

Biofilm-based delivery approaches and specific enrichment strategies of probiotics in the human gut

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ABSTRACT

The use of probiotics has been one of the effective strategies to restructure perturbed human gut microbiota following a disease or metabolic disorder. One of the biggest challenges associated with the use of probiotic-based gut modulation strategies is to keep the probiotic cells viable and stable during the gastrointestinal transit. Biofilm-based probiotics delivery approaches have emerged as fascinating modes of probiotic delivery in which probiotics show significantly greater tolerance and biotherapeutic potential, and interestingly probiotic biofilms can be developed on food-grade surfaces too, which is ideal for the growth and proliferation of bacterial cells for incorporation into food matrices. In addition, biofilms can be further encapsulated with food-grade materials or with bacterial self-produced biofilms. This review presents a newly emerging and unprecedentedly discussed techniques for the safe delivery of probiotics based on biofilms and further discusses newly emerging prebiotic materials which target specific gut microbiota groups for growth and proliferation.

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Introduction

Human body harbors trillions of diverse microorganisms (bacteria, protozoa, archaea, eukaryotes, and viruses), which reside on and within different parts of the body including the gut, skin and vagina. The human gastrointestinal tract contains the largest number and diversity of the known species repertoire of the human gut microbiota (> 70% of the human microbiota: 10^{13} to 10^{14}), termed “gut microbiome”¹. A stable gut microbial community of the host plays a key role in host’s innate and adaptive immune system,² metabolism, and health.³ Disruptions in the gut microbiota may occur owing to antibiotic use, changes in lifestyle, dietary habits, infection, or ageing, which lead to variegated pathogenic, metabolic, and inflammatory conditions. Today, there is almost no disease which has no relevance with the human gut microbiota. From intestinal bowel diseases (Crohn’s disease and ulcerative colitis),⁴ cancer,⁵ hypertension,⁶ and diabetes⁷ to mental health including anxiety

and depression⁸ and metabolic syndrome and atopy.⁹

In appreciation of microbial signatures of health and disease, microbiome-modulating interventions, especially the use of probiotics, prebiotics, postbiotics, synbiotics, live biotherapeutic products, and faecal microbiota transplants (FMT) (see definitions in Box 1), have gained escalating interest in microbiome research and clinical and healthcare settings.¹⁰ Out of these approaches, FMT is considered as the most effective therapy which greatly supports microbiota colonization; however, its complicated procedure, high cost, and risk of overt immune reaction and transmission of potential pathogens are critical hurdles.¹¹ Probiotic therapy (mainly orally) is also considered as a great intervention strategy for gut microbiota modulation and indeed a promising strategy for clinical translation because of being less costly, safer, and controllable.

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Box 1. Definition of the terms probiotics, prebiotics, postbiotics, synbiotics, biotherapeutic products, and fecal microbiota transplant

Probiotics	"Live microorganisms which when administered in adequate amounts confer a health benefit on the host"	17
Postbiotics	"Preparation of inanimate microorganisms and/or their components that confers a health benefit on the host"	18
Prebiotics	"A substrate that is selectively utilized by host microorganisms conferring a health benefit"	19
Synbiotic	"A mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host"	20
Biotherapeutic products	"Live organisms designed and developed to treat, cure, or prevent a disease or condition in humans"	21
Fecal microbiota transplant	"A treatment that involves administration of minimally manipulated microbial community from stool of a healthy donor into the patient's intestinal tract"	22

Probiotics are not only used to aim restructuring of microbial dysbiosis but also they impart many useful functions to the gut including modulation of innate immune system (mainly by gut microbiota modulation), prevention of pathogen colonization, enhancement of gut barrier function, mucin production, and increased expression of tight junction proteins. Benefits of probiotics on several human health outcomes are unanimously acknowledged and reported. Probiotic enrichment and delivery in the human gut is crucial to achieve health benefits, but these techniques have met with substantial challenges for therapeutic applications and thus it is imperative to consider the principles that govern the successful establishment and persistence of probiotics in the gut environment. Many health benefits of probiotics are attenuated mainly due to a substantial loss in the viability during the gastrointestinal transit.¹² The key to improving colonization of a bacterial species in the gut is to deliver it in a material resistant to many environmental stressors (for example, pH and bile salts) or to convert it into its biologically resilient form (e.g., biofilms). A biofilm is an assemblage of surface-adhered microbial cells embedded in self-produced extracellular polymeric substances (EPS) containing proteins,

extracellular DNA (eDNA), polysaccharides, and lipids.¹³ Any exogenously introduced gut bacterium which does not form biofilm into a new environment is likely to be eliminated quickly, even when delivered in abundance; this is the reason behind the low efficiency of many probiotic strains.¹⁴

Several reviews have been published on the encapsulation of probiotics with a focus on several techniques (e.g., extrusion, emulsion, electrospinning, freeze drying, spray chilling, fluidized bed, spray drying, spray-freeze drying, coacervation) and materials, such as alginate, microbial polysaccharides, prebiotics, gums, mucilages,¹⁵ and bacterial spore coat nanomaterial.¹⁶ The focus of this review is on novel biofilm-based encapsulation systems and biofilm-based strategies to enhance oral bioavailability and gastrointestinal delivery of probiotics. We have also emphasized on the need of developing specific prebiotics which proliferate and enrich specific bacterial groups in the human gut.

Probiotics and the human gut microbiota

Human gut microbiota contains a large diversity of bacteria, viruses (mostly bacteriophages), and fungi which live together in an intricate balance and dynamic equilibrium. The symbiotic relationship between gut bacteria and host is very old as these symbiotic microorganisms and animals have evolved together as a result of the co-evolution that dates million years back.^{23,24} Bacterial colonization of humans starts from the intrauterine period when the fetus is exposed to the microbiota from mother through transplacental passage into amniotic fluid.²⁵ Birth method (full term vaginal delivery or caesarean) also affects the acquisition and development of the gut microbiota of new born babies.²⁶ Microbiota is further affected by introduction of oral liquid feedings (breast milk or formula milk) and food intake, and its structure keeps changing until approximately 3 years when gastrointestinal tract represents mature microbiome.²⁵ Once established, the composition of the gastrointestinal microbiota remains relatively stable throughout adult life, but factors like disease conditions, use of antibiotics, surgical treatments, lifestyle, and long-term changes in diet cause a shift in microbiota.

The microbiota-host synergy guards the balance of the gut microbiota against invasion of pathogens. Thus, the balanced microbial community of the human gut has implications for health, immunity, and diet and nutrition. Perturbations of the gut microbiota, termed gut dysbiosis, are clearly associated with many diseases including colitis, liver diseases, diabetes mellitus (including type 2 diabetes), obesity, cardiovascular diseases, irritable bowel syndrome, allergy, asthma, protein conformational diseases, many neurodegenerative disorders, food allergies^{3,27-31} as well as viral infections.³² Disruption of the gut microbiota may foster dominance of harmful pathogens over beneficial bacterial groups, for example, short chain fatty acid (SCFA)-producing or butyrogenic bacteria. Enterobacterial blooms have been widely reported in gut dysbiosis in various conditions related to inflammation in the gut.³³ A recent study showed that colonization of the *Caenorhabditis elegans* gut with Gram-negative enteric bacterial pathogens led to aggregation and proteotoxicity of polyglutamine across several tissues of the body; whereas replacement of the microbiota with butyrogenic bacteria reversed the adverse effects by increasing host proteostasis and suppressing proteotoxicity pathogen-mediated aggregation of polyglutamine.²⁸

Administration of bacteriocin-producing *Pediococcus acidilactici* strains to mice led to an increase in *Ruminococcus* and *Lactobacillus* and a decrease in *Blautia* sp, which shows probiotics may also affect gut microbiota dynamics, possibly due to their metabolites, among many other factors.³⁴

Probiotics are used to restore the original gut microbiota structure which has been shown to have ameliorative effects on several metabolic disorders and diseases. Intake of probiotics does not necessarily mean enrichment of a specific bacterial group in the human gut, but microbial synergistic interactions foster growth of other bacterial groups too or in many cases trigger targeted bacterial growth, which would not have been possible otherwise. Sadiq et al. demonstrated that co-occurrence of four bacterial species of the human gut was complimentary for enhanced colonization on mucin and better growth.³⁵ Another recent study showed that treatment with multiple probiotics (three strains of *Lactiplantibacillus plantarum*)

diversified the gut microbiome of the subjects and resulted in an average increase of the beta diversity by 37.2% between-individual as a result of synergistic and modulatory effects of the specific strains in combination with the gut microbiota.³⁶ Similarly, a recent study showed that feeding with a mixture of four *Bifidobacterium* species to mice resulted in a more favourable gut ecosystem with a significant increase in the abundance of *Lactobacillus*, leading to mitochondrial fitness and the IL-10-mediated suppression of Treg cells.³⁷

