Chapter

Common Methods to Understand and Develop Indigenous Probiotics Yeast for Ruminant

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Abstract

Probiotic yeast enhanced the ruminal gut microbial balance by producing intercellular effectors and important metabolites. The impact of yeast addition on animal health is influenced by different interlinked factors including animal genomics, its gut microbiota, and environment. Therefore, all factors should be considered regarding achieving the maximum outputs from animal probiotic yeast. In the situation of a high feeding cost, microbial feed supplements provide a suitable nutritional approach, which allows increased nutrient digestion rate and accordingly improves animal performance. Many yeast products are commercially available, but their efficiency as probiotic dietary addition in a particular breed is mostly questionable. Therefore, identification of ideal probiotic yeast strain is of great interest in this context. Innovative methods in relation to develop new probiotic are mainly focused on the exploring novel microbial strains from indigenous sources. It has been noted that for the identification of best probiotic strain for the host, a linkage between culture-independent and culture-dependent methods is a functional step. In this chapter, we will discuss the mode of action of probiotic yeast on animal lower gut microbiota and identification of ideal probiotic yeast by using advanced molecular methods.

Keywords: indigenous probiotic yeast, lower gut, microbiota, molecular methods

1. Introduction

Over the past decades, the livestock industry has been revolutionized toward the use of microbial feed additives due to an increasing awareness of the stockholders on the beneficial role of probiotics in production and gut health status [1, 2]. There are several probiotic products that are commercially available and marketed for animal use [3]. Most probiotic products at the moment do not go through pre-market approvals and are commonly used for a much wider range of scenarios in which their efficacy is not well established. Similarly, latest molecular methods such as gene sequencing and phylogenetic analysis are not used to identify the probiotic strains as feed supplements. For the selection of best probiotic product, it is highly important to determine the real probiotic potential of the microbial strain by using latest molecular methods. In this contract, locally isolated and validated probiotic strains

will be better than any unauthorized local available strain. The competitive advantage and adaptability to local microbial ecosystem will allow local probiotic strain to grow and adhere well in the local animal breed. Literature showed that probiotic strains should specifically prepare according to purpose and function related to the milk enhancement in local breed [4, 5]. Nowadays, it is highly accepted that probiotic yeast is highly productive in terms of milk and meat for large animals [6, 7]. Probiotic yeast improves the ruminal gut microbiota which may increase the nutrient digestibility and leads to improve animal productivity [8]. In large animals, ingested feed digested by numerous microbial species is present along the gastrointestinal tract [9]. This microbial community consists of 1014 members, mainly composed of fibrolytic bacterial species [10]. Literature highlighted that gut microbiota plays important role in the feed digestion and utilization. The gut microbial populations in cow have been identified in almost 90% of the total microbial community [11]. On the other hand, a certain fraction of the GI tract bacterial community has yet to be identified due to less knowledge of the microbial community in gut microbial ecosystem because majority of the 16S rRNA gene sequences from feces are taken from unidentified species, and many modern methods of genomic analysis of communities to determine changes in microbiota have been used by many scientists [12]. Studies have utilized culture-independent sequencing techniques, 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing and many more have added a new era to determine the microbial diversity of the GI tract [13]. Research noted that the culture-independent methods deliver a comprehensive assessment of the microbial community composition, while the culture-dependent methods provide the structural and functional diversity of the microbial strains [14]. In this chapter, a detailed discussion on the effects of probiotic yeast in ruminant's well being, production performance, uses of different omics methodologies for the discovery of ideal animal probiotic strains and development of indigenous probiotic yeast for ruminant will be employed.

2. Yeast: an ideal microbial feed supplement for ruminants

The *Saccharomyces cerevisiae* (baker's yeast) is the first eukaryotic sequenced genome. The sequencing of first whole eukaryotic genome was a challenging task for the scientists, but the efforts of more than 600 scientists from Europe, North America, and Japan made it possible. The entire sequence of the yeast was released in 1996. The size of the baker's yeast genome is 12.1 Mb containing 16 chromosomes and 5400 coding genes approximately. The sequence information of yeast is available at *Saccharomyces* Genome Database (SGD), Yeast Protein Database (YPD), and Munich Information Center for Protein Sequences (MIPS) [15] (**Table 1**).

Ruminant nutritionists have been pondering to improvise new methodologies for ameliorating the roles of microflora in ruminants and enhance processes of

Yeast genome	
Genome size	12.1 Mb
Chromosomes	16
Genes	5300–5400
Base pairs	12 million base pairs
Databases	SGD, MIPS, YPD

Table 1.
Details of first eukaryotic sequenced genome (yeast).

digestion and fermentation along with augmented nutrients usage and bioavailability using feed supplementation. One of the commonly used methods was the use of growth promoters (antibiotics) to restrict the pathogenic effect on productivity of ruminants [16]. Nevertheless, antibiotics have been reported to cause serious health challenges to consumers and environmental implications. Thus, their usage has been banned in 2006 due to emerging antibiotic resistance. In the light of these concerns, consumer preferred more natural product. A super alternate of feed additives was the use of probiotics [17]. Probiotics are living microorganisms confined in animal feed that affect the host by improving the digestion [18]. Other definition includes probiotics as microorganisms (viable) that functions in gaining weight and feed conversions along with reducing diarrheal incidence [19]. Probiotics have been deployed as one of the recent exploited proposals in ensuring efficiency of production systems and safety to both consumers and environment [20, 21]. In ruminant nutrition, yeast probiotics are commonly being used because of their efficient roles in rumen stabilization and maintaining microbial communities specifically fibrolytic bacteria [22]. The yeast cells function in maintaining throughout viability of the digestive tract [23]. Yeast supplementation as probiotics enhanced feed conversion, efficient fermentation, and fiber digestion in the rumen, maintained ruminal pH, increased milk production [24, 25] and feed intake and production of organic acids and vitamins to activate the growth of the lactic acid bacteria (LAB) [26]. The commonly used yeast probiotic is *Saccharomyces cerevisiae*. Numerous literatures on *Saccharomyces cerevisiae* as supplement are available that dated back to the 1950s and continued under study till today [27]. Significant role of yeast supplementation (live) in diet has been stated for lactating and growing ruminants. Recent studies confirmed that they increase the ruminant's milk production early lactation period by altering the fermentation of food inside the GIT of ruminants[28]. Latest beef and dairy production systems demand active muscle growth and high milk yield via feeding animal at high ruminal ferment ability rates. This would result in increased risk of metabolic disorders such as acidosis due to dysbiosis in ruminal microbial environment resulting in abnormal functioning in rumen which further leads to poor feed intake, health, and decreased productivity [29]. Therefore, yeast supplementation in ruminant diet is beneficial in the ruminal functioning and overall animal health and maintenance. The ameliorating functions of yeast probiotic on digestibility of high forage diets also underscore the potential use of yeast supplementation to optimize the use of lower quality feeds.

