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Review Article

A Review of the Advancements in Probiotic Delivery: Conventional *vs.* Non-conventional Formulations for Intestinal Flora Supplementation

Mershen Govender,[1] Yahya E. Choonara,[1] Pradeep Kumar,[1] Lisa C. du Toit,[1] Sandy van Vuuren,[1] and Viness Pillay[1,2]

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Abstract. Probiotic delivery systems are widely used nutraceutical products for the supplementation of natural intestinal flora. These delivery systems vary greatly in effectiveness to exert health benefits for a patient. Probiotic delivery systems can be categorized into conventional, pharmaceutical formulations, and non-conventional, mainly commercial food-based, products. The degree of health benefits provided by these probiotic formulations varies in their ability to deliver viable, functional bacteria in large enough numbers (effectiveness), to provide protection against the harsh effects of the gastric environment and intestinal bile (*in vivo* protection), and to survive formulation processes (viability). This review discusses the effectiveness of these probiotic delivery systems to deliver viable functional bacteria focusing on the ability to protect the encapsulated probiotics during formulation process as well as against harsh physiological conditions through formulation enhancements using coatings and polymer enhancements. A brief overview on the health benefits of probiotics, current formulation, patient and legal issues facing probiotic delivery, and possible recommendations for the enhanced delivery of probiotic bacteria are also provided. Newer advanced *in vitro* analyses that can accurately determine the effectiveness of a probiotic formulation are also discussed with an ideal probiotic delivery system hypothesized through a combination of the two probiotic delivery systems described.

KEY WORDS: conventional and non-conventional formulations; drug delivery systems design; intestinal flora; nutraceutical products; probiotics.

INTRODUCTION

The Need for Intestinal Flora Supplementation

Probiotic bacteria are living supplementary organisms that have been shown to provide beneficial health effects to the host by replenishing natural gastrointestinal flora (1–5). The human intestinal system is thought to contain over 500 microbial species and approximately 10^{14} functional bacterial cells and also include fungi, yeasts, viruses, and protozoa. Some bacteria, such as *Streptococci* and *Staphylococci* spp., are known to cause infectious diseases in humans (6–9). These organisms are usually depleted for various reasons such as stress, infection, antibiotic use, and environmental factors. Other patients that require probiotic therapy are babies born of caesarean section and do not gain the bacteria it would naturally while travelling down the birth canal (9). Probiotic supplementation in these patients has been shown to be vital in the prevention of potentially fatal, pathological infections (10–12). A full representation of the microbial content of the human gastro-intestinal system can be found in Fig. 1.

Probiotic bacteria have also been widely recognized to have other health benefits to the patient such as effects on immunological functions, aiding in digestion, as well as protection against pathogenic bacteria such as *Salmonella typhimurium*, *Helicobacter pylori*, and *Escherichia coli* (12,14–17). Other functions of probiotics include improvement of lactose intolerance, decreasing cholesterol levels, treatment of Crohn's disease, ulcerative colitis, IBS, and replenishment of intestinal flora after antibiotic therapy to prevent antibiotic-induced diarrhea (18–23). Intestinal flora are also important in entero-hepatic recycling which results in the metabolizing of a variety of drugs. Probiotics, by definition, should adhere to the intestinal cells, not promote or encourage antibiotic resistance, not as themselves be pathogenic in nature, and must be able to co-aggregate as part of the natural gut flora (12,24). The degree at which the functional benefits are received are dependent on the type of bacteria delivered as well as the number of viable bacteria that is delivered to the gastro-intestinal system (25,26). A basic schematic diagram on the properties of an ideal probiotic can be found in Fig. 2.

Probiotic Formulations

The use of intestinal flora supplementation can be dated back decades with the first documented article of bacterial supplementation published in the early twentieth century by

[1] Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, 2193, Johannesburg, South Africa.
[2] To whom correspondence should be addressed. (e-mail: Viness.Pillay@wits.ac.za)

