Designer Probiotics in Metabolic Disorders 12

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Abstract

Microbes play several vital physiological and metabolic functions in human body. It has been observed that alteration in human gut microbiota has resulted in various chronic and acute metabolic diseases such as obesity, hypertension, neurogenic diseases (Parkinson's and Alzheimer), diabetes, etc. Hence, re-establishment of microbial population, with the help of commensal probiotic bacteria, to improve the gut dysbiosis, has always been the topic of interest. Currently, with the growing knowledge of synthetic biology, genetic engineering, metabolic engineering, and other advanced tools, researchers are attempting to design recombinant probiotic strains, which are capable of carrying therapeutic molecules to the target site. These designer probiotics will enhance the efficacy of the carried molecule without showing any side effects. However, currently, the consumer acceptance of such "Designer Probiotics" is very low. The current chapter envisages a brief introduction about designer probiotics, their developmental strategies, applications of designer probiotics in regulating metabolic diseases, and the challenges in the path of their development discussing examples of few designer probiotic strains. Overall, this chapter intends to provide insight towards the development of designer probiotics to improve the human health.

Keywords

Phenylketonuria · Patho-biotechnology · Receptor mimicking · Synthetic oligosaccharides · Anti-microbial peptide

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12.1 Introduction

In human body ecosystem, microbes are found most abundantly in the gastrointestinal (GI) tract, and these gut microbiota plays a significant beneficial role in human life by participating in various physiological functions advantageous to the host (Tlaskalova-Hogenova et al. 2004; Raghuwanshi et al. 2015; Kristensen et al. 2016). Here, the body works as a host, which provides a suitable condition for growth, while the commensal microbes perform their counterparts by preventing pathogens (Hand 2016), increasing host-immunity (Round and Mazmanian 2009; Patel and DuPont 2015; Macpherson et al. 2017; Raghuwanshi et al. 2018), enhancing the stimulus for GI-hormones (Saulnier et al. 2013), and controlling brain behavior (De Palma et al. 2014; Steenbergen et al. 2015; Kristensen et al. 2016; De Palma et al. 2017). The uniqueness of these gut microbiota is their capability evolve naturally and inhabiting every potential tissue.

Though the normal gut microbiota are essential for several vital processes, however, occasionally they fails, which can be the result of gut microbiota manipulation through hygiene, lifestyle changes, and diet, for example, diet can cause an impact to promote phylogenetic variations in the microbiota (Graf et al. 2015). Moreover, physical activity is also known to affect the gut-microbiome diversity as it is evident that athletes have a more diverse gut microbiome than non-athletes (Clarke et al. 2014). Besides these passive factors, the active manipulators of gut microbiota are antibiotics. The use of antibiotics has been linked to dysbiosis (Langdon et al. 2016), leading to low diversity and evenness among gut microbes (Dethlefsen and Relman 2010; Francino 2016). Moreover, presence and expression of microbial genes are altered following antibiotic therapy, which also lead to detrimental functions of microbiota (Reijnders et al. 2016).

All these factors for gut dysbiosis including overly use of antibiotics, passive lifestyle, use of pesticides in farms, etc., may lead to antibiotic resistance against pathogens, increase obesity epidemic, inflammation, resistance to insulin, diabetic condition, heart diseases (CVDs), brain-related disorders, dyslipidemia, pathophysiological conditions such as allergy, intestinal inflammatory diseases, and even cancers (Amaral et al. 2012; Benbouziane et al. 2013; Andreu and Torrent 2015; Aydin et al. 2015). However, enhancing the functional repertoire of probiotics, the commensal organisms that can be harnessed for therapeutic benefit, is a promising approach to combat these issues. Probiotics can affect the host either directly or through their products, or even can influence the activity of resident bacteria in the host (Scott et al. 2015). The first health benefit report of probiotics was discussed by Russian scientist, Eli Metchnikoff (1907), according to whom "*the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes with useful microbes*." Later, WHO and FAO defined the probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (WHO/FAO 2006; Hill et al. 2014). There are several reports of beneficial impacts of probiotic organisms in metabolic disorders, inflammatory disorders, CNS related disorders, pathogen infections, dyslipidemia, and regulating

