Enteric infections account for high morbidity and mortality and are considered to be the fifth leading cause of death at all ages worldwide. Seventy percent of all enteric infections are foodborne. Thus significant efforts have been directed toward the detection, control and prevention of foodborne diseases. Many antimicrobials including antibiotics have been used for their control and prevention. However, probiotics offer a potential alternative intervention strategy owing to their general health beneficial properties and inhibitory effects against foodborne pathogens. Often, antimicrobial probiotic action is non-specific and non-discriminatory or may be ineffective. In such cases, bioengineered probiotics expressing foreign gene products to achieve specific function is highly desirable. In this review we summarize the strategic development of recombinant bioengineered probiotics to control enteric infections, and to examine how scientific advancements in the human microbiome and their immunomodulatory effects help develop such novel and safe bioengineered probiotics.

Introduction

Enteric infections account for about 1.5 billion episodes of diarrheal diseases with 2.2 million deaths (mostly children) annually and are the fifth leading cause of death at all ages worldwide. Children under 5 y of age are most susceptible and the disease burden is the greatest in developing countries. Consequences of childhood enteric infections are impaired physical growth and cognitive development. Enteric infections may be caused by bacterial, viral, parasitic, or fungal agents, which disrupt intestinal function with or without causing dehydrating diarrhea. Seventy percent of all microbial diarrheal diseases are foodborne, and foodborne illnesses are a serious public health concern (Table 1). The global burden of foodborne illness is currently unknown; however, the World Health Organization (WHO) reported that 1.8 million people died from diarrheal diseases in 2005, largely due to contaminated food and water. In the US, the Centers for Diseases Control and Prevention (CDC) estimates that each year there are about 48 million cases of foodborne infections with 128,000 hospitalizations and 3,000 deaths. There are over 200 known microbial, chemical, or physical agents that can cause foodborne illness. CDC estimates that all the foodborne infections, 44% of the hospitalizations and deaths are attributed to 31 known pathogens. In light of this serious public health crisis, efforts have been directed toward the detection, control, and prevention of food-borne pathogens and diseases. It is estimated that a reduction in foodborne illness by 10% would keep about 5 million Americans from getting sick each year. With increasing trend in consumer preference for safe and wholesome food, probiotics offer an effective and alternative intervention strategy to control foodborne illnesses. Among the microbial etiologies responsible for enteric infections, WHO has prioritized around 22 infectious agents for surveillance based on their higher prevalence, morbidity and mortality. These include Brucella spp., Campylobacter spp., Clostridium botulinum, enterogroups, E. coli (EaggEC), Enteropathogenic E. coli (EPEC), enterotoxigenic E. coli (ETEC), Shiga-toxin producing E. coli (STE), Helicobacter pylori, hepatitis A virus, hepatitis E virus, Listeria monocytogenes, Mycobacterium bovis, Vibrio cholerae O1/O139, non-cholera Vibrio spp., norovirus, rotavirus, prions, Salmonella spp. (non-typhoidal), Salmonella enterica serovar Typhi, Shigella spp., and Yersinia spp., and toxins from Staphylococcus aureus, Clostridium perfringens, and Bacillus cereus. Various strategies have been employed to control enteric pathogens in foods, food producing animals, and humans. Antibiotics are used in meat animal production as prophylactic to control disease and improve growth rate and efficiency. However, increasing concerns about antibiotic resistance has led to research efforts to use naturally occurring antimicrobials as alternatives. Antimicrobials may include organic acids, essential oils and plant extracts, bacteriocins, probiotics, and bacteriophages. Antibiotics (acidic, lactic, and citric acids) are commonly used to rinse animal carcasses, fruits, and vegetables. To enhance antimicrobial efficacies, acids are also used in combination with oxidizing agents such as hydrogen peroxide. In addition, thermal (misting radiations and heating) and non-thermal treatments such as high hydrostatic pressure, high-intensity pulsed electric fields, oscillating magnetic fields, intense light pulse, photosensitization, or a combination of above (hurdle approach) are also effective.
Probiotics