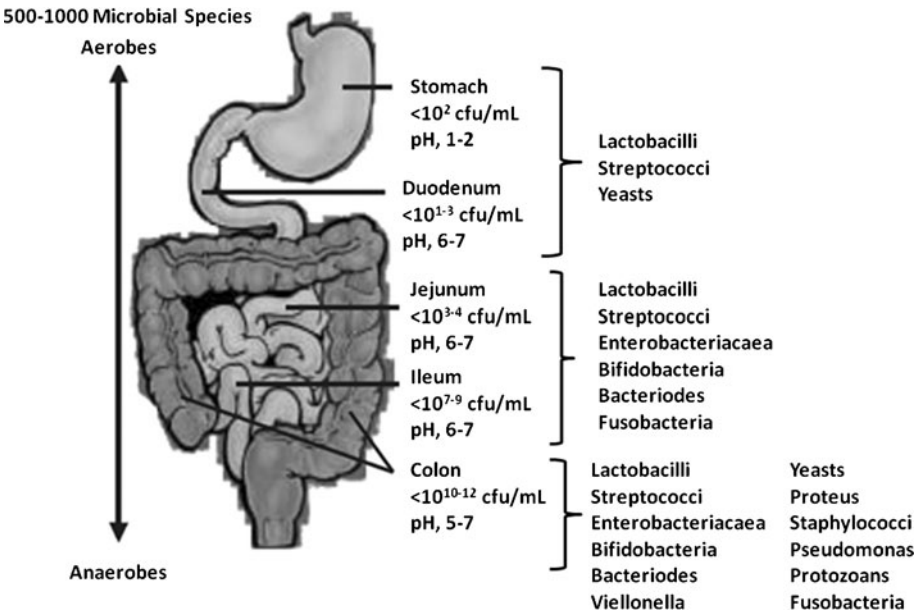

Fig. 1. Summary of the microbial content found in a healthy human GIT. [Image adapted with permission from Iannitti and Palmieri (12) © 2010 Elsevier Ltd, Microbial species adapted with permission from Holzapfel *et al.* (13) © 1998 Elsevier Science B.V.]

Elie Metchnikoff. Probiotic research has, however, greatly increased in the last decade with over 5,000 publications detailing their health benefits as well as their ability to delivery viable functional probiotic bacteria (27). The main species of bacteria used in probiotic formulations are *Lactobacillus* and *Bifidobacterium* spp., which are classified as anaerobic bacteria and therefore require an oxygen-free environment for growth to occur (16,28–32). Potentially advantageous properties of probiotic bacteria such as gastric-resistance vary from species to species and within strains. For example, *Lactobacillus* spp. are more viable in gastric conditions compared to other probiotic species, making it the most ideal probiotic (active ingredient) for dosage forms that do not provide gastro-protection (33). Some species of bacteria are also found in greater numbers in certain parts of the GIT and provide a more functional role when they colonize these areas. An example of this is *Bifidobacterium* which is found in large numbers in the colon and serves as a digestion aid to ferment and digest complex carbohydrates from the host's diet (34). The advantage of this property is that it allows for targeted, species-specific probiotic delivery systems which will result in greater health benefits to the patient.

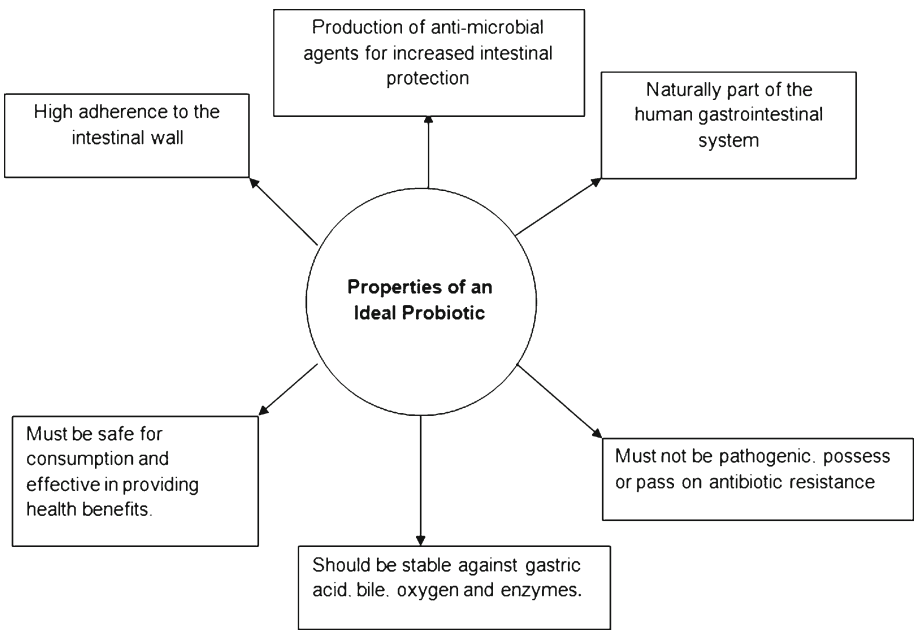

Fig. 2. A basic schematic detailing the properties of an ideal probiotic bacteria (12,24)