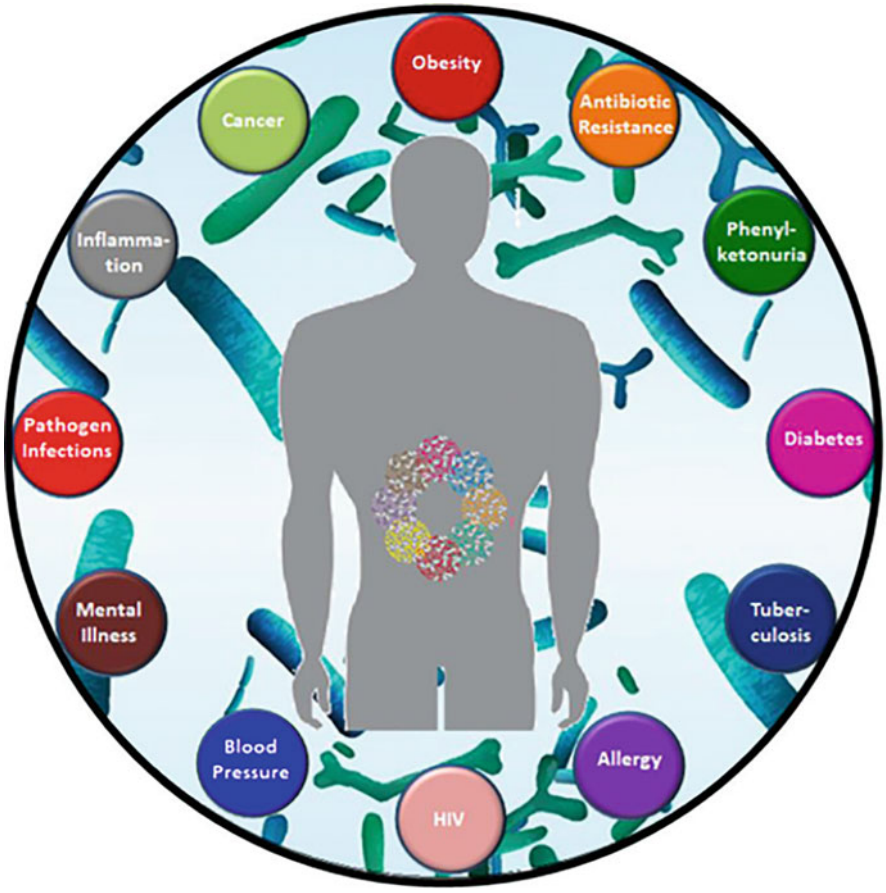

Fig. 12.1 Schematic illustration of health benefits of probiotics

mucosal immune response (Fig. 12.1) (Miettinen et al. 1996; Kwon et al. 2010; Yin 2010; Chen et al. 2011; Yan and Polk 2011; Klaenhammer et al. 2012; Asemi et al. 2013; Kim et al. 2013; Plaza-Diaz 2014; Reichold et al. 2014; Savcheniuk et al. 2014; Wang et al. 2014; Kasińska and Drzewoski 2015; Di Cerbo et al. 2016; Kobyliak et al. 2016; Nazemian et al. 2016; Wallace and Milev 2017).

12.2 Why Designer Probiotics?

The probiotics are categorized into mono-strain or multi-strain probiotics and it has been well documented that multi-strain probiotics poses significant positive effects due to symbiosis among the strains used in formulation (Timmerman et al. 2004). A list of few multispecies probiotic consortium and their positive impacts are shown in Table 12.1. However, recognition of the importance of microorganism–receptor

Table 12.1 List of probiotic consortium and their role in improving human diseases