3. Understanding of the ruminant microbial community for development of ideal probiotic yeast for ruminants

Rumen microbial manipulation by using the probiotics to improve the ruminant feed digestion is a promising production improvement strategy. A better understanding of the rumen microbiology is an important step to select and prepare a new yeast strain affecting on functional specific microbes. Latest molecular techniques have provided the opportunity to study the rumen microbiota in detail for development of the ideal probiotic.

3.1 Digestive system of ruminants

Digestive system of ruminant is composed of four parts: reticulum, rumen, omasum and abomasums. The rumen is that part of the digestive system in which fermentation is carried out [30]. The rumen can also be defined as a complex ecosystem in which nutrients consumed by different microorganisms are digested

anaerobically. Microbial biomass and volatile fatty acids are most common end products of fermentation which are then used by ruminant host. Interaction of host animal and microorganisms is a symbiotic relationship that helps the ruminant hosts in digestion of fiber-rich and protein-low diets. Rumen microorganisms provide enzymes that are necessary for fermentation processes, which in turn allow ruminants to obtain energy contained in forage [31]. Growth and activity of ruminal microorganisms are influenced by different factors including pH, temperature, osmotic pressure, buffering capacity, and redox potential. These factors are determined by environmental factors. Temperature of the rumen is in the range of 39–39.5°C. But when animal eats, fermentation occurs that generates heat due to which temperature increases up to the limit of 41°C [32, 33]. Short-chain fatty acid generation along with their absorption, saliva production, feed intake level and type, as well as exchange of phosphates and bicarbonates through epithelium of the rumen are the factors that affect pH [34]. In the reticule ruminal environment, these factors determine the buffering capacity as well as pH. There is a constant change in pH but mostly it remains in the range of 5.5–7.0 [35]. When there is an acidic environment in the cell, bacterial intracellular pH decreases. Microbial enzymes are very much sensitive to pH, i.e., bacterial growth is inhibited when there is an acidic pH. This is due to the disproportion of intracellular hydrogen ions [36]. In the rumen, ions and molecules affect osmotic pressure due to which gas tension is created. Fermentation process in the rumen depends upon the environmental factors and the diet due to which these factors also affect rumen osmotic pressure [37] (**Figure 1**).

3.2 Microbial community of GIT

Bacteria are more in number than any other microbes. It is noted that there are five groups of rumen bacteria: (1) free-living in liquid phase, (2) loosely attached with feed, (3) firmly attached with feed, (4) attached with rumen epithelial lining, and (5) attached with protozoa/fungi. The bacterial species inside the rumen are 99.5% obligatory anaerobic. Mostly rumen bacteria are involved in the fermentation of fibers, starch, and sugar present in the feed and converted into volatile fatty acid, H_2, and CO_2 [38]. Most of the bacteria are responsible for degradation of different types of dietary components [39] (**Table 2**).

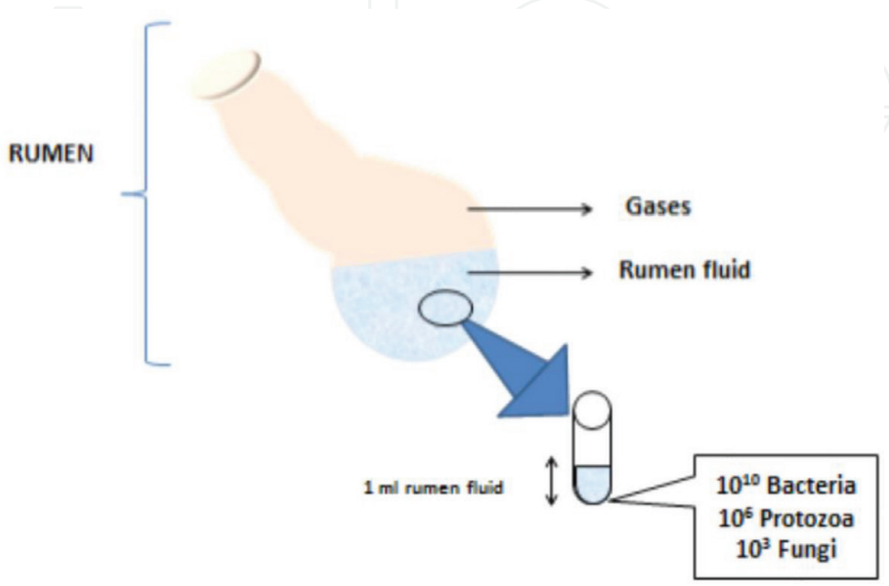

Figure 1.
Rumen ecosystem: different types of microbial flora present inside the rumen. The most abundant microbes are bacteria.

Majority of anaerobic rumen fungi is from order *Neocallimastigales* within the *phylum Neocallimastigomycota*. On the phylogeny basis, six genera have been identified, which are *Piromyces*, *Neocallimastix*, *Caecomyces*, *Anaeromyces*, *Orpinomyces*, *and Cyllamyces* [40]. In fiber digestion, fungi play a very important role because of the vegetative thallic rhizoids. The main functions of the rumen fungi are the lignin and fiber degradation by producing different types of enzymes [41] (**Table 3**).

3.3 Mechanism of action of probiotic yeast in the rumen

The rumen is the first part of the ruminant stomach which has a well-developed microbial ecosystem containing different types of microbes (bacteria, fungi, protozoa, and bacteriophages). These microbes coexist in ecological equilibrium

Bacteria	Species
Carbohydrate-utilizing bacteria	*Fibrobacter succinogenes* *Ruminococcus flavefaciens* *Ruminococcus albus* *Clostridium cellobioparum* *Clostridium longisporum* *Clostridium lochheadii* *Eubacterium cellulosolvens* *Cillobacterium cellulosolvens* *Butyrivibrio fibrisolvens* *Prevotella ruminicola* *Bacteroides ruminicola* *Eubacterium xylanophilum* *Bacteroides uniformis*
Nitrogen-utilizing bacteria	*Prevotella ruminicola* *Ruminobacteramylophilus* *Clostridium bifermentans*
Lipid-utilizing bacteria	*Anaerovibriolipolytica*

Table 2.
Bacterial diversity of the rumen microbial ecosystem.

