

# Designer Probiotics: The Next-Gen High Efficiency Biotherapeutics

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## Abstract

The probiotic engineering is a cutting edge technology for improving disease diagnosis, treating gastrointestinal disorders and infectious diseases, and improving nutrition and ecological health. Use of bioengineered microorganisms in animals has different targets and prospects owing to differences in their anatomy, physiology, and feeding habits. In ruminants, the bioengineered microorganism is primarily aimed to enhance nutrient utilization, detoxify toxic plant metabolites, and lessen the enteric methanogenesis, while in non-ruminants, the bioengineered microorganisms are aimed to enhance nutrient utilizations, confer protection against pathogens, and inhibit infectious agents.

## Highlights

- The microorganisms can be engineered to enhance their metabolic efficiency
- The bioengineered microorganisms could solve the burgeoning problem of drug-resistant pathogens
- The recombinant probiotics are promising therapeutic agents against infectious diseases.

## Keywords

Designer probiotics · Live therapeutics · Recombinant AMPs · Oral vaccines · Livestock applications

## 7.1 Introduction

Availability of quality forage is the major problem impeding livestock production in most developing countries. As humans and animals have requirement for grains and protein-rich bioresources, the demand has increased for production of pulse crops and animals used for meat.

The long-range goal should be to develop genetically engineered microbes which are able to colonize GI tract of animals and enhance the supply of nutrients to animals. It is necessary so that nutrient requirements of high-yielding animals can be met from low-quality forages or silages, and dependence on grains or protein concentrates is minimal. In addition, genetic engineering has important applications such as detoxification of anti-nutritional phytometabolites present in native forages, crop residues, and agro-industrial byproducts used as animal feed, and minimizing methane emissions. Despite huge task to be solved, the success achieved is limited, and much remains to be done in future.

The quantity of nutrients required in high-yielding cattle or buffaloes is more than the nutrients available in conventional forages and metabolic capacity of rumen microorganisms. This is because high-yielding animals require higher amounts of readily fermentable carbohydrate and protein-rich diets. The recombinant microbes used as feed additives can fill this gap.

As the development of therapeutics to prevent bacteria, fungi, and viruses infectious diseases is

a routine activity of researchers, academicians, and veterinarians associated with livestock industry, this article is confined to developments in microbial therapies against infectious agents of GI, genitourinary and respiratory tract of livestock species. Importance of important body microbial niches with reference to their relevance to animal performance is highlighted.

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## 7.2 The Era of Bioengineered Microorganisms

The bioengineering of microorganisms intended for use as feed supplements or probiotics is known to commence with the development of probiotics targeting enteric pathogens (DeGrandis et al. 1989). Applications of computational engineering, recombinant DNA technology, and synthetic biology have offered enormous possibilities to utilize microorganisms and eukaryotes for producing a variety of therapeutics and nutraceuticals targeting a range of humans and veterinary applications (Challinor and Bode 2015; Ozdemir et al. 2018; Pedrolli et al. 2018).

Studies have reported recombinant probiotics to improve nutrition (Amiri-Jami et al. 2015), and human and veterinary health (Trombert 2015; Yang et al. 2017).

*Butyrivibrio fibrisolvens* OB156 originating from rumen was transformed by electroporation with fluoroacetate dehalogenase gene (*HI*), from *Moraxella* sp. The genetically modified *B. fibrisolvens* OB156 could detoxify fluoroacetate, a naturally occurring plant toxin. The recombinant *B. fibrisolvens* was found to establish in the rumen of experimental sheep for longer period and protect them against fluoroacetate poisoning (Gregg et al. 1994, 1998).

With the development of a *Lactococcus lactis* for delivery of recombinant anti-inflammatory cytokine interleukin-10 (IL-10), intended to treat inflammatory bowel disease (IBD) (Steidler et al. 2000), the field bioengineering of probiotics has advanced swiftly as a premier area of research in nutrition and antibacterial and anti-viral therapies.