Generic Name	Microorganisms	Role	Reference
De Simone Formulation	*Streptococcus thermophilus, Eubacterium faecium, Bifidobacterium breve, B. infantis, B. longum, Lactobacillus acidophilus, L. plantarum, L. casei, and L. delbrueckii subspecies bulgaricus*	Ulcerative colitis, gestational diabetes mellitus (GDM)	Venturi et al. (1999), Timmerman et al. (2004), Jafarnejad et al. (2016)
EcologicR tolerance/ SyngutTM	*B. lactis, L. acidophilus, L. plantarum, L. lactis*	To strengthen the gut barrier function, have beneficial effects on post-immunological induced stress, inhibit Th2, and stimulate IL-10 levels, thus providing beneficial effects in patients with food intolerance	Besseling-van der Vaart et al. (2016)
Ecologic AAD	*B. bifidum, B. lactis, B. longum, E. faecium, L. acidophilus, L. paracasei, L. plantarum, L. rhamnosus, L. salivarius*	Reduced diarrhea-like bowel movements when administered in healthy volunteers taking amoxicillin	Koning et al. (2008)
Multispecies probiotic consortium	*L. acidophilus, L. casei, L. rhamnosus, L. bulgaricus, B. breve, B. longum, S. thermophilus*	Prevented rise in fasting plasma glucose (FPG), to decrease high sensitivity C-reactive protein (hs-CRP), and to increase plasma glutathione (GSH) in diabetic patients	Asemi et al. (2013)
Ecologic® 641	*L. acidophilus, L. casei, L. salivarius, L. lactis, B. bifidum and B. infantis*	Bacterial translocation, morbidity and mortality in a rat model of acute pancreatitis	van Minnen et al. (2007)
Fermented milk	*L. rhamnosus, Propionibacterium freudenreichii, B. lactis*	Effect of a multi-strain probiotic on IBS: abdominal symptoms, quality of life, gut microbiota, inflammatory markers	Kajander et al. (2008)
Multistrain probiotic consortium	*B. longum, B. bifidum, B. lactis, L. acidophilus, L. rhamnosus, and S. thermophilus*	Inhibiting pathogen growth and atopic dermatitis, suggesting further application on other diseases like IBD	Yoon et al. (2013)

interactions in the pathogenesis of disease and limited probiotic strain diversity was the main drawback (Marotz and Zarrinpar 2016). To combat such issues, researchers used synthetic biology and genetic engineering approaches to develop recombinant probiotic strains, commonly called as "Designer Probiotics." Moreover, it was also postulated that genetic engineering of target specific probiotic strains or development of probiotics as a vehicle to carry vaccine and drug molecules is a promising approach (Braat 2006; Paton 2012; Kumar 2016; Maxmen 2017). Such designer probiotics as vaccine vehicle also offer an advantage of no possibility of reversion to a virulent phenotype, which always remains a threat with the attenuated pathogenic strains (Seegers 2002).

Moreover, according to researchers (Paton et al. 2006; Sleator and Hill 2008) this approach has several other advantages as well.

(i) Oral administration of probiotic,
(ii) Well characterized receptors recognized by enteric pathogens/toxins,
(iii) Inhibition of pathogen adherence leading to lower infection,
(iv) Sequestration of a toxin by the improved host immune system which prevent clinical symptoms,
(v) It does not apply a selective pressure on the pathogen, so development of resistance against it is very unlikely.

12.3 Strategies of Developing Designer Probiotics

Among various strategies to develop designer probiotics, few main approaches are as below:

12.3.1 Patho-Biotechnology Based Designer Probiotics

Patho-biotechnology describes the concept of using a pathogenic organism for beneficial use in the biotechnological application. This stands for the use of their ability to adapt against stress, their strong host invasion system and virulence related abilities to other different areas. Recently, it has been suggested to use them in the field of development of probiotics as food supplements (Sleator and Hill 2006). In addition, patho-biotechnology can also improve strain's resistance during manufacturing process and storage period for improved probiotic response. The most common example is the use of *Listeria monocytogenes, L. salivarius*, and *Bifidobacterium breve* for the development of targeted designer probiotics. For instance, the betaine transporter gene (betL) from L. monocytogene resulted in reducing the stress and improved the survival rate of probiotics (Hoffmann et al. 2013; Sleator et al. 2003a).