Microbial species	Rumen	Fecal
Bacteria	*Bulleidia* *Roseburia* *Prevotella* *Ruminococcus* *Acidaminococcus* *Megasphaera* *Succiniclasticum*	*Bacteroides* *Bifidobacterium* *Clostridium* *Collinsella* *Blautia* *Dorea* *Lactobacillus* *Peptostreptococcus* *Treponema* *Succinivibrio* *Faecalibacterium*
Fungi	*Caecomyces* *Orpinomyces* *Piromyces*	*Caecomyces* *Orpinomyces* *Piromyces*
Archaea	*Methanobrevibacter* *Methanosphaera*	*Methanobrevibacter* *Methanosphaera*

Table 3.
Bacteria, fungi, and archaea present inside the rumen and feces of dairy cows.

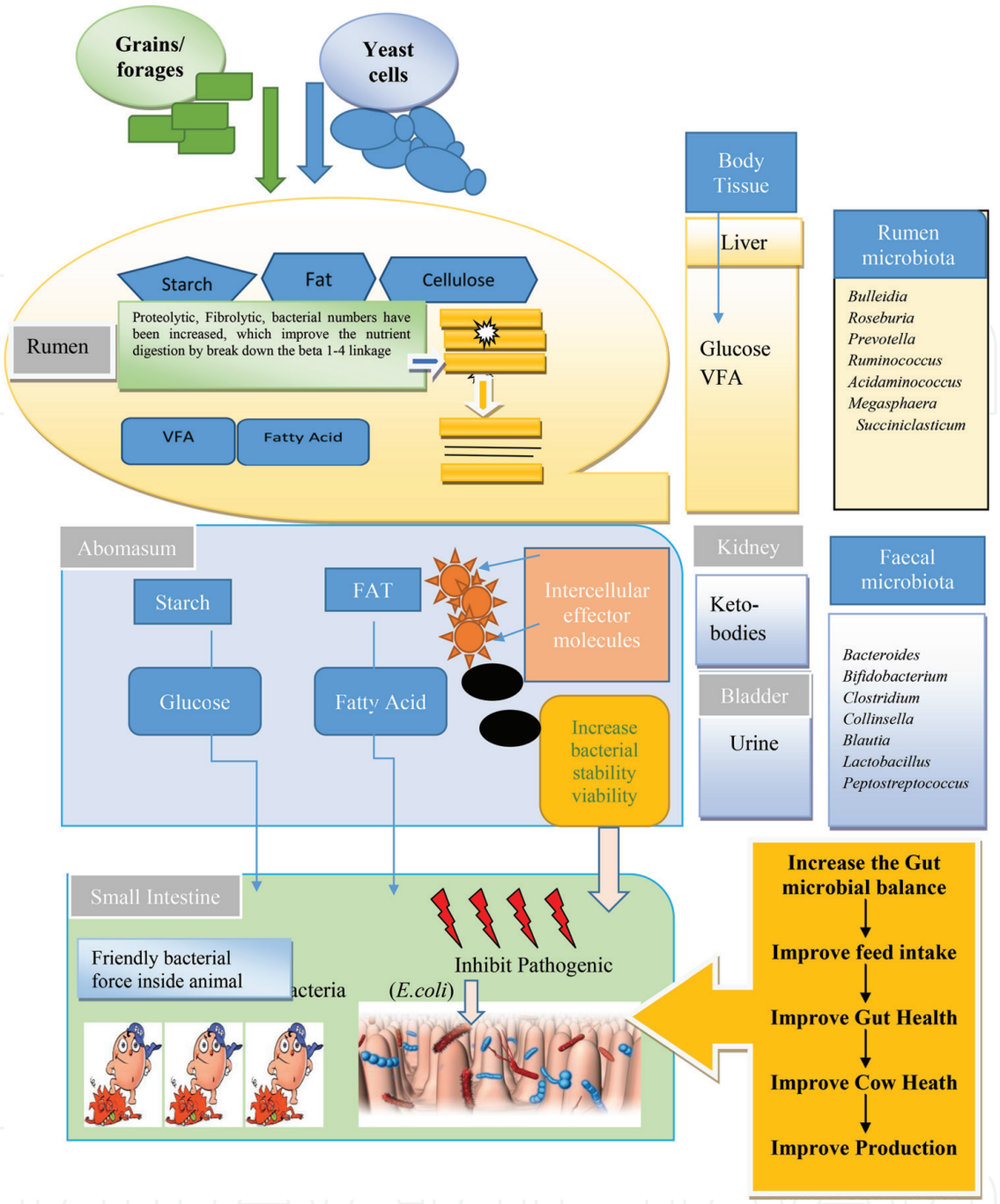

Figure 2.
Representative scheme of effect of live yeast on the microbial flora of the gastrointestinal tract in ruminants: live yeast improves carbohydrate, protein, and lipid digestion rates by improving the production of cellulolytic, hemi-cellulolytic, and proteolytic and lipolytic bacteria and fungi.

in unique symbiotic relationship between cows and rumen microbes. The cows supply food to the rumen microbes which in turn digest the feedstuff to provide cows the essential nutrients in the form of microbial protein as organic acid energy sources. The microscopic view of rumen ecosystem showed that it is consisted of a number of bacteria, protozoa and fungi [42]. Bacteria make the largest population in this diverse microbial world. Their function is to digest the fibers, starch, sugar acids, and protein to give useful compounds and elements necessary for the growth and productivity of the cows. The role of protozoa and fungi is less clear. However, these microbes do provide help in digestion of feed. The structure and function of microbial community are influenced by feed composition