## 7.3 Recombinant Bacteria for Nutrient Utilization

Interspecies transinoculation of rumen bacteria is a well-known phenomenon to alleviate forage toxicity in animals. The recombinant probiotics are tailored to produce proteins to induce cytotoxicity or tumoricidal activity in internal tumors, or for delivering therapeutic proteins to tumors that are inaccessible for delivering therapeutics (Singh et al. 2017; Ma et al. 2018). In animals, the emphasis is on developing genetically modified probiotics to enhance nutrient utilization and confer protection against infectious agents.

Pig, poultry, and fish are the non-ruminants. They are least efficient to utilize certain feed components such as cellulose, phytic acid, saponins oxalates, etc. The unutilized components released in feces into environment act as pollutants. Therefore, efforts are made to develop recombinant microorganisms that can be used as microbial feed supplements in pig, poultry, or fish.

The plasmid carrying *Bacillus subtilis* phytase genes, i.e., (*phyA*) codon was introduced into *Lactobacillus acidophilus*, *L. gasseri*, and *L. gallinarum*. The phytase activity was confirmed in lactobacilli strains, which when fed to poultry birds increased body weight gain in broilers (Askelson et al. 2014). A broiler gut-origin recombinant *Lactobacillus reuteri* expressing endoglucanase gene (*celW*) of *B. subtilis* WL001, and phytase gene (*phyW*) of *Aspergillus fumigatus* WL002, was developed to enhance feed utilization in poultry. The recombinant *L. reuteri* improved feed conversion ratio when fed to broiler and exerted antimicrobial activities against undesirable bacteria such as *Bacteroides vulgates*, *Escherichia coli*, and *Veillonella* spp. and promoted beneficial intestinal commensals like lactobacilli and bifidobacteria (Wang et al. 2014).

*Lactococcus lactis* containing recombinant phytase gene (*appA2*) insert obtained from *Escherichia coli* revealed phytase activity (4U/ml) in cell extract and 19 U/ml phytase activity in culture medium supernatant. The

recombinant *L. lactis* improved growth performance of broilers (Pakbaten et al. 2018). Recombinant bile salt hydrolase (BSH) from *Lactobacillus johnsonii* La1 exhibiting anti-giardial activities might have prospects to prevent widely spread, but neglected infectious giardial infections in humans and veterinary subjects (Allain et al. 2018). Some other relevant examples are summarized in Tables 7.1 and 7.2.

## 7.4 Beneficial Probiotic Metabolites

Bioengineered microorganisms have a pivotal role in nutrition, therapeutics, and health industry. Besides delivering drugs and gene-therapy vectors with efficiency and site-specificity, the bioengineered microorganisms have provided a lot of valuable products for diagnosis and treatment of diseases. Genome engineering and

**Table 7.1** Summary of recombinant microorganisms developed for applications to livestock nutrition health augmentation

Microorganisms	Specific features/genes introduced	Possible inferences and implications
<i>Butyrivibrio fibrisolvens</i>	Genes for fluoroacetate dehalogenase	Developed to alleviate toxicity caused by fluoroacetate present in some forages, could establish in rumen of sheep and prevent fluoroacetate toxicity (Gregg et al. 1994, 1998)
	<i>Neocallimastix patriciarum</i> xylanase	Improvement in fiber digestion by recombinant bacteria (Krause et al. 2001)
	<i>N. patriciarum</i> xylanase	Enhanced (28.7%) degradation of neutral detergent fiber compared with normal or control strains. The study explores the feasibility of expressing rumen fungus genes in rumen bacteria to enhance fibre digestion (Gobius et al. 2002)
<i>Escherichia coli</i> BL-21	Phytase genes from <i>Selenomonas ruminantium</i>	The study explores commercial scale production of BL-21 recombinant phytase (Chi-Wei Lan et al. 2014)
<i>Lactobacillus acidophilus</i> , <i>L. gasseri</i> , <i>L. gallinarum</i>	<i>B. subtilis</i> phytase (phyA) codon	Phytase gene detected in the lactobacilli, phytase degradation activity of the recombinant strains increased due to recombinant phytase. Phytase-expressing <i>L. gasseri</i> increased body weight gain in the broilers (Askelson et al. 2014)
<i>Lactobacillus reuteri</i> (from broiler GI tract)	<i>B. subtilis</i> WL001 endoglucanase gene (celW), and <i>Aspergillus fumigatus</i> WL002 phytase gene (phyW) mature peptide (phyWM)	Endoglucanase and phytase activities detected in culture medium of recombinant <i>L. reuteri</i> . The recombinant <i>L. reuteri</i> improved feed conversion ratio when fed to broiler, and exerted antimicrobial activities against <i>Bacteroides vulgates</i> , <i>Escherichia coli</i> , and <i>Veillonella</i> spp. and promoted intestinal lactobacilli and bifidobacteria (Wang et al. 2014)
<i>Lactococcus lactis</i>	Phytase gene ( <i>appA2</i> ) insert of <i>Escherichia coli</i>	The transgene could express in <i>L. lactis</i> as revealed from phytase activity (4U/ml) in cell extract, and supernatant maximal phytase activity being 19 U/ml (Pakbaten et al. 2018)

