Chapter

Understanding the Mechanism of Action of Indigenous Target Probiotic Yeast: Linking the Manipulation of Gut Microbiota and Performance in Animals

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Abstract

The gut associated microbiota of animal plays crucial rule in the conversion to accessible nutrients for improve animal health and well-beings. Probiotic yeast (PY) is commonly use to manipulate the gut microbial balance by inhibits the disease-causing microbes and increase the number and function of desirable microbes. PY produce many fermentation metabolites, intercellular effectors, minerals and enzymes that make it an idea nutritive feed supplement for ruminants. The mode of action of the PY is depends on the animal biological inheritance, breed, managemental condition and microbial feeding type. Therefore, PY must formulate using same ecological origin, alone with desirable target; as it would be more compatible with gut ecoysytem and would yield maximum outputs as compare to non-target or foreign probiotic (FP). Therefore, for development of the Indigenous Target Probiotic (ITP), the isolation source must be same ecological region with desirable target like improve animal health and productivity. In the situation of the increase food storage around the world, ITP may provide a useful feed supplements to improve the food production in cost effective manner as compare to FP. Probiotic effectiveness is considered to be population/breed/target specific due to difference in the feed intake, change gut microflora, different food habits and different host-microbial interactions. In this chapter, we will highlight the preparation of the ITP yeast and its mode of action on animal gut microbiota.

Keywords: indigenous target probiotic (ITP), *Saccharomyces cerevisiae*, mode of action, gastrointestinal tract, fiber digestion

1. Introduction

Probiotic are the live microbial feed supplements which provide the beneficial impact on the host by producing the useful metabolites [1]. Many probiotics have been available in the market for improving animal and human health in safe and healthy way. The commercially available probiotic product contains mostly lactic acid bacteria (*Lactobacillus plantrum, L.casei etc.*) and yeast (*Saccharomyces cerevisiae*) strains [2]. The beneficial impact of present probiotics is often limited and do not provide equal affects to each host. The positive impact of the probiotic product is based on the site of action, its dose, the stability/viability of the microbial strain; host genome and its environmental condition and health [3]. Mode of action of the microbial strains is one of the majors determines of the probiotic yeast usefulness. Latest molecular methods must be used for identification of the unique microbial strains for development of target-based probiotic yeast. During the last decades, probiotic yeast (*Saccharomyces cerevisiae)* has been extensively used as ruminant health promoter [4]. The beneficial outcomes from probiotic product mostly depends on the host and microbial interaction, therefore, pre-plan steps must follow for isolation of the best performance (target) microbial strains for development of the unique/true animal probiotic yeast [5]. Ruminants have a unique microbial flora which is responsible for breakdown of the fibrous and non-fibrous feed particles. The number and function of the gut microbes is highly affected by biochemical and microbial properties of the rumen [6]. The gastrointestinal tract microbial flora has a crucial role on upgrade nutrient utilization and feed digestion leads to the improve animal production and health status. Animal eat different types of feed (high energy & low energy), that determine the number and function of the microbes in the gastrointestinal tract. The gut microbiota is highly changeable due to the addition of useful microbial feed supplements in safe and healthy way as compare to any antibiotic [7]. Animal blood profile also plays an important role in the animal health and its production performance. PY brings changes in the concentration of rumen volatile fatty acid (VFAs) propionate, butyrate and valerate leads to the reduced synthesis of triglyceride and cholesterol in the liver cells and might be change the lipid profile in blood. These polysaccharides reduce the total cholesterol of serum in ruminants. Therefore, the blood chemistry and the fecal microbiota must be manipulated for better animal health and performance. Literature showed that the

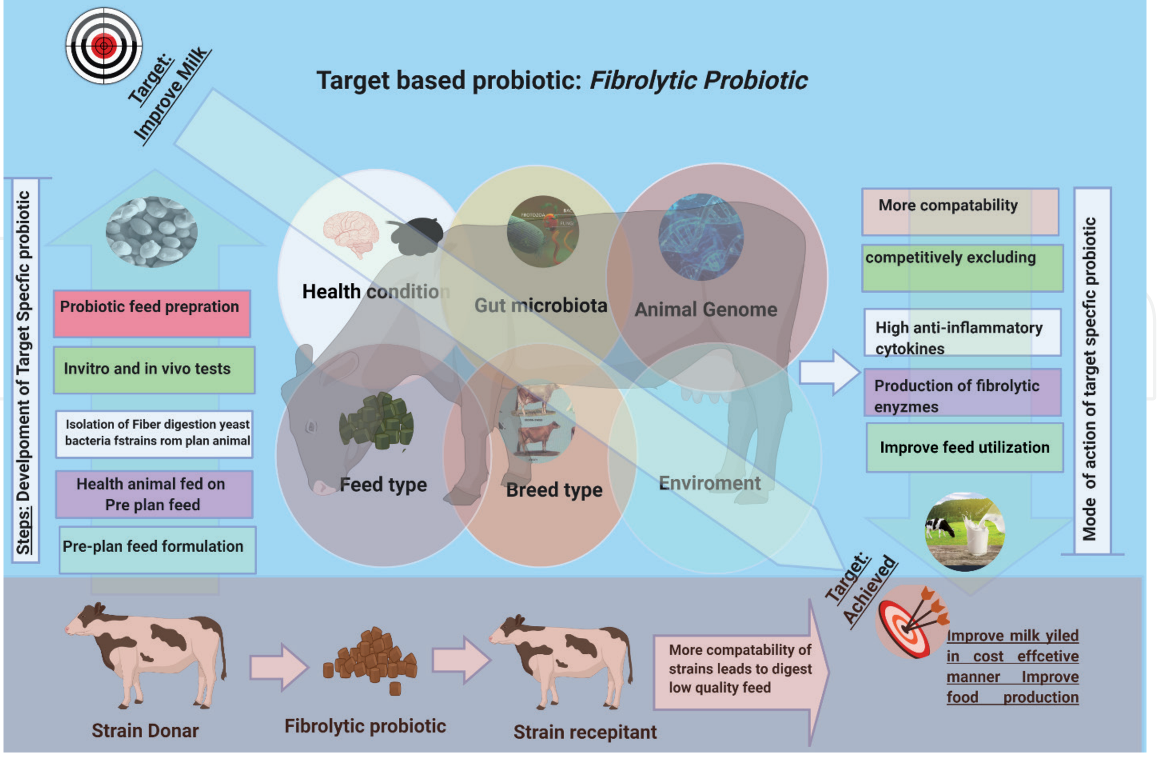

Figure 1.

