



Gut Microbiota and Advances in Microbiome Sequencing-Based Technologies: Opportunities for Potential Biologics Discovery in Meat Animals[‡]

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Abstract: The gastrointestinal (GIT) microbiome of food animals represents a promising source of biologically active compounds with applications in animal health, nutrition, and sustainable production. Recent advances in 16S rRNA gene sequencing have transformed microbiome research, enabling detailed taxonomic profiling of microbial communities across diverse animal hosts. This review explores the potential of GIT microbiome-derived biologics—including short-chain fatty acids, antimicrobial peptides, and probiotics—as alternatives to traditional feed additives and antibiotics, as well as the potential impact on meat quality. While short-read sequencing remains foundational, long-read platforms such as PacBio, Oxford Nanopore, and LoopSeq offer enhanced taxonomic resolution and support the identification of functionally critical microbial strains. Practical considerations for sequencing method selection, database compatibility, and bioinformatics challenges are discussed, emphasizing the importance of curated, system-specific reference datasets. Ultimately, multi-omics approaches are necessary to characterize microbial activity and host-microbial interactions to unlock the microbiome’s functional potential. These strategies pave the way for precision microbiome engineering and novel biologics tailored to specific species and production systems. The review concludes with recommendations to standardize methodologies, invest in functional validation, and align microbiome research with the evolving needs of sustainable meat animal agriculture.

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Introduction

The gastrointestinal (GIT) microbiome of food animals represents an untapped resource for biologically active compounds and microorganisms, representing a significant potential for improving animal health, nutrition, and production efficiency (Flint et al., 2012; Oakley et al., 2014). These microbially-derived biologics—including enzymes, antimicrobial peptides, and metabolites—have been

shown to enhance feed conversion, support gastrointestinal health, and reduce pathogen loads (Stanley et al., 2014; Maki et al., 2019). Traditional culture-based methods were inherently limited in capturing the full scope of microbial ecosystems (Ricke, 2015). Unlocking these benefits requires advanced molecular tools. Continued advances in 16S rRNA gene sequencing have revolutionized our capacity to investigate the taxonomic composition, diversity, and functional attributes of the GIT

microbiome with unprecedented depth (Jami et al., 2013; Kim and Isaacson, 2015; Ricke et al., 2017; Dittoe et al., 2022; Weinroth et al., 2022). Next-generation sequencing and robust bioinformatics pipelines have enabled high-throughput, culture-independent profiling of microbial communities and their metabolic capabilities. These techniques have illuminated species-specific microbiota and critical microbial-host interactions, revealing promising candidates for biologics development (Caporaso et al., 2011; Xiao et al., 2021).

Biologics, broadly defined as naturally derived molecules or organisms that exert therapeutic or functional effects, follow a development pipeline that includes discovery, isolation, characterization, and scaling for commercial application (Walsh, 2018; Ricke et al., 2025). Historically, biologics in food-animal production have included tissue-derived products such as plasma proteins or hormones collected during meat processing (Boland et al., 2013). However, emerging research has identified the GIT microbiome as a rich source of microbial-based biologics with potential for use as feed additives, immunomodulators, and pathogen control agents. The GIT microbial ecosystem—comprising bacteria, archaea, fungi, protozoa, and bacteriophages—plays essential roles in nutrient metabolism, immune development, and disease resistance (Neish, 2009). Microbial metabolites such as short-chain fatty acids (SCFAs), antimicrobial peptides, and bioactive intermediates offer tangible applications in animal health and feed innovation (Spivak et al., 2022). These contributions vary across species; ruminants rely on foregut fermentation, especially within the rumen, to extract energy

from fibrous feedstuffs (Henderson et al., 2015; Krause et al., 2003), while monogastric animals such as poultry, swine, and aquatic species rely on hindgut microbial fermentation (Looft et al., 2014). Each species harbors distinct microbial consortia that support SCFA production, immune regulation, and fiber degradation (Oakley et al., 2014).

In this context, biologics in meat animal production refer to naturally derived microbial cells, metabolites, or functional molecules that promote animal growth, health, and product quality. Originating from the GIT microbiome, these include probiotics, enzymes, SCFAs, bacteriocins, and immune-modulating compounds. Biologics offer targeted, sustainable, and microbiome-aligned alternatives to synthetic feed additives or pharmaceuticals. As outlined in Table 1, biologics represent a distinct category of animal products, differentiating themselves by their microbial origin, ecological compatibility, and potential to reduce reliance on antibiotics and other synthetic inputs. Importantly, the identification and classification of biologics are increasingly driven by sequencing technologies, particularly 16S rRNA gene sequencing and metagenomics, which enable researchers to pinpoint beneficial taxa, track functional pathways, and inform mechanism-based applications. This integration of molecular tools with functional outcomes is key to developing next-generation biologics tailored to meat animal systems. The objective of this review is to discuss gut microbiota-derived biologicals and their potential application to meat animal production, followed by a description of current microbiome sequencing technologies that would enhance the discovery of a wider range of useful biologicals.

Table 1. Comparative overview: Biologics vs. other additives in meat animal production

Category	Definition	Source	Mechanism of Action	Common Applications	Key References
Biologics	Naturally derived functional molecules or microorganisms	Microbiome, animal tissues	Modulate GIT microbiota, immunity, or metabolism	Probiotics, SCFA-producing bacteria, microbial enzymes	Walsh, 2018; Ricke et al., 2025; Ben Lagha et al., 2017; Louis and Flint, 2017
Pharmaceuticals	Chemically synthesized or purified drugs	Synthetic or semi-synthetic	Target specific pathogens or physiological systems	Antibiotics, vaccines, hormones	Boland et al., 2013; Walsh, 2018
Nutraceuticals	Feed-derived compounds with health benefits	Plants, marine organisms	Antioxidant, anti-inflammatory, and metabolic effects	Essential oils, polyphenols, and omega-3 fatty acids	Maki et al., 2019; Oakley et al., 2014
Feed Additives (non-biologic)	Non-living, non-microbial nutritional additives	Minerals, vitamins, chemicals	Nutrient supplementation, growth promotion	Ionophores, amino acids, mineral premixes	Stanley et al., 2014; Ricke et al., 2017
Chemical Interventions	Synthetic substances for pathogen control	Lab-synthesized	Surface decontamination, carcass rinses	Peracetic acid, chlorine, acidifiers	Feye et al., 2020

Applications of 16S rRNA Profiling in Meat Quality Research

The gut microbiota and their respective metabolic products influence host physiology beyond gut health, including effects on carcass traits and meat quality through nutrient metabolism, SCFA production, and host-microbe signaling. A growing body of research demonstrates that shifts in gut microbiota—whether driven by feed additives, diet, or host genotype—can shape intramuscular fat, fatty acid composition, and muscle growth across poultry, swine, cattle, and small ruminants (Kim et al., 2023; Li et al., 2025; Petri et al., 2018; Mizoguchi and Guan, 2024). Table 2 summarizes key studies that integrate 16S-based microbiome profiling with meat quality outcomes, illustrating how taxonomic and functional shifts in the gut microbiota translate into phenotypic traits of economic relevance.