**Table 7.2** Summary of recombinant microorganisms developed as oral vaccines or for protection against bacterial viral infections

Microorganisms introduced	Specific features/genes	Possible inferences and implications
<i>Escherichia coli</i>	Expressing recombination activating gene 2 (RAG2) in <i>E. coli</i>	Successful expression of recombinant fusion protein, and its purification. Using purified fusion protein to produce polyclonal antibodies (Jin et al. 2017)
<i>Lactobacillus plantarum</i>	Porcine epidemic diarrhea virus S gene fused to a DC-targeting peptide	The mice immunized by lavage administration of recombinant <i>L. plantarum</i> (NC8-pSIP409-pgsA'-S-DCpep) NC8 strain lead to increased secretion of cytokines, namely IFN- $\gamma$ , IL-4 and IL-17, thereby indicating that recombinant strain triggered humoral immune response, and that recombinant strain could be used for producing novel oral vaccine against porcine epidemic diarrhea virus (Huang et al. 2018)
	Expression of 3M2e-HA2 influenza virus proteins in <i>L. plantarum</i>	The chicks immunized with N/pgsA'-3M2e-HA2 exhibited characteristic humoral, and T cell-mediated immune response against avian influenza virus. The study is emphasized as a novel approach and effective vaccine to promote mucosal immunity (Yang et al. 2018b)
	Expression of TGEV antigen(S) to dendritic cells (DCs) via dendritic cell-targeting peptides (DCpep)	Induction of high levels of B7 molecules on DCs, plus high levels of IgG, secretory IgA, IFN- $\gamma$ , and IL-4. Expression of DC-targeted antigens could induce cellular, mucosal, and humoral immunity in murine models, indicating that recombinant <i>L. plantarum</i> could be used as oral vaccine against TGEV (Yang et al. 2018a)
	Expression of spike (S) protein originating from TGEV, fused to DC-targeting peptides (DCpep) in <i>L. plantarum</i>	The recombinant <i>L. plantarum</i> (NC8-pSIP409-pgsA'-S-DCpep) expressing S fused with DCpep enhances the MHC-II <sup>+</sup> CD80 <sup>+</sup> B cells, and CD3 <sup>+</sup> , CD4 <sup>+</sup> T cells of ileal lamina propria with simultaneous increase in levels of secretory IgA in feces, and IgG in serum, indicating that recombinant bacterium is suitable for use as oral vaccine against the virus (Jin et al. 2018)

synthetic biology that allow design and construction of genetic circuits, and precise fine-tuning of transgene expression, have opened up new frontiers to augment the therapeutic and nutritive potential of the probiotics.

Postbiotics, the microbial metabolites, are important factors accounting for efficacy of the probiotics. Chromatography coupled with tandem mass spectrometry and Fourier transform ion cyclotron resonance mass spectrometry with

direct infusion is recommended to identify and characterize bacterial metabolites or postbiotics such as AMPs, bacteriocins, fatty acids, purines, sphingolipids, and oligosaccharides (Kok et al. 2013). Currently, fluorescent in situ hybridization (FISH), DNA pyrosequencing, microarrays (PhyloChip), and quantitative PCR assays, and analysis of conserved 16S rRNA genes for phylogenetic analysis are used to analyze complex microbial ecosystems.