Representative scheme of development of target-based probiotic (TBP): The right side covers the main steps involving in the preparation of the TBP, the internal part covers the legalistic evidence of the interrelationship between, host and microbes. The left side covers the mechanistic activity of the TBP; including the improve gut microbial balance which leads to the improve feed digestion resultantly improve host health and production in cost effective manner.

microbial diversity of the animal GIT is very important in feed digestion processes. Ruminants has a big anaerobic chamber/vat called rumen. Inside rumen, three main microbial species, i.e. bacteria, fungi and protozoa are present for feed digestion. Rumen microbial flora digests the lignocellulosic biomass and release the energy (VFAs) for animal use. Rumen microbial flora are animal best friends. If required specialized gut microbial flora are not present, the food digestion process can be shut down and death of the animal can occur. For colonization of the best microbial flora inside the rumen, we must formulate animal feed after clear understanding of the rumen ecosystem, and host genetic (**Figure 1**) [8, 9].

In the situation of high animal feed cost, we must identify the cost-effective probiotic by using the concept of ITP to improve poor quality feed into high quality milk and meat. We had already given the concept of indigenous probiotic yeast our previous book chapter [31]. A clear understanding regarding the proposes guidelines to develop the ITP to improve gut microbiota resultantly improve milk and meat production. This book chapter will discuss the identification of the microbial strain from local ecological breed and its mode of action for preparation of target based probiotic products. We will also support our concept of ITP with our lab conducted experiments.

2. Yeast: promising microbe for development of target probiotic for animal use

Yeast is a very useful microorganism with broad range of industrial application, because of their unique genetics and physiology. Yeast cells have many useful metabolites (protein, carbohydrate, vitamins; vitamin B6, thiamin, biotin, riboflavin, nicotinic acid and pantothenic acid and minerals; zinc and magnesium) [10]. The utilization of the naturally prepared yeast would be accelerated in coming years due to the nature-oriented mind set of the consumers. Therefore, research on the isolation of the nutritious rich yeast strains for preparation of probiotic product has rapidly increased [11, 12]. Yeast is an important single cell microorganism, belongs to fungus family and it multiplies by cell division. The genetics and physiology of the yeast are very unique, and, therefore, a broad range of research work in biological sciences is being carried out on this microbe. The yeast cell size is composed of 5×10 μm and the size of the baker's yeast genome is 12.1 Mb containing 16 chromosomes and 5400 coding genes approximately [13]. Members of the order Saccharomycetales are mainly used for the animal probiotics when serves as reliable and economical source of essential amino acids, vitamins, carbohydrates, and minerals from yeast cell. Thiamin, Riboflavin, Niacin and Biotin are present in yeast [14]. The antagonistic ability of the yeast to block bacterial pathogenicity is also makes its very useful [15]. Yeast cell has competition for nutrients, pH changes in the medium, high concentrations of ethanol production, secretion of antibacterial compounds and release of antimicrobial compounds are major antagonistic steps. Yeast cell has many useful fermentation metabolites (protein, vitamins, carbohydrates) which makes it important microbial feed supplement. Yeasts are naturally present ($1.3 \text{ X } 10^5$ yeasts ml-1) inside the rumen fluid [16]. Literature showed that, yeasts (*Sac. Cerevisiae*) are not significant members of the rumen microbial flora, but mostly, entering inside the rumen with fibrous feed [17]. Therefore, we claim that the viable yeast rich diet can improve the its numbers and function inside the rumen. Now a days, *Saccharomyces cerevisiae* (live yeast) has been extensively used as animal probiotic to improve milk production and its composition. Many researchers have given different types of conclusion related to the mode of action of yeast and its impact on host animal. Mostly all researchers agreed that the improved live bacterial count inside rumen is the most reproducible impact of PY [18–24]. Based upon a research, it is being hypothesized that probiotic effectiveness is considered to be population-specific due to differences in the feed, gut microflora composition, food habits and host-microbial interactions. We can isolate and identify the target yeast strain from animal gut and can used that strain for preparation of the animal probiotic yeast.

3. Probiotic yeast for neonatal and growing ruminant diet

The role of the probiotic yeast in dairy animal is well studied [25]. They have been extensively used for improve milk yeast and its composition in cost effective manners. The benefits to cost ratio of probiotic yeast is 4:1 in dairy animals. They have also used as preventer against digestive problems, and rumen acidosis.

The main target of the PY used in new born ruminate diet are; (a) improvement in the rumen maturation; (b) stop the pathogenic bacterial growth; (c) establishment of the normal growing animals like microbial flora [26–28]. Microbial based feed can improve the rumen development during the growing phase of the dairy animals. The new born gut is sterile and have no germ [29]. After 6 months of age the rumen is colonized with diverse microbial flora. PY provides beneficial metabolites and enzymes like thiamine for fast growth of the fungi. The poor fungal growth of the animal fed on PY might be due to the low production of thiamine [30]. At the same time, the animal plays an important role in the maximum colonization of the beneficial microbial population [31]. If there is any imbalance bacterial species, it would result in digestive problems and leads to the economic loss. The establishment of the useful bacterial strains results in the development of strong and balanced rumen which resultantly strong immunity and health condition [32, 33]. PY provide the improve the rumen maturation and its microbial flora is also in strong balance. PY provide the useful bacterial species for feed digestion, like cellulolytic bacterial species and ciliate protozoa [34]. The balance in rumen microbial flora plays a crucial role in feed utilization and could result in better animal productivity [35]. PY remove oxygen from rumen and provides a more anaerobic environment for its growth of key beneficial microbial groups [36]. The newborn gut can easily be modulating by PY. The new born key beneficial microbial *Bacteroides-Prevotella* and the *C. coccoides -E. rectale* group easily be grown with PY presence by removing the oxygen inside rumen [17]. Under filed conditions, crossbred animal are usually underfed, which results in deficiencies of certain nutrients and ultimately reflected in the levels of certain biochemical constituents Literature showed that the use of PY may enhance the blood and fecal biomarkers leading to to improved health status in dairy animals [37–40].