12.3.2 Receptor-Mimicking Based Designer Probiotics

In this approach, the probiotics are developed by engineering the expression of host-receptor-mimics on the surface of a commensal bacterium. Paton and group developed a designer probiotics for the prevention of gastrointestinal infections using a strategy involving the expression of host cell receptor-mimics on the surface of probiotic strains (Paton et al. 2006; Sleator and Hill 2008). Expression of two galactosyl-transferase genes (*lgtC* and *lgtE*) from *Neisseria gonorrhoeae* into *Escherichia coli* strain generated a lipopolysaccharide terminating in Gal(α1, 4) Gal(β1, 4)Glc, which mimics Shiga-toxin (*stx*) receptor and was found effective against shigatoxigenic *E. coli* (STEC) (Paton et al. 2000). Using a similar strategy to that mentioned above, an *E. coli* strain was engineered to produce a chimeric *LPS* receptor mimic capable of binding a heat-labile enterotoxin (Paton et al. 2005). Similarly, a probiotic strain with an altered *LPS* was designed by Focareta et al. (2006). This altered *LPS* receptor terminates in a structure that mimics the GM1 ganglioside terminus, which is the binding receptor for cholera toxin (Focareta et al. 2006). Similar approaches have also been used to develop probiotics for enterotoxigenic *E. coli* (ETEC) (Paton et al. 2005).

12.3.3 Synthetic Oligosaccharide-Based Designer Probiotics

In this approach, oligosaccharides in specific conformation or in multivalent interaction correspond to a given receptor epitope to inhibit the ligand binding (Zopf and Roth 1996; Mulvey et al. 2001). Using this strategy, a probiotic ("Synsorb-pk") comprising silica particles linked to synthetic Gal(α1,4)Gal(β1,4)Glc oligosaccharide was developed for severe gastroenteritis, which can progress to hemolytic uremic syndrome (HUS) (Paton and Paton 1998). Similarly, probiotic (Synsorb 90) displaying a Gal(α1,3)Gal(β1,4)GlcNAc epitope was developed against *Clostridium difficile* (Heerze et al. 1994). Merritt et al. (2002) developed a probiotic for cholera toxin consisting of a pentacyclic core, each displaying m-nitrophenyl-α-d-galactoside, which showed enhanced binding to the toxin. "SUPERTWIGS" having dendrimers with multiple tri-saccharides was also developed using similar strategy against an O157:H7 STEC (Nishikawa et al. 2002; Nishikawa et al. 2005; Watanabe et al. 2004).

12.3.4 Anti-Microbial Peptides Based Designer Probiotics

Probiotics produced from commensal bacteria are capable of expressing antagonism against pathogenic microorganisms. Expression of anti-microbial peptides with potential to overcome the antibiotic resistance among pathogens resulted in combined benefits of anti-microbial peptides as well as of probiotics (Proctor 2011; Reid et al. 2015). This can be achieved by cloning and expression of anti-microbial peptide specific genes in the probiotic bacteria.

12.4 Designer Probiotic Strains

Most of the probiotics as well as probiotic consortium are developed from *Bifidobacterium*, *Lactobacillus* species, and other lactic acid bacteria (LAB) or specific yeast strains (*Saccharomyces cerevisiae, S. boulardii, Kluyveromyces lactis,* and *Pichia pastoris*) (Govender et al. 2013). While on the other hand, when we consider designer probiotics, there are some specific promising bacterial species that are currently under consideration.

12.4.1 Faecalibacterium Prausnitzii

F. prausnitzii is a bacterium of Clostridium cluster IV (Martín et al. 2017), and its anti-inflammatory response proposed its usage as targeted anti-inflammatory drugs for Crohn's disease (Quévrain et al. 2016). In addition, it can induce the Clostridium-specific IL-10-secreting regulatory T cell subset, and reduce IL-12 and IFNg production, to maintain the gut barrier immune function (Quévrain et al. 2016). Also it is reported to reduce the severity of trinitrobenzene sulfonic acid (TNBS) colitis and correct the associated dysbiosis (Sokol et al. 2008). These studies suggest that *F. prausnitzii* can be regarded as a potential probiotic candidate for chronic gut inflammation and Crohn's disease (Sokol et al. 2008; Martín et al. 2017).

12.4.2 Akkermansia Muciniphila

Schneeberger et al. (2015) reported the impact of *A. muciniphila* in lipid metabolism, inflammatory markers in adipose tissue, regulation of glucose level, resistance to insulin, and occurrence of plasma. This led to investigate the role of this bacterium in adipose tissue homeostasis and metabolism. A study conducted by Dao et al. (2016) suggested *the A. muciniphila* is associated with body fat distribution and glucose homeostasis. While the effect of *A. muciniphila* in reversing the atherosclerotic lesson and improving the metabolic endotoxemia-induced inflammation and controlling the dysbiosis has also been reported (Li et al. 2016). All these prospects put *A. muciniphila* in the category of potential designer probiotics (Dao et al. 2016).