The word “probiotic” is derived from the Greek word meaning “for life.” Probiotics are live nonpathogenic microorganisms that are administered to maintain and improve intestinal microfloral balance and protect host from infective agents. Physiologically, these microbes are endowed with certain characteristics that enable them to survive in the gut environment and colonize mucosal surfaces. The rationale for the use of probiotics in the prevention of enteric infections and treatment of diarrhea are associated with three major factors: (1) maintenance of the epithelial gut barrier, (2) modulation of innate and acquired immunity, and (3) inhibition of pathogen growth by producing bacteriocins, hydrogen peroxide and other antimicrobials. Besides, probiotics also help prevent chronic enteric infection associated with stunted growth, abnormal low body mass indices, and impairment of cognitive function in children.

The use of probiotics, prebiotics, and synbiotics (combination of probiotics and prebiotics) has also gained increased interest in recent years. The use of microflora to reduce pathogen load in the gut is termed as a probiotic strategy. Probiotic techniques involve the use of microflora to reduce pathogen load in the gut, with the goal of limiting and allowing the growth of a specific subset of the gut microflora. The use of probiotics, prebiotics, and synbiotics can provide a nutrient (prebiotic) that is limiting and allows the growth of specific microorganisms.

Beneficial attributes of probiotics are broad and well documented. Table (2) includes lactose metabolism, improved digestion, increased nutritional value, production of antimicrobial factors, anti-inflammatory effects, anti-carcinogenic properties, immunologic enhancement, production of short-chain fatty acids, anti-atherogenic, and cholesterol-lowering attributes, regulatory role in allergy, protection against vaginal or urinary tract infections, maintenance of epithelial integrity and barrier, stimulation of repair mechanism in cells, and maintenance and reestablishment of a well-balanced indigenous intestinal, respiratory, and urogential microfloral communities.

Prevention and Control of Enteric Infections Using Wild Type Probiotics

Enteric viral infections. Probiotics have been used to control viral infections. Rotavirus is responsible for 20–25% of the diarrheal diseases worldwide. Gastrointestinal pigs fed with Lactobacillus acidophilus and L. reuteri enhanced IFNγ and IL-4 levels in serum and decreased rotavirus infection. Probiotics are also effective against Norovirus, which is responsible for 58% of foodborne illnesses. Probiotic fermented milk containing L. casei Shirota strain was effective in controlling norovirus gastroenteritis in a health service facility. A controlled double-blind study using a probiotic formulation (VSL#3) was shown to significantly reduce stool frequency and requirement for oral rehydration in children.