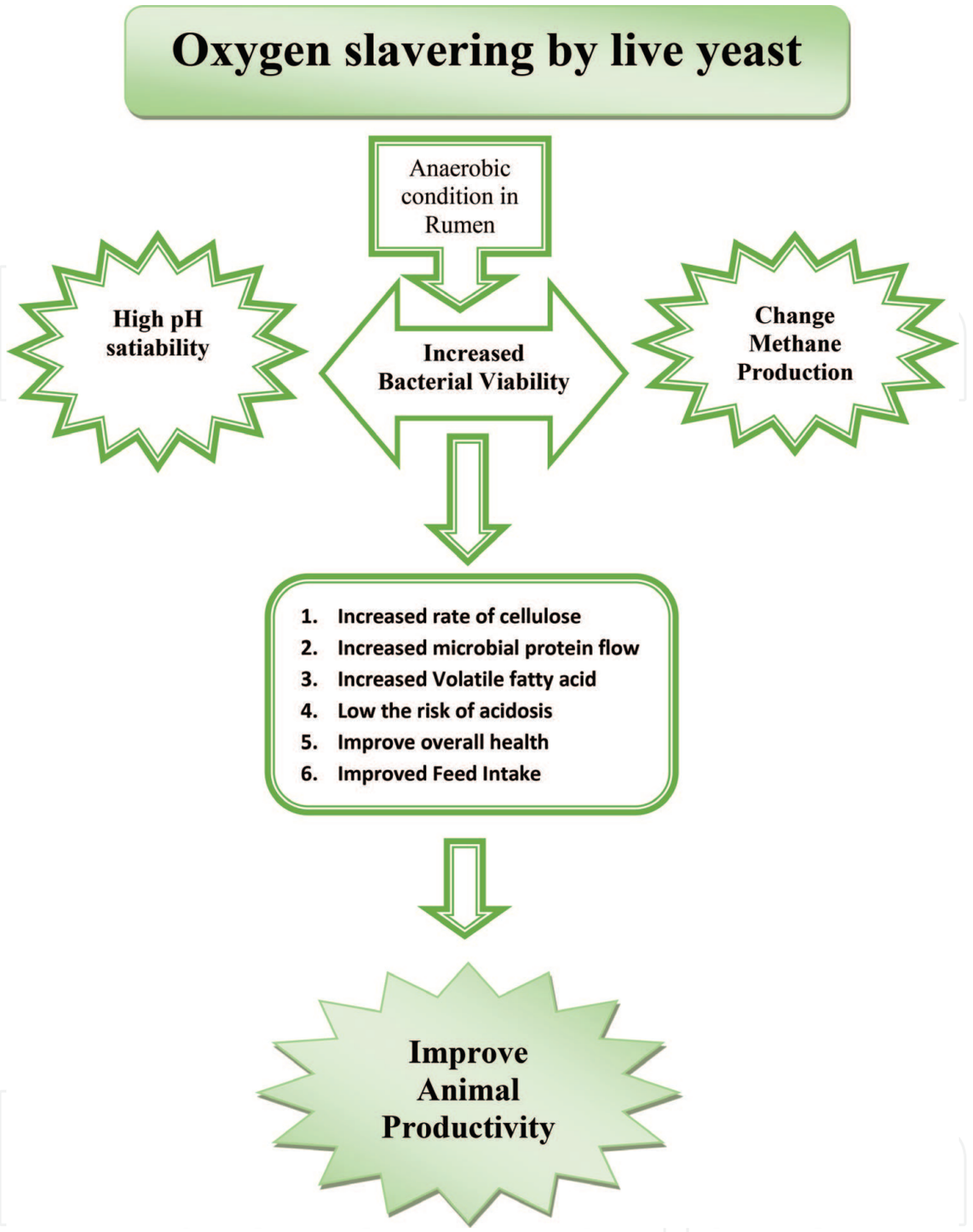

Figure 3.
A scheme describing the mode of action of yeast culture: improved the gut microbial balance is related to the O_2 slavering by live yeast cells.

and mainly by the host genetic potential. *Prevotella* and *Succinivibrionaceae* are the dominated rumen bacterial communities, cellulolytic and fibrolytic genera; *Neocallimastigaceae* are the dominant fecal and rumen fungal communities; and *Methanobrevibacter* are the dominant fecal and rumen archaeal communities in the adult ruminants. *Bacteroidetes* and *Firmicutes* are the dominant phyla of bacterial communities. *Bacteroidaceae*, *Lachnospiraceae*, *Prevotellaceae*, *Ruminococcaceae*, *Succinivibrionaceae*, and *Veillonellaceae* are the most abundant bacterial families in adult ruminant [43]. The term "yeast" is originally derived from the Dutch word

gist, which basically refers to the foam that formed during beer fermentation. A variety of roles is played by yeast in veterinary practices, livestock feeding, and medicine as well as in biomedical and pharmaceutical industries [44]. Hayduck first discovered the inhibitory activity of yeast. Probiotics such as yeast or fungi have been extensively used in ruminant feed for the improvement of growth, health, and lactation due to their impact on rumen pH, intake of dry matter, and digestibility of nutrients [45]. Probiotic yeast has potential beneficial effects on the rumen. In the cattle, the ability of live yeast for enhancement of milk yield as well as weight gain is due to the fact that yeast is responsible for stimulating bacterial activity in the rumen [46]. Mechanism of action of yeast mainly stimulates the growth of cellulatic and hemicellulatic bacteria [47]. Increase in the number of bacteria in the rumen is due to the reproducible effects of probiotic yeast. Yeasts remove oxygen from the rumen due to which bacterial performance improves in the rumen. To maintain the metabolic activity, yeast cells consume available oxygen on the surface of freshly ingested feed in the rumen. Few studies showed that there is a significant decrease in redox potential, up to -20 mV by providing yeast supplementation (**Figure 2**).

Better conditions have been created by this change for the growth of anaerobic cellulolytic bacteria which in turn stimulates their attachment to forage particles as well as increases the initial rate of cellulolysis. Recalcitrant plant lignocellulosic material is not degraded by ruminants on its own. They rely on rumen microbial flora for its degradation [48]. The main components of the fiber are cellulose, hemicellulose, and lignin. It has been estimated that 20–70% of the ruminant feed is composed of the cellulose and hemicellulose [49]. The most abundant carbohydrate in plant cell wall is the cellulose which makes up to 40% of the plant cell wall. The microbial cellulolytic enzymes have the capability to digest the β-1,4 links present inside the cellulose, glucose molecules [50] (**Figure 3**).

