

## New insights into the bioactivity of peptides from probiotics

Santi M Mandal<sup>1</sup>, Bikas R Pati<sup>1</sup>, Ranadhir Chakraborty<sup>2</sup>, Octavio L. Franco<sup>3</sup>

<sup>1</sup>Anti-Infective Research Lab., Department of Microbiology, Vidyasagar University, Midnapore 721102, WB, India. <sup>2</sup>Department of Biotechnology, North Bengal University, Siliguri- 734013, Darjeeling, India, <sup>3</sup>Department of Biology, Institute of Biological Sciences, Graduate Program in Genetics and Biotechnology, Federal University of Juiz de Fora, Juiz de Fora–MG, 36036-900, Brazil

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Gut homeostasis
4. Evidence of effectiveness
5. Strategies for re-engineering probiotic surface for anti-infective therapy
6. Conclusion
7. References

### 1. ABSTRACT

Probiotics are unique bacteria that offer several therapeutic benefits to human beings when administered in optimum amounts. Probiotics are able to produce antimicrobial substances, which stimulate the body's immune responses. Here, we review in detail the anti-infective peptides derived from probiotics and their potential immunomodulatory and anti-inflammatory activities, including a major role in cross-talk between probiotics and gut microbiota under adverse conditions. Insights from the engineered cell surface of probiotics may provide novel anti-infective therapy by heterologous expression of receptor peptides of bacterial toxins. It may be possible to use antigenic peptides from viral pathogens as live vaccines. Another possibility is to generate antiviral peptides that bind directly to virus particles, while some peptides exert anti-inflammatory and anticancer effects. Some extracellular polymeric substances might serve as anti-infective peptides. These avenues of treatment have remained largely unexplored to date, despite their potential in generating powerful anti-inflammatory and anti-infective products.

### 2. INTRODUCTION

It is universally accepted that some antimicrobial peptides, such as bacteriocins, which are derived from lactic acid bacteria (LAB), may be a potential food preservative as they inhibit target organisms. The application of bacteriocins and their effectiveness has been described in detail in several review articles, and their synthesis and mode of action are also well described (1-4). The application of antimicrobial/anti-infective peptides depends upon the chemical composition of peptides and types of host (5).

Dynamic applications of probiotics has been extensively explored in conferring several health benefits, such as maintaining gut homeostasis, reducing inflammation, improving immunity, as well as antibacterial, antiviral and anticancer properties. Indeed, it has been noted that extracellular peptides produced by probiotics are important in controlling a number of acute or chronic infections.

The perception of peptide promiscuity, where multiple functions are associated with a single peptide, is currently gaining ground (6). For example, endogenous anti-infective peptides, derived from potential probiotics, have also been reported to serve as immunomodulators of the cellular immune response. Strategies like re-engineering of the probiotic cell surface with heterologous expression of new sugars and proteins are necessary to increase the viability and stability of anti-infective peptides derived from probiotics in the gut.

Furthermore, self-assembling strategies are important phenomena that may stabilize the peptide molecules in harsh conditions like high salt and acidic environment. In the self-assembling strategy, several weak interactions between molecules or atoms forming a complex supramolecular architecture may play a fundamental role (7). This binding has a strong dependency on balancing the forces of attraction and repulsion between the molecular building blocks that form supramolecular structures (8,9). These non-covalent bonds, including Van der Waals, electrostatic and hydrophobic interactions, as well as hydrogen and coordinate bonds, are formed between the assembled molecules and the substrate surface, and also between the molecules in the adjacent layers.

With the advances in peptide research, novel strategies may thus evolve to make them stable for longer periods. Attempts have also been made to design and develop potential receptors for bacterial exotoxin(s), such as cholera toxin and shigella toxin, among many others (10). Instead of binding to the target region of epithelial cells, exotoxin(s) with the respective receptor peptide(s) may be captured. Thus, probiotics are very promising in therapeutic applications with re-engineered anti-infective peptides.

### 3. GUT HOMEOSTASIS

The human gastro-intestinal tract (GIT) consists of a complex community of microorganisms with highest density of natural bacterial population among other body parts. Bacterial density in the colon ranges from  $10^{11}$  ml<sup>-1</sup> to  $10^{12}$  ml<sup>-1</sup> content (11). The predominant gut bacterial phylotypes belong to two divisions — the Bacteroidetes (48%) and the Firmicutes (51%). The residual phylotypes are distributed among the Proteobacteria, Verrucomicrobia, Fusobacteria, Cyanobacteria, and Spirochaetes (12). Interestingly, the balance between the two major phylotypes, Bacteroidetes and Firmicutes, in the gut bacterial community, influences host health. Shifts or imbalance in the composition of the two are associated with multiple pathogeneses, including diabetes, obesity, bowel diseases, chronic inflammation and gastrointestinal cancer, as well as stress and autism. Such imbalances are often brought about by diet or usage of antimicrobials.