Bacterial enteric infection. Among enteric pathogens that cause diarrhea, Campylobacter jejuni is responsible for about 400 million cases every year in both industrialized and developing countries. Several probiotic strains have been evaluated for their efficacy in controlling Campylobacter infection. Lactobacilli and Bifidobacteria were shown to enhance colonization resistance in mice that were infected by C. jejuni or Salmonella. Probiotics also increased proliferation of lymphocytes against Salmonella antigens and reversed pathogen-induced immunosuppressive activity. Synbiotics consisting of probiotic galacto-oligosaccharide and probiotic Bifidobacterium longum significantly reduced C. jejuni load in poultry feces. Vibrio cholerae causes acute dehydration watery diarrhea with 1.8 million cases and 27,000 deaths annually. Experimental administration of L. acidophilus BKM 2020 orally in mice and suckling rabbits prior to infection prevented cholera. Probiotic L. plantarum AT1 attached efficiently to cultured cell lines (HT-29) and reduced V. parahaemolyticus attachment by competitive exclusion and displacement. Probiotics are also found to be effective against diarrhea causing E. coli including STEC and ETEC. L. acidophilus, L. casei, L. fermentum, L. plantarum, and Enterococcus fecalii significantly reduced E. coli O157:H7 shedding by sheep. Bifidobacteria caused reduced Shiga toxin production by STEC in mice and protected against E. coli O157:H7 infection. Nonpathogenic probiotic E. coli
strains 1307 and Nisole also inhibited STEC growth and Shiga
toxin production.\textsuperscript{54} Furthermore, pre-exposure to \textit{L. paracasei}
resulted in an upregulation of dendritic cells, activation of
helper T cells and antibody production, and downregulation of
proinflammatory cytokines resulting in enhanced intestinal
integrity and protection against enteric infection.\textsuperscript{55} Probiotics
have been widely tested to control \textit{S. enterica} colonization and
infection. Administration of one or several probiotic strains in
broiler chicks inhibited \textit{Salmonella} contamination.\textsuperscript{38} A commer-
cial probiotic cocktail significantly reduced \textit{Salmonella} counts
in the tonsils and ceca of chickens and poults.\textsuperscript{39} Furthermore,
administration of \textit{L. reuteri} strain significantly reduced
\textit{Salmonella} populations and increased the survival rate in
chicks.\textsuperscript{40} In vivo study using a mouse model demonstrated that
continued administration of \textit{L. casei} CRL diminished \textit{Salmonella}
counts in the intestine and extraintestinal dissemination.\textsuperscript{41}
\textit{L. casei} Shirota strain also protected mice against lethal infection
with multi-drug resistant \textit{S. Typhimurium} DT104.\textsuperscript{42} Besides the
antimicrobial effects, probiotics also increased the performance
and feed conversion in chickens and turkey poults. Probiotics
were also effective against other enteric pathogens such as \textit{Shigella}
sonnei, \textit{Staphylococcus aureus}, \textit{Enterococcus faecalis}, \textit{Proteus mira-
bilis}, and \textit{Pseudomonas aeruginosa}.\textsuperscript{43} A bacteriocin (Microcin S)
producing probiotic \textit{Escherichia coli} G3/10 also suppressed EPEC
adherence and pathogenesis.\textsuperscript{44}

\textbf{Recombinant Bioengineered Probiotics}

As discussed above probiotics can be effective in the prevention
and treatment of intestinal diseases. However, probiotic action
is non-specific and non-discriminatory or ineffective in certain
hosts.\textsuperscript{57} This is in part due to broad mode of action and strain
variability (Table 2). Probiotics differ from one another, there-
fore, the beneficial attributes of one strain or a cocktail of strains
may not be reproducible and may vary from person to person.\textsuperscript{64}
Additionally, the probiotic strain, dose, route of administration,
and the formulation of probiotic preparation can also affect the
efficacy of a probiotic.\textsuperscript{65} Furthermore, the manufacturing process

| Health benefits | Proposed mechanism |
|-----------------|--------------------|
| Resistance to enteric pathogens | Antagonism |
|                   | Increased antibody production |
|                   | Colonization resistance |
|                   | Limiting access of enteric pathogens (pH, bacteriocins, antimicrobial peptides, lactic acid production) |
| Aid in lactose metabolism | Bacterial lactose hydrolase lactose in the small intestine |
| Small bowel bacterial overgrowth | Decrease toxic metabolite production |
| Immune system modulation | Strengthening of non-specific and antigen-specific defense |
|                   | Regulate/influence Th1/Th2 cell activation |
| Anticancer effect | Antimutagenic and anticarcinogenic activity |
|                   | Detoxification of carcinogenic metabolites |
| Decreased detoxification/excretion of toxic microbial metabolites | Increased bifidobacterial cell counts and shift from a preferable protein-to-carbohydrate-metabolizing microbial community |
| Anti-Allergic activity (eczema or atopic dermatitis, asthma) | Prevention of antigen translocation into blood stream |
| Blood lipids, heart disease | Prevent excessive immunologic responses to increased amount of antigen |
| Urogenital infections | Assimilation of cholesterol by bacterial cell |
|                   | Alteration in the activity of bile salt hydrolase (BSH) |
| Necrotizing enterocolitis | Decrease in TLRs and signaling molecules and increase in negative regulations |
|                   | Reduction in IL-8 response |
| Rotavirus gastroenteritis | Increased IgA response to the virus |
| Inflammatory bowel disease | Enhancement of mucosal barrier function |
| Crohn disease | Reduction in proinflammatory cytokines production |