4. Manipulation of ruminal gut microbiota by target probiotic (*Fibrolytic probiotic*)

For clear understanding of the ruminal gut microbiota using latest genomic methods to get useful information for preparation of specific probiotics. The ruminants feed consists of concentrate, silage, seasonal fodders etc. There diet mostly contains cellulose, hemicellulose starch and water-soluble carbohydrate. The rumen microbes play an important role in feed digestion. The animal feed is digested inside rumen and then energy is released for animal use. Cow and its microbes are mutually benefiting each other (**Figure 2**). Rumen is the first and the largest anaerobic chamber of the cow GIT. The temperature inside the rumen chamber is between

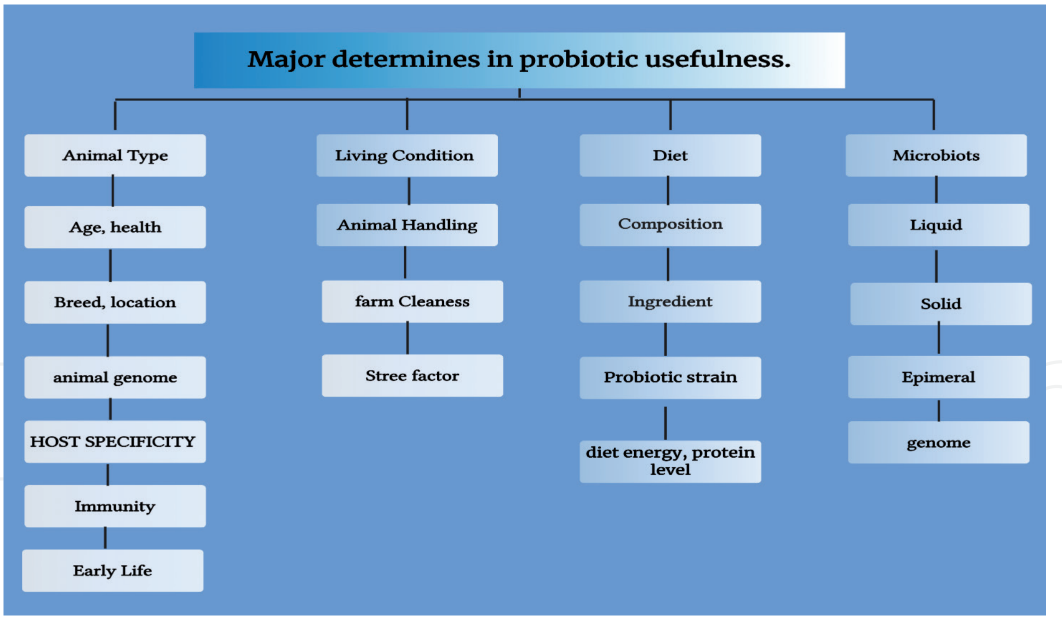

Figure 2.
Major factors effects on the mode of action of probiotic.

38 to 41 °C, with 6-7 pH (depends on feed type). There are three different types of microbes present inside the rumen including, bacteria, fungi and ciliated protozoa [41–44] . The location and size of the rumen microbes depends on the feed formulation and host genetic. Mostly, bacteria are associated with fibrous feed particles; fungi, protozoa [45, 46]. Some are freely living and some are bound with rumen mucous membrane. 1 ml of the rumen is composed of 109 to 1010 per ml bacteria with 200 different species, 10^4 to 10^6 per ml protozoa with 20 different species, and 10^3 per ml fungi with 20 different species [47]. The rumen bacteria are gram negative 1-2 micrometer in size and cocci, and rod shaped mostly. Rumen bacterial are mostly non-spore producing, facultative anaerobes. 1- 5 % of the bacterial cells in rumen are cellulose digesters [48]. The rumen fungi (gut fungi) also play an important role in fiber digestion by stimulating growth of fibrolytic bacteria [49]. The rumen microbial features are heritable; moreover, animals age, feed and genome plays an important role in the microbial colonization. The composition of the diet describes the type of gut microbial species [50]. Therefore, the rumen microbiota can be manipulated by using the yeast-based probiotic to obtained the useful products. The feed must be targeted for modulating the rumen microbiota (**Figure 3**).

The modulation of the rumen microbiota is mostly for the enhanced colonization of the fiber digesting microbiota [35, 36]. Literature showed that, animal diet has an important role in the manipulation of the rumen microbiota. Low amount of fibrous feed builds up fast working microbes (fibre-degrading

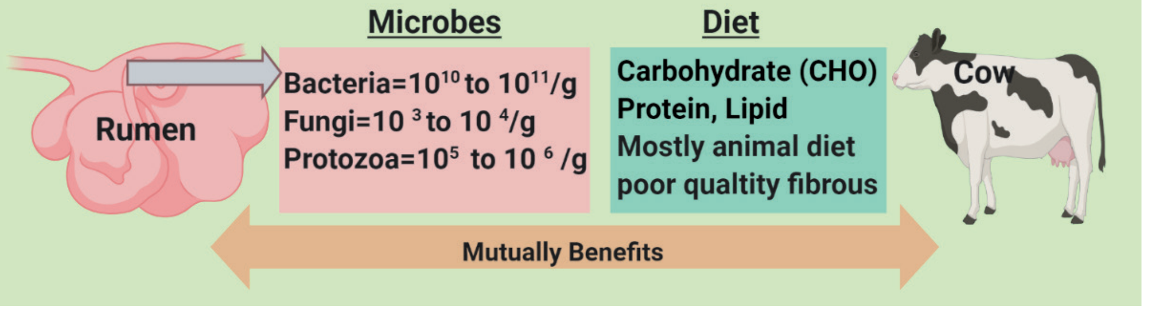

Figure 3.
A scheme describing the mutually benefits between host microbes.