In swine, for example, Khanal et al. (2020) demonstrated that specific heritable microbial taxa were associated with carcass traits such as intramuscular fat (IMF), pH, and meat color. Kim et al. (2023) reported that enzyme supplementation led to increased microbial diversity and enrichment of beneficial taxa, resulting in improvements in pork tenderness, lipid profile, and color. Tiezzi et al. (2024) advanced this field by incorporating microbiome features into genomic prediction models to

enhance the accuracy of selection for meat quality traits. Niu et al. (2023) further showed that dietary silage altered microbial composition in ways that improved oxidative stability and water-holding capacity in pork.

Similar associations have been reported in poultry and ruminants. Li et al. (2025) used an integrated 16S and metabolomics approach in ducks to demonstrate that prebiotic-driven microbiota shifts enhanced SCFA production and amino acid metabolism, improving meat quality and mineral deposition. In ruminants, rumen-based studies have linked microbial taxa, such as *Butyrivibrio*, to polyunsaturated fatty acid biohydrogenation and the formation of conjugated linoleic acid (CLA), directly impacting meat lipid profiles (Kepler et al., 1971; Lourenço et al., 2010; Petri et al., 2018; Mir et al., 2004). Additional work has shown that grazing systems, dietary oils, and forage composition drive microbial shifts that enhance CLA content and promote healthier fat profiles (Vargas et al., 2017; Bainbridge et al., 2018; Dewanckele et al., 2018).

Notably, several of these studies highlight the functional importance of low-abundance taxa such as *Butyrivibrio* and *Turicibacter*, which, despite their modest relative abundance, have been linked to fatty acid biohydrogenation, amino acid metabolism, and SCFA production (Kepler et al., 1971; Kim et al., 2023; Lourenço et al., 2010). These examples

Table 2. Representative studies linking 16S rRNA-based gut microbiota profiles to meat quality outcomes

Study/Reference	Animal Model	16S rRNA Usage	Gut Microbiota Link	Meat Quality Outcome
Khanal et al., 2020	Swine	Microbiability of meat quality traits estimated via gut microbiota profiles	Heritable microbial taxa associated with carcass composition and intramuscular fat	Fat content, color, pH, IMF correlated with specific gut taxa
Kim et al., 2023	Swine	Gut microbiome analysis post enzyme feed additive	Altered microbiota diversity, increased beneficial bacteria	Improved meat color, tenderness, and fat profile
Li et al., 2025	Duck	16S and metabolomics to track GIT microbiota changes due to inulin	Microbiota shifts linked to SCFA and amino acid metabolism	Enhanced meat color, IMF, and mineral deposition
Mizoguchi and Guan, 2024	Beef cattle	Review of microbiome-based strategies	Associations of microbial taxa with marbling, growth, feed efficiency	Potential to enhance marbling and tenderness
Wen et al., 2023	Multiple	Review on gut-muscle axis	Microbial metabolites regulate muscle fiber and lipid metabolism	Linked to muscle growth and IMF deposition
Petri et al., 2018; Mir et al., 2004; Lourenço et al., 2010; Kepler et al., 1971	Ruminants	Rumen microbial analysis using 16S and biochemical assays	Identified microbes (e.g., <i>Butyrivibrio</i>) driving Polyunsaturated fatty acids (PUFA) biohydrogenation and CLA formation	Enhanced CLA/vaccenic acid in fat; modified fatty acid profiles
Vargas et al., 2017; Bainbridge et al., 2018; Dewankle et al., 2018	Ruminants	Microbial changes tracked under different oil or grazing treatments	Diet-induced microbial shifts influence fatty acid biohydrogenation routes	Improved healthy fat profiles and increased CLA content
Tiezzi et al., 2024	Swine	Integrated microbiome and genomic prediction	Microbial features used as predictor traits for quality phenotype	Enabled enhanced accuracy of meat quality trait prediction
Niu et al., 2023	Swine	Microbiome sequencing after dietary silage intervention	Altered microbial composition linked with antioxidant and metabolic regulation	Improved meat water-holding, pH, and oxidative stability

underscore the need to move beyond broad diversity metrics or the most abundant operational taxonomic units (OTUs) toward more mechanistic insights that reflect microbial contributions to host physiology and meat phenotype (Khanal et al., 2020; Tiezzi et al., 2024; Wen et al., 2023). As sequencing resolution improves and multi-omics tools continue to mature, the ability to pinpoint specific taxa, microbial pathways, and metabolites involved in carcass trait development will expand. These approaches are crucial for translating microbiome profiles into actionable strategies for improving meat quality in food-animal systems, whether through precision feeding, microbial selection, or biologic interventions.

Microbial Ecology and Biologics Potential from Meat Animal GITs

Ruminants rely on a rumen GIT compartment for microbial fermentation of fibrous plant material. Primary work on cellulolytic microorganisms laid the groundwork for understanding fermentation dynamics and microbial interactions (Bryant, 1959; Hungate, 1990; Hungate, 1966). These communities produce fermentation byproducts such as short-chain and branched-chain fatty acids, which serve as energy sources and support immune function (Flint et al., 2012; Macfarlane and Macfarlane, 2011). Methanogens, key hydrogen-utilizing archaea, play a significant role in methane production through interspecies hydrogen transfer, affecting both fermentation efficiency and greenhouse gas output (Wolin and Miller, 1982; Saengerksub and Ricke, 2014). Shifting fermentation toward propionate production can improve feed efficiency while lowering methane

emissions (Hook et al., 2010). Microbial products such as bacteriocins are being explored as biologics due to their antimicrobial and anti-inflammatory properties (Ben Lagha et al., 2017). Though the considerable focus is on bacteria, other organisms, such as fungi and archaea, also contribute to fiber degradation and metabolite synthesis and are essential targets for precision microbiome modulation.

In poultry, the cecum is a primary fermentation site, where genera such as *Lactobacillus* and *Clostridium* aid in SCFA production, pathogen suppression, and immune modulation (Stanley et al., 2014). Dietary strategies can enrich these beneficial microorganisms, while fungi, archaea, and bacteriophages offer additional potential for microbiome engineering. Competitive exclusion approaches have reduced pathogens such as *Salmonella* and *Campylobacter*, and may be enhanced by incorporating nonbacterial taxa. In swine, microbial fermentation in the hindgut supports nutrient absorption, immunity, and metabolic regulation. Enzymes and peptides produced by the GIT microbiome may include casomorphins and lactotripeptides that affect gut-brain signaling and offer potential for functional feed development (Meisel and FitzGerald, 2003). Aquatic species such as fish and shrimp also rely on microbial fermentation, particularly those lacking a stomach, with microbiota playing a vital role in digestion and immune defense (Egerton et al., 2018). Across all systems, probiotics, prebiotics, and microbial enzymes are increasingly used to improve feed efficiency and reduce disease incidence.