Malnutrition in children has been related to perturbations in the normal development of the gut microbiota. Specific probiotic strains of the gut origin could potentially be exploited to resolve undernutrition syndromes through modulating the gut microbiota.³⁸ Table 1 shows evidence from different studies where probiotic strains ameliorated different diseases and metabolic conditions through modulation of the human gut microbiota. In addition to their role in gut microbiota modulation, many probiotics produce complimentary enzymes required for normal metabolism as without certain bacterial taxa in the human gut, many food components may remain indigestible owing to the paucity of alimentary enzymes.⁶¹ For instance, enzymes to digest xyloglucans are only encoded by a single, complex gene locus present in *Bacteroides ovatus*.⁶²

Most of the probiotics that are used in either commercial probiotic development or probiotic research mostly belong to limited genera, such as *Bifidobacterium* and *Lactobacillus* and less commonly to *Saccharomyces*, *Bacillus*, *Escherichia*, and *Weissella*. With advancements in gut microbiome science, we are on the cusp of a new era of research on probiotics and synthetic microbial communities to abolish intestinal imbalance as a result of diseases and to meet consumer's demands. An astounding range of candidates for probiotics have emerged many of which have yet to adapt the gut environment. Disease-specific next-generation probiotics have rapidly attracted much more attention than traditional probiotics with non-specific effects.⁶³ In 2008, a clinical study on humans demonstrated the role of *Faecalibacterium prausnitzii* in inflammatory bowel disease (IBD) pathogenesis, where the pathological conditions of colitis were significantly related to low count of *F. prausnitzii*

Table 1. Role of probiotics in ameliorating various diseases and metabolic conditions through modulation of the gut microbiota.

Name of the probiotic	Targeted disease/ metabolic condition	Targeted gut microbiota	Type of the model	Reference
<i>Lactobacillus acidophilus</i> EG004	Cognitive conditions	An increase in the Firmicutes and Proteobacteria phyla	A mouse model	39
<i>Pediococcus pentosaceus</i>	Colorectal cancer	Increased the abundance of Akkermansia-Verrucomicrobia clade	A mouse model	40
EcologicBarrier consisting of <i>Lactobacilli</i> , <i>Lactococci</i> , and <i>Bifidobacteria</i>	Stress	Increased the abundance of <i>Butyrivibrionas</i> , <i>Parabacteroides</i> , <i>Alistipes</i> , <i>Christensenellaceae_R-7_group</i>	Human subjects	41
<i>Lactobacillus helveticus</i> R0052 and <i>Bifidobacterium longum</i> R0175	Anxiety-like behavior	An increase in the population of <i>Prevotella</i> and <i>Bifidobacterium</i> species	Hamster model	42
<i>Lactiplantibacillus plantarum</i> TWK10	Aging-related disorders	Increased population of SCFA-producing bacteria	A mouse model	43
<i>L. plantarum</i> CCFM8610	Irritable bowel syndrome	Increased population of butyrate-producing bacteria (e.g., <i>Anaerostipes</i> , <i>Anaerotruncus</i> , <i>Bifidobacterium</i> , and <i>Butyrivibrionas</i>), and decreased the abundance of bacterial genus causing bloating (<i>Methanobrevibacter</i>)	A mouse model	44
<i>L. Plantarum</i> PL-02	Fatigue and tiredness	Increased the abundance of <i>Akkermansia muciniphila</i> and reduced the population of <i>Blautia coxoides</i> and <i>Pedobacter kwangyangensis</i>	A mouse model	45
<i>L. Plantarum</i> P8	Stress	Increased the abundance of <i>Bifidobacterium adolescentis</i> , <i>Faecalibacterium prausnitzii</i> , and <i>B. longum</i>	Human fecal samples	46
<i>Lactobacillus paracasei</i>	Type-2 diabetes	A significant increase in <i>Bacteroidales_S24-7_group</i> and the families <i>Ruminococcaceae</i> and <i>Lachnospiraceae</i>	Type 2 diabetic (T2D) rat model	47
<i>Lactobacillus rhamnosus</i> LRa05	Hyperglycemia	An increase in short-chain fatty acid producing bacteria (<i>Bacteroides</i> and <i>Alloprevotella</i>) and a decrease in proinflammatory bacteria (<i>Mucispirillum</i> and <i>Odoribacter</i>)	Type 2 diabetic mice	48
<i>Lactobacillus reuteri</i> FGSZY33L6 and <i>L. rhamnosus</i> FJ5YC4-1	Metabolic syndrome	Increased the relative abundance of <i>Desulfovibrio</i> whilst decreasing the abundance of <i>Romboutsia</i>	Mice with a high-fat diet	49
<i>Bifidobacterium breve</i> , <i>Bifidobacterium bifidum</i> , <i>B. longum</i> , and <i>Bifidobacterium lactis</i>	Colitis	Increased the abundance of <i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Kosakonia</i> , and <i>Cronobacter</i>	A mouse model	37
<i>L. plantarum</i> DR7	Stress and anxiety	Increased alpha diversity and an increase in bacteria associated with dopamine and serotonin (<i>Bacteroides</i> and Firmicutes)	-	50
<i>B. breve</i> , <i>B. bifidum</i> and <i>B. longum</i>	Dextran sodium sulfate induced colitis	Decreased the abundance of pathobionts such as <i>Klebsiella</i> , <i>Haemophilus</i> and <i>Lachnospira</i>	A mouse model	51
<i>L. plantarum</i> HT121	Obesity	Restored Firmicutes/Bacteroidetes ratio	A mouse model	52
Probiotic-4 (<i>B. lactis</i> , <i>B. bifidum</i> , <i>Lactobacillus casei</i> , and <i>L. acidophilus</i>)	Memory deficit	Decreased the abundance of Proteobacteria, <i>Pseudomonas</i> and <i>Lachnospiraceae</i> NK4A136 group	A mouse model	53
<i>L. reuteri</i>	-	-	-	-
<i>B. bifidum</i> CCFM16 and <i>L. plantarum</i> CCFM8610	Atopic dermatitis	Increased the proportion of <i>Bacteroidetes</i>	Randomized controlled human trial	54
<i>Bifidobacteria adolescentis</i>	Atopic dermatitis	Increased the abundance of <i>Lactobacillus</i> species and decreased the abundance of <i>Dorea</i> and <i>Pediococcus</i>	A mouse model	55
<i>L. reuteri</i>	Asthma	<i>Lactobacillus</i> and <i>Enterococcus</i> abundance	A mouse model	56
<i>L. Plantarum</i> ZDY2013	Colitis	Increased the abundance of <i>Lactobacillus animalis</i>	A mouse model	57
-	-	Stabilized the abundance of <i>Alistipes</i> , <i>Bacteroidetes</i> and <i>Alloprevotella</i>	-	-
<i>L. Plantarum</i> LIP-1	Hyperlipidaemia	Reduced lipopolysaccharide-producing bacteria (e.g., <i>Sutterella</i> , <i>Oscillibacter</i> , and <i>Bilophila</i>) and increased the abundance of SCFA-producing bacteria such as <i>Lactobacillus</i> , <i>Ruminococcus</i> , <i>Eubacterium</i> , <i>Alloprevotella</i> and <i>Coprococcus</i>	Rats	58
<i>L. plantarum</i>	Chronic undernutrition	The gut microbiota which interacts with the somatotrophic hormone axis	A mouse model	59
<i>Lactobacillus curvatus</i> HY7601 and <i>L. plantarum</i> KY1032	Obesity	Increased the abundance of 11 species of phylum firmicutes	A mouse model	60

⁶⁴ and this was further supported by subsequent studies.^{65,66} The therapeutic potential of this commensal bacterium (as probiotic) and its supernatant (as postbiotic) has been demonstrated in chronic colitis and colitis reactivation in a TNBS (2,4,6-Trinitrobenzene sulfonic acid)-induced acute colitis mouse model.^{67,68} Ameliorative effects of *F. prausnitzii* through gut microbiota modulation have now been reported in many metabolic diseases such as, obesity⁶⁹ and liver diseases.⁷⁰ In addition, many other next-generation probiotics have shown beneficial roles in the prevention of carcinogenesis by maintaining intestinal barrier integrity, improving immunotherapy efficacy, producing beneficial metabolites, and acting against pathogens.⁷¹

Controlled delivery of probiotics

Transplanting probiotics to the gut microbiome can positively modulate bacterial population dynamics and structure, and thus probiotic intervention strategies remain of great interest for disease prevention and treatment. The biggest challenge in the development of probiotic-based dietary and therapeutic supplements and in achieving their clinical efficacy is related to their targeted delivery to specific sites in the human body. Orally administered probiotics encounter several challenges during the gastrointestinal transit, and these include host and microbial enzymes (e.g., saliva in the mouth), digestive enzymes (e.g., acidic gastric fluids in the stomach, pancreatic juice and bile in the small intestine), and colonization resistance (e.g. in the colon where bacterial density is 10^{11} to 10^{12} CFU/ml) from commensal bacteria.¹²

Therefore, effective probiotics delivery systems and bioinspired strategies are needed to effectively use the potential of probiotics through improved tolerance during the gastrointestinal transit and rapid colonization.