## 7.5 Diversity of Genitourinary Microbiota

The female reproductive is specific in terms of immunological organization, epithelial barrier, sensitivity to estrogen, and microbial ecosystem (Petrova et al. 2018). The affirmation that normal genitourinary microbes protect the host against pathogens has encouraged the researchers to decipher uterine tract microbiota to manage reproductive health and infertility (Zhou et al. 2004; Martin et al. 2013).

Until recently, the microbes inhabiting urinary tract were studied by empirical culture-dependent methods that are unable to depict the complete outline of microbiota. Since the cultivable microorganisms merely represent a fraction of total microbial diversity, it is envisaged that the microorganisms that are not cultured, and therefore remain unexplored, play important role in the health of the mother and neonates (Hyman et al. 2014).

Deciphering how the microorganisms interact with each other and with uterine epithelium is fundamental for a more complete understanding of feminine reproductive health (Singh et al. 2013). These culture-independent approaches have provided information about novel, previously unidentified uterine microbiota in different endocrinological milieus, and support a shift in the microbiome during assisted reproduction and pregnancy outcomes.

In this context, it is necessary to analyze complex microbial niches, genes, and metabolic pathways therein for developing alternatives therapies that are safer and effective against antibiotic-resistant microorganisms (Thallinger et al. 2013; Cardona et al. 2015).

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## 7.6 Recombinant Antimicrobial Peptides

One of the major challenges in modern medicine is paucity of alternative therapies against drug-resistant pathogens. Genitourinary tract infection is one of the many major causes of infertility in dairy and beef cattle. The vaginal

microbiota dominated by LAB confers protection against bacterial infections. Recombinant microbicides may offer a target specificity ensuring prolonged protection against genitourinary infections.

Infertility in livestock is a matter of serious concern. The major factors declining livestock productivity include infectious diseases, nutritional imbalance, climatic stress, pests, and parasitic infestation. Besides, the brucellosis, chlamydiosis, infectious bovine rhinotracheitis, leptospirosis, listeriosis, corynebacteriosis, campylobacteriosis, neosporosis, trichomoniasis, and viral infections are primarily responsible for causing infertility in animals.

However, little is known about microbial communities of reproductive tract of animals and aberrations or dysbiosis in cow and buffalo leading to infertility. The females unable to conceive and calving are then abandoned which pose a threat to humans by causing fatal accidents and spreading parasites and zoonotic pathogens through urine, uterine discharge, and defecation.

The information is sparse on microbiota of reproductive tract microbes and their role in reproductive health and infertility in animals (Durso et al. 2015). Culture-dependent microbial analysis and 16S rRNA sequencing of cow and ewe ectocervicovaginal lavages has revealed a large difference in their vaginal microbial composition (Swartz et al. 2014). Similar reports are available from analysis of Nellore cattle uterine metagenome (Laguardia-Nascimento et al. 2015). Antimicrobial peptides (AMPs) and bacteriocins which act via membrane active mechanisms are widely used in food industries, feed industry and also be used in personal care products, surmounting the pathogens (Ong et al. 2014; Juturu and Wu 2018). As genitourinary tract microbiota is least explored, it is important to study the reproductive ecosystem of livestock species to understand microbial causes and cure of infertility. The bioengineered probiotics producing high titers of AMPs could enhance benefits of probiotics as well as antimicrobial peptides *vis-a-vis* their role in reviving reproductive health of animals.

## 7.7 Health Threats to Porcine and Poultry Industry

Besides, nutritional deficiencies and toxicity, the poultry and pigs suffer from several parasitic, bacterial, and viral infections. In addition, wild and abandoned animals serve as important sources of infections to healthy animals and zoonotic diseases. Viral infections such as porcine transmissible gastroenteritis virus (TGEV), belonging to coronaviruses (CoVs) (Yang et al. 2018a; Jin et al. 2018), and porcine epidemic diarrhea virus (PEDV) (Huang et al. 2018) cause severe economic losses in pork industry worldwide. In addition to infecting poultry, the animal viruses pose a serious threat to humans (Walker et al. 2018). To prevent viral infections, therapeutics developed should be safer, and no residual remains are left in meat or milk intended for human consumption, and confer long term protection.