Microbiome-derived biologics—such as SCFAs, bacteriocins, exopolysaccharides, and bioactive peptides—represent some of the most promising alternatives to conventional feed additives (Table 3).

Table 3. Microbiome-derived biologics in food-animal systems

Biologic Compound	Microbial Sources	Function	Application in Animal Production and Processing	Key References
Short-Chain Fatty Acids (SCFAs)	<i>Clostridium</i> spp., <i>Faecalibacterium</i> spp.	Energy source, GIT barrier support, anti-inflammatory	Feed efficiency, gut health, pathogen exclusion	Flint et al., 2012; Dittoe et al., 2022; Macfarlane and Macfarlane, 2011
Butyrate	<i>Roseburia</i> spp., <i>Butyrivococcus</i> spp.	Colonocyte energy, immune modulation	Probiotic/feed additive for GIT integrity	Louis and Flint, 2017; Ben Lagha et al., 2017
Bacteriocins	<i>Lactobacillus</i> spp., <i>Enterococcus</i> spp.	Antimicrobial against Gram-positive bacteria	Antibiotic alternative in feed or carcass interventions	O'Toole and Cooney, 2008; Vieco-Saiz, 2019
Exopolysaccharides	<i>Bifidobacterium</i> spp., <i>Weissella</i> spp.	Prebiotic, biofilm modulation	Functional feed additive or protective coating	O'Toole and Cooney, 2008
Bioactive peptides	Gut microbes via protein fermentation	Gut-brain signaling, blood pressure regulation	Appetite, gut motility, and metabolic balance	Meisel and FitzGerald, 2003; FitzGerald et al., 2004
Hydrogenase enzymes	Methanogens, acetogens, sulfate reducers	Regulate fermentation end-products	Feed strategies to improve efficiency & reduce methane	Le Van et al., 1998; Hook et al., 2010
Lipases and proteases	<i>Clostridium</i> spp., <i>Bacillus</i> spp.	Enhanced digestion of lipids and proteins	Enzyme supplements for growth promotion	Oakley et al., 2014; Agyekum and Nyachoti, 2017

Advances in 16S rRNA gene sequencing, metagenomics, and other molecular tools have been instrumental in identifying the microbial taxa responsible for producing these compounds and in linking their presence to beneficial host outcomes. For example, taxa such as *Butyricoccus*, *Faecalibacterium*, and *Bifidobacterium* have been associated with SCFA production, biofilm modulation, and host immune benefits—insights enabled by culture-independent sequencing approaches. Functional annotations derived from sequencing data have revealed metabolite pathways linked to host performance, antimicrobial activity, and gut barrier integrity. As sequencing resolution and database accuracy improve, so too does our ability to pinpoint microbial strains and metabolites with biologic potential. Table 2 summarizes key microbiome-derived compounds, their microbial origins, and their documented applications in food-animal production systems. These contributions vary by species: ruminants rely on foregut fermentation, while monogastric animals such as poultry, swine, and aquatic species depend on hindgut fermentation. Each host has a distinct microbiota capable of SCFA production, fiber degradation, and immune regulation (Oakley et al., 2014). High-throughput sequencing continues to accelerate the discovery of these functionally relevant microorganisms, expanding the toolbox for precision biologics development across animal agriculture.

The GIT microbiota and their respective metabolic products influence host physiology beyond gut health, including effects on carcass traits and meat quality through nutrient metabolism, SCFA production, and host-microbe signaling. A growing body of research demonstrates that shifts in gut microbiota—whether driven by feed additives, diet, or host genotype—can shape intramuscular fat, fatty acid composition, and muscle growth across poultry, swine, cattle, and small ruminants (Kim et al., 2023; Li et al., 2025; Petri et al., 2018; Mizoguchi and Guan, 2024). Table 3 summarizes key studies integrating 16S-based microbiome profiling with meat quality outcomes. Notably, several of these studies highlight the functional importance of low-abundance taxa, such as *Butyrivibrio* and *Turicibacter*, which have been linked to fatty acid biohydrogenation, amino acid metabolism, and SCFA production, despite their modest relative abundance (Kepler et al., 1971; Kim et al., 2023; Lourenço et al., 2010). These examples underscore the need to move beyond broad diversity metrics or top OTUs toward mechanistic insights that capture microbiota contributions to meat composition and phenotype (Khanal et al.,

2020; Tiezzi et al., 2024; Wen et al., 2023). As sequencing resolution improves and multi-omics integration advances, these approaches will be essential to pinpoint microbial taxa and metabolites that directly or indirectly contribute to meat quality in food-animal systems.

Advancements in 16S rRNA Gene Sequencing for GIT Microbiome Research

16S rRNA gene sequencing: Microbiome profiling

The advent of 16S rRNA gene sequencing has become a cornerstone of microbiome research in animal and food agriculture, offering a cost-effective, high-throughput method for taxonomic classification and community profiling (Ricke et al., 2017). Although traditional culture-based techniques provided foundational knowledge, they were inherently limited by their reliance on selective enrichment and growth media, which excluded many fastidious or unculturable microorganisms (Rodriguez-R and Konstantinidis, 2014; Ricke, 2015). Advances in sequencing technologies and bioinformatics have evolved microbial ecology studies, enabling comprehensive, culture-independent characterization of GIT microbial communities. Studies now routinely utilize 16S rRNA gene sequencing to examine the composition and diversity of complex microbiomes in most food-animal production systems, processing, and food products (Sabater et al., 2021; Olson et al., 2022; Ricke et al., 2017; Ricke et al., 2022a; Ricke et al., 2022b; Ricke et al., 2022c; Weinroth et al., 2022). Most importantly, these technologies support the discovery of biologics by identifying beneficial microorganisms and their ecological roles, microbial shifts in response to dietary interventions, pathogen exposure, and other environmental or physiological variables.