Butyrivibrio fibrisolvens and F. succinogenes) and high amount of fibrous build up slow working fiber degrading microbes *(M. elsdenii, S. bovis, S. ruminantium, and P. bryantii)*. On the fibre mat of the rumen, the slow working fibre digestion microbes reside. The fast working microbes are present on the rumen fluid, for sugar and starch digestion. Microbes digest feed into end product so, the balance in the rumen microbiota must be improved. The animal diet containing the rapidly degradable starch facilitates the removal of ciliated protozoa populations (Entodinium) from the rumen fluid. On the other hand, high concentrate diets lead to the low ruminal pH which more detrimental to growth and survival of the fiber degrading bacterial species. The low pH can have negative impact of the growth of ruminal fungi [36–47]. Similarly, zoospores by Caecomyces decreases by addition of the more soluble sugar [35]. At the same time, the best growth of the fungal spores occurs between 39-40 °C. High-fibre diet might facilitate the growth of diverse fungal species in rumen. Therefore, the host animal is highly affected by the diet formulation its nutrient composition. Rumen fungi growth is alo affected by animal breed, its age and breed type. Gut fungi are the only fungi for which no oxygen is required for completion of their life cycle and the presence

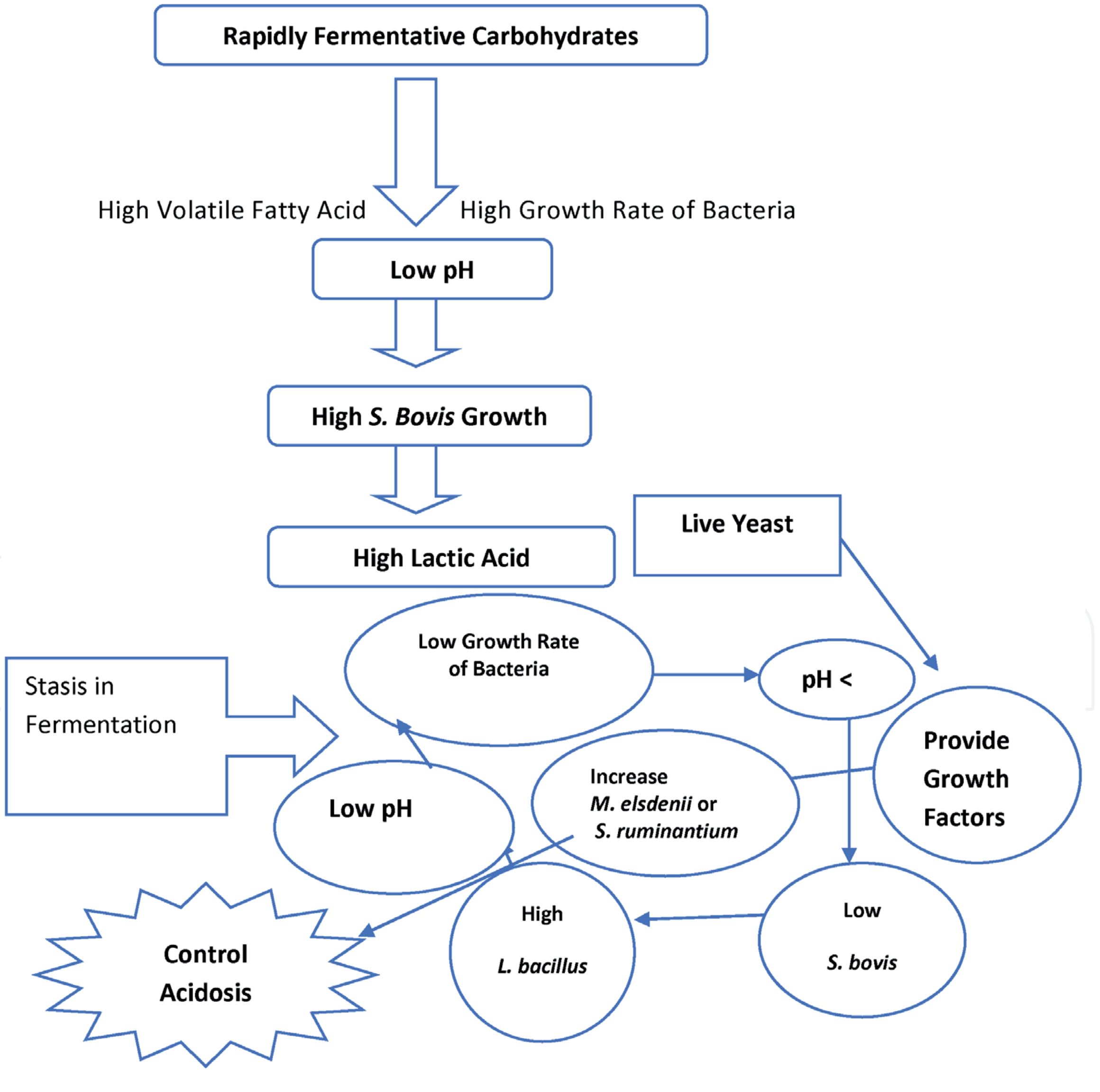

Figure 4.
Potential mechanisms of microbial ruminal acidosis: This figure suggested that, the live yeast supply different growth factors (amino acid, peptides, vitamins and organic acids). These growth factors have the knock-on impact of increases the stimulation and metabolism of lactic acid utilizing anaerobic bacteria, such as M. Elsdenii or S. ruminantium (that control the acidosis). Yeast cells has a affinity for sugar which outcompete S.bovis for the utilization of sugar.