Adapted from Nagpal et al.\textsuperscript{23}

\textbf{Table 2. Health benefits of probiotic bacteria and their proposed mechanisms}
and probiotic delivery system have been shown to modify exo-
poly saccharide production by the probiotics and thereby modify
their efficacy.47–49 Recent studies on the gut microbiome di-
versity have revealed that the variability in the indigenous flora
among different populations may also affect probiotic efficacy.
50 These limitations reinforce the need for novel and innovative
approaches to design and create genetically modified probiotic
strains to exclusively target a specific pathogen or toxin to be used
either as a vaccine or for drug delivery.51,52
Over the last decade recombinant probiotics have been gener-
ated for mucosal delivery of therapeutic and prophylactic mole-
ecules including DNA, peptides, single-chain variable fragments,
cytokines, enzymes, and allergens.53–56 The major advantages of
probiotic bacteria as delivery system are their (1) ability to colo-
nize mucosal surface, (2) tolerance to gastric acid and bile salts
enabling survival and transit through the gastrointestinal tract
(GIT), and (3) sustained colonization and prolonged protection
against pathogen.57–59 Furthermore, oral recombinant probiot-
ics offer several advantages: direct delivery of active molecule to
the mucosal surface without the need for bio-separation of the
active molecules, increased shelf-life and stability, low delivery
costs, and ease of technology transfer following prototype devel-
opment. This led to the concept of “biodrug” that is based on the
oral administration of live recombinant microorganisms for the
prevention and treatment of various diseases.60
In order to create therapeutically effective bioengineered
recombinant probiotics, certain physiologic attributes are essen-
tial: (1) tolerance to stressors encountered during product manu-
facturing and storage, and during oral delivery, (2) strong mucosal
colonization, (3) expression of target antigen under the gastroin-
testinal environment, and (4) potent antipathogenic action.

Bioengineering of Probiotics

to Improve Stress Tolerance

Probiotics encounter stress during manufacturing, storage,
and passage through the host GIT, namely temperature, acid-
ity, salts, and water activity.53 Physiologically, accumulation of
compatible solutes helps stabilize protein function at low tem-
peratures and prevent plasmolysis under low water activity. To
improve stress tolerance in probiotic strains, the betaine trans-
porter gene (betD) from Listeria monocytogenes was cloned into
Lactobacillus salivarius under the control of the nisin inducible
promoter.61 Thus accumulation of betaine in recombinant L. sali-
varius enabled it to be osmo tolerant (7% NaCl) and cryo- and
baro-tolerant. Similarly, cloning of the trehalose synthesis gene
(tsuAB) from E. coli into Lactococcus lactis protected recombinant
bacteria from freeze-drying, bile toxicity, and resistance to gastric
acid.62 Furthermore, cloning of betD into Bifidobacterium breve
UCC2003 significantly improved its survival in gastric juice thus
improving its therapeutic attributes.63