Immunological and anti-inflammatory aspects probiotic biofilms

Bacterial biofilms are communities of bacteria derived from either a single or multiple bacterial species, which grow on biotic or abiotic surfaces, or remain free floating as an aggregate in liquid or at

interfaces. Biofilm is a bacterial life phase which is remarkably different from the same bacteria in planktonic form in terms of physiology, genetics, and sometimes morphology and whereby specific metabolic activities are juxtaposed. Biofilm-dwelling cells remain protected from various environmental stressors (for example, antimicrobials) and bacteriophages because of complex self-produced or host-derived polysaccharide sheets, which comprise extracellular DNA (e-DNA), proteins, and lipids.⁷² Due to multifaceted material properties of probiotic bacterial biofilms (for example, chemically active, resilient, hydrophobic, sticky, slimy, conductive, self-healing, and safe), they can be used in a broad range of applications. In addition, properties of biofilms can be modified and improved further. For instance, porosity of biofilms can be changed which improves the biofilm permeability toward nutrients and electrons and it ultimately improves the viability of bacterial cells enclosed within the matrix. Various biologically important compounds can be integrated into the biofilm matrix such as minerals, nanoparticles, enzymes, and metal ions which make the biofilm functionally diverse and induce crosslinking effects. Biofilms can be further encapsulated with polymers and minerals to make them further tolerant towards environmental stresses.⁷³

Probiotics delivered in the form of biofilm confer several advantages on the host (Figure 1), compared to their conventional delivery in the planktonic form. Probiotic bacteria can be effectively delivered in their biofilms where they remain viable during the gastrointestinal transit and show more adherence characteristics. Probiotics in the form of biofilms remain unaffected or at least less affected by saliva, gastric fluid, pancreatic juice, bile acids, antimicrobial agents, laminar/turbulent fluid forces, effectors of the host immune defence system, as well commensals, pathogenic bacteria and bacteriophages. In an *in vivo* swine study, clinical *Bacillus subtilis* probiotics which were coated with self-produced biofilms exhibited a 17-times higher intestinal colonization and 125-fold greater oral bioavailability compared to uncoated bacteria.⁷⁴ Similarly, *Lactobacillus reuteri* when delivered in a biofilm formulation showed increased survival during the gastrointestinal transit and proved to be more effective in reducing NEC in a rodent

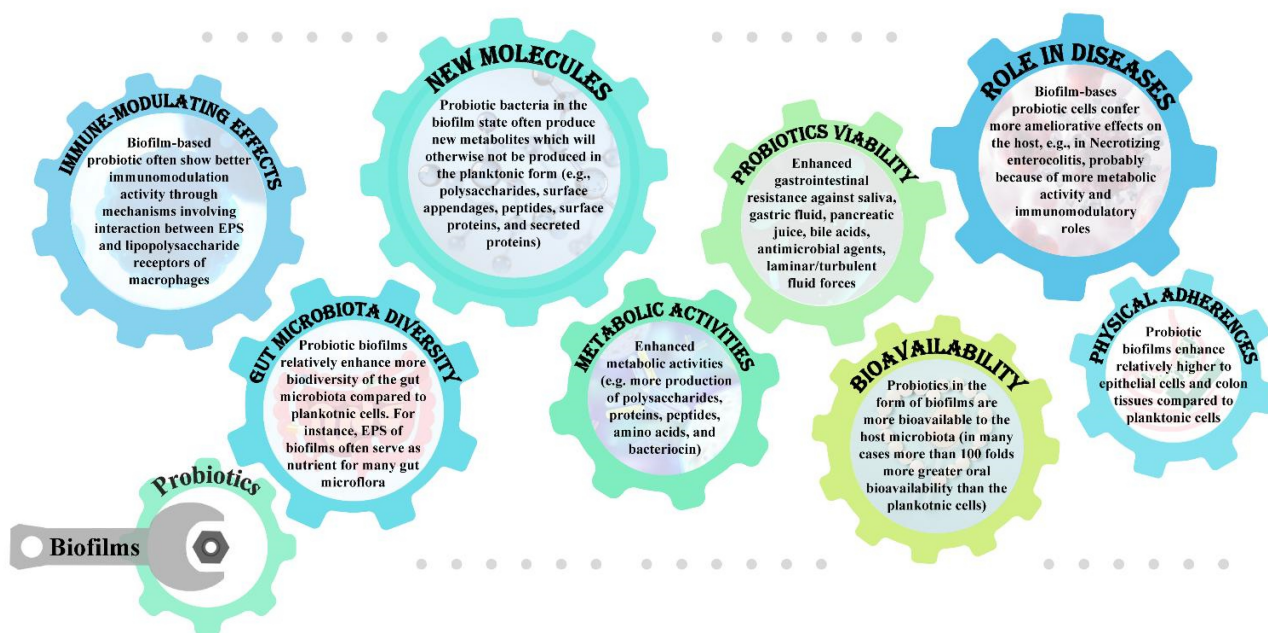


Figure 1. Major advantages of administering probiotics in the form of biofilms.

model.⁷⁵ Biofilm-dispersed cells may show more adherence potential compared to their counterpart planktonic cells. This has been evidenced from *in vitro* and *in vivo* study of *Klebsiella pneumoniae* cells spontaneously dispersed from biofilm. Biofilm-dispersed cells showed greater adhesion ability on biotic and abiotic surfaces.⁷⁶

Bacterial delivery in the form of biofilms is not only ideal because of enhanced physical adhesion and chemical barrier functions, but several reports indicate many beneficial effects of probiotic biofilms in terms of bacterial metabolic activities. Biofilms may exacerbate the beneficial effects of probiotics to host cells and in many cases probiotic bacteria in the form of biofilms may confer functions potentially more advantageous to the host. Bacterial cells in biofilms can also produce new molecules or larger quantities of certain molecules (e.g., polysaccharides, surface appendages, peptides, surface proteins, and secreted proteins) than counterpart planktonic cells.⁷⁷ EPS provide additional functional advantages to the host which could not be achieved in the planktonic probiotic deliveries. Using untargeted metabolomics, it was recently reported that amino acids and carbohydrate metabolisms were greatly enhanced when the probiotic strain *Lactobacillus paraplantarum* L-ZS9 was delivered in the form of biofilms compared to its planktonic dose. Further, biofilm-based

probiotic doses showed better immunomodulation activity and enhanced intestinal diversity of *Lactobacillus* microbiome in the dogs.⁷⁸ Biofilm exopolysaccharides are produced abundantly in probiotic biofilms, like other bacterial biofilms, and thus protect bacterial species and confer many other metabolic advantages to the host. A fourfold increase in exopolysaccharide production by *B. bifidum* was reported in its biofilm compared to planktonic cells in a study involving untargeted metabolomics.⁷⁹

There are several ways in which probiotic bacteria in the form of biofilms have better immunomodulatory and ameliorative effects compared to planktonic cells, and these are mainly related to better survival in biofilms and more EPS production (Figure 2). EPS from probiotic biofilms may interact with several carbohydrate recognition receptors present on the intestinal epithelial cells and such interactions are involved in modulating immune response. A plethora of immunomodulating effects of EPS produced by *Lactobacillus* and *Bifidobacterium* strains has been described.⁸³

Polysaccharides of bacterial biofilms interact with toll-like receptors (TLRs: TLR2 and TLR4 and C-type lectins) and induce the immune response in macrophages.^{81,84} It has been shown that EPS of bacterial biofilms can induce T cell-