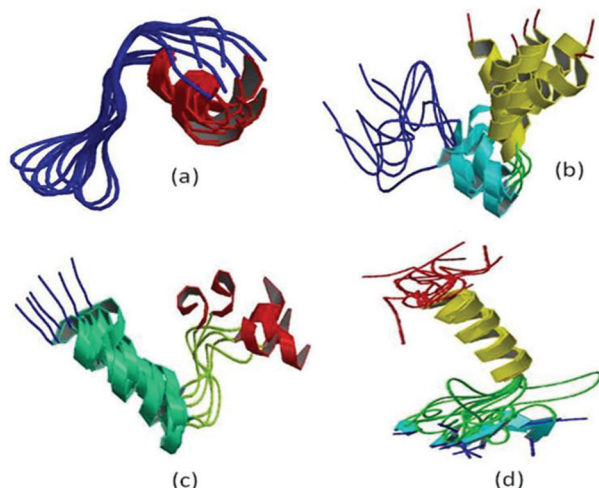
Gut bacteria may also afford an efficient protective barrier against different pathogens, through a phenomenon known as colonization resistance (13). Fulfillment of very important physiological functions like the development of the digestive system, immune system maturation and antigen tolerance are dependent on the interactions between commensal bacterial species and the host (14). Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis (15). In the GIT, certain antimicrobial peptides (AMPs) have been identified as essential molecules for the maintenance of intestinal barrier and immune homeostasis. Most of the human defensin peptides (including cathelicidin, LL-37) are expressed in gut mucosa, such peptides are also known to be expressed in the paneth cells in the small intestine or epithelia of gastrointestinal tissues, stomach, small intestine and colon (16). Some defensin molecules are also released upon proteolytic degradation by enzymes like trypsin, chymotrypsin etc. (18). In addition to these AMPs of human origin found in the GIT, another class of AMPs has been identified as derived from gut microflora. The molecular mechanisms by which the inhabitant microbial community inhibits the development of invading microbes remain obscure, but there is increasing evidence that direct microbe-microbe interactions could play a critical role in this specific process (19).

Gut microbiota have several beneficial aspects to health in different ways but the imbalances in this community, dysbiosis can poses a threat to host health. In this context, probiotics can restore the balance once again. They are produced by bacteria for the fulfillment of the defense mechanism. The common beneficial features of antibacterial peptides include being nontoxic to humans and the possession of specific antibacterial activity. These peptides have recently been extensively used as food preservatives, especially those produced by LABs (18). However, LABs are not the only species of probiotics that produce antimicrobial peptides. There are some other species of *Bacillus* and *Bifidobacteria* that are endowed with the same ability to produce AMPs, but two genera, *Lactobacillus* and *Bifidobacteria*, constitute the majority of the probiotics (20). Antimicrobial peptides are produced exclusively by probiotics, and so their impact on gut homeostasis is essential in restricting pathogen numbers in the GIT.

### 4. EVIDENCE OF EFFECTIVENESS

Antibacterial peptides, probiotic characteristics and biopreservative efficacy of *Bacillus* species have been studied for several years. The antibacterial compounds from *B. subtilis* and *B. licheniformis* strains exhibited the highest inhibitory activity against *M. luteus* ATCC 9341. Peptides whose isolation or synthesis is pH dependent but stable in a wide pH range (4.0–10.0.) have found applicability in controlling spoilage of diverse acidic or alkaline fermented foods. An example of the commercial use of such peptides is nisin, a bacteriocin that acts in acidic conditions and is practically insoluble at pH 8.0. Since there have been claims that many *Bacillus* species are probiotic in nature, it is vital to characterize these organisms for their gastrointestinal persistence before they are prescribed as probiotic. To be finally prescribed as a potential probiotic, the bacterial culture must pass the test of tolerance to both acid and bile, which would permit survival and growth in the adverse conditions encountered in the gut, before conferring a beneficial health effect (21). A few *Bacillus* strains have shown tolerance to a high acidic condition at pH 3.0. Among the strains capable of producing bacteriocins that would have application as a bio-preservative, there are some *B. subtilis* strains and the Ec1 and lactic acid bacterial (LAB) strains (22).

The majority of bacteriocins have been discovered so far and summarized in the Bactibase database, a few of the representative structures are shown in Figure 1. The antimicrobial activities of bacteriocins E 50–52 and B 602 against antibiotic-resistant strains involved in nosocomial infections were studied in detail (23). The bacteriocin-producing strain, *Enterococcus faecium*, was isolated from the cecum of a Russian broiler chicken. Synthesis of the matching bacteriocin E 50–52 was accomplished by



**Figure 1.** The representative structure of some anti-infective peptides from *Lactobacillus* probiotics. Subtilisin, an antimicrobial peptide including antiviral activity produced by *Bacillus amyloliquefaciens* (a); antibacterial peptide Curvacin A from *Lactobacillus acidophilus* (b); Lactococcin-G  $\beta$  from *Lactococcus lactis* (c) and Sakacin P from *Lactobacillus sakei* (d). The pdb files of NMR solved structures were downloaded from Bactibase (A database dedicated to bacteriocin).

growing the isolate in *Brucella* broth at pH 6.8–7.2. The other bacteriocin, B 602, produced by *Paenibacillus polymyxa* B 602, was also isolated from a Russian broiler chicken's cecum. Based on multiple sequence alignment analyses, these peptides were assigned as pediocin-like bacteriocins or class IIa bacteriocins.

Bacteriocins E50–52 and B 602 were shown to possess a wider activity spectrum against both Gram-positive (*S. aureus*) and Gram-negative bacteria (*Acinetobacter baumannii*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus* spp.), compared to the previously characterized class IIa bacteriocins. Additionally, the potency of these peptides was tested against 10 MRSA isolates with the MDR phenotype (resistant to several classes of antibiotics, such as beta-lactams (oxacillin), aminoglycosides (gentamicin), fluoroquinolones (ciprofloxacin), tetracyclines (doxycycline), erythromycin, clindamycin, chloramphenicol, and rifampicin). The MRSA isolates were found to be very sensitive to both B 602 (MIC < 0.0.2.5.  $\mu\text{g/ml}$ ) and E 50–52 (MIC ranged from 0.0.5. to 0.2.  $\mu\text{g/ml}$ ) bacteriocins. The non-fermenting Gram-negative rods, *A. baumannii* and *P. aeruginosa*, were also found to be sensitive to B 602 (MIC: 0.0.2.5.  $\mu\text{g/ml}$  for *A. baumannii*; MIC range: 0.1. to 1.6.  $\mu\text{g/ml}$  for *P. aeruginosa*) bacteriocin. The said bacteriocins, B 602 and E 50–52, were also found to be effective against several members of *Enterobacteriaceae* (*E. coli*, *K. pneumoniae*, *C. freundii*, and *Proteus* spp.).