3.4 Mechanism of action of probiotic yeast in the lower gut

The lower gut microbial population is affected by dietary supplementation of the probiotic yeast. The probiotics provide a desirable microbial balance due to shift in the balance of friendly and pathogenic microbiota. The GIT having healthy microbial populations are often related with improved host performance and its immune system. In the lower gut, the pathogenic microbial species reduces due to the production of the antimicrobial material (bacteriocin) and the attachment of the friendly microbes to the gut wall, via the competitive exclusive method. The most common modulation of the GIT microflora is provided by probiotics [51].

4. Modern methods to understand and develop fibrolytic probiotics for ruminants

Latest researches have improved our understanding related to the mode of action of probiotic yeast inside the rumen. Well-designed animal studies have verified that target-specific probiotic strains have health and production benefits in the ruminants. These studies have made the livestock industry to accept and understand the probiotic concept [52]. On the other hand, current probiotic has not been chosen for definite purposes in the animal feed. Therefore, some unique molecular methods are needed for selection and characterization of target-specific probiotic strains [53]. It has been noted that during stress conditions, some portion

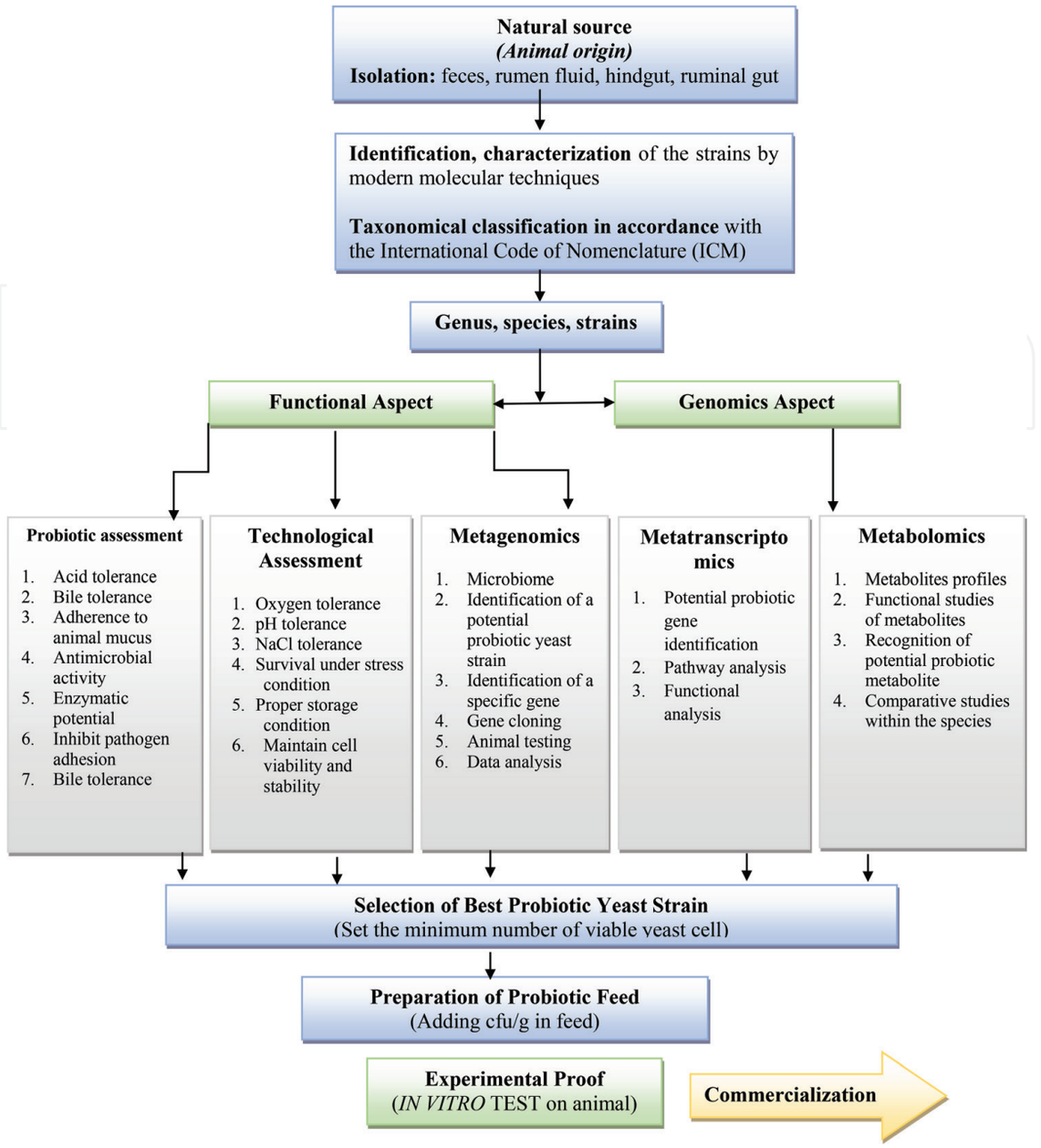

Figure 4.
Probiotic preparation: general steps for the isolation and characterization of probiotic yeast strains for local animal breed.

of the live probiotic microbial strain enters in the dormant but metabolically active state called viable but nonculturable (VBNC) state. These microbial cells have an ability to replicate when acclimated to a favorable condition inside the host [54]. Uses of molecular techniques have changed the study of the rumen ecosystem. First is the PCR which is more sensitive than growth on traditional selective media in determining small differences in population sizes in response to dietary changes or upon the inclusion of an additive to the diet and thus may identify changes or shifts within levels of the microbial population which may have been previously overlooked [55] (**Figure 4**).

In response to various feeding sources, changes within the microbial population can be studied by DNA fingerprinting (DGGE, TTGE, and TGGE). Probiotic can be classified into three different types, like mono-probiotic, poly probiotics, and combined probiotics depending on the probiotic strain function [55] (**Figure 5**).