However, repeatability in 16S rRNA microbiome studies remains a widely acknowledged limitation, particularly when analyzing low-biomass or spatially heterogeneous samples. Technical and biological replicates can yield noticeably different community profiles due to small-scale variations in DNA input, extraction efficiency, and sequencing noise (Olson et al., 2024; Schmidt et al., 2018). Even duplicate swabs or tissue biopsies collected from adjacent sites may differ in microbial composition, highlighting the impact of microenvironmental variability and sampling

error. These issues can be compounded by amplification bias, stochastic read dropout, and taxonomic assignment inconsistency, especially when microbial load is low or diversity is high. To mitigate these concerns, standardization of DNA extraction protocols, rigorous sample homogenization, and the use of negative/positive controls and technical replicates are recommended (Nearing et al., 2021). Moreover, increasing sequencing depth and applying compositional data analysis tools may improve the detection of true biological signals amid technical variability.

Initially proposed by Carl Woese, the 16S rRNA gene is a highly conserved component of the bacterial genome with variable regions that permit phylogenetic differentiation (Olsen and Woese, 1993; Clarridge, 2004). Amplification and sequencing of these variable regions enable broad-range microbial detection and taxonomic resolution down to the genus or species level, depending on sequencing depth and primer selection (Caporaso et al., 2011). Despite its utility, 16S rRNA sequencing has known limitations. One of the most obvious is that it cannot distinguish between live and dead cells, and the data generated are compositional, reflecting relative abundances rather than absolute counts (Gloor et al., 2017; Weinroth et al., 2022). Additionally, as a housekeeping gene, 16S rRNA does not directly affect functional capacity or metabolic activity (Weinroth et al., 2022).

Microbial diversity analyses using 16S data typically involve alpha diversity (within-sample richness and evenness) and beta diversity (between-sample dissimilarity). Alpha diversity indices such as Shannon, Pielou's Evenness, and Chao1 measure species richness and evenness, while beta diversity utilizes distance metrics (e.g., UniFrac, Bray-Curtis) to assess differences in community composition (Callahan et al., 2019; IMPACCT Investigators, 2022). Analytical

platforms such as QIIME2, Mothur, and R facilitate these calculations, utilizing PERMANOVA or Kruskal-Wallis tests to determine significance and pairwise differences (Estaki et al., 2020; Bolyen et al., 2019). Taxonomic classification of sequencing reads is performed using machine learning algorithms, such as Naive Bayes classifiers or Random Forest models, against curated reference databases such as SILVA or Greengenes (Bokulich et al., 2018; Yang et al., 2019). Differential abundance analysis is commonly conducted using tools such as ANCOM, DESeq2, and LefSe. ANCOM employs log-ratio transformations to account for compositionality and control false discovery rates (Mandal et al., 2015), while DESeq2 applies negative binomial models suited for count-based data (Love et al., 2014). LefSe uses linear discriminant analysis to identify taxa that differ significantly across treatment groups (Segata et al., 2011).

Although often applied for taxonomic surveys, 16S rRNA sequencing also supports biologics discovery by enabling researchers to track candidate probiotic strains, SCFA producers, and other beneficial taxa. Integrating functional prediction tools and downstream validation forms the foundation for precision microbiome engineering. As summarized in Table 4, 16S rRNA gene sequencing continues to enhance understanding of GIT ecology and supports the pipeline for identifying and developing novel microbiome-derived biologics.

Short-length 16S rRNA gene sequencing and database selection

Short-read 16S rRNA gene sequencing remains a widely adopted strategy for microbiome profiling due to its affordability, high-throughput capacity,

Table 4. Role of 16S rRNA gene sequencing in biologics discovery

Step in Biologic Discovery	16S rRNA Gene Sequencing Contribution	Example Application	Key References
Microbial profiling	Identify dominant, rare, and functional taxa	Discovery of novel SCFA producers in poultry and swine	Dittoe et al., 2022; Ricke et al., 2017
Ecological diversity analysis	Assess richness/evenness and treatment effects	Diet-based shifts in fermentation pathways via SCFA tracking	Weinroth et al., 2022; Callahan et al., 2019
Targeted candidate selection	Guide isolation of functionally beneficial strains	<i>Lactobacillus</i> and <i>Clostridium</i> as next-gen probiotics	Ben Lagha et al., 2017; Louis and Flint, 2017
Predictive functional insights	Use of PICRUSt2 or Tax4Fun to infer gene pathway potential	Predict butyrate synthesis or antimicrobial peptide genes	Douglas et al., 2020; ABhauer et al., 2015
Feed/microbiome intervention validation	Detect community shifts from in vitro/in vivo interventions	Prebiotic or fiber-driven changes to improve animal performance	Feye et al., 2020; Olson et al., 2022
Taxonomic resolution of beneficial taxa	Long-read 16S resolves species-level differences	Distinguishing probiotic vs pathogenic <i>Clostridium</i> spp.	Callahan et al., 2019; Souza et al., 2023

and compatibility with established sequencing platforms and bioinformatics tools. 16S approach typically targets one or more hypervariable regions of the 16S rRNA gene, most commonly the V3 to V4 (~460 bp) or V4 (~250 bp) regions, which offer adequate taxonomic resolution for most microbial communities while maintaining manageable sequencing costs and error rates (Klindworth et al., 2013). Illumina platforms such as MiSeq and NovaSeq are commonly used for short-read sequencing. MiSeq, for example, generates 2 × 250 bp paired-end reads that overlap to produce highly accurate consensus sequences (Pollock et al., 2018). While short-read sequencing reliably resolves taxonomy at the genus or family level, it typically lacks the discriminatory power to differentiate closely related species or strains. Moreover, primer selection can introduce amplification biases, as the variable regions differ in taxonomic resolution across microbial lineages (Johnson et al., 2019). Despite these limitations, short-read sequencing remains foundational in microbiome studies, particularly when paired with robust bioinformatics workflows such as QIIME2 and DADA2. These pipelines offer reproducible data processing, denoising, and diversity analysis accessible to a broad research community.

Accurate taxonomic classification of short-read 16S sequences depends heavily on the reference database. The Ribosomal Database Project, SILVA, and Greengenes are the most commonly employed databases, each with specific advantages (Quast et al., 2013; DeSantis et al., 2006; Bokulich et al., 2018). Using a Naive Bayesian classifier, the Ribosomal

Database Project (RDP) is optimized for short-read classification. It is fast and efficient, lacks full-length sequences, and is less comprehensive than other options. SILVA is the most phylogenetically curated and up-to-date database. Although SILVA supports short-read and full-length 16S rRNA sequencing and environmental microbiome research, it requires greater computational resources. Although no longer actively updated (last release in 2013), Greengenes remains helpful in working with older datasets and is still integrated with tools such as QIIME and PICRUSt for functional prediction. The study objectives should guide the selection of the appropriate database. As sequencing methods evolve, so must database curation and compatibility with emerging tools to ensure reliable classification and biologics discovery. Numerous studies have leveraged short-read 16S rRNA sequencing to explore GIT microbiota across food-animal species, providing insights into host-microbial interactions, production traits, and potential probiotic candidates (Table 5).