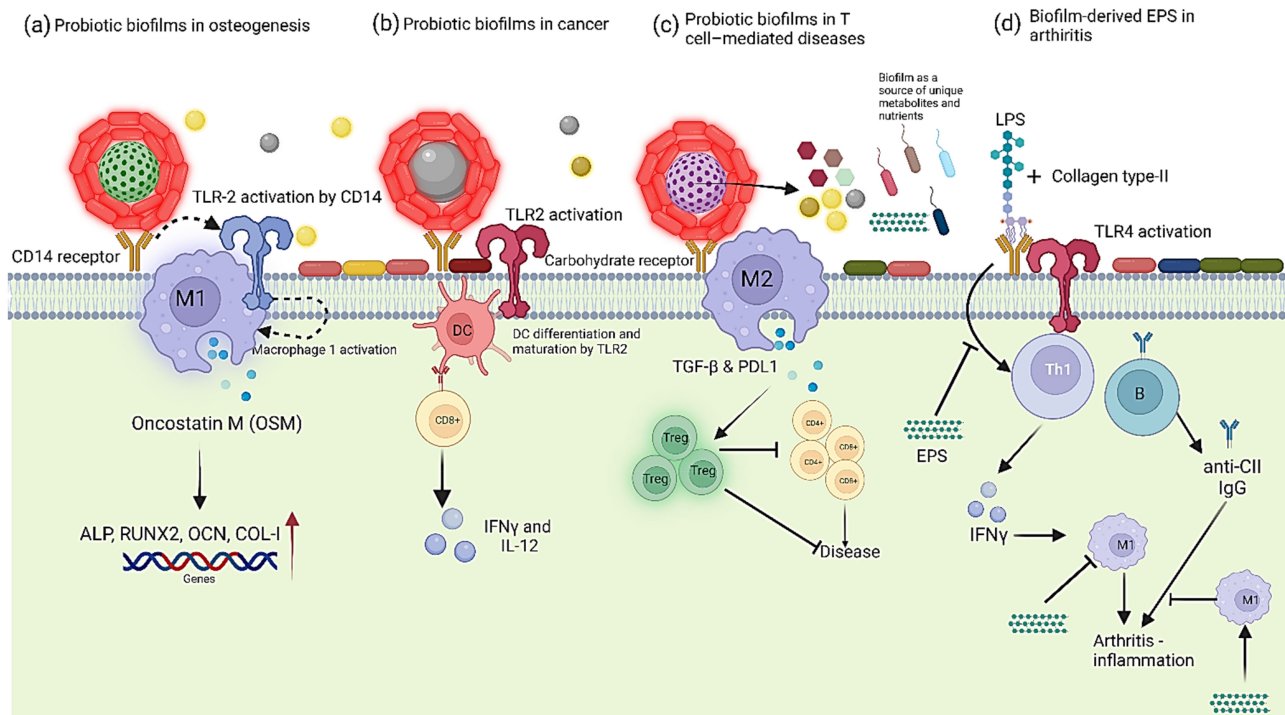


Figure 2. Probiotics in the form of biofilms are more effective than planktonic probiotic doses in several ways, especially in ameliorating diseases, as shown above in five different sections. (a) Polysaccharides on the surface of probiotic biofilms attach on CD14 of macrophages which leads to the activation of toll-like receptor cells (TLRs) signalling pathway. CD14 (cluster of differentiation 14) activates TLR2 to boost M1 macrophages phenotype which results in enhanced production of osteoinductive cytokines such as oncostatin M (OSM) to improve osteogenesis and it is evidenced by upregulation of osteogenic-related genes: runt-related transcription factor 2 (RUNX2), osteocalcin (OCN), and type I collagen (COL-1)⁸⁰ (b) Smectite laden with lactic acid bacteria biofilms inhibit tumor growth by activating dendritic cells (DCs) via Toll-like receptor 2 (TLR2) signalling (c) Exopolysaccharides from probiotic biofilms can induce M2 macrophages that inhibit CD4+ and CD8 + T cells by producing TGF- β and PD-L1 and possibly by induction of Tregs to prevent T-cell mediated diseases⁸¹ (d) EPS from some LAB strain may inhibit T-cell proliferation and production of IFN- γ which leads to the polarization of M2 macrophage (an anti-inflammatory effect) and facilitates suppression of arthritogenic CII-specific IgG (T cell-dependent humoral response) – adapted from Nowak and colleagues.⁸² In addition, it has also been shown EPS from biofilms serve as a source of nutrients for commensals and probiotic biofilms also produce biofilm-specific metabolites which are not produced by planktonic doses.

dependent immune responses in case of several inflammatory diseases. For instance, EPS produced by probiotic *Lactobacillus rhamnosus* KL37⁸² and *B. subtilis*⁸¹ inhibited T-cell proliferation and stimulated the production of IFN- γ . Lipopolysaccharides, on the outer surface of Gram-negative bacteria, are the main ligand for TLR4. Binding of LPS to TLR-4 leads to the activation of adaptor proteins like MyD88 and molecules like TIR-domain-containing adaptor-inducing β -interferon (TRIF) which are involved in intracellular signalling TLRs – especially TLR4. Adapter proteins phosphorylate and translocate NF- κ B to the nucleus.⁸⁵ NF- κ B induces the expression of various genes involved in pro-inflammatory processes (e.g., cytokines and COX2), for examples those encoding cytokines and chemokines, and they also regulate

inflammasome.⁸⁶ EPS compete with LPS to bind to the surface of TLR-4 in the cell membrane (Figure 3). Biofilm-derived EPS from various probiotics has shown to downregulate TLR and inhibit the LPS-induced inflammatory responses, for examples EPS from *L. plantarum*,⁸⁷ *B. subtilis*,⁸¹ *L. rhamnosus*,⁸² and *Lactobacillus casei*⁸⁰ can inhibit the LPS-induced inflammatory responses. EPS from bacterial biofilms also repress the expression of iNOS (inducible nitric oxide synthase) which causes oxidative stress by upregulating NRF2/HO-1⁸⁷ (Figure 3).

Osteointegration ability of probiotic biofilms on medical implants is associated with their immunoregulatory and anti-infection properties. In case of osteogenesis, exopolysaccharides from inactivated food-grade *L. casei* biofilms interact with LPS

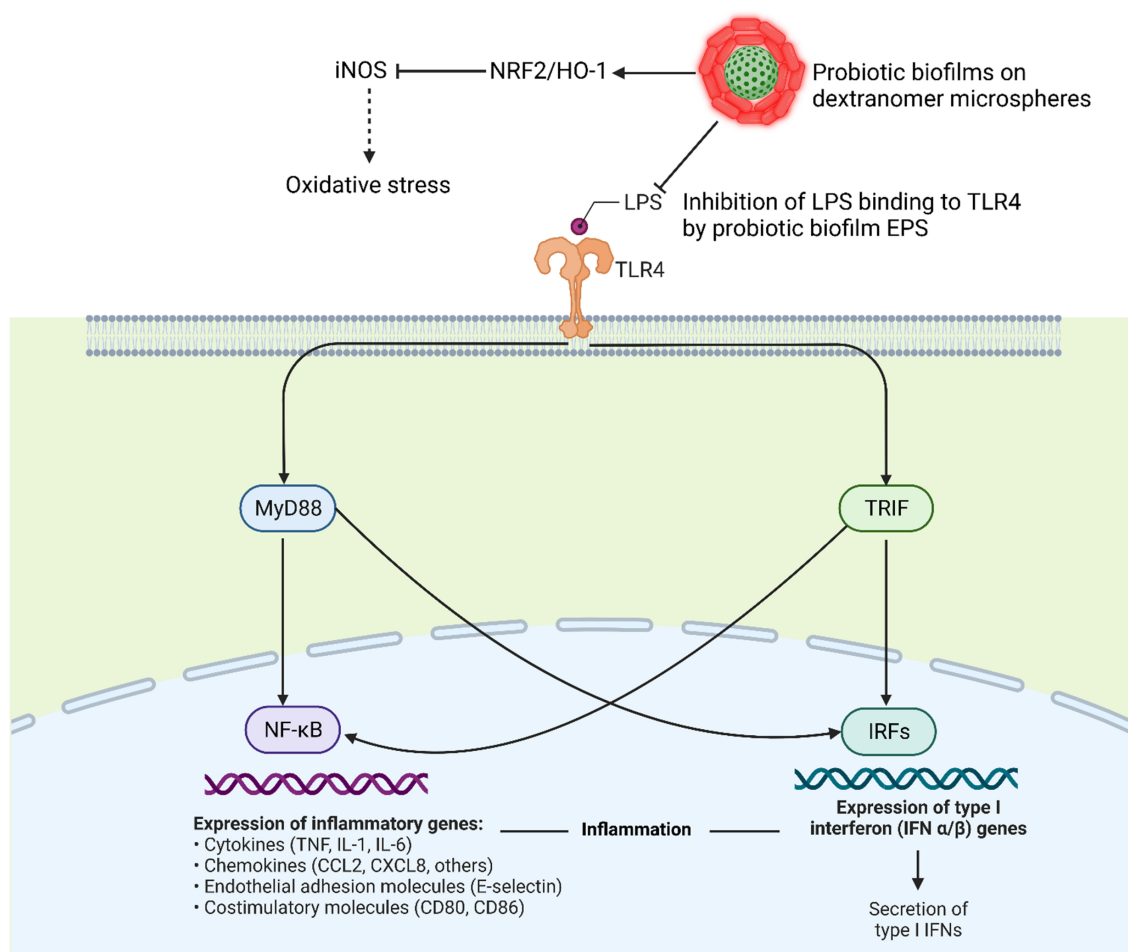


Figure 3. Probiotic biofilms (usuallu carbohydrate moities) compete with lipopolysaccharides on the surface of Gram-negative bacteria to bind to toll-Like receptor 4 (TLR4) cells, activation of which through MyD88 and TRIF pathways leads to the production of inflammatory factors. EPS from bacterial biofilms also repress the expression of iNOS (inducible nitric oxide synthase) which causes oxidative stress by upregulating NRF2/HO-1.⁸¹

receptors of macrophages, resulting in the activation of the TLRs pathways which stimulate the production of osteogenic cytokines (e.g., oncostatin M) by macrophages. EPS produced by *L. casei* biofilms on heat-treated titanium has shown to stimulate macrophages to produce larger quantities of osteogenic cytokines (e.g., oncostatin M), which helps to improve osteointegration of the titanium implant.⁸⁰ High histamine production by probiotics in the biofilm state⁷⁹ may be a possible reason behind these observed phenotypes as histamine has been shown to be involved in upregulating oncostatin M in human macrophages in M1 macrophages.⁸⁸ Apart from the role of EPS, another role of inactivated *L. casei* biofilm on the surface of titanium implants is their effective antibacterial potential against methylene resistant *Staphylococcus aureus*.⁸⁰

