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Rumen microbes: Exploring its potential for productivity and commercial use

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ABSTRACT

Ruminant animals are known for their dairy and meat products worldwide. They are the best converters of poor quality fibrous feed ingredients, and presence of rumen, the anaerobic chamber that harbours vast category of microbes, is attributable to this phenomenon. The microbes include bacteria, fungi, protozoa, archaea and bacteriophages that work on synergistically for optimal performance of ruminant animals. These microbes help not only in digestion of fibrous materials, but also involved in various biological functions, such as probiotic activity, antimicrobial metabolite production, synthesis of health promoting bioactive fatty acid molecules, biomass conversion, etc. Earlier, the probiotic organisms used in food animals (calves, sheep, goat, swine and poultry) were mainly originated from dairy products but today organisms of autochthonous origin are being used, as they show better adaptability. Since, rumen do possess organisms with probiotic and fibre utilising activity, these organisms are now explored for their suitability as a probiotic and fibrolytic agent in monogastric food animals. Diversity of rumen microbes was not properly understood through the conventional culture methods, however with advancement in 'Omic' technologies, researchers could identify new class of organisms from the rumen and their potential use for the commercial and industrial purposes.

Keywords: Biotechnological use, Biological functions, Gut health, Microbiota, Probiotics, Rumen

The concept of consuming ideal protein for optimal lifestyle and other activities has led to an increase in consumption of animal proteins globally. Due to its higher bioavailability and variety, the animal products are attracting more consumers. This shift in consumption pattern together with shrinkage in land availability for agriculture, more pressure has been laid on the animal productivity. This has led to making of animal production system as more and more intensive. Due to high productivity as a result of genetic improvement and intensification, the food animals are more prone to sub-clinical infections (He *et al*. 2022), which could cause more economic losses than clinical conditions, to the farm producers. To counteract these effects, producers started to use sub-therapeutic levels of anti-microbial, which gradually resulted in residues and resistance (Marshall and Levy 2011). Even though, the European Union has banned the sub-therapeutic use of antibiotics in food animals by 2006 (Regulations 2003), recent studies indicates presence of high level of antibiotic resistant genes in manure and environment of livestock production system (Zalewska *et al*. 2021, Haulisah *et al*. 2022). Presence of bacteria with antimicrobial resistant genes is now being considered as new type of environmental pollutant (Zalewska *et al.* 2021), which emphasize sub-therapeutic level of antibiotics for food animal production. To comprehend their use, importance of gastro-intestinal system (GIT), its role, micro-environment and its functions needs to be understood. The GIT is the major system which comes in direct contact with environment through consumption feed and water. Intestinal tract serves the function of digestion and absorption of vital nutrients for the growth and development of animals. In addition to nutrient absorption, they are the host for various microbes (bacterial, virus, fungi, protozoa, archaea) (Matijašic *et al.* 2020). Ideal animal performance is based on these microbial balances in the intestine and any disturbance/imbalance leads to various conditions like reduced performance, immunity, diarrhoea, disease and contamination (Kober *et al.* 2022). Even the probiotics were used prior to antibiotic ban in food animals; their importance has attracted much attention following the ban. Ruminant animals, like cattle, sheep and goats, have a large anaerobic chamber as rumen which harbours vast microbial diversity. Recent studies are targeted towards isolation and identification of rumen origin microbes for better performance and reducing neonatal mortality (Singh *et al.* 2021). With this background, the current paper was developed to discuss the characteristics of rumen microbiota, its biological functions as probiotics and other commercial and industrial scope and applications.

Rumen microbiota: Microbe-host interactions are

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essential for normal development and physiology of mammalians (Alipour *et al.* 2018). This interaction is more predominant in ruminant animals due to the presence of rumen. Rumen is an anaerobic fermentation chamber, which harbours diverse group of microorganisms with the bacterial population of about 10^{10}-10^{11} cells/ml with a diversity of 7000 species, but still about 30% rumen bacteria are need to be identified (Patra and Yu 2012). Anaerobic environment is maintained inside the rumen with the help of microorganisms, which provide *p*H of 5.5 to 6.9 and temperature between 38 to 41ºC. Favorable condition inside the chamber allows the microorganisms to attain ideal growth and transform the feed particle into desirable products. The microbiota in the rumen includes bacteria, archeae, protozoa, fungi and bacteriophages. Normal micoflora will eliminate the pathogen; increase the host defense mechanisms against pathogens (Pinloche *et al.* 2013). The microbial population in gastro intestinal tract of ruminants plays a prominent role in well-being of host (Stover *et al.* 2016).