In another study, isolated peptides from *L. rhamnosus* were found capable of inhibiting

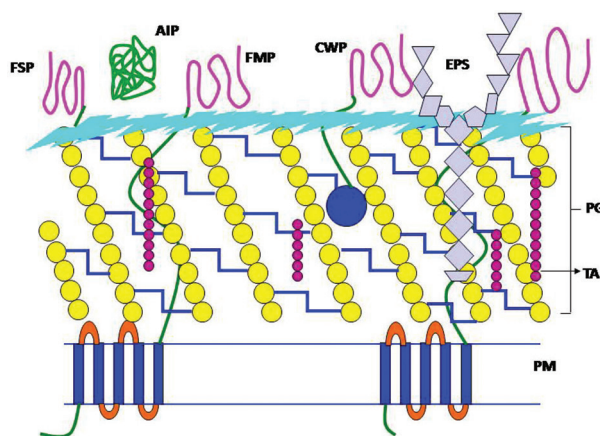
the growth of a wide range of bacterial pathogens comprising *E. coli*, *E. aerogenes*, *S. typhi*, *Shigella* sp., *P. vulgaris*, *P. aeruginosa*, *Serratia marcescens*, *S. aureus*, *K. pneumoniae*, *H. pylori*, *Campylobacter jejuni*, *Micrococcus luteus* and *Listeria monocytogenes* (24). Growths of the clinical strains of *Candida albicans* and *S. aureus* were found to be inhibited by lectins produced by the probiotic strains of *Lactobacillus* and *Bifidobacterium*. It was reported that lectins were effective against nystatin-resistant *C. albicans* strains of clinical origin. It has been envisaged that the presence of probiotic bacterial lectins results not only in preventing fungal growth in the earlier stage of infection (primary anti-fungal effect) but also in the relatively late stages of biofilm formation and colony lysis phase (secondary or prolonged antifungal effects). Acidic *Bifidobacterial* lectins were also shown to have antifungal effect at sub-cyto-agglutinating concentrations (from 0.0.1.–0.0.8.  $\mu\text{g mL}^{-1}$ ) (25).

## 5. STRATEGIES FOR RE-ENGINEERING PROBIOTIC SURFACE FOR ANTI-INFECTION THERAPY

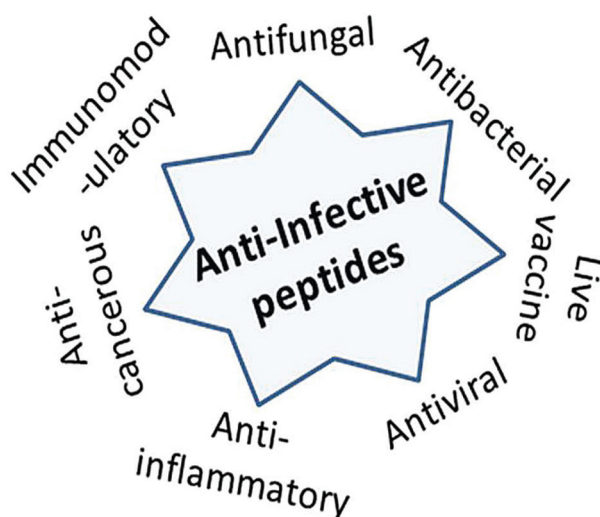
Re-engineering the bacterial cell surface via recombinant technology is an acceptable molecular method to re-make far more potent tools for biotechnological or biomedical applications (Figure 2). The plausible cell surface-expressed proteins may have potential application in biotech-based industry, such as immobilized enzyme(s), biocatalysts, biosensors, and biosorptive materials; and in the biomedical sector they can be a good candidate for vaccine development or anti-cancer agents (26). In the process of vaccine development, the pathogen-associated antigenic part is required to develop the adaptive immune response in the cell. Hence, the expression of a pathogen-associated antigen on the cell surface of probiotics, with the aid of recombinant DNA technology, is important to improve immunogenicity. It is known that surface expression of proteins produces a multivalent display of antigens and so can be easily cross-linked to B-cell receptors (27). Surface-engineered probiotic cells open up a novel opportunity for delivery along with inactivated bacterium in different food grades or as a live bacterial vector. Recombinant live probiotics expressing a pathogen-associated antigen may lead to the generation of antigens at the mucosal level, which will in turn greatly influence delivery to the APC (28).

Moreover, several probiotics are also capable of producing antiviral peptides. For example, the bacteriocin subtilisin, the antimicrobial peptide produced by *Bacillus amyloliquefaciens*, has shown remarkable activity against Herpes simplex virus type 1 (HSV-1) (29). There are several possibilities for probiotics to act as antiviral agents through different modes of action. Generally, probiotic bacteria inhibit virus attachment by directly binding to the host cell receptors. When probiotics adhere to the





**Figure 2.** Schematic cell surface architecture of Probiotics. The cell wall of lactobacilli is composed of different macromolecules together with techniques for the heterologous expression of cell surface proteins. The abbreviations are as CWP: Cell wall associated protein; PG: Peptidoglycan; PM: Plasma membrane; FSP: Fusion to S-layer protein; FMP: Fusion to membrane protein; EPS: Exopolysaccharides; TA: Teichoic acid; AIP: Anti-infective peptides/proteins.



**Figure 3.** Versatile applications of anti-infective peptides derived from probiotics.

epithelial surface blocking the viral attachment, they may also induce intestinal production of mucins. Mucins in turn interfere with the viral adherence to epithelial cells by neutralizing the viruses. All these cellular phenomena may finally activate CD8+ T lymphocytes to destroy virus-infected cells. Thus, the use of probiotics in antiviral therapy would be of intense interest.