12.4.3 Bacteroides Fragilis

Bacteroides species are gram −ve, obligate anaerobe, and non-spore forming commensal bacteria, which constitutes approximately 25% of our gut microbiota (Wexler 2007). They passed from mother to infant during vaginal delivery and thus are considered as gut's primary colonizers. Among various Bacteroides species, *B. fragilis* is the most common and this produces an immunomodulatory molecule, polysaccharide A (PSA), which play a vital role in homeostasis and development of the host immune system and preserves the balance between T cell types (Troy and Kasper 2010; Round et al. 2011).

12.4.4 Bacteroides Uniformis

Oral administration of *B. uniformis* has shown considerable improvement in lipid profile, glucose insulin and leptin levels, TNF-a production, and phagocytosis in mice studies (Gauffin Cano et al. 2012). Moreover, it has also been reported that their administration can improve immunological dysfunction and metabolic disorder related to gut dysbiosis (Gauffin Cano et al. 2012; Yang et al. 2016).

12.4.5 Eubacterium Hallii

E. hallii is an anaerobic bacterium that resides in our gut and affects the gut metabolism (Engels et al. 2016). It is a natural butyrate producer and is supposed to lower mucosal inflammation and oxidative status, enhance the host–gut microbiota homeostasis, strengthen the gut barrier, increase energy metabolism, improve insulin sensitivity, and act as energy (Canani et al. 2011; Engels et al. 2016). Moreover, it has also been regarded safe as its high dose did not cause any negative impact (Udayappan et al. 2016).

12.4.6 Clostridium Cluster Members

Patients suffering from inflammatory bowel disease (IBD) have reduced number of bacterium related to Clostridium spp. clusters IV and XIVa, which are supposed to be exceptional Tregs (Regulated T cells) inducer in the colon (Atarashi et al. 2011). These Tregs are also considered as potential therapeutic agents for allergies and IBD (Atarashi et al. 2011). Later, Atarashi et al. (2013) again reported that these Clostridia clusters XIVa, IV, and XVIII were also playing role in Treg cell differentiation and accumulation (Atarashi et al. 2013). Moreover, these Clostridium clusters were also reported to produce short chain fatty acids (SCFAs) to improve the gut dysbiosis conditions (Atarashi et al. 2013).

12.4.7 Listeria Monocytogenes

It is a pathogenic bacterium, which as auxotrophic mutant or after selective elimination of virulence genes can be used as novel vaccine and drug delivery vehicles (Zhao et al. 2005). Also these bacterium can be used to incorporate stress tolerant genes into non-pathogenic probiotic strains (Sleator and Hill 2006). Such genes can support the survival of probiotic strain in stressful conditions such as gastric juice, bile juice, low pH, etc. (Mattila-Sandholm et al. 2002; Sleator and Hill 2007a). *L. monocytogenes* serves as an ideal candidate for this concept as its genome has been fully sequenced and can be manipulated to resisting numerous stresses and also eliciting a strong host immune response in probiotics (Glaser et al. 2001; Hamon et al. 2006; Gray et al. 2006; Lecuit 2005). *L. monocytogenes* has three solute uptake

genes *betL*, *gbu,* and *opuC* (Wemekamp-Kamphuis et al. 2002). Among them, *betL* is reported to be a betaine transporter gene (Sleator et al. 1999; Sleator et al. 2000; Hoffmann et al. 2013), whose cloning has contributed to probiotic survival under a variety of stresses (Sheehan et al. 2006; Sleator et al. 2003a).

12.4.8 Bifidobacterium Breve

B. breve UCC2003 strains expressing *betL* were shown to exhibit significantly increased tolerance to simulated gastric juice (pH 2.5) as well as osmotic stress. Moreover, the heterologous expression of the *BilE* system from *L. monocytogenes* would increase bile tolerance and subsequent gastrointestinal persistence of the probiotic strains (Sheehan et al. 2007). Interestingly, *B. breve* UCC2003 expressing *betL* gene has improved survival rate even in gastric juice (Sheehan et al. 2007), which may be attributed to the fact that improving compatible solutes accumulation leads to more physiologically robust probiotic strains (Sleator et al. 2003b).