Similarly, EPS produced by *L. rhamnosus* KL37 showed immunomodulatory properties in case of collagen-induced arthritis because of its potential to inhibit either one or both possible inflammatory signalling pathways: Th1 → IFN-γ → M1 inflammatory macrophages → arthritis and/or Th1 → IFN-γ → B cells → arthritogenic antibodies → arthritis.⁸²

Biofilms on the surface of smectite have been reported to reduce tumor growth. Biofilm-laden smectite particles activate dendritic cells by the classical TLR2 signalling pathway and suppress tumor growth and increase the efficacy of chemotherapy, compared to planktonic doses of the same probiotic. In an *in vitro* trial, smectite with LAB showed to exhibit anti-tumor characteristics and enhanced the efficacy of chemotherapy and

immunotherapy, when used in combination with these therapies.⁸⁹

Necrotizing enterocolitis (NEC) is an inflammatory necrosis of the gut of premature infants which leads to the appearance of bloated and sensitive abdomen and results in feeding intolerance, and bloody diarrhoea and thus it results in a significant surgical emergency in neonates. A large number of meta-analyses have evaluated the effect of probiotics (e.g., *Bifidobacterium* and *Lactobacillus*) in NEC, concluding that probiotic intervention decreases the risk of NEC and reduces mortality in premature infants.^{90,91} A recent study using planktonic and biofilm forms of *L. reuteri*, on maltose loaded dextranomer microspheres (DM), to treat NEC in a rat model reported no significant effect of the planktonic doses of *L. reuteri* on NEC, compared to its biofilm formulation which reduced NEC in rats and this effect was attributed to more pronounced anti-bacterial and anti-inflammatory effects of *L. reuteri* in its biofilm form, mainly due to more production of reuterin and histamine.⁹² It is known that *L. reuteri* produces more abundant quantities of anti-bacterial and anti-inflammatory factors, especially reuterin, in its biofilm state, compared to its planktonic state.⁷⁷

Another earlier study also reported the ameliorative effects of *L. reuteri* ATCC 23272 biofilm formulation developed on DM supplemented with biofilm-promoting substances (maltose and sucrose) against NEC. The biofilm-loaded microspheres improved mucosal barrier and inactivated the production of proinflammatory cytokines in a single prophylactic dose because the better survival of the bacterium during the gastrointestinal transit and enhanced adherence to gastrointestinal epithelial cells, compared to the delivery of the same bacterium in its planktonic form.⁹³ In many other studies, *L. reuteri* biofilms developed on DM loaded with maltose have proved to be more effective, compared to planktonic cells, in ameliorating harmful effects of NEC in a rat model, which shows that the biofilm form of probiotics has more clinical potential for the deleterious effects of NEC.^{94,95}

Human rotavirus (HRV) is one of the major causes of diarrhoea in children, causing a large-scale morbidity and mortality. *Escherichia coli* Nissle 1917 has been widely used in the treatment

of many enteric diseases in humans; however, its clinical efficiency faces constraints due to limited stability and persistence of the strain. *E. coli* Nissle 1917 biofilm developed on DM reportedly enhanced innate and B cell immune responses after HRV infection and reduced diarrhoea as a result of HRV infection in malnourished pigs.⁹⁶

In addition to the above-mentioned advantages of biofilm-based delivery of probiotics in different diseases/pathological conditions, EPS of biofilms also serve as a source of nutrients for some gut commensals as well as antagonists of pathogens in the gut.⁹⁷

Another advantage of biofilm-based delivery system of probiotics is the encapsulation of many probiotic species/strains in the form of mixed biofilms that remain in close proximity because of bacterial interspecific interactions. Some gut species have been known to co-exist as a result of co-evolutionary processes. For example, *L. reuteri* has been reported to coexists in the form of mixed species biofilms with species which are part of the *Lactobacillus johnsonii* cluster (*L. johnsonii*, *Lactobacillus gasseri*, and *Lactobacillus taiwanensis*).⁹⁸ This implies the idea of developing mixed species biofilm-based probiotic doses to target the human gut microbiota.

Effective biofilm-based probiotic delivery models

One of the most effective approaches for the safe delivery and complete dissemination of probiotics in the human gut is the exploitation of safe, food-grade, non-allergic, biocompatible materials as carriers for the delivery of biofilms. Polysaccharide-based materials (e.g., DM and calcium alginate beads), minerals (e.g., smectite), and food fibres (e.g., grape seeds and starch fiber of chickpea milk) have been successfully used for the development of probiotic biofilms and the use of many of these materials have been reported in successful biofilm-based probiotic delivery systems in several trials as shown in [Figure 4](#). Probiotics delivered in the form of biofilms have been reported to exhibit immunomodulatory and ameliorative effects evidenced from different research trials ([Table 2](#)).

The use of hollow semi-permeable, biodegradable and biocompatible DM has shown to be very

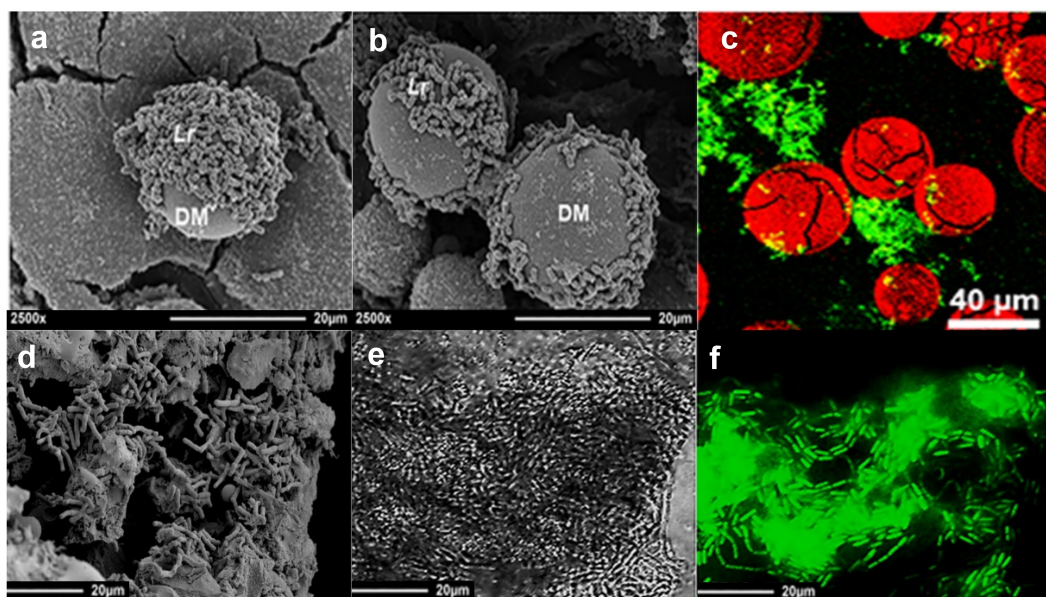


Figure 4. (a) Scanning electron microscopy image of *Lactobacillus reuteri* (Lr) biofilms on the surface of dextranomer microspheres (DM), (b) shows extracellular polymeric substances joining the two microspheres (Olson, Navarro et al. 2018), (c) shows sucrose-filled DM (stained red with Congo Red) with biofilms of *L. reuteri* (stained green with SYTO₉) (Navarro, Mashburn-Warren et al. 2017). (d) shows *Bifidobacterium bifidum* biofilms on the surface of grape seed flour, whereas (e and f) show biofilms of *Bacillus subtilis* on starch fiber of chickpea milk. *B. subtilis* is stained intense green with SYTO₉, whereas starch fiber of chickpea milk is shown as faint green.⁹⁹

Table 2. Immunomodulatory and ameliorative effects of probiotics in the form of biofilms developed on different surfaces and trailed in different models.