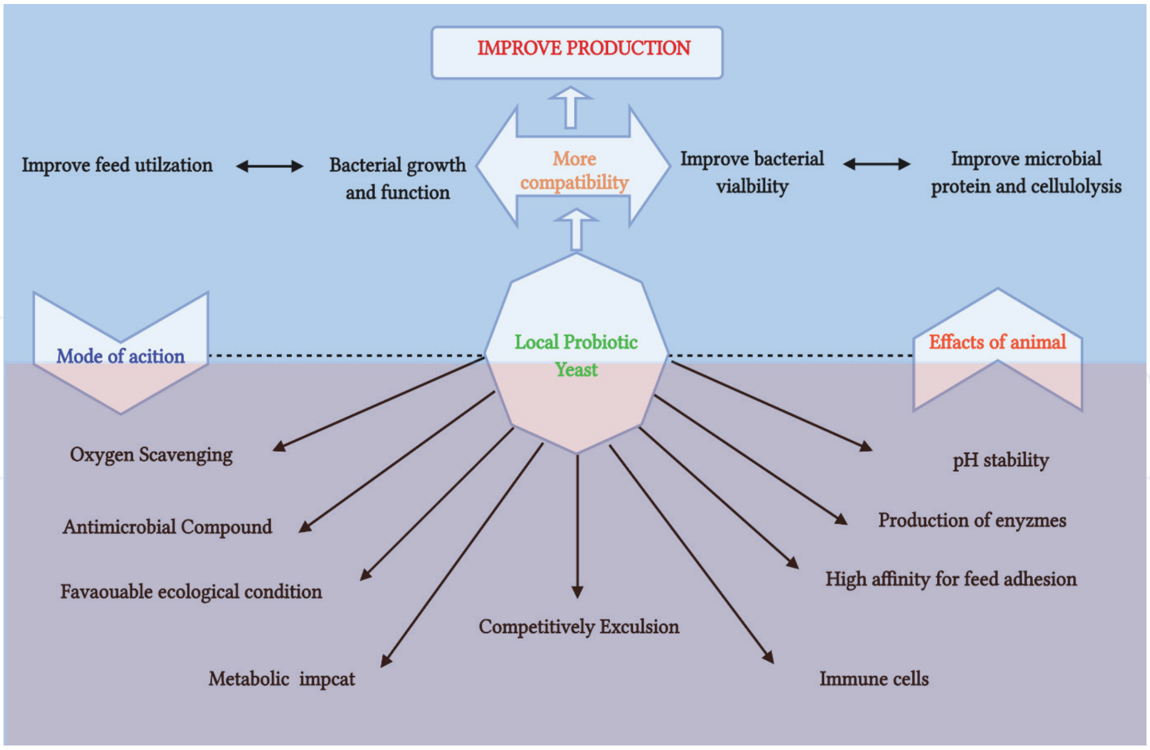

Figure 5.
A proposed flowsheet to explain mechanistic pathway of IPY: Steps involved in the mode of action of PY and its impact on animal.