Short-read vs. full-length 16S rRNA gene sequencing: Considerations and advancements

The 16S rRNA gene comprises 9 hypervariable regions (V1 to V9) interspersed with conserved sequences. In short-read amplicon sequencing applications, researchers target subsets of these hypervariable regions depending on the sample matrix and specific

Table 5. 16S rRNA gene sequencing applications in food-animal GIT

Animal	Key Findings	Candidate Microbial Biologics	Use of PICRUSt	Reference
Chicken	Identified distinct cecal microbiota associated with <i>Campylobacter</i> colonization resistance	<i>Lactobacillus</i> , <i>Faecalibacterium</i> , <i>Clostridiales</i> spp.	No	Oakley et al., 2014
Pig	Found differences in GIT microbiota between high- and low-feed-efficiency pigs	<i>Prevotella</i> , <i>Roseburia</i> , <i>Treponema</i>	No	Kim et al., 2011
Cattle	Microbiome associated with feed intake and gain in steers	<i>Ruminococcus</i> , <i>Butyrivibrio</i> , <i>Oscillospira</i>	No	Myer et al., 2015
Chicken	Tracked succession of microbiota during development	<i>Enterococcus</i> , <i>Lactobacillus</i>	No	Videnska et al., 2014
Swine	Tracked weaning transition in piglets, noting taxa associated with health outcomes	<i>Bacteroides</i> , <i>Clostridium</i> XIVa, <i>Blautia</i>	No	Holman et al., 2017
Cattle	Examined rumen microbial response to diet	<i>Succinivibrio</i> , <i>Megasphaera</i> , <i>Lachnospiraceae</i>	No	Mao et al., 2015
Various livestock	Applied PICRUSt and 16S rRNA functional characterization to predict co-digestion strategies of various animal manures for biogas production	Not specified	Yes	Ijoma et al., 2021
Lambs and goat kids	Combined 16S rRNA sequencing and metabolomics to characterize gut microbiota, metabolism, and immune status in diarrheic lambs and goat kids	<i>Lactobacillus</i> , <i>Acinetobacter</i>	Yes	Zhang Y. et al., 2023

research goals. For instance, the V3 to V4 sub-region is recommended when assessing microbial members of the poultry digestive system, given its propensity to classify prokaryotic gut inhabitants and enteric pathogens (Ricke et al, 2017; Feye et al., 2020). Moreover, the V1 to V2 and V3 to V5 sub-regions have demonstrated inferiority in classifying phyla Proteobacteria and Actinobacteria sequences, respectively. At the same time, V1 to V3 well-classified sequences of the genus *Escherichia/Shigella*, V3 to V5 sequences of *Klebsiella*, and V6-V9 sequences of *Clostridium* and *Staphylococcus* (Johnson et al. 2019). Short-read platforms (e.g., Illumina) are subsequently used to sequence libraries encompassing the selected sub-regions, rendering data that can be matched against reference databases for taxonomic identification and subsequent microbiome exploration (Pollock et al., 2018).

Despite the widespread use of short-read amplicon sequencing (Pollock et al. 2018), multiple limitations can influence study reproducibility and biological inference. Among the most documented shortcomings is achieving taxonomic resolution at the species level, as sequencing 1 or 2 hypervariable regions (approximately 100 to 500 bp; Callahan et al. 2019; Bailén et al. 2020) commonly constrains taxonomic assignment beyond genus due to intraspecific sequence homology (Gupta et al. 2019; Johnson et al. 2019; Usyk et al. 2023). This limitation can present a notable challenge for prokaryotic genera exhibiting symbiotic plasticity, which may encompass mutualistic, commensal, and pathogenic species and subspecies that are difficult to delineate. With the addition of primer bias, these biases can result in over- or underrepresentation of taxon groups, distorting the accurate composition of a microbial community (Wang and Qian 2009; Kumar et al. 2011; Guo et al. 2013). Further limitations of short-read amplicon sequencing include putative uncertainty in taxonomic assignment (Wang et al. 2007; Guo et al. 2013) and the inability to sequence the entire 16S rRNA gene in a single read, as different hypervariable regions offer varying levels of taxonomic resolution (Graspeuntner et al. 2018; Johnson et al. 2019).

Full- and near-full-length 16S rRNA gene sequencing approaches have gained traction in microbiome studies, given the potential to capture all 9 hypervariable regions and thus vastly improve the prokaryotic taxonomic assignment. However, their adoption has been impeded historically by relatively high error rates (~10%), platform cost, and limited availability (Pollock et al. 2018; Callahan et al. 2019; Callahan

et al. 2021). Improvements in sequencing chemistries and error correction algorithms mitigate these challenges, making long-read applications increasingly viable. A comparative summary of short- and long-read sequencing platforms—including technical parameters, resolution, cost, and suitability for microbiome biologics discovery—is provided in Table 6. The following section reviews the primary techniques available for full-length 16S rRNA gene sequencing, emphasizing those commercially available. It further highlights their applications in profiling food-animal-associated microbiomes and addresses the unique considerations necessary for robust long-read amplicon workflows.

Comparison of Long-Read Sequencing Platforms

Pacific Biosciences single-molecule real-time

Pacific Biosciences (PacBio) sequencing employs single-molecule real-time (SMRT) technology, which enables the generation of highly accurate long reads by sequencing circularized DNA molecules (Wagner et al. 2016). To generate these reads, hairpin adapters (SMRTbells) are ligated to the ends of double-stranded DNA, circularizing the linear DNA molecule. Circularization allows the sequencing polymerase to read the DNA multiple times, generating circular consensus sequences (CCS) (Au et al. 2012; Schloss et al. 2016). While raw PacBio reads can reach lengths exceeding 100 kb, the CCS approach enhances accuracy by producing shorter reads, typically ranging from 1 to 20 kb (~1,500 bp for full-length 16S rRNA gene applications), with per-base error rates comparable to short-read sequencing, approximately 0.5% (Callahan et al. 2019; Wenger et al. 2019). The method's accuracy is particularly notable when coupled with analysis in DADA2 software (Callahan et al. 2016), resolving ASVs (Callahan et al. 2017) with single-nucleotide precision (Callahan et al. 2019). Such resolution enables species- and even subspecies-level taxonomic classification, a significant improvement compared to short-read approaches. Thus, the PacBio SMRT technology is becoming increasingly popular for high-resolution profiling of food-animal microbiomes despite its elevated cost relative to short-read and other long-read sequencing platforms (Jeong et al. 2021; Yu et al. 2022).