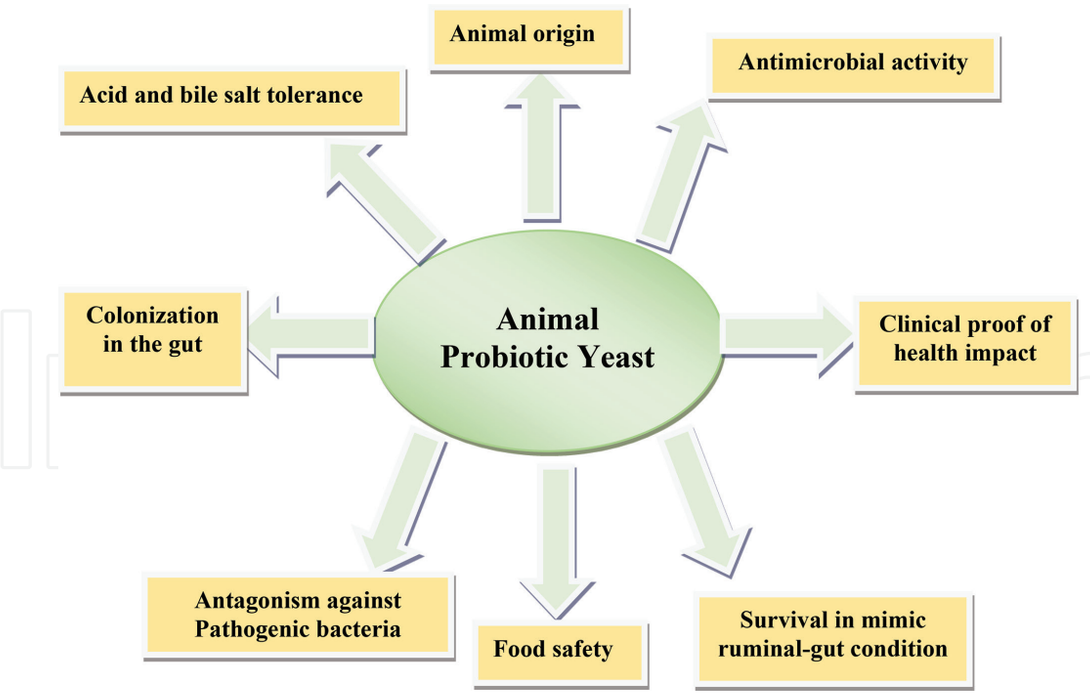

Figure 5.
Potential characteristics of typical animal probiotic yeast.

5. Common methods used to identify indigenous probiotic yeast

Yeasts and fungi are the ideal organisms and have been used in vast genetic studies and comparative genomic studies in eukaryotes because of their small and compact genomes.

We have sketched sampling approaches and finalized the protocols that will guide researchers in identifying the most ideal probiotics for animal use. Livestock is under increasing threat of antimicrobial resistance genes; therefore, continued optimization of protocols is urgently needed so that these threats can be reduced through the use of probiotics. Two sequence-based methods are commonly used for the identification of yeast. The first and the most common method used for the identification is PCR amplification of internal transcribed spacer (ITS) of nuclear ribosomal variable region that has been recognized as the universal barcode for the identification of fungi. The second and the advanced approach to identify fungal species or strains is shotgun metagenomics [56]. Microbes are very vital to life present on the earth. Their significance is increasing day by day as their beneficiary potential has been recognized in the field of health and medicine. There are two methods which have been utilized till now for the identification of the microorganisms present in microbial community.

- Culture-dependent method
- Culture-independent method

Both approaches have their own significance. Culture-based methods are considered effective for the morphological, physiological, and functional characterizations of a particular strain, while culture-independent technology is preferred to unravel the microbial diversity along with genomic and genetic identification of microbial communities. Studies have also indicated that there is a loss of 99% microbes in the laboratory-dependent culturing methods. Culturing-independent

method has been recognized as an effective and efficient method to isolate the DNA of a number of microbes from an environmental sample which seems impossible using the cultural methods. The linkage of culture-dependent and culture-independent data has been recognized as a crucial step for the identification of probiotics [57]. For identification of the potential probiotic strains, researchers should use the latest molecular methods, and the probiotic strains should be deposited in some recognized microbial culture collection. Proteomics and metabolomics may also be used for choosing the best yeast species [58]. By utilizing strain's proteome and metabolome, which are argued to yield a positive influence upon ruminal fermentation, it may be possible to identify specific traits, characteristics, and secondary growth metabolites that play a potential role to enhance the growth of target-specific microorganisms inside the rumen. Even accounting for the potential bias of latest molecular methods, it is obvious that these methods are the dominant tools recently accessible for monitoring the gut for bacterial diversity of dairy animals and developing new yeast strain [59]. Extensive use of molecular methodologies may give insights into the new era where such microbial studies are no longer limited to a handful of laboratories with an abundance of funding and labor. It is noted that the specific yeast strains of known origin act more precisely and efficiently as compared to the yeast strain obtained from any unknown origin [60]. As we note all ruminates live in different parts of the world; therefore, upon the ruminal fermentation different yeast strains may exhibit markedly different effects. Therefore, we should identify new yeast strains for getting best results on the rumen fermentation. Uses of molecular techniques have changed the study of the rumen ecosystem. First is the PCR which is more sensitive than growth on traditional selective media in determining small differences in population sizes in response to dietary changes or upon the inclusion of an additive to the diet and thus may identify changes or shifts within levels of the microbial population which may have been previously overlooked. In response to various feeding sources, changes within the microbial population can be studied by DNA fingerprinting (DGGE, TTGE, and TGGE). To select best yeast strains, proteomics and metabolomics may also be used. By characterizing the proteome and metabolome of microbial isolates endowed with the ability to have a positive impact on the rumen fermentation, it may be possible to identify specific traits, characteristics, and secondary growth metabolites which play genuine role in the improvement of the growth of some important microbial species [61] (**Figure 6**).

5.1 Culture-dependent techniques

Cultural approach is the widely used method in microbiology to grow a microbe in a laboratory. Sampling is the basic and the crucial step for the identification of the indigenous probiotic yeast. The second step is isolation of the pure yeast strain under laboratory conditions which requires a series of inoculation steps of the microbes on the selective media. After purification of the yeast isolate on the OGA media, the biochemical tests are performed to identify the distinct features of the pure isolates. Morphological features of the isolate are determined by using electron microscope. The next step is the molecular identification of the yeast via 18S rRNA gene sequencing. The probiotic characterization is usually performed according to the standards defined by the WHO [62]. The best probiotic strain is retrieved among all the selected potential candidates, and in vivo experiments are performed using an animal model. After functional testing, all technological and safety measures are accessed, and the probiotic yeast strain is ready for probiotic product and packaging [63].