Antimicrobial Action of Bioengineered Probiotics

Receptor mimicry system and toxin neutralization. To achieve
pathogen and/or toxin-specific activity, several strategies were
employed to create bioengineered probiotics. Patton and col-
leagues64 cloned and expressed toxin-specific host cell receptor on
probiotic E. coli thus creating a competitive environment for toxin
binding to host cells. They cloned glycosyltransferase genes from
either Nisseria meningitidis or C. jejuni on the surface of non-
pathogenic probiotic E. coli to express chimeric lipopolysaccha-
dride that mimics host cell receptor (ganglioside) for cholera toxin
or ETEC heat labile toxin, LT. During infection enterotoxins are
sequestered by the probiotic E. coli thus protecting host against
diarrheal infection. In another study, L. reuteri was engineered to
express ETEC heat stable (ST) and heat labile (LT) enterotoxins
under the nisin inducible promoter. This recombinant probiotic
successfully bound to the enterotoxins and prevented enterotox-
ocity in a mouse model. Furthermore, orally immunized mice with
the toxin secreting recombinant L. reuteri increased serum IgG and
muco sal IgA levels and protected animals from ETEC
infection.65

Prevention of colonization. Cloning and expression of
adhesins, toxins, or secretory systems of pathogens may serve as
potential targets for the development of therapeutics to prevent
infection. Several strategies were employed to enhance probiotic
adhesion to mucosal surface using gene products of target patho-
gen to create a competitive environment for pathogen coloniza-
tion. Probiotics expressing adhesion factor LAP (Listeria adhesion
protein) from L. monocytogenes was able to exclude probiotic col-
onization and prevented pathogen induced cell damage.66 LAP is an
adhesion factor in L. monocytogenes that interacts with the host
cell receptor, heat shock protein 60 (Hsp60),64,66 and promotes
listerial adhesion and transspatial translocation during intesti-
nal phase of infection.67 Pre-exposure of intestinal monolayers
to the recombinant probiotic Lactobacillus paracasei expressing
LAP followed by L. monocytogenes infection led to a reduction
in adhesion, invasion, and transspatial translocation by 44, 45, and
46%, respectively68 (Fig. 1). The recombinant probiotic also
protected the epithelial monolayers from L. monocytogenes mediat-
ed cytotoxicity and tight junction compromise.

Similarly, S. enterica attachment was inhibited by using recombinant probiotic bacteria. Lactococcus lactis expressing Fga-
gellin of a probiotic strain of Bacillus cereus CH4 adhered strongly
to mucin-coated polystyrene plates in an in vitro experiment and
competitively inhibited the adhesion of pathogenic E. coli and S.
enterica to the same molecule.69

A recombinant L. acidophilus strain carrying the K99 fim-
brie from ETEC was able to reduce the attachment of ETEC
to porcine intestinal brush border in a dose dependent manner.70

Similarly, L. casei was bioengineered to express ETEC adhesins
K99 or K8871 and the efficacy of the recombinant probiotic to
protect host from ETEC infection was verified in a mouse model.

Oral vaccination of mice with the recombinant strain resulted in
high levels of mucosal IgA in bronchoalveolar lavage and intesti-
nal fluids and systemic IgG response. The recombinant probiotic
protected more than 80% of the vaccinated mice after challenge
with a lethal dose of ETEC.70 Likewise, L. casei expressing adhe-
sion protein ( intimin of EPEC) induced systemic and mucosal
antibodies in mice and the antibodies inhibited the adhesion of
EPEC in an in vitro epithelial cell culture model.71 Employing

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a similar approach, a recombinant L. casei strain expressing S. Enteritidis flagellar antigen FlG induced antigen specific protective immune response against S. Enteritidis in a mouse model.\textsuperscript{75} Similar strategies have also been adopted for viral pathogens. L. casei was engineered to secrete simian immunodeficiency virus (SIV) specific cyanoovin-N and the recombinant probiotic strain reduced the SIV infection by 62.9\% in the Chinese macaque model.\textsuperscript{75,76}

Regulation of virulence gene expression. Pathogenic bacteria have the ability to control the expression of virulence genes by sensing signals (termed quorum sensing) from their own species, other bacteria or their environment. Since the quorum sensing system senses population density, mediates colony-wide coordinated behavior, and controls virulence pathways,\textsuperscript{77} interruption of quorum sensing pathway may serve as a viable option for disease prevention. V. cholera release cholera autoinducer-1 (CAI-1) and autoinducer-2 (AI-2) that accumulate when the population density increases at which point bacteria produce virulence factors. An AI-2 producing E. coli Nissle strain was engineered to co-express CAI-1, which suppressed virulence gene expression in V. cholera leading to its reduced lethality on infant mouse.\textsuperscript{78}