For instance, PacBio sequencing of the full-length 16S rRNA gene uncovered more rare bacterial species

Table 6. Comparison of short- and long-read 16S rRNA gene sequencing platforms used in microbiome research relevant to meat animal biologics discovery

Platform	Description	Pros	Cons	Read Length and Sequencing Depth		Taxonomic Resolution	Error Rate and Accuracy	Throughput and Cost per Sample	Library Preparation Complexity	Application Suitability	References
				Sequencing Depth	Read Length						
Illumina	Short-read sequencing targeting specific hypervariable regions. Used for high-throughput microbiome studies.	Cost-effective; high sequencing depth; well-established bioinformatics pipelines; suitable for genus-level classification.	Limited taxonomic resolution; primer bias affects results; cannot sequence full-length 16S.	150–300 bp per read; high sequencing depth.	Up to genus level.	~0.1–1% per base.	High throughput, low cost per sample.	Simple and well-established protocols.	Best for large-scale microbiome studies.	Weinroth et al., 2022; Pollock et al., 2018; Caporaso et al., 2011	
PacBio SMRT	Long-read sequencing using circular consensus sequencing (CCS) to generate high-accuracy full-length 16S rRNA gene sequences.	High taxonomic resolution; reduced primer bias; improved rare species detection.	Higher cost per sample; requires higher DNA input; complex library preparation.	1,500 bp (full-length 16S); moderate sequencing depth.	Species- and subspecies level.	~0.5% per base (with CCS for correction).	Lower throughput, higher cost per sample.	Requires higher DNA input and complex library prep.	Best for high-resolution taxonomic profiling.	Callahan et al., 2019; Schloss et al., 2016; Myer et al., 2016	
Oxford Nanopore (ONT)	Long-read sequencing using nanopores to directly sequence native DNA molecules, enabling real-time analysis.	Real-time sequencing; portable and low-cost instruments; ultra-long reads possible (>2 Mb).	Higher error rates; requires post-sequencing correction; no DADA2 support for ASVs.	Full-length 16S (>1,500 bp, variable); lower sequencing depth.	Species level possible (dependent on error correction).	~5–38% raw-read error (requires correction).	Medium throughput, lower instrument cost, but higher per-base error correction cost.	Rapid but variable quality, needs post-processing.	Best for rapid field-based diagnostics.	Dumschott et al., 2020; Zhang T. et al., 2023; Heikema et al., 2020	
LoopSeq	Synthetic long-read sequencing reconstructs full-length sequences from short reads using unique molecular identifiers.	High accuracy (~0.005% error rate); full-length 16S sequencing on short-read platforms; improved taxon identification.	Proprietary workflow; complex library preparation; requires short-read sequencing platforms for data acquisition.	Full-length 16S (~1,500 bp, short-read-based reconstruction).	High-resolution species-level classification.	~0.005% (extremely low error rate).	Medium throughput, moderate cost.	Proprietary workflow, requires additional steps.	Best for combining high accuracy and taxonomic depth.	Callahan et al., 2021; Jeong et al., 2021; Chung et al., 2020	

in the dairy cow vaginal microbiome relative to Illumina sequencing of the V4 sub-region, coupled with a higher percentage of classified reads (Souza et al., 2023). Similar findings were realized between the targeted sub-regions/sequencing platforms while characterizing the intestinal microbiota of equine (Di Pietro et al. 2021). Earlier work with the PacBio RSII instrument showed improved taxonomic resolution of the steer ruminal microbiome with V1 to V8 CCS reads compared to V1 to V3 Illumina reads (Myer et al. 2016). Biologically relevant insights have been realized with PacBio full-length 16S rRNA gene sequencing for other animal-associated microbiomes, including those of poultry (Ivulic et al. 2022; Dai et al. 2022), meat sheep (Li et al. 2024), swine (Peng et al. 2023), dairy goats (Hu et al. 2024), meat goats (Luo et al. 2023), and fish (Klemetsen et al. 2019; Sumithra et al. 2024).

Oxford Nanopore Technologies

Oxford Nanopore Technologies (ONT) sequencing utilizes biological nanopores to sequence native DNA or RNA molecules directly. In this process, single nucleic acid strands pass through a nanopore embedded in a membrane under an electric field. As the strand translocates through the pore, each nucleotide causes characteristic disruptions in the ionic current, which are recorded as electrical signals (Cao et al., 2017). Specialized base-calling algorithms then decode these current shifts to infer the nucleotide sequence (Deamer et al. 2016). The ONT sequencing chemistry produces ultra-long reads (greater than 2 mb), thus making it suitable for sequencing complex sequences such as the full-length 16S rRNA gene or highly repetitive genomic regions (Dumschott et al., 2020).

Oxford Nanopore Technologies has become an attractive option for full-length 16S rRNA gene sequencing due to its on-site accessibility with portable devices (MinION Mk1b, Mk1c, and Flongle), low platform and reagent costs relative to other long-read sequencing technologies, and real-time data analysis capabilities (Winand et al., 2019 and references therein). However, ONT's higher error rates (5 to 38.5%; Heikema et al., 2020) present a significant limitation, often falling below the 97% and 99% identity thresholds needed for reliable genus- and species-level taxonomic assignments, respectively (Zhang T. et al., 2023). Improvements in ONT sequencing chemistry have increased raw-read accuracy progressively—from approximately

64% with Nanopore R7 (Ashton et al. 2015), approximately 87% with R9 (Minei et al. 2018), and approximately 92% with R9.4.1 (Huang et al. 2021), to approximately 99% with R10.4.1, which has enabled high-quality species-level identification of prokaryotes in environmental samples (Zhang T. et al., 2023). Yet, consensus sequence quality often requires post-sequencing correction for robust taxonomic resolution. For example, Zhang T. et al. (2023) analyzed over 4 million reads generated with R10.4.1 for a commercial standard, finding an average of 96.5% raw-read accuracy, which remains below the ideal genus and species classification thresholds. Moreover, because ONT lacks clustering methods like DADA2, nanopore reads cannot generate ASVs, limiting alpha and beta diversity analyses typically performed in short-read amplicon workflows. Unique molecular identifier (UMI)-based and consensus sequence approaches have been developed to improve sequence quality across both older ONT chemistries (Li et al. 2016; Calus et al. 2018; Volden et al. 2018; Karst et al. 2021) and R10.4+ (Deng et al. 2024; Lin et al. 2024), providing higher-accuracy options for full-length 16S rRNA gene sequencing. However, these methods often require additional instrumentation and bioinformatics resources, making ONT best suited for rapid diagnostics and preliminary microbiome assessment.