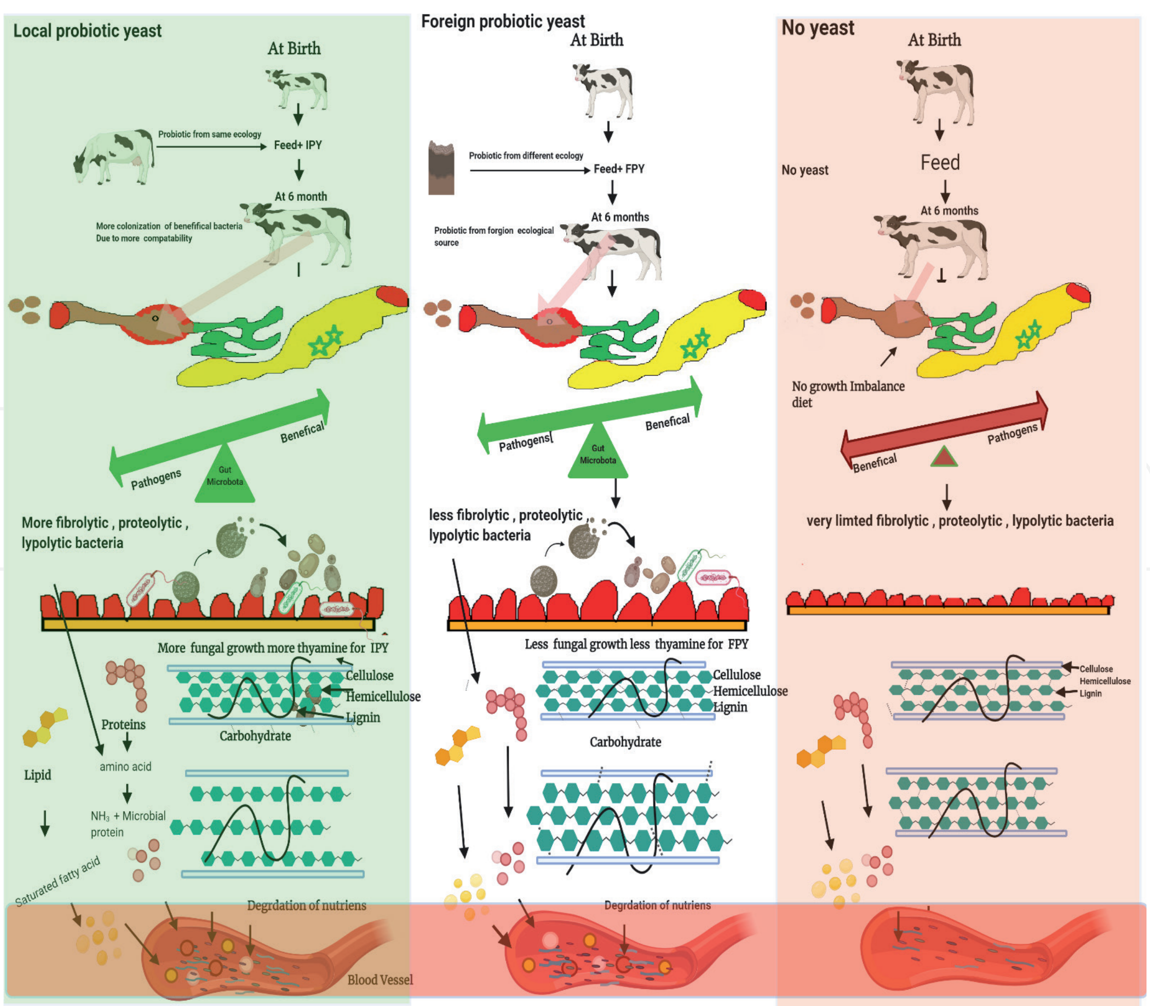

Figure 6.
A simple scheme proposed to explain mode of action of probiotic yeast in gut: IPY improve carbohydrates, protein and lipid digestion rate by improving the production of cellulolytic, hemi- cellulolytic and proteolytic and lipolytic bacteria and fungi as compare to FPY and no yeast animal.

of oxygen is toxic [35–56]. These rumen microorganisms can degrade complex plant fibers and polysaccharides and produce volatile fatty acids (VFAs), microbial proteins, and vitamins, which provide nutrients to meet the host's requirement for maintenance and growth [35, 36] Manipulation of the rumen gut microbiota could be done for obtaining the required fermentation product and improve animal production [57, 58] Rumen manipulation could be made by change/manipulated the feed intake, and some microbial supplement/probiotic [35, 36] As far as lipid part is noted that lipid are organic compounds that are insoluble in water but soluble in organic solvents. Fat and oil are nutrition important lipids [57–59]. The high forages diet leads to high rumen pH which in turn results in high amount of the cellulolytic and hemicellulolytic bacteria and protozoa, On the other hand high concentrate diet leads to lower rumen pH which results in lower number of cellulolytic and hemicellulolytic and amylolytic bacteria and lower number the rumen protozoa (**Figure 4**) [59, 60]. Probiotic change rumen environmental condition through manipulation of rumen microbiota for our required fermentation end product. The animal feed must be kept constant to build up the required rumen microbiota [61] Cow microbiota established after some weeks of birth, and the microbial diversity increases day by day [62]. The animal feed, the managemental condition, genetics plays important role in the establishment of the animal gut microbiota [63, 64] Once established, if the feed and the life style same, the number and function of the rumen microbiota mainly same throughout life. But we can manipulate the GIT microbiota for our own purpose. If we isolate the fiberlytic yeast strains from the rumen, we can prepare the best and unique probiotic yeast for improve animal feed digestion (**Figures 5** and **6**).

5. Prepration of indigenous probiotic yeast: right choice for maximum outcomes

The gut microbiota can digest the animal feed and produce nutrients for improve host health and well beings. Animal feed and host genetics play important role in shaping and composition of gut microbiota [18]. Same is the case of the rumen microbiota, which is highly variable and is depended on various factors like animal breed, physiology, feed type and geographical location. It has been commonly accepted that commercially available probiotic yeast may not showed equal impact to all animal breeds [65, 66] The compatibility of PY could be variable among animals. The local prepared yeast probiotic isolated from same ecological niche may have more beneficial impact than any exotic probiotic yeast [3]. The local isolated probiotic yeast may have fast adaptability and colonization in the local rumen ecosystem [24]. The origin of the probiotic strain determines the best prepared probiotic product. The strain selection is the most important step for the development of right probiotic for animal. Being precise during the strain's selection could yield positive outcomes from the probiotic. The probiotic yeast may use for the rumen microbial manipulation [67] Different types of PY have been used for improve animal health and production [7–68]. Some PY strains produced beneficial results in animals while others did not. The difference of that variable results of PY may be explained by different host and PY associated factors [69–71]. These factors are; animal age, breed, sex, feeding dose, PY strains isolation source and some unknown factors [3]. The major factors might be the low compatibility of the exotic probiotic yeast strain with animal having diverse biological inheritance and gut microbial composition. The right probiotic strain should be novel, so we must use latest molecular methods to isolate the target specific/local isolated microbial strains. The local isolated and molecular identified probiotic strains may have more impact on local animals in cost effective manners. The probiotic are species specific by targeting the indigenous strains and local dairy farms can get the cost-effective probiotic product for improve milk production and composition.

The main steps involved in the preparation of the breed specific probiotic yeast are as following [3].

- Pre-plan ruminate diet for isolation of probiotic yeast
- Identification of yeast strain based on the molecular techniques
- Probiotic potential of selected yeast strains
- In vitro probiotic potential
- Safety assessment/In-vivo animal model

6. Mode of action of the IPY Vs FPY inside the rumen and post-ruminal GIT

The first mode of action of the probiotic yeast is competitive exclusion (CE) [27]. The CE is a probiotic mode of action that involves the colonization of the beneficial microbial strains to GIT tract to reduce the addition of disease-causing microbial flora [18–74]. The ability of probiotic yeast cell to fight with other useless microbial flora can improve growth and function of beneficial microbial flora. The IPY has the indigenous strain, which has the advantage that it drives from animal of interest (Cow). IPY has an environmental modification capability. The concept of co-evolution of host microbial has been seen in case of IPY mode of action. The local strain gains an advantage because of its ability to adjust/modify itself in new environment by producing the antimicrobials e.g (lactic acid) to make its less suitable for its competitors. The FPY has the foreign origin strain, which has the less environmental modification capability less, competition for available nutrients, and mucosal adhesion sites. Second mode of action of the PY is reported as a good pH stabilization. Rumen microbial flora can work under stable pH [75]. Rumen pH is highly affected by animal feed intake and its composition. Ruminants eat different types of feed, like high energy concentrate diet, fodder, and silage. These types of feed have a quick impact on rumen pH. If rumen pH is not stable, the animals may have different types of metabolic diseases [76] Literature showed that PY has a stabilizing effect on the rumen pH. [77, 78] Some studies reported a rise rumen pH when animal was fed on diet with high energy supplemented with PY. Sometimes, the increased pH might be due to the decreased VFAs inside the rumen [3–79]. The lower pH leads to the rumen acidosis, PY can prevent the acidosis condition of the dairy animals [7]. The third proposed mechanism is that yeast cell provides the anaerobic condition inside rumen by removing the oxygen thus facilitated the useful feed digestion microbes [35, 36]. The main microbial flora are bacteria fungi and protozoa. These microbial species have a fiber digestion role by secreting the cellulase and hemicellulase enzymes. Fiber is the main part of the ruminant diet. Therefore, fiber digestion, nature blessed them with unique fibrolytic digestion bacteria (*Fibrobacter succinogenes, Ruminococcus flavefaciens, Ruminococcus albus),* fungi *(Necallimastix*) and protozoa. That complex fibrolytic microbes catalyze the rumen fiber digestion and improve feed intake. The yeast supplementation provides the useful metabolites which stimulate the growth of fiber degrading bacteria [18–47].