Production of antimicrobial factors. Some probiotics produce several antimicrobial compounds and peptides as a defense mechanism against pathogens. Engineering of probiotics to detect pathogen signals for timed production of antimicrobials would be a novel approach. Saadat et al.\textsuperscript{79} engineered a commensal E. coli to detect signals from the pathogen for the production of bacteriocin.\textsuperscript{80} Pseudomonas aeruginosa quorum sensing system (Las/Lqsr) controls virulence gene expression. Las produces homoserine lactone that activates LasR and leads to virulence gene expression. A bacteriocin producing probiotic E. coli strain was engineered to express LasR (to detect homoserine lactone) under the control of the luxR promoter and E7 lytic protein to aid in release of the bacteriocin. Co-culture of P. aeruginosa and recombinant E. coli led to a decrease in P. aeruginosa growth and biofilm formation by 99\% and 90\%, respectively.\textsuperscript{81}

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Figure 1. Inhibition of Listeria monocytogenes transepithelial translocation through epithelial barrier by bioengineered probiotic, Lactobacillus paracasei expressing Listeria adhesion protein (LAP). (A) Immunofluorescence staining of LAP expression by Listeria monocytogenes\textsuperscript{79} and recombinant L. paracasei\textsuperscript{80} (LbpLAP) showing about 46\% reduction in L. monocytogenes translocation through epithelial barrier while, Lbp paracasei\textsuperscript{80} (LbpWT) or L. paracasei containing empty vector (LbpLAP-) had no effect. Figures adapted from Koo et al.\textsuperscript{80}
pseudotuberculosis, S. epidermidium, and Streptococcus pneumoniae infections. Similar recombinant vaccine was developed using L. acidophilus engineered to express protective antigen (PA) of Bacillus anthracis to activate dendritic cell to protect host against anthrax. Likewise, expression of PA in L. gastricus also provided 100% protection against anthrax in a mouse model.

Probiotics were also engineered to deliver vaccines to the mucosal surfaces. The first recombinant probiotic oral vaccine was developed by expressing the tetanus toxin fragment C in Lactococcus lactis. Recombinant Lc. lactis strain expressing Internal A protein of L. monocytogenes enabled this non-invasive probiotic to invade the small intestine and to deliver the immunostimulatory molecule inside the epithelial cells.

To control rotavirus infection, several live attenuated vaccines using the human and/or bovine rotavirus strain have been developed; however, these were ineffective due to lack of robust mucosal immune response. To help elicit strong mucosal immune response, recombinant L. paracasei expressing the variable domain of llama heavy-chain antibody was developed against rotavirus. This antibody expressing probiotic was able to markedly reduce disease length, severity, and viral load in a mouse model. In another study, recombinant L. lactis expressing rotavirus spike-protein VP8 induced mucosal IgA and anti-VP8 antibodies at both intestinal and systemic levels in a mouse model and provided 100% protection against rotavirus challenge.