Notwithstanding error rate limitations, ONT sequencing has proven valuable for microbiological analyses within food-animal production. In poultry, ONT has been used successfully for on-farm detection of *Campylobacter jejuni* (Marin et al. 2022), microbiome profiling of gut and fecal environments through both 16S rRNA gene sequencing and shotgun metagenomic approaches (Lundberg et al. 2021; Ndotono et al. 2022; Peng et al. 2023), and for characterizing pathogen antibiotic resistance genes (Peng et al. 2023). Similar applications have advanced the dairy cattle industry, where ONT has enabled 16S rRNA-based milk microbiome profiling (Catozzi et al. 2020; Shinozuka et al. 2021; Urrutia-Angulo et al. 2024), identification of bacteria linked to mastitis (Usui et al. 2023; Urrutia-Angulo et al. 2024), and analysis of antimicrobial resistance genes in *Staphylococcus aureus* (Chakrawarti et al. 2024). Beyond poultry and dairy, ONT applications have expanded into other food-animal sectors, including the characterization of microbiomes in cultured fish (Toxqui-Rodríguez et al. 2023), beef cattle (Miura et al. 2022), meat and dairy

sheep (Reinoso-Peláez et al. 2023), and swine (Chen et al. 2022; Vereecke et al. 2023).

LoopSeq

LoopSeq by Element Biosciences is a synthetic long-read (SLR) sequencing chemistry developed to overcome limitations inherent to amplicon-based microbiome profiling. The technology first assigns UMIs to each parent DNA molecule in a sample, which are distributed intramolecularly before enzymatic fragmentation. Following sequencing, short reads that share a UMI are assembled *de novo* to reconstruct the full-length sequence of the original molecule (up to approximately 6kb; Callahan et al. 2021). LoopSeq's consensus-driven error correction mechanism renders long reads with a remarkably low error rate of 0.005%, as only dominant read structures within a UMI group contribute to the final consensus sequence (Callahan et al., 2021). Although proprietary aspects of library preparation and SLR reassembly can add complexity to the workflow (Deng et al. 2024), LoopSeq has demonstrated superior sequencing accuracy and taxon identification over other short- and long-read 16S rRNA gene methods across synthetic communities (Chung et al. 2020; Callahan et al. 2021; Deng et al. 2024; Lin et al. 2024), soil (Yu et al. 2022), and human gut microbiomes (Jeong et al. 2021) at a lower per-Mb cost than PacBio CCS (Yu et al. 2022). Coupled with the ability to sequence libraries on accessible short-read instruments, such as the Illumina MiSeq (the most widely used instrument for 16S rRNA gene sequencing; Pollock et al. 2018) and the avidity chemistry-based AVITI platform (Arslan et al. 2024), it is likely that LoopSeq-based 16S rRNA gene sequencing will gain prominence in food-animal microbiome research.

Although relatively new, LoopSeq has already demonstrated value for characterizing microbiomes within food-animal production. In poultry, LoopSeq has been applied to profile the broiler chicken litter microbiome (Gupta et al. 2021) as well as the GIT microbiome following infection by *Clostridium perfringens* (Fathima et al. 2024) and *Campylobacter jejuni* (Al Hakeem et al., 2024a; Al Hakeem et al., 2024b). In beef cattle, it has facilitated the identification of bacterial taxa associated with fertility in the uterine microbiome (Walker et al., 2023). The platform has also been employed in dairy, including microbiome assessments of fermented milk (Baldeh et al. 2022) and biosurveillance efforts targeting animal-derived pathogens in food products (Callahan et al. 2021; Grinevich et al. 2024).

Practical Considerations for Long-Read 16S Sequencing: DNA Input, Costs, and Bioinformatics Challenges

DNA input requirements and cost considerations

Selecting long-read 16S rRNA gene sequencing requires careful evaluation of research goals, sample type, and resource availability. Although this approach offers improved taxonomic resolution - sometimes reaching species or even subspecies levels—it comes with increased demands for DNA input quality and quantity (Tedersoo et al., 2021). These demands can pose significant challenges in food-animal research, where microbial biomass is often low (e.g., reproductive tracts, rinsates, and lavage samples) (Weinroth et al., 2022). Additionally, long-read platforms such as PacBio and Oxford Nanopore remain more expensive than short-read technologies, which can limit their accessibility in large-scale studies. Researchers must weigh the value of higher-resolution taxonomic data against these practical limitations. Short-read sequencing targeting hypervariable regions with higher replication may provide more statistical power and more precise biological insights for studies that detect broad compositional shifts or treatment effects. Conversely, long-read sequencing may be advantageous for specific applications requiring species-level resolution, such as identifying novel probiotic strains, tracing antimicrobial resistance lineages, or profiling microbiome-derived biologics with high taxonomic specificity. Table 4 provides a comparative summary of short- and long-read sequencing platforms, including technical parameters, resolution, cost, and suitability for microbiome biologics discovery.

Bioinformatics challenges and the importance of curated databases

Long-read sequencing technologies have spurred the development of bioinformatic pipelines capable of processing full-length 16S rRNA gene sequences. However, most reference databases used for taxonomic classification, such as SILVA, RDP, and Greengenes, were initially optimized for short-read data and often lack the resolution or completeness required for full-length reads (Ciuffreda et al., 2021; Tedersoo et al., 2021). This limitation was evident in recent studies where PacBio long-read sequencing identified greater

bacterial richness in equine and bovine samples but still failed to consistently classify taxa at the species level (Di Pietro et al., 2021; Souza et al., 2023). These challenges underscore the need for system-specific, full-length reference databases. For example, DAIRYdb significantly outperformed general-purpose databases in resolving dairy-associated microorganisms (Meola et al., 2019), and custom-built databases incorporating all 16S alleles have enabled subspecies-level identification of key pathogens such as *Escherichia coli* and *Salmonella enterica* (Grinevich et al., 2024). Recent efforts to address this gap have resulted in the development of curated, full-length databases for broader applications across livestock and environmental microbiomes (Dueholm et al., 2022; Escapa et al., 2020; Graf et al., 2021; Seol et al., 2022; Krabberød et al., 2024; Walsh et al., 2024). These resources are essential for maximizing the taxonomic and functional insights gained from long-read sequencing platforms and enabling precise detection of biologically relevant taxa.

While the technical challenges of long-read sequencing are real, they must be weighed against the growing demand for precision in microbiome applications. In the context of biologics discovery, long-read sequencing enables more accurate tracking of probiotic strains, identification of novel antimicrobial producers, and differentiation of functionally distinct microbial lineages. This level of resolution can inform feed additive formulation, pathogen diagnostics, and microbial therapeutics in food-animal systems. Choosing between short- and long-read sequencing depends on study objectives, available resources, and desired taxonomic resolution. By combining sequencing strategy with system-specific database development and clear application goals, researchers can unlock the full potential of microbiome data to advance sustainable animal production, health, and food safety.