Ruminant animals could thrive under fibrous residues (lingo-cellulosic biomass) which are made of complex polysaccharides due to presence of mesophilic organisms (Deng *et al.* 2017). Presence of cellulolytic bacteria, *Ruminococcus flavefaciens, Ruminococcus albus* and *Fibrobacter succinogenes*, in rumen has been attributed to be the primary agents for utilization of these agricultural by-products. These bacteria convert the complex polysaccharides to oligosaccharides then to hydrogen and volatile fatty acids (Deng *et al.* 2017, Ozbayram *et al.* 2017). End products fibre fermentation in rumen includes VFAs, mainly acetate, propionate and butyrate, also small amount of valeric, caproic, isobutyric and isovaleric acids; which serve as the major source of energy for the ruminant animals (Dijkstra *et al.* 2005). In addition to microbes of fibre degradation, rumen also harbours various species *Bacillus, Prevetella, Selenomonas, Streptococcus, Megashera, Lactobacillus*, etc. These bacteria could perform various biological functions-probiotic, enzymatic, antimicrobial, electricity generation and even industrial applications. Using traditional culture techniques (isolation, enumeration and characterisation), one could probably identify 10-20% of the rumen microbial population (Sylvester *et al.* 2004). But, with the advancement of omics research especially metagenomics, vast categories of bacteria are being identified and could be exploited for various commercial purposes. Since, the role of rumen microbes on *in situ* fibre utilization has been widely studied; current mini review discusses the possible uses of microbes from rumen origin.

Biological functions

Probiotic candidates: Metchnikoff (2010) observed that the Bulgarian peasants have good health and longevity due to the consumption of fermented milk which contained beneficial bacteria and termed them as probiotics. Over the years, there were numerous authors/researchers defined the probiotic organisms according to their observations. However, the modern definition for probiotics was proposed by the FAO and WHO in 2001 and in 2014, International Scientific Association for Probiotics and Prebiotics suggested grammatical correction to bring an end to the ambiguity by defining it as the live microorganisms, which if administered in adequate amounts, confer a health benefit to the host (Hill *et al.* 2014).

It is important to know the role of each microorganism present in the rumen. Studies have proven that probiotics, which are used as feed additives in farm animals showed enhanced production performance (growth), digestion of nutrients, reduction in incidence of diarrhoea and decreased serum lipid levels (Cavalheiro *et al.* 2015). In addition to above, consumption of probiotics in humans and farm animals, the immune system is also positively influenced. Some of the ruminal microbes have also been used as feed additives for animal production or development (Das and Wensheng 2012). Ruminal *Streptococci* can inhibit the pathogenic microbes by releasing a range of bacteriocins (Whitford *et al.* 2001). Lactic acid bacteria isolated from rumen includes *Lactobacillus ruminis* and *Streptococcus equinus*, which are regarded as true rumen inhabitants. In rumen transient bacteria, such as *Lactobacillus plantarum* and *Lactococcus lactis* that have been introduced with the feed and water also exhibited probiotic functions but their existence is short lived (Stewart 1992). Some of the studies on lactic acid utilizing bacteria such as *Megasphaera elsdenii* (Klieve *et al.* 2003), *Propionibacterium freudenreichii* (Raeth-Knight *et al.* 2007) used as direct fed microbials showed improvement in lactic acid metabolism in ruminants. Microbes isolated from rumen showed higher tolerance to bile salts than dairy product isolates. Table 1 shows some of the microbes isolated from rumen and used as probiotics.