Probiotics are gaining high-priority status and importance due to their tremendous beneficial effects on health and disease management. The claims are substantiated by various scientific reports resulting from clinical trials since 1999 (30). Antimicrobial peptides

derived from probiotics in the GIT can diffuse through the mucus layer that finally triggers the immune cells. It was found that *Bifidobacterium* in the colon has the properties to mitigate inflammatory bowel disease, rheumatoid arthritis or lupus, irritable bowel syndrome, and infections by enteropathogens (31,32). The molecular signals generated from probiotics have the ability to activate several genetic cascades which modulate the expression level and make necessary physiological changes. These signals are relayed to the nucleus receptor through different pathways by means of mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3-kinase (PI-3K), and glycogen synthase kinase-3 (GSK-3) (33).

The mining of information with respect to cellular receptors, which are responsible for the recognition of antimicrobial peptides derived from probiotic bacteria, is still inadequate. It can be imagined that AMPs may be acquainted with the Toll-like receptors (34,35) because TLR-2 is responsible for recognizing different lipoproteins (36); presumably, peptides derived from probiotics would also follow a similar recognition process. This speculation has been strengthened by a recent report which mentioned the possibility of the C-type lectin receptor (CLR) of dendritic cells (DCs) and macrophages recognizing several compounds of probiotic bacteria (37). However, future studies are indispensable to shed more exact light on the correct pathway for the involvement of other receptors specific to AMP.

Probiotics have proved their successful use in the treatment of inflammatory bowel disease and acute diarrhea. GIT associated diseases, such as inflammatory bowel disease (IBD) and chronic relapsing inflammatory disorders occur from the convergence of several reasons, including genetic, immunological, microbial, and environmental factors. The definite role of the gut microbiome in the etiopathogenesis of such diseases has been elucidated from data emanating from a number of clinical and genetic experiments. While all humans host many species of microorganisms, each human host has a unique make-up of these microorganisms. As a result of this diversity, different humans may respond differently to diseases and treatments. Since every individual has his or her unique background, designing a treatment contingent with probiotics may require different permutations of strains of probiotics, nutrients and nutritional supplements or a combination of the above with other medications or treatments, such as antibiotics or chemotherapy.

Novel opportunities, leading to finding effector molecules capable of eliciting definite responses in the human intestine, have been provided in the post-genomic era. With such advances it would be possible in the near future to define causes of IBD conditions and improve therapy by personalized intervention to restore the proper gut microbiome. Bacteria from the infected gut of

**Table 1.** Probiotic-derived anti-infective peptides

Source	Compound	Activity	Ref.
<i>Lactobacillus sakei</i>	Sakacin G	Anti-listerial	39
<i>Lactobacillus rhamnosus</i>	Lactocin 160	Gardnerella vaginalis	40
<i>Streptococcus salivarius</i> K 12	Salivaricin A and salivaricin B	Infections of the human oral cavity	41
<i>Enterococcus faecium</i>	Bacteriocin E 50–52 and bacteriocin B 602	Multi-drug resistant nosocomial infections	42
<i>Lactobacillus johnsonii</i>	Peptide extract	<i>Helicobacter pylori</i>	43
<i>Lactobacillus casei</i>	Peptide extract	<i>Helicobacter pylori</i>	44
<i>Lactobacillus gasseri</i>	Peptide extract	<i>Helicobacter pylori</i>	45
<i>Lactobacillus acidophilus</i>	Peptide extract	<i>Helicobacter pylori</i>	46
<i>Lactobacillus amylovorus</i>	Peptide extract	<i>Helicobacter pylori</i>	47
<i>Bifidobacterium longum</i> subsp. <i>longum</i>	Serpin (AAN23973)	Inhibition of pancreatic and neutrophil elastases	48
<i>B. longum</i> subsp. <i>infantis</i>	CHWPR peptide	Anti-inflammatory and decreases the colonic permeability in IL-10-deficient mice	49
<i>B. breve</i>	Unidentified proteins	Prolonged survival and maturation of DCs; increased IL-10 and IL-12 production by DCs	50
<i>L. rhamnosus</i> GG	NPSRQERR and PDENK	Antimicrobial activity	51
<i>L. acidophilus</i> PZ 1138, <i>L. fermentum</i> PZ 1162	Unidentified secreted proteins	Induction of hBD2 production in epithelial cells	49
<i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. casei</i> and <i>L. delbrueckii</i> subsp. <i>bulgaricus</i>	Unidentified secreted proteins	Induction of mucin secretion	47
<i>L. rhamnosus</i> GG	Unidentified secreted proteins	Increase of the production of HSP25 and HSP72 in YAMC cells	52
<i>L. acidophilus</i> and <i>L. rhamnosus</i>	Unidentified secreted proteins	Increase of the chloride/hydroxyl exchange activity in Caco-2 cells	53
<i>L. rhamnosus</i> GG	p40 (homologous to gi 116493594)	Growth promotion	54
<i>L. rhamnosus</i> GG	p75 (homologous to gi 116493849)	Reduction of the injuries caused by TNF- $\alpha$ ; attenuation of the TER decrease induced by hydrogen peroxide	55
<i>L. rhamnosus</i> GG	Supernatant containing P40 and p75	Decrease of IL-8 production in epithelial cells	54
<i>L. acidophilus</i> NCFM	SlpA (YP_193101.1.)	Induction of IL-10 production in DCs; DC immunomodulation	54
<i>Lactococcus Lactis</i>	Pediocin PA-1 and Lactococcin A	Antimicrobial/antifungal	55
<i>Lactobacillus plantarum</i>	Unknown	Rotaviral Infection/induces endotoxin tolerance capacity	56
<i>L. acidophilus</i> , <i>B. adolescentis</i> , <i>B. bifidum</i>	Acidic and basic lectins	<i>Candida albicans</i> and <i>Staphylococcus aureus</i>	57
<i>Bifidobacterium</i> sp.	Peptides	<i>Helicobacter pylori</i>	58
<i>L. rhamnosus</i>	Peptides	<i>Escherichia coli</i> , <i>Enterobacter aerogenes</i> , <i>Salmonella typhi</i> , <i>Shigella</i> sp., <i>Proteus vulgaris</i> , <i>Pseudomonas aeruginosa</i> , <i>Serratia marcescens</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> , <i>Helicobacter pylori</i> , <i>Campylobacter jejuni</i> , <i>Micrococcus luteus</i> and <i>Listeria monocytogenes</i>	54
<i>Lactococcus lactis</i> subsp. <i>cremoris</i>	Nisin	<i>L. monocytogenes</i> and <i>S. aureus</i>	59
<i>Lactococcus lactis</i> GI3	Lactocin GI3	<i>L. monocytogenes</i> and <i>S. aureus</i>	60