12.5 Applications of Designer Probiotics in Metabolic Disorders

Enzymes are the crucial factors in cellular metabolism and their deficiency can result in metabolic disorder. Manipulating the gut ecosystem by designer probiotics expressing therapeutic biomolecules such as enzymes might serve as alternative approaches against metabolic disorders (Singh et al. 2017; Isabella et al. 2018; Kurtz et al. 2019).

12.5.1 Diabetes

Diabetes is a condition of hypo-secretion of insulin resulted in higher blood glucose levels in the body, which may subsequently create several acute health concerns such as cardiovascular disease and Alzheimer, etc. (Li et al. 2015). Diabetes are of 2 types, type 1 is related to the impaired cells in the pancreas and type 2 occurs due to insulin resistance (Klöppel et al. 1985). Compared to conventional insulin injection, use of probiotics capable of secreting pro-insulin or cytokines would be a better alternative because of less pain and negligible side effects (Vinay et al. 2005; Van Belle et al. 2011; Bluestone et al. 2010). Recently, several attempts have been made to engineer probiotics carrying interleukins (ILs), pro-insulin, glucagon like proteins (GLPs), and other therapeutic protein, which have shown proven records in combating diabetes in mouse models. Liu (2016) developed a probiotic strain of *Lactococcus lactis* expressing HSP65-6IA2P2 protein (pro-insulin autoantigen), which showed significant improvement in diabetes mellitus conditions among diabetic mice (Liu 2016). Oral administration of IL-10, and this designer probiotics with anti-CD3 showed stability against diabetes in 59% of tested mice (Liu 2016). Similarly, engineered *L. lactis* NZ9000 expressing fusion protein HSP65-6P277

was found effective against diabetes (Ma et al. 2014). Moreover, *L. lactis* harboring auto-antigen GAD65370-575 and IL-10 brought about stabilization in pancreatic inflammation (Robert et al. 2014). While designer probiotic strain of *Lactobacillus casei* induces SP (Usp45)-INS-specific antibodies, which improve the levels of IL-4 and protect them from pancreas injury (Schwenger et al. 2015). GLP-1 (1–37) fused with USP45-LEISS secretion marker and polyhistidine tag, expressed in *Lactobacillus gasseri* ATCC 33323 showed improved insulin secretion by converting rat cells into insulin-secreting cells (Duan et al. 2015). In another report, *Lactobacillus paracasei* expressing exendin-4 peptide also enhanced insulin secretion (Zheng et al. 2017).

12.5.2 Phenylketonuria (PKU)

Phenylketonuria (PKU) another metabolic disorder is caused by defect in the phenylalanine hydroxylase (PAH) that prevents the action of phenylalanine ammonia lyase (PAL), which breakdown the phenylalanine into ammonia and cinnamic acid (Wang et al. 2005). The rise in level of phenylalanine results in acute health concerns resulting in reduced intellectual ability, seizures, etc. (Mitchell et al. 2011). Researchers are working for developing probiotic strains expressing PAL gene to be a potential solution for PKU. Overexpressing the PAL gene from *Rhodosporidium toruloides* in *E. coli* significantly reduced the phenylalanine level in mouse model (Sarkissian et al. 1999). Similar results were obtained when *Lactobacillus reuteri* having PAL gene from *Anabaena variabilis* was administered (Durrer et al. 2017). Recently, *E. coli* Nissle 1917 was genetically engineered to overexpress PAL and L-amino acid deaminase to convert phenylalanine into phenylpyruvate, which substantially lowered the level of phenylalanine in blood (Isabella et al. 2018).

12.5.3 Hyperammonemia

Hyperammonemia is caused due to an enzymatic defect in metabolizing free ammonia to urea resulting in increased ammonia level in blood (Leonard and Morris 2002). This condition can be reversed using lactulose or antibiotics; however, the use of antibiotics may have many side effects (Auron and Brophy 2012). Recently probiotic strain SYNB1020 was developed from *E. coli* Nissle 1917 by deleting *thyA* and *argR* genes (−ve regulators for arginine translocation) and incorporating an *argA215* gene (N-acetyl glutamate) to convert ammonia into L-arginine. This designer probiotics on administration resulted in 50% improvement in the survival rate of hyperammonemia suffering mice model (Kurtz et al. 2019).