Bioconversion of lignocellulosic waste: Lignocellulosic biomass of about 200 billion tons across the globe is one of the most abundant renewable energy resources (Nguyen *et al.* 2019). This biomass has the untapped potential to be the future biofuel but its structural complexity is the major constraint for its bioconversion to fuel or other valuable products (Bhujbal *et al.* 2022). Production biofuel from these substances requires either enzymatic or chemical processing. However, ruminant animals, like cattle, sheep, goat, could effectively utilise the lingo-cellulosic biomass due to the presence of microbes (bacteria and fungi). For better/efficient bioconversion, combination effect of microbial degradation of rumen origin followed by enzymatic action for biofuel production could be exploited.

Biofuel (bioethanol) are currently produced from the fermentation of process of food ingredients especially grains however, use of fibrous non-food cellulose biomass for biofuel production could serve the purpose of environmental protection as well as sparing of valuable food grains. Fujimoto *et al.* (2011) isolated *Bacillus licheniformis* from bovine rumen and observed they possess high cellulose degrading activity. These isolates are more heat-resistant than conventional soil isolated microbes

Table 1. Microbes isolated from rumen origin and being used as probiotics in food animals

Microorganism	Origin	Response	Reference
L. salivarius L100 *L. fermentum* L120	Cattle	Improve the silage quality of alfalfa	Guo *et al*. (2020)
Lactic acid bacteria	Cattle	Used as a starter in rice straw silage with damage level not reaching 50%.	Foeh *et al*. (2019)
Butyrivibrio fibrisolvens *Streptococcus spp.* *Clostridium aminophilum*	Cattle	Superior and have potential to be used as microbial feed additive in ruminants if fed in higher quantity	Das and Wensheng (2012)
Enterococcus hirae	*Bos primigenius* cattle	Exhibited antibacterial and anti-inflammatory activity	Arokiyaraj *et al*. (2014)
Shigella flexneri G3	Rumen liquid	Could be used for hydrogen production, waste treatment	Wang *et al*. (2011)
Streptococcus bovis	Rumen liquid	Reduces the coliform count in ruminants	Aphale *et al*. (2019)
Lactobacillus pentosus *Lactobacillus plantarum* *Lactobacillus paraplantarum* *Lactobacillus fermentum* *Pedicoccus pentosacum*	Goat	Exhibited antimicrobial property against food pathogens	Ladha and Jeevaratnam (2018)

(*Trichoderma, Aspergillus, Penicillium),* which are heat-sensitive and could be maintained under micro-aerophilic condition (Fujimoto *et al.* 2011). The biofuel production through high temperature process is more advantageous, as it reduces the cost associated with cooling systems, higher saccharification yield and lower cross contamination (Abdel-Banat *et al.* 2010, Phong *et al.* 2022).

Similar to cellulose, hemi-cellulose is vastly available among all the cereal crop by-products. These are chemically heterogeneous polymers of xylan consisting of β 1→4 linkages (Velazquez *et al.* 2004). These linkages can't be broken down by the mammalian enzyme system. Velazquez *et al.* (2004) isolated a new group of spore forming bacilli *Paenibacillus favisporus* spp., from the dung of cattle, which exhibits xylanolytic activity. This bacterium produces diverse extracellular polysaccharide hydrolyzing enzymes. Studies have underlined that the use of rumen liquor from health animals have higher *in vitro* fibre degradation than the individual organisms; which indicates the synergistic action between rumen bacteria and fungi (Yue *et al.* 2013, Giménez *et al.* 2017). Among the food animals, chickens are the poor utiliser (approximately 40%) of crude fibre in their diet (Ginindza *et al.* 2017). Use of these bacteria isolated from the rumen could be either used for production of non-starch polysaccharides enzymes (for food industry or commercial feed enzymes) or as a direct fed microbes through their diet could improve their fibre digestibility and also reduce the production costs.