(Contd..)

Table 1. (Continued)

Source	Compound	Activity	Ref.
<i>Lactococcus lactis</i> BFE	Nisin-like bacteriocin	<i>L. monocytogenes</i> and <i>S. aureus</i>	59
<i>Lactobacillus curvatus</i> LTH	Pediocin A	<i>L. monocytogenes</i> and <i>S. aureus</i>	60
<i>Lactobacillus acidophilus</i> LF221	Curvacin A	<i>L. monocytogenes</i> and <i>S. aureus</i>	61
<i>Lactobacillus bavaricus</i> MN	Bavaricin MN	<i>L. monocytogenes</i> and <i>S. aureus</i>	62

an individual can be cultured and enriched with bacteria constituting a healthy microbiome (38). Such an enriched culture used for therapy may be more effective than probiotics administered according to the one-size-fits-all concept. Hence, in the context of recent knowledge, management of the gut microbiome by probiotics or a mixed culture of useful bacteria is becoming a pragmatic strategy in therapeutics and prophylactics to counter many infectious and inflammatory diseases within the gut.

## 6. CONCLUSION

Owing to the colonizing nature of probiotics in the GIT, they can be an effective tool in biotechnological applications. Antimicrobial peptides originating from different sources have been well studied on the basis of their structure-function relationship. Probiotics are the most important flora in the human gut, and several beneficial aspects have been tested, ranging from *in vitro* studies to clinical trials. However, the enumeration of the total molecules of probiotics has not been presented yet, particularly for anti-infective peptides derived from probiotics with multifunctional activities (Figure 3). Molecular information from synthesis to heterologous expression is inadequate. The signaling mechanisms and physiological changes induced by antimicrobial peptides in host cells are not well described. In the light of this discussion, a combined and urgent drive is required from researchers in diverse disciplines to design protocols to use probiotics successfully for sustainable health. Moreover, chemically and genetically re-engineered probiotics will serve as a novel tool in modern biomedical technology.

## 7. REFERENCES

1. J Cleveland, TJ Montville, IF Nes, ML Chikindas: Bacteriocins: safe, natural antimicrobials for food preservation. *Int J Food Microbiol*, 71, 1-20, (2001)  
DOI: 10.1016/S0168-1605(01)00560-8
2. T Abee, L Krockel, C Hill: Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *Int J Food Microbiol* 28, 169–185, (1995)  
DOI: 10.1016/0168-1605(95)00055-0
3. J. Delves-Broughton, P Blackburn, RJ Evans, J Hugenholtz: Applications of the bacteriocin, nisin. *Antonie van Leeuwenhoek* 69, 193–202, (1996)  
DOI: 10.1007/BF00399424  
PMid:8775979
4. S Wessels, B Jelle, I Nes: Bacteriocins of lactic acid bacteria. Report of the Danish Toxicology Centre, Denmark (1998)
5. B Sánchez, MC Urdaci, A Margolles: Extracellular proteins secreted by probiotic bacteria as mediators of effects that promote mucosa-bacteria interactions. *Microbiology*, 156, 3232-3342 (2010)  
DOI: 10.1099/mic.0.044057-0  
PMid:20864471
6. OL Franco: Peptide promiscuity: an evolutionary concept for plant defense. *FEBS Lett*, 585, 995-1000, (2011)  
DOI: 10.1002/bip.21602  
PMid:21328576
7. A Roy, OL Franco, SM Mandal: Biomedical exploitation of self assembled peptide based nanostructures. *Curr Protein Pept Sci*. 14, 580-587, (2013)  
DOI: 10.2174/1389203711209070687  
PMid:23968344
8. A Roy, D Mahata, D Paul, S Korpole, OL Franco, SM Mandal: Purification, biochemical characterization and self-assembled structure of a fengycin-like antifungal peptide from *Bacillus thuringiensis* strain SM1. *Front Microbiol*, 21, 4, 332, (2013)  
DOI: 10.1016/j.seppur.2013.05.010  
DOI: 10.1016/j.seppur.2012.12.014
9. G Barbarella, F Di Maria: Supramolecular Oligothiophene Microfibers Spontaneously Assembled on Surfaces or Coassembled with Proteins inside Live Cells. *Acc Chem Res*. 18, 48, 2230-2341, (2015)  
DOI: 10.1021/acs.accounts.5b00241  
PMid:26234700