Name of the probiotic	Biofilm type	Any known effect	Model	Reference
<i>Lactiplantibacillus plantarum</i>	Biofilm in microtiter plates	Enhanced antibacterial activity of probiotic biofilms against <i>Pseudomonas aeruginosa</i> and <i>Listeria monocytogenes</i>	<i>In vitro</i> trials	100
<i>Lactobacillus</i> and <i>Bifidobacterium</i>	Biofilms on smectite (clay minerals)	Anti-cancer chemo/immunotherapy	Mouse model	89
<i>Lactobacillus rhamnosus</i> and <i>L. plantarum</i>	Biofilms developed on polystyrene surfaces	Biological activity against pathogens	<i>In vitro</i> trials	100
<i>Lactocaseibacillus paracasei</i> ATCC334	Biofilms on calcium pectinate beads	Attenuated the effects of the dextran sulfate sodium (DSS)-induces colitis	Mouse model	101
<i>Lactobacillus casei</i>	Biofilm on the surface of titanium	Antibacterial activity against multidrug resistant <i>Staphylococcus aureus</i> and osteointegration ability	<i>In vitro</i> trials	102
(A) <i>reuteri</i>	Biofilms on sucrose- or maltose-loaded dextranomer microspheres	Attenuated the effects of necrotizing enterocolitis	Mouse model	93
<i>L. plantarum</i> and <i>Lactobacillus fermentum</i>	Biofilm supernatant	Enhanced immunomodulatory effects	Zebrafish embryos	103
<i>Lactobacillus casei</i> ATCC334	Biofilm supernatant	Enhanced immunomodulatory effects	Zebrafish larvae	104
<i>L. reuteri</i>	Biofilm in microtiter plates	Modulation of cytokine output and production of the antimicrobial reuterin	-	77

effective in biofilm-based probiotic delivery systems. The interactions between probiotic biofilms and cross-linked dextran are mediated by glucosyl-transferase-dependent adherence. Probiotic biofilms on the surface of DM have proven to be highly tolerant towards several harsh gastrointestinal conditions and resulted in successful delivery

of probiotics to targeted sites with greater abilities to adhere to epithelial cells and mucosal surfaces.

DM is a porous material and thus many nutritious prebiotic substances can be loaded within the microsphere lumen, which may remain metabolically available only to probiotics and can successfully pass through the gastrointestinal tract. For

instance, disaccharide (sucrose and maltose) may be metabolized and absorbed during the gastrointestinal transit if they are not protected by a suitable material. Lumen of DM can be load with growth promoting disaccharides that are discriminatively

available for the probiotics adhered to the surface of DM and the presence of these growth-promoting factors reportedly enhances adherence of probiotics on the surface of DM (Figure 5A).¹⁰⁶ Probiotic strains on the surface of DM produce EPS which

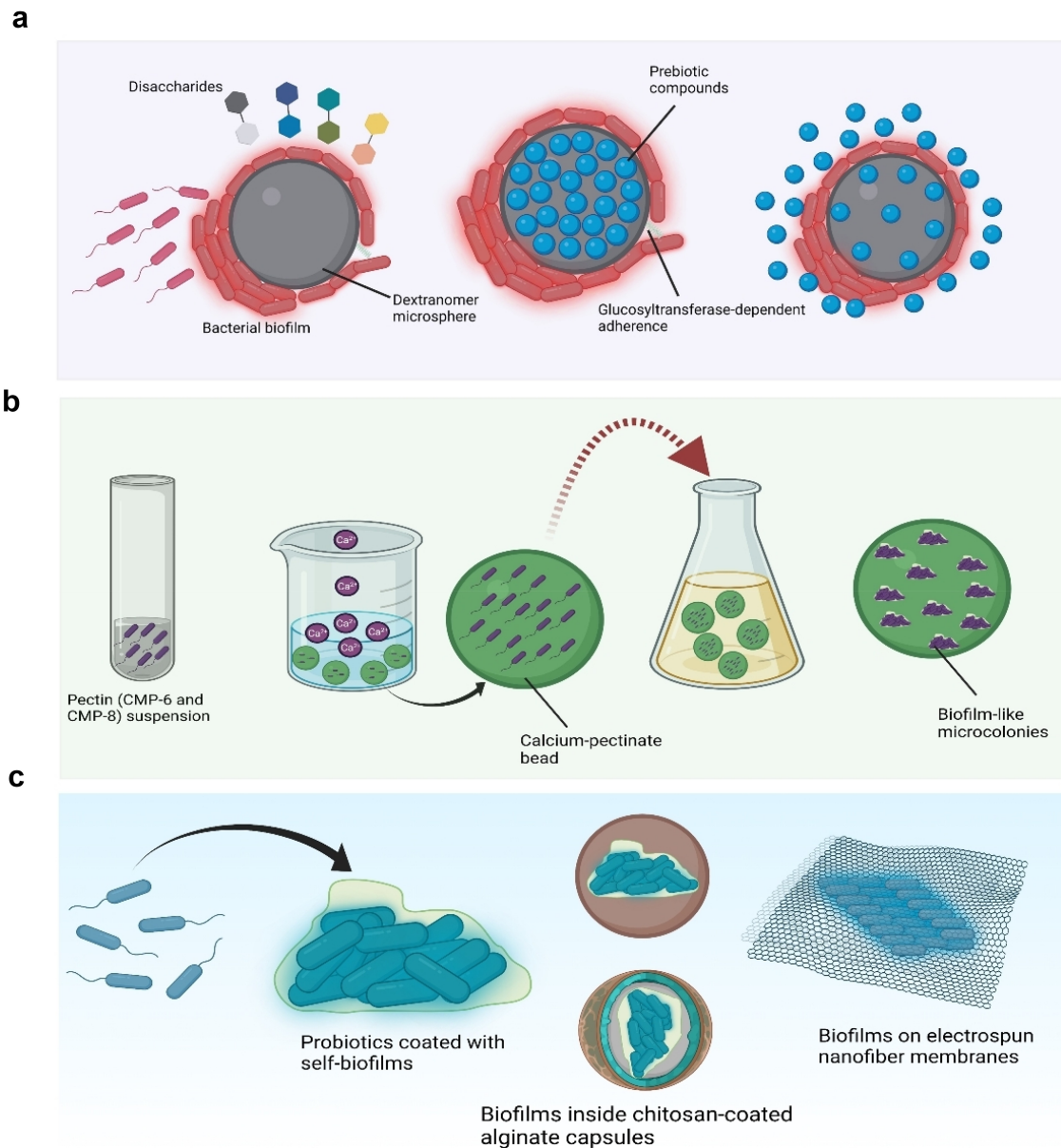


Figure 5. Biofilm-based probiotic delivery systems. Part A shows delivery of probiotic biofilm formed on the surface of a dextranomer (DM) where the addition of disaccharides, as growth-promoting factors, to the lumen of DM results in more probiotic growth and enhanced biofilm formation. The bright red colour outside the strains adhering to DM shows the produced extracellular polysaccharides (EPS) which help them to adhere to the surface of DM. The lumen of DM is shown to be loaded with nutritious prebiotic substances which remain available to the adhered probiotics and can survive the gastrointestinal transit. Part B shows encapsulation of probiotic cells into calcium pectinate capsules which promoted its growth into biofilm-like microcolonies and provided extra protection against environmental stressors. Part C shows coating of probiotic cells with their own biofilms containing EPS as a bioinspired strategy for oral doses of gut microbiota. Probiotic biofilm can be further encased in specific material as shown (chitosan-coated alginate capsules). Biofilms are further shown to be entrapped in electrospun cellulose acetate nanofiber membranes which has been reported to have great gastrointestinal resistance and it is ideal for the growth and biofilm formation of probiotics such as *Lactoplantibacillus plantarum* as shown in part C.

can help to stick two different DM particles together (Figure 5A and 5B). Interestingly, DM containing biofilms of *L. reuteri* can be loaded with L-histidine which serves as a unique method for the delivery of L-histidine to *L. reuteri* in the form of biofilms.¹⁰⁶ Probiotic-biofilm containing DM particles assure a high density of probiotics on the surface of epithelial cells in a single dose – the density which could only be achieved by frequent repetition of planktonic doses.⁹³ DM-based probiotic biofilm delivery system has also been reported to increase the probiotic potential of bacterial strains and maximize associated health benefits.

Smectite (a type of silicate clay) has a large reactive surface area and it been reported to induce biofilm formation in many bacterial species.¹⁰⁷ It remained ignored as a great bioactive material for the development of probiotic biofilms for a long time. The ion-exchangeable microstructure of smectite clay promotes biofilm formation, both *in vitro* and *in vivo*, by *Lactobacillus* and *Bifidobacterium* species on its surface. Organic acids produced by LAB supposedly give positive charge to the surface of smectite, which facilitates bacterial absorption because of their negatively charged cell wall as a result of teichoic acids.⁸⁹ It is known that smectite can adhere to tissue surfaces such as mucus and helps to maximize bacterial contact with gastrointestinal organs. Smectite-laden biofilms have been shown to have ameliorative effects on tumors as discussed in the above section. Given the limited survival of probiotics in the gastrointestinal tract, the smectite clay, being a safe material, has potential as an ideal carrier for the delivery of dense probiotic biofilms to the gut, where it may also enhance the growth of other LAB. Role of smectite laden probiotic biofilms have clinical implications in case of chemotherapy as discussed in the above section.