7. Experimental proofs: who is better; indigenous or foreign microbe as animal probiotic?

7.1 Experiment: impact of probiotic yeast on blood fecal biomarkers in dairy heifers and growing animals

Based upon the above discussion, we have conducted two research experiments on dairy animals by using the IPY concept to improve the gut health. In experiment 1, eight dairy heifers (87 ± 5 kg and 6–7 months) were divided into two equal groups (control n = 4 and probiotic n = 4) [80]. Control group animals fed on NRC recommended diet and probiotic group animals fed control diet FPY (Yea-Sac[1026]; 5 g/animal). After 120 days results showed that the FPY significantly affected the serum glucose, and urea levels in dairy heifers [24].

Items	Feeding regime		p-Value
	Control[2]	FPY[3]	
Urea (mg/100 ml)[1]			
Before treatment[4]	30.10 ± *0.711	31.14 ± 0.974	0.012
After treatment[5]	33.34 ± 0.432	29.23 ± 0.494	0.01
Glucose (mg/100 ml)			
Before treatment	62.67 ± 4.04	60.86 ± 2.80	0.605
After treatment	63.31 ± 2.60	65.47 ± 2.84	0.600

[1]n = 4 per treatment.
[2]Control feed without yeast.
*[3]Probiotic feed compose of control feed supplemented with 2.5×10 [07] cfu/g commercially available probiotic yeast (Yac-Sac[1026]) at the rate of 5 g per animal/day * ± Standard error of the mean.*
[4]Before treatment (day 0).
[5]After treatment (day 120).

Table 1.
Blood serum metabolites (Means ± SEM) in dairy heifers fed on control and foreign probiotic yeast.

Parameters	Feeding regime		
	Control[2]	IPY[3]	FPY[4]
Urea (mg/100 ml)1			
Before treatment[5]	14.55 ± *0.57	14.18 ± 0.21	15.54 ± 0.32
After treatment[6]	14.18[a] ± 0.58	12.31[b] ± 0.22	13.68[ab] ± 0.90
Glucose (mg/100 ml)			
Before treatment	75.70 ± 1.24	73.99 ± 2.51	75.08 ± 2.30
After treatment	73.84[b] ± 0.71	77.42[a] ± 1.28	78.97[a] ± 0.54

[a],[b] Values on the same row with different superscripts differ significantly ($P < 0.05$).[1]n = 3 per treatment.
[2]Control feed without yeast.
[3]LAB-Probiotic feed compose of control feed supplemented with 3.13 × 10[07] cfu/g laboratory produces probiotic yeast (QAUSC03) at the rate of 8 g/day/animal.
[4]COM-Probiotic feed compose of control feed supplemented with 2.5×10 [07] cfu/g commercially probiotic yeast (Yac-Sac[1026]) at the rate of 10g/day/animal.
[5]Before treatment (day 0).
*[6]After treatment (day 120) * ± SEM = standard error of the mean.*

Table 2.
Effect of indigenous Vs foreign probiotic yeast on blood parameters (Means ± SEM) in lactating dairy cattle.

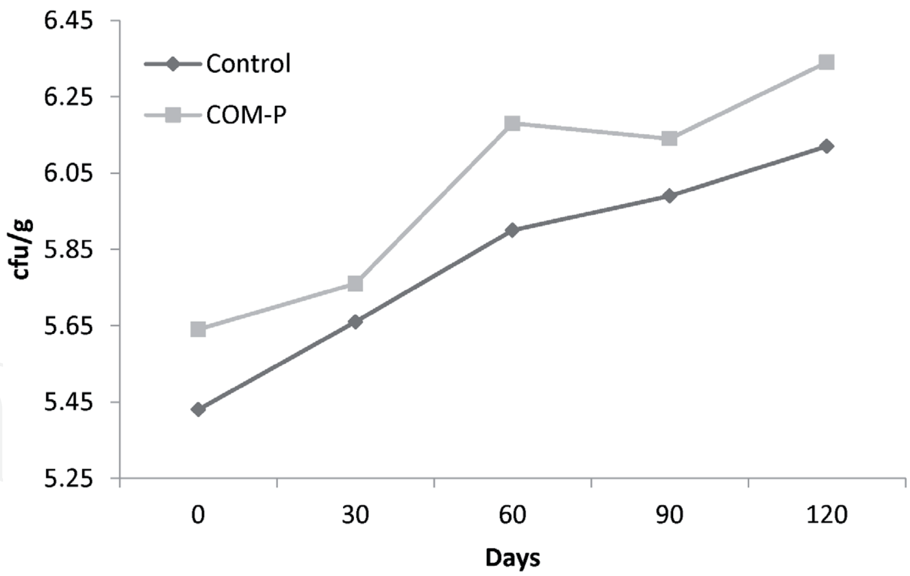

Figure 7.
Total Lactococcus count (CFU/g) in the ruminal gut of dairy heifers fed on control feed (control, ♦; no yeast) or commercial probiotic feed (COM-P, ■; control feed plus commercial yeast) (n = 4).