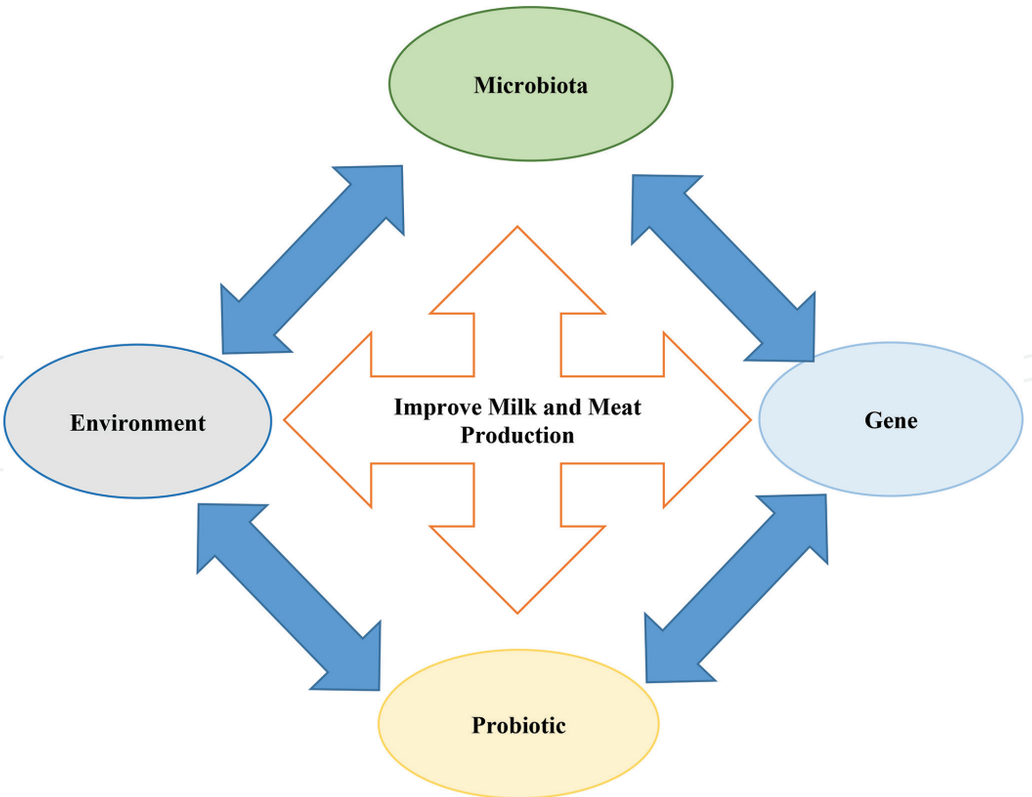

Figure 6.
Interlinked factors involved in the application of probiotic in the ruminant nutrition.

5.2 Culture-independent techniques

The use of omics approach has been emphasized to study the microbiome of microbes. To identify the potential probiotic strains among the microbial community present in any environment, it is very important to identify all the microorganisms in microbiota and determine their structural and functional differences at genomic level. Below are the currently available omics approaches for the identification, screening, and selection of probiotic strains of indigenous yeast [64] (**Figure 7**).

5.2.1 18S amplicon sequencing

Amplicon sequencing refers to the sequencing of a specific fragment of interest of a microbe using high-throughput sequencing technique. 18S amplicon sequencing is specifically used to determine the most prevalent fungal yeast species present in microbiota [65]. The methodologies used in the recent researches for the identification of bacterial probiotics can be applied in the recognition of indigenous probiotic yeast strains. The comparative and detailed analysis of 18S amplicon sequencing data can help the scientists in the isolation of potential probiotic after the identification of functional and structural characteristics of the indigenous yeast in microbiota. Further experiments and testing would be required to maximize the production and ability of probiotic yeast in the gut of an animal [66]. Furthermore, the 18S amplicon sequencing does not only help in the indigenous yeast identification, but it also reveals the diversity of microeukaryotes when 18S rRNA gene is sequenced [67].

5.2.2 Shotgun metagenomics

Shotgun metagenomics is one of the most advanced techniques of sequencing in which the entire microbiome of microbiota is sequenced. The data generated