Several food matrices serve as natural scaffolds for the adherence and biofilm formation of many probiotics which could be added in food or delivered directly. Different probiotic bacteria have been tested for their ability to develop biofilm on dietary fibres. Liu and others reported the biofilm-forming potential of six *Bifidobacterium* species on grape seed flour (insoluble dietary fibre with porous structure), where *Bifidobacterium pseudo*,

Bifidobacterium longum, and *Bifidobacterium breve* formed strong biofilms (more biofilm mass) with high tolerance under low pH limited nutrients.¹⁰⁸ Wheat bran is considered as a beneficial dietary fibre with a well-known prebiotic potential. Wheat bran fraction reportedly induce microbiota changes in the human gut, specifically targeting colon butyrate producers.¹⁰⁹ Development of probiotic biofilms by *L. plantarum* 8-RA-3 on wheat bran has been reported which opens further avenues of utilising wheat bran as a probiotic carrier. Survival and tolerance of *B. subtilis* can be enhanced by developing its biofilms on starch fibres of chickpea milk with significantly more production of pulcherimin (an antimicrobial pigment for some pathogens).^{99,110} The development of *B. subtilis* biofilms on starch fibres confers effective adaptation strategy to the bacterium on food matrices for enduring harsh gastrointestinal conditions as well as during food processing and storage, as a part of functional food ingredients. The fibre can ultimately be used by the colonized probiotic strains for their proliferation.

Another approach for the delivery of probiotics in the form of biofilms is to encapsulate them in a material that may stimulate their biofilm formation and keep bacteria entrapped in the form of biofilms. When *Lactobacillus acidophilus* LMG9433T, *L. rhamnosus* LMG25859, and *L. casei* LMG6904T were encapsulated in microcapsules produced using low-methoxyl pectins (CMP-6 and CMP-8) and calcium as the wall material and allowed to grow further in a suitable growth medium, they changed into biofilms and better survived the gastrointestinal transit and heat and cold shock, compared to their planktonic cells,¹¹¹ as shown in Figure 5B.

Self-coating probiotics with biofilms has also been reported as an important bioinspired strategy for oral doses of gut microbiota (Figure 5C). *B. subtilis* cells coated with self-produced biofilms endowed the transplanted gut microbiota with superior tolerance (a 125-fold higher oral bioavailability) and enhanced mucoadhesion capacity (17 times higher) in mice and swine models.⁷⁴ Apart from developing biofilms on different surfaces as discussed above, bacterial biofilms can be encapsulated in suitable materials, usually polysaccharide-based capsules, which can successfully release the

entrapped probiotics in the colonic environment. In one of the earlier investigations on probiotic biofilm delivery systems, chitosan-coated alginate capsules were proved to confer *L. rhamnosus* biofilms more protection against several environmental stressors (e.g., low pH and temperature).¹¹² Heumann and others showed the successful release of biofilm-like microcolonies of *Lactocaseibacillus paracasei* ($10 \log$ (CFU/g) encased in calcium-pectinate beads in the gastrointestinal tract. The resulting interaction between three-dimensional network of calcium pectinate and bacterial exopolysaccharides kept bacteria adhered to the surface.¹⁰¹ Electrospun nanofiber membranes have three-dimensional porous structure with high bio-reactive surface area which make them ideal for the growth and proliferation of bacterial cells. Biofilms formed by a probiotic strain of *L. plantarum* was entrapped in electrospun cellulose acetate nanofiber membranes, which showed excellent

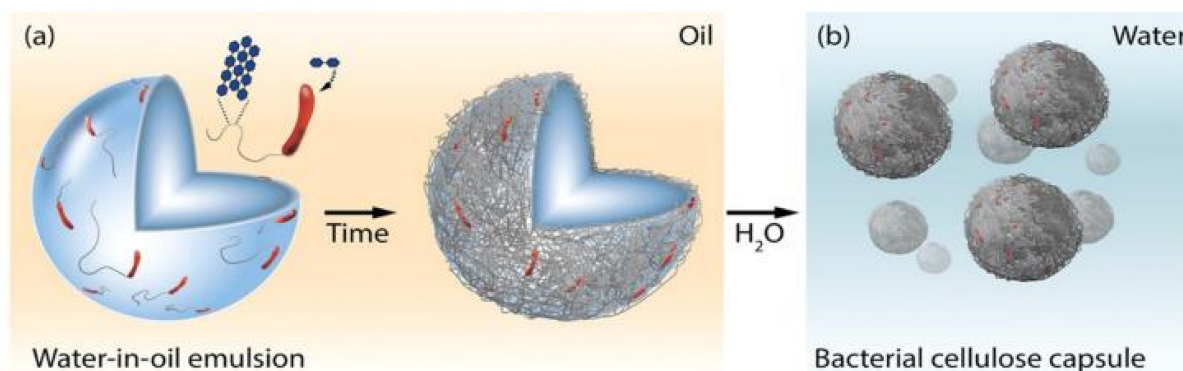
gastrointestinal tolerance compared to bacterial cells in the planktonic form¹⁰⁵ (Figure 5C).

A recent study reported a new method for the development of probiotics capsules based on cellulose produced by self-aggregated biofilms of *Gluconacetobacter xylinus*¹¹³ as demonstrated in Figure 6. This principle of self-produced EPS can be used for many biofilm-producing probiotic strains. Probiotic cellulose does not only protect enclosed probiotic cells, but it also serves as an antibacterial agent against many pathogens.¹¹⁴

Mixed biofilms for probiotic delivery

Most of the probiotic bacterial species or the gut commensals have been reported to co-exist in mixed biofilms where their growth, biofilm mass and metabolic characteristics are enhanced because of inter-species interactions. Bacterial interaction in co-cultures may also lead to enhanced

a



b

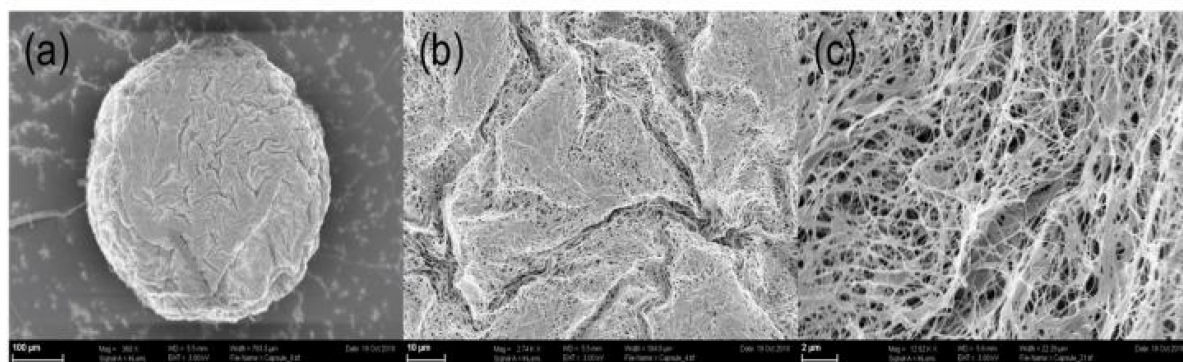


Figure 6. Probiotic self-grown capsules based on cellulose derived from self-assembled biofilms in an oil-water emulsion. Part A shows water-in-oil emulsion loaded with nutrients and the bacterium *Gluconacetobacter xylinus*, enclosed in the water phase (a) and his ultimately leads to the formation of bacterial self-produced cellulose capsules (b). Part B (a, b, and c) shows scanning electron microscopy images of the biofilm-based cellulose capsule with visible cellulose fiber network under three different magnifications (100, 10, and 2 μm). This figure is reproduced with permission.¹¹³

production of several beneficial metabolites. Increased production of bacteriocin was reported in a co-culture of *Lactobacillus* and *Pediococcus* species.¹¹⁵ There are many probiotic strains which do not form biofilms and thus it presents a challenge to increase their viability and probiotic potential based on the model discussed hereby. One way to deal with this challenge is to mix non-biofilm forming probiotics with some biofilm-forming probiotic strains so that they can get protection from the EPS of other strains. For instance, a few strains of *B. subtilis* have emerged as potential probiotics and they produce abundant EPS which can protect other probiotic bacteria and keep them entrapped within the matrix.¹¹⁶ Some species trigger biofilm formation in other non-biofilm forming species in combinations and thus mixed-species biofilms can be used as probiotics and can further be encapsulated based on the reported techniques discussed above. Sadiq, Wenwei, Heyndrickx, Flint, Wei, Jianxin and Zhang³⁵ demonstrated the existence of four different gut species together in the form of mixed-species biofilms where individual species were not able to form biofilms but existed together because of probably synergistic interactions. Species from different kingdoms (bacteria and yeasts) may also co-exist in the form of biofilms and thus this delivery system may help to deliver doses of mixed probiotics from different kingdoms. Interactions between *Lactobacillus kefir* and *Saccharomyces cerevisiae* is probably the best examples of the existence of trans-kingdom probiotics in the form of biofilms because of interactions between bacterial surface layer proteins (SLPs) and yeast mannans.¹¹⁷

Novel probiotics to enrich specific bacterial groups in the gut

Responses of the human gut microbiota to dietary components are mechanistically not well understood because of complexities involved in the interactions of the gut microbiota affecting metabolic capabilities of species. The last one decade has witnessed a surge in the development of novel probiotics and effective delivery and enrichment strategies of probiotics. According to the consensus statement of the International Scientific Association for Probiotics and Prebiotics,

a prebiotic substance must be selectively utilized and have adequate evidence of health benefit for the target host. Apart from an astounding range of established carbohydrate-based prebiotics (such as, inulin, fructooligosaccharides, galactooligosaccharides, and lactulose), several novel prebiotics have emerged which target specific microbial groups in the human gut, for instance, xylooligosaccharides, chitooligosaccharides, isomaltooligosaccharides, lactosucrose,¹¹⁸ protein-based prebiotics,^{119,120} some dietary fibres,¹²¹ and prebiotics derived from fungal¹²² and algal sources, such as seaweed polysaccharides.¹²³ Table 3 mentions many studies which reported the effectivity of selected prebiotic in promoting the growth of targeted bacterial groups in the gut.