Probiotic Delivery Mechanisms

Many systems have been developed for the delivery of probiotics to the gastrointestinal system which include both conventional pharmaceutical systems and non-conventional commercial products. These commercial formulations consist mainly of food-based products, many of which use probiotic bacteria in their production with others having added these bacteria as an adjunctive health benefit of ingesting the product. These products account for 90% of probiotic formulations and with the large amount of research into improvement of commercial food-based products for delivery of functional probiotic bacteria, their ability to act as probiotic delivery systems cannot be ignored. Non-conventional probiotic formulations range from cheeses, yogurts, creams, chocolates, milk, and meat, among others. They have been around for decades and are sold to the general public with little or no regulation and control (35–38). A brief overview on the non-conventional probiotic delivery systems can be found in Fig. 3. Due to their easy availability and convenience, they are good delivery systems that, if effective, can be beneficial to the patient. A few of these products have the ability to deliver viable probiotic bacterial cells to the human intestine but as with pharmaceutical formulations, they differ greatly. This difference is as a result of various reasons ranging from formulation processes, viability of dosed bacteria, as well as variability in the ability of different species of bacteria to survive physiological conditions and adhere to the intestinal wall.

Conventional pharmaceutical products tend to be more effective in this regard and are much more characterized compared to commercial food-based carrier systems. Examples of pharmaceutical formulations for the delivery of probiotics currently include, among others, beads, capsules, and tablets (32,39). These formulations, as with the commercial products, vary in their effectiveness and ability to deliver viable functional bacteria in the numbers required for health benefits to be seen. While each type of formulation has been found to possess advantages in the delivery of probiotics, with each one delivering varying amounts of viable probiotic bacterial cells, differences in their effectiveness to deliver the correct amount of bacteria to the human intestinal system as well providing protection to the dosed probiotic bacteria differs. Formulation processes also seem to affect the ability of dosage forms to deliver the correct numbers of viable bacteria with processes, such as lyophilization, affecting the stability of probiotic bacteria. With these issues, various pharmaceutical changes have been incorporated into formulations to improve the survival rate of probiotic bacteria, not only during formulation processes, but also in both *in vitro* and *in vivo* studies that seek to improve on the issues of physiological viability of probiotic bacteria.

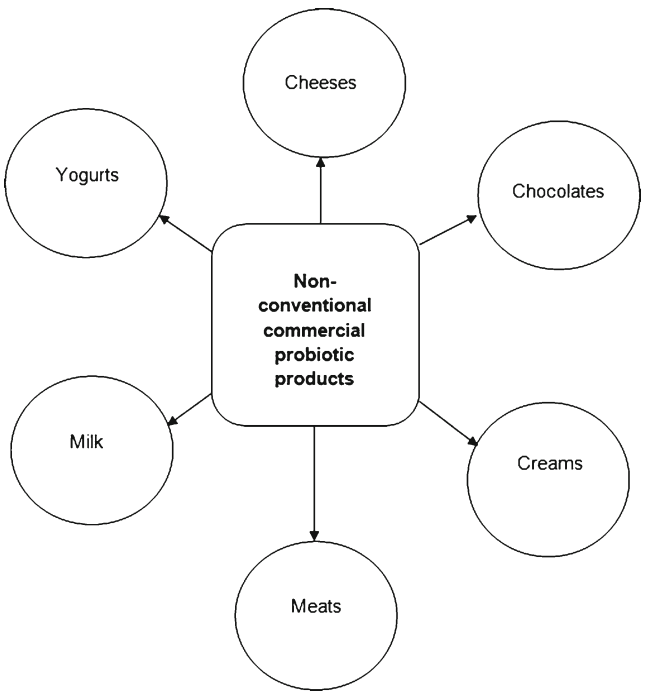

Fig. 3. An overview of the available non-conventional delivery systems used for probiotic delivery

Challenges do exist in both categories of delivery systems and have been thoroughly researched. Many newer systems seek to alleviate these issues and provide effective functional delivery systems that will provide health benefits to the patient. These challenges that exist in delivery of probiotics in both the pharmaceutical and commercial products include lack of protection in the harsh gastric environment, delivery of inadequate amounts of viable bacteria at the time of administration, delivery of the incorrect strains of probiotic bacteria as well as little protection against the concurrent delivery of antibiotics (40–43). Studies have shown that at least 10^8-10^9 viable cells must reach the intestine for health benefits to be achieved for the patient (44–46). Many formulations tend to deliver species of probiotic bacteria that have more gastro-resistant properties compared to other species of bacteria. *Lactobacillus* spp. tend to have a greater resistance to gastric acid than others species such as *Bifidobacterium* spp. and are found more often in probiotic formulations in comparison with other probiotic species (12,47,48). *Enterococci* have also been found to be more resistant to gastric conditions compared to other bacteria, with 66% of tested bacteria surviving 60mins at pH 3.0 and 40% in pH 2.0. This was considered acceptable in the delivery of bacteria to the stomach as the average human stomach is between pH 2.0 and 3.0 (49). Bile tolerance is also an important property required in probiotic bacteria due to the interaction of the bacteria with bile on entry into the small intestine. This issue, however, is not seen with naturally occurring probiotic bacteria as they have developed bile tolerance being commonly exposed to bile salts in the intestinal system (49).