12.5.4 Parkinson's Disease

Parkinson's disease is linked with the low level of dopamine, a neurotransmitter, in the brain cells. The medicine, which is used for the treatment of Parkinson's disease,

is Levodopa. This levodopa is converted to dopamine on decarboxylation mediated by an enzyme acid decarboxylase (AADC) (Bergmann et al. 1974). However, besides brain, this levodopa can also be decarboxylated in gut causing side effects and reduced its bioavailability. Recently, pyridoxal-5-phosphate dependent tyrosine decarboxylase from *Enterococcus faecalis* was reported to decarboxylate the L-dopa, while *Eggerthella lenta* was found to have dopamine dehydroxylase (*Dadh*) gene for conversion of L-Dopa to m-tyramine in gut (Rekdal et al. 2019). They also observed that in dopamine dehydrogenase enzyme the variants have arginine at 506th position could metabolize L-Dopa while the variants with Serine at 506th position could not. This indicated that an SNP mutation in enzyme can predict the L-Dopa metabolism in complex gut microbiota (Rekdal et al. 2019).

12.5.5 Alzheimer's Disease

Alzheimer's disease is a chronic neurodegenerative condition caused by abnormal accumulation of amyloid and tau protein in and around the brain cell, which decreased the level of acetylcholine and resulted in memory loss and cognitive loss (Selkoe and Hardy 2016). Recently, its progression was found to be linked with dysbiosis in gut microbiota, which caused accumulation of amino acids. This amino acid accumulation further activates the M1 microglia that is responsible for cognitive loss (Wang et al. 2019). For the reversal of this condition, a drug molecule (GV-971), a mixture of acidic linear oligosaccharides with varied degree of polymerization, was used to improve cognition by re-establishing the gut flora (Wang et al. 2019). This suggests that designer probiotic strains linked to these oligosaccharide molecule will be a potential candidate to improve this metabolic disease.

12.5.6 Obesity

Obesity is now considered a worldwide pandemic, which is associated with alteration in gut flora (Robert et al. 2014). Researchers have attempted to engineer probiotics having potential to deliver therapeutic molecules such as leptins and N-acylphosphatidyl-ethanolamines (NAPEs) to reduce the obesity (Steidler et al. 2000). Stritzker and Szalay (2013) reported reduction in the level of obesity in mice administered with *E. coli* expressing NAPEs. Similarly, *E. coli* Nissle 1917 expressing NAPEs was found to reduce obesity in mice (Chen et al. 2014), while those expressing pyrroloquinoline quinone and fructose dehydrogenase facilitate in treating fructose induced hepatic steatosis in mice (Somabhai et al. 2016).

12.5.7 Angiotensin Level Linked Hypertension

Angiotensin is a hormone, which functions as vaso-contractor, i.e., it narrows the blood vessels, which resulted in hypertension (high blood pressure conditions),

however if the receptors for angiotensin are blocked, this condition can be reversed. In this aspect, angiotensin-converting enzyme inhibitory peptides (ACEIPs) are reported to reduce the blood pressure by relaxing the blood vessels. Yang et al. (2015) engineered a probiotic strain *Lactobacillus plantarum* NC8 expressing ACEIPs, which on administration to mice was observed to decrease the angiotensin levels and subsequently reduced the blood pressure.

12.6 Challenges in Development of Designer Probiotics

The use of designer probiotics has the potential to significantly impact the mortality/morbidity rates. However, being genetically engineered, these designer probiotics contain additional genetic elements for inducing antigenicity, immunomodulation, and effect on normal metabolic pathways, and hence safety of bioengineered probiotics is an important issue (Singh et al. 2016). Besides the issue of biological containment, the stress due to change in water activity and temperature are other challenges for probiotics (Sleator and Hill 2007b), Also, the viability percentage of the bacterial strain during product manufacturing and storage processes is also a matter of concern (El Hage et al. 2017).