Multi-omics approaches: Functional and metabolic insights

While 16S rRNA gene sequencing provides critical taxonomic information, it lacks insight into microbial function. Bioinformatic tools such as PICRUSt2 and Tax4Fun offer statistical predictions of functional potential. However, they heavily rely on available reference genomes and may miss underrepresented or novel taxa (Abhauer et al., 2015; Douglas et al., 2020). To move beyond inference, researchers are increasingly integrating complementary -omics approaches—metagenomics, metatranscriptomics, metaproteomics, and metabolomics—to characterize

the functional and metabolic activity of the microbiome in more detail.

Holo-omics are multi-omics strategies that aim to uncover host-microbiome interactions and support precision interventions in livestock and poultry (Nyholm et al., 2020; Dehau et al., 2022). Metagenomics enables comprehensive analysis of microbial genetic potential, including biosynthetic pathways, resistance genes, and enzymes relevant to animal health and nutrition. However, it does not capture gene expression or actual metabolic activity, necessitating integration with 1) metatranscriptomics, which reveals active gene expression under specific conditions (Khodadadian et al., 2020). 2) Metaproteomics, which quantifies the proteins produced by microbial communities (Andersen et al., 2021; Karaduta et al., 2021). 3) Metabolomics, which profiles small molecules and metabolic intermediates to illuminate real-time host-microorganism interactions and biochemical states (Goldansaz et al., 2017; Chatman et al., 2024). Large-scale integration of microbiome data with host genomics, transcriptomics, and metabolic phenotypes can enable researchers to identify candidate microbial strains for probiotic development, link specific microbial metabolites to improved performance traits, and guide the design of microbiome-informed feed formulations and therapies.

A high level of resolution is essential for translating microbiome data into actionable tools for animal production. Correlating functional microbial signatures with desirable production outcomes, multi-omics approaches can pave the way for next-generation biologics tailored to specific species, production systems, and health challenges. Future studies should prioritize cross-disciplinary data integration, investment in functional validation platforms, and the development of curated multi-omics databases specific to food-animal systems.

Conclusions

The GIT microbiome of food animals remains a largely untapped reservoir of biologically active compounds with far-reaching implications for not only animal health, nutrition, and biotechnology but also other host properties such as meat quality. Advances in short- and long-read 16S rRNA gene sequencing have significantly expanded our capacity to explore this complex ecosystem. Short-read platforms continue to dominate due to their cost-effectiveness and throughput. In

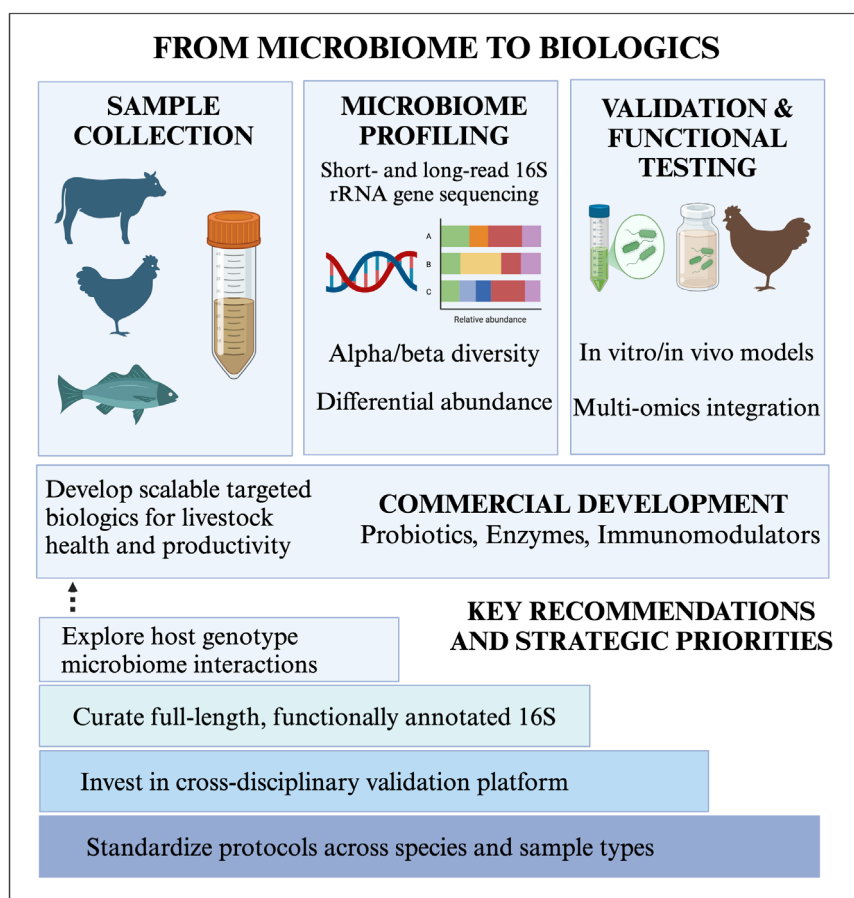


Figure 1. The integrated pipeline from microbiome sampling to biologics development, alongside strategic priorities necessary to advance microbiome-based innovations in food-animal systems. Created in BioRender. <https://BioRender.com/o96towk>

addition, long-read technologies provide the taxonomic resolution necessary to more precisely identify functionally important microbial strains. Yet, realizing the full functional potential of the GIT microbiome requires moving beyond taxonomic inventories. Integrating multi-omics approaches can help researchers uncover and validate the metabolic pathways that drive microbial contributions to host physiology. Combining these tools is essential for identifying SCFA production mechanisms, antimicrobial peptide biosynthesis, immune modulation, and other key functions that form the basis for biologic development.

Future efforts must prioritize the identification of candidate strains, microbial consortia, and bioactive metabolites that can be validated and developed into scalable biologics for livestock applications. Promising innovations include next-generation probiotics, fiber-degrading enzymes, and precision feed additives designed to enhance animal performance and potentially improve meat quality. To translate microbiome insights into actionable tools for animal agriculture, [Figure 1](#) presents a framework that links

the biologics discovery pipeline with strategic research priorities. Ultimately, aligning advanced sequencing technologies with multi-omics integration and application-focused research will catalyze a shift from microbiome profiling to biological innovation. As the livestock industry advances toward sustainable, antibiotic-free production, microbiome-derived biologics will be central to achieving healthier animals, improved productivity, and enhanced meat quality.

Declaration of Competing Interest

Brett Hale is employed by AgriGro®. The authors declare that there are no commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contributions

E. G. Olson: conceptualization, literature review and data curation, writing—original draft, writing—review and editing, visualization; B. M. Hale: literature review and data curation, writing—review and editing, visualization; C. C. Chatman: literature review and data curation, writing—review and editing; H. C. Mantovani: literature review and data curation, writing—review and editing; E. L.-W. Majumder: literature review and data curation, writing—review and editing; S. C. Ricke: conceptualization, writing—review and editing, supervision and guidance, funding acquisition.

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