Besides the use of heterologous antigens to stimulate immune responses, expression of cytokines can also help in immunostimulation. Several probiotic strains have been engineered to express cytokines and other anti-inflammatory molecules to help suppress intestinal inflammation and provide cytoprotection. Murine IL-10, an immunosuppressive and anti-inflammatory cytokine was cloned and expressed in Lc. lactis strain, and the recombinant strain reduced inflammation and colitis in 40% of the mice. Oral administration of IL-10 secreting probiotic in a colitis murine model resulted in a reduction in inflammatory symptoms. Human interferon-β (huIFN-β) is immunomodulatory and increases IL-10 expression. Lc. lactis secreting huIFN-β was shown to significantly reduce microbial colitis and inflammation. In addition to huIFN-β, heme oxygenase-1 (HO-1) has also been shown to modulate the anti-inflammatory effect of IL-10. Lc. lactis secreting HO-1, when administered in rats, prevented mucosal injury by LPS, reduced LPS-induced endotoxemia, and significantly increased survival rate in rats. Oral immunization of mice with L. casei expressing IL-1β and heat-killed S. Enteritidis (SE) enhanced anti-SE antibodies demonstrating adjuvant properties of recombinant probiotics. Recombinant L. plantarum surface displaying invasin protein of Y. pseudotuberculosis served as a potent activator of NF-κB and was demonstrated to be a promising mucosal delivery vehicle for vaccine antigen. L. acidophilus was engineered to express hemagglutinin of the avian influenza virus H5N1 and induced strong mucosal and serum antibody response to H5N1.

Safety of Probiotic Therapy and Biocontainment

The ultimate goal of developing a recombinant probiotic is its use in humans and animals. Prior to the approval of a recombinant probiotic for human use, it is essential that the bacteria be screened for potential pathogenicity and virulence traits. Providing evidence for the absence of virulence properties is relatively straightforward in elucidating the pathogenic potential. Besides phenotypic characterization, it is also essential to genetically screen potential candidates for use as probiotics. Another critical consideration is the scope for antimicrobial resistance. In addition to being sensitive to antibiotic selection, it is also essential to evaluate whether the probiotic bacteria do not carry any transferrable antibiotic resistance genes, which can serve as genetic reservoirs for other potentially pathogenic bacteria. Besides acquisition of antibiotic resistant genes, there is also the risk for uptake of virulence genes from pathogens that co-inhabit the intestinal tract at the same time. However, there is no evidence in the literature for such event taking place in the gut. This could partly be due to the transient colonization of the gut by probiotics. Considering all these factors that are essential in assessment of safety of probiotic therapy, it is paramount that the general conclusion “probiotics are safe” cannot be broadly made. Prior to the use of a probiotic or probiotic cocktail in foods or dietary supplement, they need to be determined to be safe for the general population. Therefore, when intended for use as drugs, the safety assessment must balance risk with benefit.

Another important consideration for genetically modified probiotic is preventing its accumulation in the environment and preventing lateral dissemination of the genetic material to other bacteria. The best approach to address this concern is to use a biological system that is propagated along with the probiotic termed as biological containment systems. Biocontainment systems can be active or passive. Active containment involves the conditional production of a bacterial toxin through tightly regulated gene expression that is controlled by an environmental cue. Passive containment results in growth dependence on the complementation of an auxotrophy or gene defect, by supplementing another gene or essential metabolite. The approach to contain recombinant Streptococcus mutans: They deleted the alp gene necessary for di-aminopimelic acid synthesis that is essential for biosynthesis of cell wall. Similarly, Fu and Xu developed a containment system for recombinant L. acidophilus using the thymidylate synthase gene (thyA) from L. casei as a marker for plasmid maintenance.

Conclusions and Future Perspectives

Although probiotics have been used in food to enhance flavor or to provide health benefits, currently there is an increasing trend for their use in medicine. They provide a viable alternative especially in the treatment and prevention of enteric diseases. Over the years, several probiotics have been demonstrated to be effective against enteropathogens and their mode of action has been elucidated. A better understanding of the host-pathogen interaction has also enabled the development of bioengineered probiotics that can be used for the targeted elimination of pathogens. The use of engineered probiotics helps overcome the short-half-life limitation of live microorganisms.
Although there are several hurdles in the development of safe and effective bioengineered probiotics, advancements in technologies and further refinements in techniques will continue to provide novel bio-therapeutics for the treatment and prevention of enteric infections both in rich and economically challenged countries.

Disclosure of Potential Conflict of Interests
No potential conflicts of interest were disclosed.
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