Viral infections such as avian influenza (AIV) are among serious threats to poultry industry (Yang et al. 2018b). Edible vaccines in the form of probiotics are recommended in view of their safety and ease of administration. Besides humans (Boesmans et al. 2018), the probiotic therapies are recommended to prevent infections in poultry (Kareem et al. 2016, 2017; Yazhini et al. 2018), calves (Maldonado et al. 2016, 2017; Plaizier et al. 2018), and porcine (Nordeste et al. 2017; Xu et al. 2018).

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## 7.8 Recombinant Microorganisms as Oral Vaccines

Effective public health research and awareness requires a comprehensive and thorough understanding of viruses that are at risk of transmission to humans. Vaccines, antibiotics, antibodies, and antibiotics–antibody conjugates are used to prevent animals against infections.

Edible vaccines offer advantages in animals. Oral delivery of antigens by means of recombinant probiotics, such as those expressing dendritic cell (DC)-targeting peptides, fused with virus antigens is expected to confer protection in

animals against viral infections (Wang et al. 2017).

Probiotic lactic acid bacteria, especially lactobacilli and lactococci, in view of their status as safe, and natural presence in humans, and animals including birds and insects are opted to express antigens of viruses of porcine and poultry species to produce cytokines and antibodies, for use as oral vaccines.

A recombinant *Lactobacillus plantarum*, named *L. plantarum* (NC8-pSIP409-pgsA-S-DCpep), expressing spike (S) protein originating from TGEV fused with DCpep enhanced MHC-II<sup>+</sup>CD80<sup>+</sup> B cells, and CD3<sup>+</sup>, CD4<sup>+</sup> T cells of ileal lamina propria with simultaneous increase in levels of secretory IgA in feces, and IgG in serum shows that recombinant bacterium is suitable as oral vaccine against the virus (Jin et al. 2018). In another study, 3M2e-HA2 influenza virus proteins were made to express in *L. plantarum*. The experimental chicks immunized with N/pgsA'-3M2e-HA2, exhibited characteristic humoral, and T cell-mediated immune response against avian influenza virus. The study is emphasized as a novel approach and effective vaccine to promote mucosal immunity (Yang et al. 2018a, b).

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## 7.9 Outlook and Challenges

Development of novel antibiotics to combat drug-resistant pathogens is slow compared to rise of drug resistance. Recombinant microbes are the emerging and futuristic candidates of nutrition and health management. The bioengineered microorganisms are now making their way to improve nutrient utilization, alleviate forage toxicity, and protect animals against pathogens.

It is a time for new prescription against pathogens and metabolic diseases. A broader approach to address bacterial infection is needed as new pathogens are emerging with resistance to conventional antibiotics. A combined therapy comprising of antimicrobial property, immunomodulation, and anticancer effects of bioengineered probiotics could boost the host immune system.

Engineered probiotics that are safer upon administering or feeding should be prioritized. However, the recombinant microorganisms raise certain safety concerns. The likely probiotic-mediated induction of immune response should be carefully examined. It is likely that overproduction of AMPs by recombinant probiotics may deter the normal gut bacteria that are otherwise essential to wellbeing of the host. It is necessary to prevent proliferation of genetically modified microorganisms in environment when they come out in feces and prevent transfer of transgenes into environmental or pathogenic microorganisms. Key issues, such as strain characterization, quality control, dose optimization, lateral gene transfer from recombinant probiotics to normal microflora, should be embarked upon.

## 7.10 Conclusions

Exploiting microbiota is increasingly a big business. As recombinant probiotics may rebuild microbiota and restore the health with efficiency and site-specificity, the challenges and key problems associated with their use should be thoroughly investigated to enhance their applications in animals serving as part of human food chain. The research to discover normal microbiota and developing alternative microbial therapeutics should be prioritized. The therapies require a critical evaluation before being recommended for human applications.

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