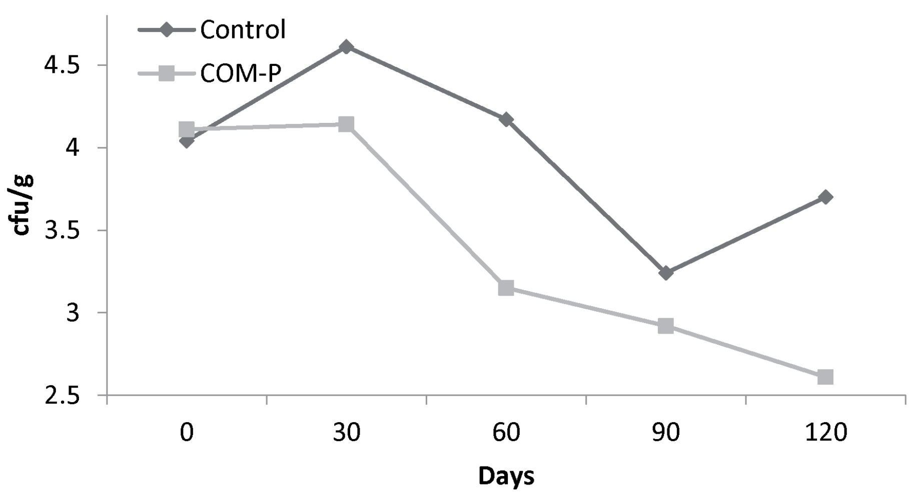

Figure 8.
Total Enterococcus count (CFU/g) in the ruminal gut of dairy heifers fed on control feed (control, ♦; no yeast) or commercial probiotic feed (COM-P, ■; control feed plus commercial yeast) (n = 4).

That means, we had a proof of positive impact of PFY on animal health. We had isolated the yeast from dairy animals fed on yeast. After careful assessment of the probiotic potential, we conducted another experiment to determine the impact of FPY Vs IPY on the health of lactating dairy cattle. Mix breed (*Sahiwal* and *Sahiwal×Jersey*, n = 9, with 4-5-liter milk per day) animal were selected for blood and fecal flora study. Animals were divided into three groups. Group 1 fed on 8 g IPY with 3.13×10^{07} CFU/g; group 2 fed on 10 g FPY with $2.5 \times 1\,0^{07}$ CFU/g FPY, group 3 fed only control diet with no probiotic (Figure). After 90 days, results showed that the gut associated microbial flora and blood biochemical parameters were improved in the presence IPY as compare to the FPY (**Tables 1** and **2**).

We highlighted that improved animal health condition might be due to improved digestive enzymes produced from well propagated IPY. The VFAs have a capability to reduce the triglycerol and cholesterol in liver cells and might be change the animal lipid profile. Results of the ruminal gut microflora showed that the average, beneficial *Pediococcus* and *Weisella* species (CFU/g) counts increased while pathogenic E.coli species (CFU/g) counts decreased in IPY fed lactating cows than other groups which leads to improve GIT microbial balance (**Figures** 7 and **8**).

It can be concluded IPY improves the, gut health, and wellbeing of lactating dairy cattle in cost effective manner. IPY strain may adopt well in the cattle gut than FPY [80].

8. Conclusion

Ruminants of developing and developed countries have different types of gut microbiota due to their living standard, feeding type, their managemental style. Although from above discussion we have a clear understanding that the interlink between gut microbiota and fiber digestion plays a key role for obtaining maximum profit from dairy animals. Therefore, the PY must be target specific which give maximum outcomes in cost effective manners. For animals of specific geographical region, a unique and precise YP must be designed by isolating the local yeast strains from that population, only then maximum beneficial outputs can be obtained. The reason beings, compactivity of the local strains with normal microbiota of the rumen ecosystem (**Figure 9**).

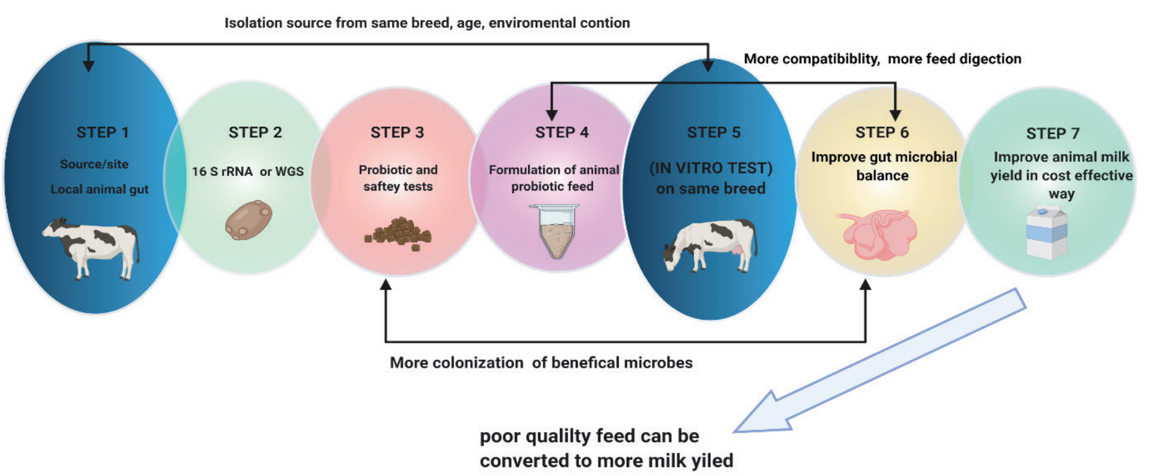

Figure 9.
Target based Probiotic Preparation strategy: This figure showed probiotic preparation of by using the local animal GIT tract as preparation of local yeast probiotic. Interlinked factors involved in the application of probiotics in the ruminant's nutrition.

9. Recommendations

The recommendations are outlined as follows;

- Pre-plane feed formulation for the manipulation of the rumen microbiota to digest the fibrous feed
- Identification of breed specific probiotic strains with same target.
- Whole genome sequencing of the probiotic strains as well as animal for maximum outputs
- Mode of action of the probiotic should studied well for understanding of the useful and useless probiotic.

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