Antimicrobial agent: Emergence of resistance microbes especially the methicillin-resistant-*Staphylococcus aureus* (MRSA) is global issue and needs urgent addressing. The microorganisms are known for production broad spectrum of antimicrobial metabolites (Clardy *et al.* 2006) and among them the genus *Bacillus* are predominant organisms, which encode 4-5% of genetic content for the production of antimicrobial substances (Stein 2005, Caulier *et al.* 2019). Recently, Baharudin *et al.* (2021) isolated *Bacillus velezensis* strains, which possess potent antimicrobial activity against MRSA from stingless bee. The cell free supernatant from the pure culture of this bacterium had MIC and MBC of 2^3 dilution factor (125 µl/mL) against MRSA and more heat and *p*H stable. Of late, gut microbiology laboratory has isolated, characterized through sequencing and standardized the growth conditions for this bacterium from cattle rumen liquor (Rajendran *et al.* 2022). The untapped microbes of the rumen microbial community could potentially serve the human society for the production potent antimicrobial substances.

Production of bioactive fatty acids: Milk and meat from ruminant animals contains a variety of bioactive fatty acids consisting of conjugated linoleic acid (CLA), short and medium chain fatty acids, branched chain fatty acids (BCFA) and odd-chain fatty acids (anteiso branched chain fatty acids) (Taormina *et al.* 2020). The CLA is known for its anti-obesity, anti-carcinogenesis, cell cycle and apoptosis, anti-atherosclerosis, anti-diabetes and immunomodulation effects in humans (Benjamin and Spener 2009). These cis-9 trans-11 rumenic acids are synthesized in the rumen during the process of partial biohydrogenation (Harfoot and Hazlewood 1988) and their concentration in the milk could be manipulated by the natural feed composition consumed by the dairy animals (Muller and Delahoy 2016). *In vitro* microbial CLA based function foods could be produced using *Propionibacterium, Lactobacillus* and *Bifidobacterium* isolated from rumen (Salsinha *et al.* 2018).

Branched chain fatty acids constituting about 2% of dairy fat, have been reported to act as bioactive molecules which are synthesized by rumen microbes (bacteria and protozoa) (Taormina *et al.* 2020). These are unique group of fatty acids namely anteiso-fatty acids (aFA), which are branched odd numbered fatty acids (Eibler *et al.* 2017). Biological functions of a FA are being studied apart from their role in obesity, anti-inflammatory, anti-carcinogenic activity and role in glucose homeostasis (Taormina *et al.* 2020). An *in vitro* study revealed that the concentration of these fatty acids are influenced by the supply of branched chain amino acids to *Bacillus* and the presence of protozoa helps in elongation of fatty acids chain length (15:0 and 17:0 *versus* 13:0) (Eibler *et al.* 2017). This BCFAs in particular a FA has been relatively untapped area and an in depth study could reveal more biological functions.

Electricity-microbial fuel cells: Energy generation from various natural processes is gaining momentum following the gradual depletion of fossil fuels. Globally, every nation is searching for alternate sources for energy generation through hydro, wind, chemical or even nuclear means. The concept of energy generation from cellulosic biomass is an interesting and sustainable one considering the abundant availability of these fibrous materials (Rismani-Yazdi *et al.* 2007). This process is being carried out using bioelectrochemical reactors, which mediate the direct conversion of stored chemical energy in organic matter (cellulosic biomass) to electrical energy by microbial means (Rabaey and Verstraete 2005). Rismani-Yazdi *et al.* (2007) demonstrated electricity generation from cellulose using rumen microorganisms as biocatalysts. They could generate around 55 mW/m^2 (1.5mA; 313mV) using cellulose as electron donor and rumen liquor from fistulated Holstein cow as catalyst. Authors further characterized the microbes responsible for this electricity generation and identified the microbes *Clostridium* spp., and *Comamonas* spp. were the most abundant.

Conclusion

Feeding of probiotics has gained global attention for its various biological functions. Over the years, use of probiotics in food animals especially in swine and poultry has also increased. However, use of probiotic microbes in ruminant animals is gaining importance especially in young ones (calves, kids and lambs) for improved performance and reduced incidence of diarrohea. Recently, studies are being carried out to develop probiotics of autochthonous origin for farm animals especially for ruminant animals. Ruminant animals are the best converter of poor quality fibrous roughages and there is huge potential in identifying organisms that possess good probiotics property and better fibre digestibility. The microbes have potential for health promoting action as well as industrial purposes such as biomass-electricity generation, value added-bioactive molecules production and environmental sustainability.

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