10. I Adkins, J Holubova, M Kosova, L Sadilkova: Bacteria and their toxins tamed for immunotherapy. *Curr Pharm Biotechnol*, 13, 1446-1473 (2012)  
DOI: 10.2174/138920112800784835  
PMid:22339216
11. WB Whitman, DC Coleman, WJ Wiebe: Prokaryotes: the unseen majority. *Proc Natl Acad Sci USA*, 95, 6578-6583, (1998)  
DOI: 10.1073/pnas.95.12.6578  
PMid:9618454 PMCID: PMC33863
12. F Backhed, RE Ley, JL Sonnenburg, DA Peterson, JI Gordon: Host-bacterial mutualism in the human intestine. *Science* 307, 1915-1920, (2005)  
DOI: 10.1126/science.1104816  
PMid:15790844
13. TD Lawley, AW Walker: Intestinal colonization resistance. *Immunology* 138, 1–11, (2013)  
DOI: 10.1111/j.1365-2567.2012.03616.x  
PMid:23240815 PMCID: PMC3533696
14. S Rakoff-Nahoum, J Paglino, F Eslami-Varzaneh, S Edberg, R Medzhitov: Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell*, 118, 229–241, (2004)  
DOI: 10.1016/j.cell.2004.07.002  
PMid:15260992
15. JL Kubinak, JL Round: Toll-Like Receptors Promote Mutually Beneficial Commensal-Host Interactions. *PLoS Pathog.* 8, e1002785, (2012)  
DOI: 10.1371/journal.ppat.1002785  
PMid:22910541 PMCID: PMC3406078
16. CL Bevins, NH Salzman: Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. *Nat Rev Microbiol*, 9, 356-368, (2011)  
DOI: 10.1038/nrmicro2546  
PMid:21423246
17. JR Mastroianni, AJ Ouellette: Alpha-defensins in enteric innate immunity: functional Paneth cell alpha-defensins in mouse colonic lumen. *J Biol Chem*, 9, 284, 27848-56, (2009)  
DOI: 10.1074/jbc.M109.050773  
PMid:19687006 PMCID: PMC2788835
18. SC Yang, CH Lin, TS Calvin, JY Fang: Antibacterial activities of bacteriocins: application in foods and pharmaceuticals. *Front Microbiol*, 5, 241, (2014)  
DOI: 10.3389/fmicb.2014.00241  
DOI: 10.3389/fmicb.2014.00683
19. WH Van der Putten, JN Klironomos, DA Wardle: Microbial ecology of biological invasions. *The ISME J*, 1, 28–37, (2007)  
DOI: 10.1038/ismej.2007.9  
PMid:18043611
20. F Bottacini, M Ventura, D van Sinderen, M O'Connell: Motherway, Diversity, ecology and intestinal function of bifidobacteria. *Microb Cell Fact*, 13, S1-S4 (2014)  
DOI: 10.1186/1475-2859-13-S1-S4
21. L Ruiz, A Margolles, S Borza: Bile resistance mechanisms in *Lactobacillus* and *Bifidobacterium*. *Front Microbiol*, 4, 396, (2013)  
DOI: 10.3389/fmicb.2013.00396  
PMid:24399996 PMCID: PMC3872040
22. CH Pohl, JLF Kock, VS Thibane: Antimicrobial compounds produced by *Bacillus* spp. and applications in food. Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas (Ed.) (2015)
23. EA Svetoch, BV Eruslanov, VV Perelygin, EV Mitsevich, IP Mitsevich, VN Borzenkov, VP Levchuk, OE Svetoch, YN Kovalev, YG Stepanshin, GR Siragusa, B S Seal, NJ Stern: Diverse antimicrobial killing by *Enterococcus faecium* E 50-52 bacteriocin. *J Agric Food Chem.* 26, 56, 1942-1948, (2008)  
DOI: 10.1021/jf073284g  
PMid:18293921
24. CH Pohl, JLF Kock, VS Thibane: Science against microbial pathogens: communicating current research and technological advances. A. Méndez-Vilas (Ed.) (2015)
25. MV Lakhtin, A Lakhtin, A Aleshkin, A Bajrakova, S. Afanasiev, V Aleshkin: Lectin systems imitating probiotics: Potential for biotechnology and medical microbiology. In: Rigobelo EC, editor. Probiotics 2012. New York: InTech; p. 417 – 32, (2012)  
DOI: 10.5772/47828
26. SM Mandal, ON Silva, OL Franco: Recombinant probiotics with antimicrobial peptides: a dual strategy to improve immune response in immunocompromised patients. *Drug Discov Today*, 19, 1045-1050, (2014)  
DOI: 10.1016/j.drudis.2014.05.019