In addition, the probiotics have also to face the regulatory issues, as worldwide they are also classified into different categories depending on their use in a particular condition. For instance, in the USA, as a dietary supplement, probiotics are considered as "food" and should be regulated by the Dietary Supplement Health and Education Act (DSHEA); and in case of therapeutic purpose, the probiotic comes under drug category and hence it must be approved by the FDA. On the other hand, in Japan probiotics are categorized as foods as well as drugs. Since, the classification and definition of probiotics by different regulatory bodies vary across world; the regulatory status of these probiotics is still unknown (El Hage et al. 2017).

12.7 Conclusions

Development of antibiotic resistance in pathogenic bacteria and increase in metabolism related diseases have led an urge to search for alternative cost-effective approaches to conventional antibiotic prescription. In this aspect, probiotics have shown proven records for health benefits by preventing illness, increasing immunity and maintaining the gut microbiome. Moreover, with the growing understanding about the synthetic biology and genetic engineering of microbes, researchers are now inclining towards development of recombinant "designer probiotics" to be used as drug delivery system, gene therapy vectors, invaders to pathogenic microbes and also career of therapeutic proteins in a more target specific manner. These designer probiotics comprising an anti-microbial, anti-inflammatory, and immunomodulatory repertoire not only help the host against infectious diseases but also their target specific nature reduce the production level and subsequently the cost. Few designer probiotics strains and their role in prevention and treatment of human diseases are listed in Table 12.2.

Table 12.2 List of designer probiotic organisms depicting their engineered element/proposed hypothesis in reducing the metabolic disorders

Metabolic disorders	Probiotic organism	Genetically modified element/ Proposed hypothesis	Reference
Hypertension	*Lactobacillus plantarum NC8*	Expressing ACEIP coding sequences from TFP and YFP joined by an arginine linker	Yang et al. (2015)
Obesity	*Escherichia coli*	N-acylphosphatidylethanolamines (NAPEs)	Chen et al. (2014)
	E. coli	N-acylphosphatidylethanolamines (NAPEs)	Stritzker and Szalay (2013)
	E. coli	Genes for pyrroloquinoline quinone and fructose dehydrogenase	Somabhai et al. (2016)
Hyperammonemia	*E.coli*	Deletion of thyA and argR genes and integration of argA215 gene	Kurtz et al. (2019)
PKU	*E. coli*	PAL gene	Sarkissian et al. (1999)
	Lactobacillus reuteri	PAL gene	Durrer et al. (2017)
	E. coli	PAL gene and L-amino acid deaminase	Isabella et al. (2018)
Diabetes	*Lactobacillus gasseri*	Glucagon like protein GLP-1 (1–37)	Duan et al. (2015)
	Lactococcus lactis	HSP65-6IA2P2 protein	Liu (2016)
	Lactococcus lactis	Fusion protein HSP65-6P277	Ma et al. (2014)
	L. lactis	Auto-antigen GAD65370-575	Robert et al. (2014)
	Lactobacillus casei	SP (Usp45)- INS-specific antibodies	Schwenger et al. (2015)
	Lactobacillus paracasei	Exendin-4 peptide	Zheng et al. (2017)
Parkinson's disease	Gut microbiota	Integration of tyrosine decarboxylase and dopamine dehydroxylase (Dadh)	Rekdal et al. (2019)
	Inactive DadH producing gut microbiota	SNP mutation (Arg506 in place of Ser506) of inactive dopamine dehydrogenase present in gut microorganisms for conversion of L-Dopa to m-tyrosine	Rekdal et al. (2019)
Alzheimer disease	Probiotic strains	Synthetic oligosaccharides used in GV-971	Wang et al. (2019)

Though this concept is gaining interest and popularity in view of long-term protection against chronic diseases, however, the issue of biological containment and consumer acceptance of recombinant probiotics is still a significant roadblock. In conclusion, we are optimistic that utilizing the advancement in technologies such

as synthetic biology, genetic engineering, and patho-biotechnology, research in this area will continue to generate stable recombinant designer probiotics with strongly regulated gene expression and clearly demonstrable medical benefits. Moreover, with the use of rigorous biological containment strategies, detailed risk analysis, scientific evidences, and consumer education, these designer probiotics will attain broader acceptance in the near future.

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