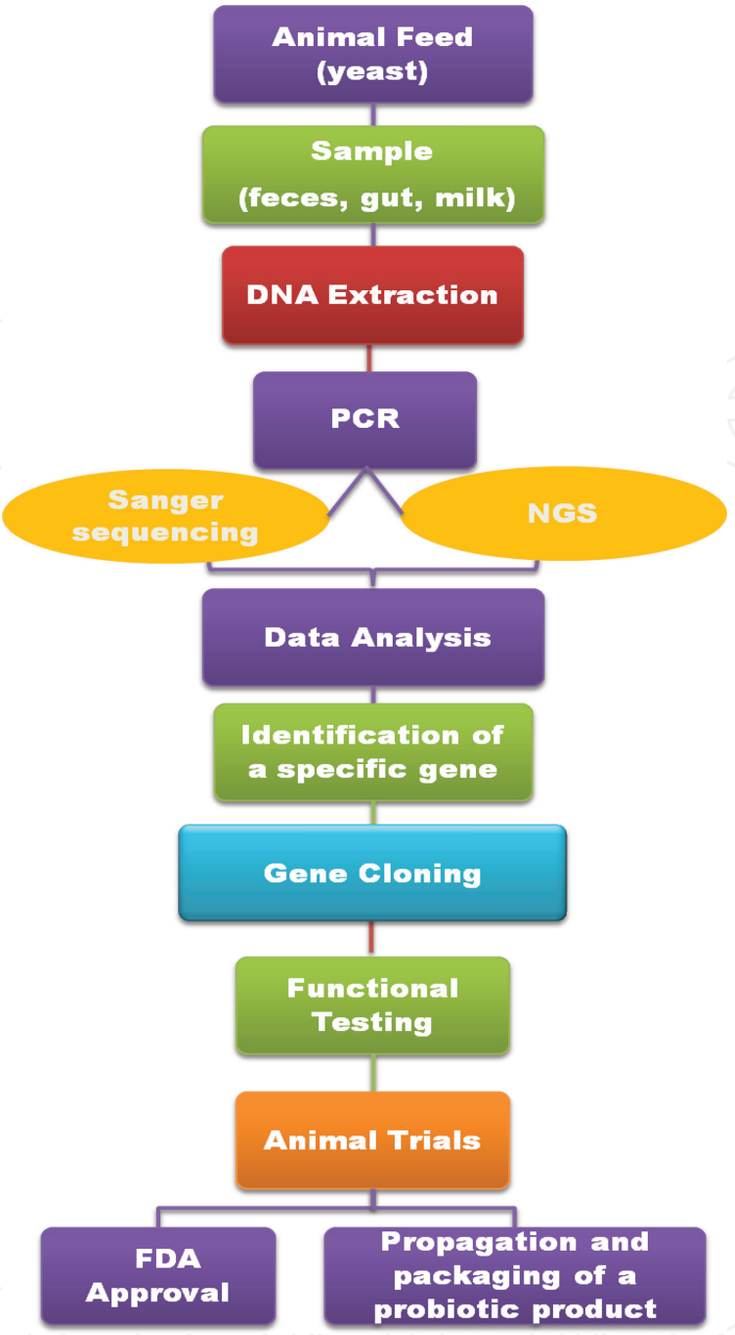

Figure 7.
Omics approaches to identify the probiotic.

using this method provides all the information about the genome of an organism [68]. Metagenomics information unravels the composition of microbial community and also indicates the genes, their functions, and associated genetic pathways. The identification of the indigenous yeast and their probiotic potential and capabilities can also be determined using the metagenomics data. Their relationship within the microbial community and their effect on the host can also be studied on the basis of the retrieved information [69].

5.2.3 Metatranscriptomics

Scientists and researchers are using metatrancriptomics to study and analyze the expression profiles of mRNA in a microbial community. The identification of genes, genetic pathways and their regulation, host-microbe interaction, and the symbiotic relation among microbes can easily be determined by using the mRNA

expression data. Metatranscriptome approach can be pursued in the identification of indigenous probiotic yeast within the microbiota of an animal. For this purpose the sampling methods and molecular techniques should be improved [70].

5.2.4 Metabolomics

Metabolomics refers to the study of the metabolites or final cellular products. This is also considered one of the useful and efficient methods for the identification of probiotic potential of a microorganism within a microbiota of an animal or selected biological sample [71]. Indigenous probiotic potential of yeast can also be determined using this technique. Studies are still needed to fully understand the function of metabolites in context of probiotic potential and other inhibitory functions of metabolic compounds. As metabolites vary in structure and function, so they could be used in the comparative studies of species and populations. A number of species with high probiotic potential could be approached using metabolomics [72].

6. Challenges in preparation of suitable probiotic yeast

- Yeast probiotics not only help to improve the performance factor of cattle, but it also enhances nutrient digestibility. However, the effectiveness of yeast-supplemented products is variable. Therefore, future studies are required to estimate the potency of these diet products as supplements for finishing beef cattle, with an objective to have healthier and productive animals without negotiating their efficiency and costs.

- The animal body is a "supraorganism" and refers to the gastrointestinal tract as a virtual organ of the human body. The ongoing research is mainly on probiotics that are used chiefly for the GI tract, whereas there is an impetus need to evaluate the progress on other regions of the body as well.

- Yeast supplementation is an effective strategy; thus, it is vital to ensure the stability and viability of yeast-supplemented diet products by developing practicable and cost-effective technologies (e.g., storage, microencapsulation, etc.), which poses marketing and technological challenges for producers at industrial level. Polysaccharides, lipids, and proteins are chiefly used for encapsulation materials in food industry. However, cost-effective production remains a challenge for production of future probiotics and formulation technologies.

- Role of yeast probiotics in combating antibiotic-associated diseases has been extensively reported through control trials and ingestion of yeast probiotics (*Saccharomyces boulardii*) and has positive therapeutic effects specifically in preventing antibiotic-associated diarrhea (ADD), but validated biomarkers for numerous target diseases are probiotic or antibiotic deficient. Therefore, in the field of probiotic investigation, the defining of validated biomarkers needs to be advanced.

- There is a dire need to understand the composition and relationship of microbial community within an animal gut for improving the production of dairy products. Advances in the high-throughput technologies, computational tools, and omics approaches give insights into the molecular and genetic potential of an organism. Studies in the omics arena are still needed to fully understand the genetic mechanisms and pathway analysis.

7. Conclusions and future research

Every living organism is different in terms of their genetic makeup. The current progresses in sequencing and functional omics techniques have delivered better understandings into the precise mechanisms underlying probiotic functionality. The emerging understanding of the animal gut microbiota allowed accurate characterization of probiotic effects on the commensal microbiota of animal in vivo. Identification of genes vital to probiotic functionality is providing scientists the capacity to genetically tailor probiotics to encounter the requirements for precise applications. The livestock sector has a larger proportion of land consumption than agriculture keeping in view both grain feed intake and grazing. This trend is expected to rise, putting pressure and competencies on land resources in the agriculture sector. Moreover, there is a high demand for quality production which Cannot be attained by traditional practices for feeding ruminants. Quality cereal feed costs high and is uneconomical for large production. Consequently, this creates an imbalance in nutrition which drastically reduces dairy production. Probiotic yeast can overcome dairy production disparity. It augments nutrient uptake and increases Immunity, overall better health and production. Utilization of probiotic yeast for health and production is influenced by many different factors including probiotic strains, age, and breed of cattle. Essentially, yeast probiotics enhance assimilation by balancing the microflora of the rumen. It facilitates fiber digestion via inducing fermentation and stabilizing high pH. Facilitating an environment that flourishes rumen microbes is one factor. Other avenues need to be explored for probiotic yeast. More probiotic yeast strains are needed to be identified. For the preparation of probiotic feed, a complete nutritional profile generation is required. Furthermore, the amino acid profile of milk produced by dairy heifers fed on yeast probiotic should be analyzed.

8. Recommendations

The recommendations are outlined as follows:

- Sampling source should be indigenous for isolation of the probiotic strains.
- The identification of the probiotic strains must be based on the international validated molecular methods.
- The identified strain name should be deposited in validated microbial culture collection.
- The probiotic as well as genetic properties of the probiotic strains should be studied. Good manufacturing practices must be applied with quality assurance and shelf-life conditions established and labeling made clear to include minimum dosage and verifiable health claims.

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