- PMid:24881782
27. MF Bachmann, GT Jennings: Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. *Nat Rev Immunol*, 10, 787-796, (2010)  
DOI: 10.4161/hv.6.7.12827  
DOI: 10.1016/j.vaccine.2010.07.030  
PMid:20659519 PMCID: PMC2939220
  28. ME Bianchi: DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol*, 81, 1-5, (2007)  
DOI: 10.1189/jlb.0306164  
PMid:17032697
  29. NI Torres, KS Noll, S Xu, J Li, Q Huang, PJ Sinko, MB Wachsman, ML Chikindas: Safety, formulation, and *in vitro* antiviral activity of the antimicrobial peptide subtilisin against herpes simplex virus type 1. *Probiotics Antimicrob Proteins*, 5, 26-35, (2013)  
DOI: 10.1007/s12602-013-9128-0  
DOI: 10.1007/s12602-012-9123-x  
PMid:23637711 PMCID: PMC3637976
  30. DK Podolsky: Inflammatory bowel disease. *N. Engl. J. Med.* 347, 41 7-429 (2002)  
DOI: 10.1056/NEJMra020831  
PMid:12167685
  31. E Owaga, RH Hsieh, B Mugendi, S Masuku, CK Shih, JS Chang: Th17 Cells as Potential Probiotic Therapeutic Targets in Inflammatory Bowel Diseases. *Int J Mol Sci.* 16, 20841-20858, (2015)  
DOI: 10.3390/ijms160920841  
PMid:26340622 PMCID: PMC4613231
  32. DW Lescheid: Probiotics as regulators of inflammation: A review. *Funct Food Health Dis*, 4, 299-311,(2014)
  33. C Hoarau, L Martin, D Faugaret, C Baron, A Dauba, CA Jacquin, F Velge-Roussel, Y Lebranchu: Supernatant from Bifidobacterium differentially modulates transduction signaling pathways for biological functions of human dendritic cells. *PLoS ONE*, 3, e2753, (2008)  
DOI: 10.1371/journal.pone.0002753  
PMid:18648505 PMCID: PMC2447180
  34. Ren, T., D. S. Zamboni, C. R. Roy, W. F. Dietrich and R. E. Vance: Flagellin-deficient Legionella mutants evade caspase-1- and Naip5-mediated macrophage immunity. *PLoS Pathog*, 2, e18, (2006)  
DOI: 10.1371/journal.ppat.0020018
  35. FH Yan, TL Cao, TL Cover, R Whitehead, MK Washington, DB Polk: Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology*, 132, 562-575, (2007)  
DOI: 10.1053/j.gastro.2006.11.022  
PMid:17258729 PMCID: PMC3036990
  36. SR Konstantinov, H Smidt, WM de Vos, SC Bruijns, SK Singh, F Valence, D Molle, S Lortal, E Altermann: S layer protein A of Lactobacillus acidophilus NCFM regulates immature dendritic cell and T cell functions. *Proc Natl Acad Sci U S A*, 105, 19474-19479 (2008)  
DOI: 10.1073/pnas.0810305105  
PMid:19047644 PMCID: PMC2592362
  37. E Bellaguarda, EB Chang: IBD and the gut microbiota--from bench to personalized medicine. *Curr Gastroenterol Rep.* 17, DOI: 10.1007/s11894-015-0439-z (2015)
  38. R Kort: Personalized therapy with probiotics from the host by TripleA. *Trends Biotechnol*, 32, 291-293 (2014)  
DOI: 10.1016/j.tibtech.2014.04.002  
PMid:24785330
  39. L Simon, C Fremaux, Y Cenatiempo, JM Berjeaud: Sakacin G, a new type of antilisterial bacteriocin. *Appl Environ Microbiol.* 68, 6416-6420, (2002)  
DOI: 10.1128/AEM.68.12.6416-6420.2002  
PMid:12450870 PMCID: PMC134399
  40. Turovskiy Y, RD Ludescher, AA Aroutcheva, S Faro, ML Chikindas: Lactocin 160, a Bacteriocin Produced by Vaginal Lactobacillus rhamnosus, Targets Cytoplasmic Membranes of the Vaginal Pathogen, Gardnerella vaginalis. *Probiotics Antimicrob Proteins.* 20,1, 67-74, (2009)  
DOI: 10.1007/s12602-008-9003-6  
PMid:20445810 PMCID: PMC2863056
  41. PA Wescombe, M Upton, KP Dierksen, NL Ragland, S Sivabalan, RE Wirawan, MA Inglis, CJ Moore, GV Walker, CN Chilcott, HF Jenkinson, JR Tagg: Production of the lantibiotic salivaricin A and its variants by oral streptococci and use of a specific induction assay to detect their presence in human saliva. *Appl Environ Microbiol.* 72,1459-1466, (2006)  
DOI: 10.1128/AEM.72.2.1459-1466.2006



- PMid:16461700 PMCID: PMC1392966
42. EA Svetoch, BV Eruslanov, VV Perelygin, EV Mitsevich, IP Mitsevich, VN Borzenkov, VP Levchuk, OE Svetoch, YN Kovalev, YG Stepanshin, GR Siragusa, BS Seal, NJ Stern: Diverse antimicrobial killing by *Enterococcus faecium* E 50-52 bacteriocin. *J Agric Food Chem.* 26, 56, 1942-1948, (2008)  
DOI: 10.1021/jf073284g  
PMid:18293921
  43. RM Duar, KJ Clark, PB Patil, C Hernández, S Brünig, TE Burkey, N Madayiputhiya, SL Taylor, J Walter: Identification and characterization of intestinal lactobacilli strains capable of degrading immunotoxic peptides present in gluten. *J Appl Microbiol.* 118, 515-27, (2015)  
DOI: 10.1111/jam.12687  
PMid:25376327
  44. L Zhang, P Su, A Henriksson, J O'Rourke, H Mitchell: Investigation of the immunomodulatory effects of *Lactobacillus casei* and *Bifidobacterium lactis* on *Helicobacter pylori* infection. *Helicobacter.* 13, 183-190, (2008)  
DOI: 10.1111/j.1523-5378.2008.00595.x  
PMid:18466393
  45. A Ushiyama, K Tanaka, Y Aiba, T Shiba, A Takagi, T Mine, Y Koga: *Lactobacillus gasseri* OLL2716 as a probiotic in clarithromycin-resistant *Helicobacter pylori* infection. *J Gastroenterol Hepatol.* 18, 986-991, (2003)  
DOI: 10.1046/j.1440-1746.2003.03102.x  
PMid:12859730
  46. YJ Yang, CC Chuang, HB Yang, CC Lu, BS Sheu: *Lactobacillus acidophilus* ameliorates *H. pylori*-induced gastric inflammation by inactivating the Smad7 and NF $\kappa$ B pathways. *BMC Microbiol.* 19, 12, 38, (2012)
  47. Q Tan, H Xu, T Chen, P Li, ZP Aguilar, D Xu, X Ming, F Xu, H Wei: Differential expression of virulence and stress fitness genes during interaction between *Listeria monocytogenes* and *Bifidobacterium longum*. *Biosci Biotechnol Biochem.* 76, 699-704 (2012)  
DOI: 10.1271/bbb.110832  
PMid:22484935
  48. B Sa'nchez, MC Urdaci: Extracellular proteins secreted by probiotic bacteria as mediators of effects that promote mucosa-bacteria interactions and *Abelardo margolles*. *Microbiology*, 156, 3232-3242 (2010)  
DOI: 10.1099/mic.0.044057-0  
PMid:20864471
  49. C Hoarau, C Lagaraine, L Martin, F Velge-Roussel, Y Lebranchu: Supernatant of *Bifidobacterium breve* induces dendritic cell maturation, activation, and survival through a Toll-like receptor 2 pathway. *J Allergy Clin Immunol.* 117, 696-702, (2006)  
DOI: 10.1016/j.jaci.2005.10.043  
PMid:16522473
  50. R Lu, S Fasano, N Madayiputhiya, NP Morin, J Nataro, A Fasano: Isolation, identification, and characterization of small bioactive peptides from *Lactobacillus GG* conditional media that exert both anti-Gram-negative and Gram-positive bactericidal activity. *J Pediatr Gastroenterol Nutr.* 49, 23-30, (2009)  
DOI: 10.1097/MPG.0b013e3181924d1e  
PMid:19465870
  51. M Hausmann: How Bacteria-Induced Apoptosis of Intestinal Epithelial Cells Contributes to Mucosal Inflammation. *Int J Inflam*, 6, 574568, (2010)  
DOI: 10.4061/2010/574568
  52. A Borthakur, RK Gill, S Tyagi, A Koutsouris, WA Alrefai, GA Hecht, K Ramaswamy, PK Dudeja: The Probiotic *Lactobacillus acidophilus* Stimulates Chloride/Hydroxyl Exchange Activity in Human Intestinal Epithelial Cells. *J Nutr.* 138, 1355-1359, (2008)  
PMid:18567760 PMCID: PMC2705118
  53. VL Moal, AL Servin: Anti-Infective Activities of *Lactobacillus* Strains in the Human Intestinal Microbiota: from Probiotics to Gastrointestinal Anti-Infectious Biotherapeutic Agents. *Clin Microbiol Rev.* 27, 167-199, (2014)  
DOI: 10.1128/CMR.00080-13  
PMid:24696432 PMCID: PMC3993101
  54. N Horn, MI Martínez, JM Martínez, PE Hernández, MJ Gasson, JM Rodríguez, HM Dodd: Production of Pediocin PA-1 by *Lactococcus lactis* Using the Lactococcin A Secretory Apparatus. *Appl Environ Microbiol.* 64, 818-823, (1998)  
PMid:9501421 PMCID: PMC106332
  55. YH Chiu, Y-C Lu, C-C Ou, S-L Lin, C-C Tsai, C-T Huang, M-Y Lin: *Lactobacillus plantarum* MYL26 induces endotoxin tolerance phenotype in Caco-2 cells. *BMC Microbiol.* 13, 190 (2013)

