteria [J]. Microbial Ecology in Health and Disease, 2004, 16(2/3): 131–136.

[63] JOST T, LACROIX C, BRAEGGER C P, et al. Vertical mother-neonate transfer of maternal gut bacteria via breastfeeding [J]. Environmental Microbiology, 2014, 16(9): 2891-904.

[64] MALDONADO N C, CHIARAVIGLIO J, BRU E, et al. Effect of milk fermented with lactic acid bacteria on diarrheal incidence, growth performance and microbiological and blood profiles of newborn dairy calves [J]. Probiotics & Antimicrobial Proteins, 2017(Suppl.1): 1-9.

[65] KAO J Y. Principles of mucosal immunology $95.00 society for mucosal immunology, principles of mucosal immunology [M]. SMITH P, MACDONALD T, BLUMBERG R. Garland Science. New York: [s.n.], 2012.

[66] COLLADO M C, LAITINEN K, SALMINEN S, et al. Maternal weight and excessive weight gain during pregnancy modify the immunomodulatory potential of breast milk [J]. Pediatric Research, 2012, 72(1): 77-85.

[67] AKYOL Ç, TURKER G, INCE O, et al. Performance and microbial community variations in thermophilic anaerobic digesters treating OTC medicated cow manure under different operational conditions [J]. Bioresource Technology, 2016, 205: 191-198.

[68] OUWEHAND A C, SAXELIN M, SALMINEN S. Phenotypic differences between commercial *Lactobacillus rhamnosus* GG and *L. rhamnosus* strains recovered from blood [J]. Clinical Infectious Diseases, 2004, 39(12): 1858-1860.

[69] LIU J, ZHAO Z, ORFE L, et al. Soil-borne reservoirs of antibiotic-resistant bacteria are established following therapeutic treatment of dairy calves [J]. Environmental Microbiology, 2016, 18(2): 557-564.

[70] BALDASSARRE M E, BELLANTUONO L, MASTROMARINO P, et al. Gut and breast milk microbiota and their role in the development of immune function [J]. Current Pediatrics Reports, 2014, 2(3): 218-226.

[71] MATSUMIYA Y, KATO N, WATANABE K, et al. Molecular epidemiological study of vertical transmission of vaginal *Lactobacillus* species from mothers to newborn infants in Japanese, by arbitrarily primed polymerase chain reaction [J]. Journal of Infection and Chemotherapy, 2002, 8(1): 43-49.

[72] RAUTAVA S, LUOTO R, SALMINEN S, et al. Microbial contact during pregnancy, intestinal colonization and human disease [J]. Nature Reviews Gastroenterology & Hepatology, 2012, 9(10): 565-576.

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