Researchers focusing on prebiotic development are more interested in developing prebiotics targeting specific bacterial groups in the human gut at the species or strain level to increase the abundance of beneficial bacteria while reducing detrimental members in the gut microbiota, and to shift the host colonic microbiota toward a healthier composition.¹⁴⁵

Bifidobacteria are important bacterial groups in the human gut which mainly rely on dietary carbohydrates for their growth and physiological activities. It is well established that some dietary fibers such as fructans and galacto-oligosaccharides enhance the fecal abundance of *Bifidobacterium* and *Lactobacillus* species.^{134,146}

Prebiotics containing levan can be specifically utilized by *Bacteroides thetaiotaomicron*.¹⁴⁷ Similarly, yeast mannan (indigestible water-soluble polysaccharide) has been reported to specifically promote the growth of *B. thetaiotaomicron* and *B. ovatus* in trials based on human colonic microbiota model.¹⁴⁸ Soluble dietary fiber (10% inulin) reportedly enhances growth and abundance of *Bacteroides fragilis* with a concomitant increase in IgA.¹⁴⁹

Some probiotics in the human gut particularly require some proteins for their growth and proliferation. Probiotics often contain sugar-binding proteins on their cell surface, and these mediate the uptake of many complex oligosaccharides. A protein delivery system in combination with sugars (galactooligosaccharide-lactoferrin hydrolysate micelles) was successfully used targeting the specific growth of *L. casei*.¹²⁰

Table 3. Selective prebiotics targeting the growth of targeted bacteria in the gut.

Name of the prebiotic	Source	Targeted bacteria	Reference
Inulin	-	<i>Bifidobacterium</i> and Lachnospiraceae	124
Omega-3 fatty acids	-	<i>Coprococcus</i> and <i>Bacteroides</i> species	124
Berberine and Curcumin	-	<i>Bifidobacterium</i> and <i>Akkermansia</i> species	125
Sulfoquinovose	Green vegetables	<i>Faecalibacterium prausnitzii</i>	126
Galactosyl- β 1,4-l-rhamnose	-	<i>Bifidobacterium longum</i> subsp. <i>Longum</i>	127
Polyphenols	Fu brick tea	<i>Akkermansia muciniphila</i> , <i>Alloprevotella</i> , <i>Bacteroides</i> , and <i>Faecalibaculum</i>	128
Levan-type fructan	<i>Erwinia</i> species	<i>Bifidobacterium</i> and <i>Eubacterium rectale</i>	129
Polyphenols	Chilean currants	<i>F. prausnitzii</i>	130
Polyphenols	Cranberry and blueberry fruit powders	<i>A. muciniphila</i>	131
Protein-oligosaccharide conjugates	The conjugates were formed by mild Maillard-reaction-based covalent conjugation of galacto-oligosaccharides to lactoferrin hydrolysate	<i>Lactobacillus casei</i>	119
Chitin-glucan	-	<i>Bifidobacterial</i> communities, especially <i>Bifidobacterium breve</i>	132
Epigallocatechin gallate, caffeine, and theanine	Green tea	<i>Bifidobacteria</i> and <i>Lactobacillus</i> species	133
<i>Lycium barbarum</i> polysaccharides	Goji berries	<i>Bifidobacterium</i> and <i>Lactobacillus</i> species	134
Proanthocyanidin	Grapes	<i>Akkermansia</i> species	135
Anthocyanins	Black rice	<i>Bifidobacterium</i> and <i>Lactobacillus</i> species	136
Triterpenoid saponins	<i>Gynostemma pentaphyllum</i> (a medicinal plant)	<i>Bacteroides</i> , <i>Lactobacillus</i> and <i>Bifidobacterium</i>	137
Yeast α -mannan	<i>Saccharomyces cerevisiae</i>	<i>Bacteroides thetaiotaomicron</i>	138
Partially hydrolyzed guar gum	-	<i>Bifidobacterium</i> and butyrate producing bacteria	139
Polyfructan levan	Produced from sucrose using Lsc3 from <i>Pseudomonas syringae</i>	<i>Bacteroides</i> , <i>Faecalibacterium</i> , <i>Escherichia</i> and <i>Streptococcus</i>	140
Galactooligosaccharide mixture	Produced by enzymatic activity of <i>Bifidobacterium bifidum</i> NCIMB 41171	<i>Bifidobacterium bifidum</i>	141
Lactulose	-	<i>Bifidobacterium</i> species	142
Xylooligosaccharide (XOS)	-	<i>Bifidobacterium longum</i> subsp. <i>longum</i> CR15	143
Pectic oligosaccharides	Sugarbeet pulp and lemon peel waste	<i>Faecalibacterium</i> and <i>Roseburia</i>	144

Some dietary components have potential to be used as prebiotics because of their associated growth impacts on targeted microbiota groups. Sulfoquinovose (SQ), a sulfonated monosaccharide highly dominant in green vegetables and can be effectively used by *Eubacterium rectale* and *F. prausnitzii* leading to the production of H₂S through acetate production in the human gut.¹²⁶ Similarly, quinones is another food component which specifically targets *Faecalibacterium* species in the human gut.¹⁵⁰ Omega-3 supplementation has been reported to result in a significant increases in *Coprococcus* and *Bacteroides* species.¹²⁴ Dietary polyphenols can be utilized by certain (poly)phenol-transforming bacteria of the human gut such as *Akkermansia muciniphila* (a bacterial species well known for its anti-obesity properties) leading to its abundance with substantial ameliorating effects on obesity.^{151,152}

Concluding remarks

Human gut harbors trillions of diverse microorganisms which live in an intricate balance, and dynamics of which are affected by host and environmental factors, including the diet and lifestyle. A perturbed gut leads to several pathological conditions and aberrant metabolism. The use of probiotics, among many other microbiome-modulating interventions, has been well acknowledged as a strategy to overcome the conditions associated with gut dysbiosis, because of their role in replenishing missing microbial groups. Development of specific prebiotics for targeted growth of bacteria at the species or strain level, in the human gut is also very important to modify the host colonic microbiota toward a healthier composition. Unique gut bacterial profiles with

dominance of specific genera and species are linked with health and disease status.

One of the challenges in probiotic research is their effective delivery and maintenance of viability across divergent product formats. Biofilms of probiotics have been argued as the most resistant form that can better survive the gastrointestinal transit, compared to planktonic probiotic forms. Probiotic biofilms can entail a mixture of probiotic species/strains with network interactions and synergistic effects that can serve as highly resistant therapeutic microbial consortia. Probiotic biofilms can be easily subjected to many processing regimes, for instance, encapsulation, coatings, structural changes, and compound enrichment which widen their use in several applications and diverse systems as functional ingredients.

Probiotic biofilms have been proven to be more effective than their counterpart planktonic probiotics in many immunological and pathological conditions mainly due to their better survival, improved gut barrier function, more adherence, EPS production, and production of biofilm-specific metabolites such as vitamins and certain enzymes.

Probiotics in a biofilm formulation increase the efficiency of chemo- or immunotherapy by activating anti-cancer immune responses carried out by dendritic cells, which is not a property of planktonic cells. Their advantages over the conventional delivery of probiotics can be evidenced by their immunomodulatory roles in osseointegration of medical implants, necrotizing enterocolitis, and collagen-induced arthritis. Probiotics in their biofilm state produce many new metabolites and many metabolites in higher concentration which promote the growth of gut microbiota and inhibit the growth of many pathogens, apart from their metabolic and anti-inflammatory effects.

Whilst considering biofilms as the appropriate form of probiotic intake, one should consider the stage of biofilm development with the view of the production of metabolites, structural stability, and viability of cells. Many studies have provided evidence of more therapeutic potential of probiotics in the form of biofilms, mainly because of enhanced resistance of the biofilm-enclosed cells, and thus probiotic-based delivery approaches should be further investigated for drug delivery systems and for the incorporation of probiotics in foods and supplements.

Disclosure statement

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