- DOI: 10.1186/1471-2180-13-190  
PMid:23937116 PMCID: PMC3751156
56. S Kandasamy, KS Chattha, AN Vlasova, G Rajashekara, LJ Saif: Lactobacilli and Bifidobacteria enhance mucosal B cell responses and differentially modulate systemic antibody responses to an oral human rotavirus vaccine in a neonatal gnotobiotic pig disease model. *Gut Microbes*, 5, 639-651 (2014)  
DOI: 10.4161/19490976.2014.969972  
PMid:25483333 PMCID: PMC4615723
  57. E Chenoll, B Casinos, E Bataller, P Astals, J Echevarría, JR Iglesias, P Balbarie, D Ramón, S Genovés: Novel Probiotic *Bifidobacterium bifidum* CECT 7366 Strain Active against the Pathogenic Bacterium *Helicobacter pylori*. *Appl Environ Microbiol.* 77, 1335–1343 (2011)  
DOI: 10.1128/AEM.01820-10  
PMid:21169430 PMCID: PMC3067243
  58. M Parapouli, C Delbès-Paus, A Kakouri, A-I Koukkou, M-C Montel, J Samelis: Characterization of a Wild, Novel Nisin A-Producing *Lactococcus* Strain with an *L. lactis* subsp. *cremoris* Genotype and an *L. lactis* subsp. *lactis* Phenotype, Isolated from Greek Raw Milk. *Appl Environ Microbiol.* 79, 3476–3484, (2013)  
DOI: 10.1128/AEM.00436-13  
PMid:23542625 PMCID: PMC3648029
  59. ME Janes, R Nannapaneni, A Proctor, MG Johnson: Rice Hull Ash and Silicic Acid as Adsorbents for Concentration of Bacteriocins. *Appl Environ Microbiol*, 64, 4403–4409, (1998)  
PMid:9797298 PMCID: PMC106660
  60. KI Garver, PM Muriana: Purification and partial amino acid sequence of curvacin FS47, a heat-stable bacteriocin produced by *Lactobacillus curvatus* FS47. *Appl Environ Microbiol*, 60, 2191–2195, (1994)  
PMid:8031103 PMCID: PMC201625
  61. J Verluyten, F Leroy, L Vuyst: Influence of Complex Nutrient Source on Growth of and Curvacin A Production by Sausage Isolate *Lactobacillus curvatus* LTH 1174. *Appl Environ Microbiol*, 70, 5081–5088, (2004)  
DOI: 10.1128/AEM.70.9.5081-5088.2004  
DOI: 10.1128/AEM.70.8.4807-4813.2004
  62. AL Kaiser, TJ Montville: Purification of the bacteriocin bavaricin MN and characterization

of its mode of action against *Listeria monocytogenes* Scott A cells and lipid vesicles. *Appl Environ Microbiol*, 62, 4529–4535, (1996)  
PMid:8953724 PMCID: PMC168279

**Key Words:** Probiotics, Anti-Infective Peptides, Heterologous Expression, Bacterial Surface Engineered, Review

**Send correspondence to:** Santi M. Mandal, Anti-Infective Research Lab., Department of Microbiology, Vidyasagar University, Midnapore 721102, WB, India, Tel: 91-3222-276554, Fax: 91-3222- 275297, E-mail: mandalsm@gmail.com