This review will therefore provide a description of the health benefits of probiotic supplementation, elaborate on the different ways in which formulations are prepared, distinguish between the differences between conventional pharmaceutical and non-conventional commercial products for the delivery of probiotic bacteria, and will discuss current issues facing probiotic delivery. In addition, newer analyses that are currently being used for the accurate determination of the effectiveness of probiotic delivery systems will be given attention as well as recommendations made for future advancements in probiotic delivery.

THERAPEUTIC ADVANTAGES OF PROBIOTIC SUPPLEMENTATION

Health Benefits of Probiotics

Probiotic bacteria are currently classified as nutraceuticals and account for billions in sales annually (32). Nutraceuticals are defined as dietary substances that deliver a concentrated form of a bioactive substance in quantities that exceed what can be obtained from food (16). Intestinal flora supplementation through the use of probiotics has been shown through many studies for the prevention and treatment of conditions caused by pathogenic bacteria. This treatment has been shown to occur due to the probiotic's competitive inhibition of pathogenic bacteria through adhesion and colonization of binding sites in the small intestine (4,50). It is this preventive role, among others, of intestinal flora that's of vital importance for the health and overall well-being of humans.

Immuno-Stimulatory Responses and Biological Activities of Probiotics

With the intestinal mucosa exposed to a variety of pathogenic bacteria, vaccines are a crucial intervention that is currently being sought for the prevention of many life-threatening conditions. Probiotic bacteria, which are organisms that are naturally part of the intestinal mucosa, have the potential to be a delivery system of vaccines for the prevention of conditions caused by pathogenic organisms in the intestine. Due to the invasive nature of current vaccine formulations, research into the use of orally induced live carriers capable of expressing specific pathogenic virulence factor-derived antigens to exert an immunological response has occurred. *E. coli* Nissle 1917 is an example of such a probiotic bacterium that has been linked to a possible use as a vaccine delivery system (51). This nonpathogenic bacterium, when utilized as a live carrier, has been shown in this study to successfully exert an immunological response in rats when coupled with model antigens. This immunological response, determined through specific antibiotic titers, however was not consistence and determined to be sub-effective to provide protection. Due to the positive results seen in this study, the use of probiotic bacteria in vaccine development further highlights the possibility of utilizing probiotic bacteria in immunological modulation. This study also highlights a highly promising area of probiotic research still to be fully undertaken with the possibility of groundbreaking discoveries to be found.

Other than the prevention of adhesion and colonization of pathogenic bacteria, probiotic bacteria have been found to provide other immuno-stimulatory responses. While the positive effects of the oral ingestion of bacteria have been widely debated, more health benefits have been shown to exist above gut-specific functions and immunological functions leading to the belief that probiotics have functional benefits at both a cellular and molecular level (52). The proposed method of treatment by probiotics is that the human gastrointestinal system routinely samples gut microflora to assert with its regulatory functions and that probiotic supplementation assists in this process (52). A study into the effects of probiotics on fish microflora has shown that probiotic bacteria assist in the expression of cytokines and other inflammatory mediators. Further analysis of the immunological effects of probiotic bacteria showed an increase in survival rates with animals exposed to *Vibrio alginolyticus* (53). The test organism (shrimp) in the study showed increases in phenoloxidase activity, phagocytic activity and clearance efficiency of the inoculated pathogen compared to shrimp that were not fed a steady probiotic diet (53). This increase in survival was, however, proportional to the number of bacteria delivered to the shrimp (53). It was therefore hypothesized that probiotic bacteria have immunological effects far beyond protection from colonization and adhesion, but may also assist in both the prevention and eradication of pathogenic bacteria.

Lactobacillus has also been shown to have anti-allergy effects in the prevention of atopic sensitization (17,54). The proposed method for this immune-modulation is that *Lactobacillus* as well as other probiotics potentiates T-helper [Th] cells influencing the formation of pro-Th cytokines. This immune-modulation has, however, been shown to be strain-specific and not applicable to all strains of *Lactobacillus* bacteria (54). Images of mice predisposed to atopic dermatitis treated with *Lactobacillus acidophilus* strain L-55 as well as with prednisolone, a common treatment for atopic dermatitis can be found in Fig. 4.

Probiotic bacteria furthermore, have been linked to cancer therapy. A wide variety of studies have been conducted to

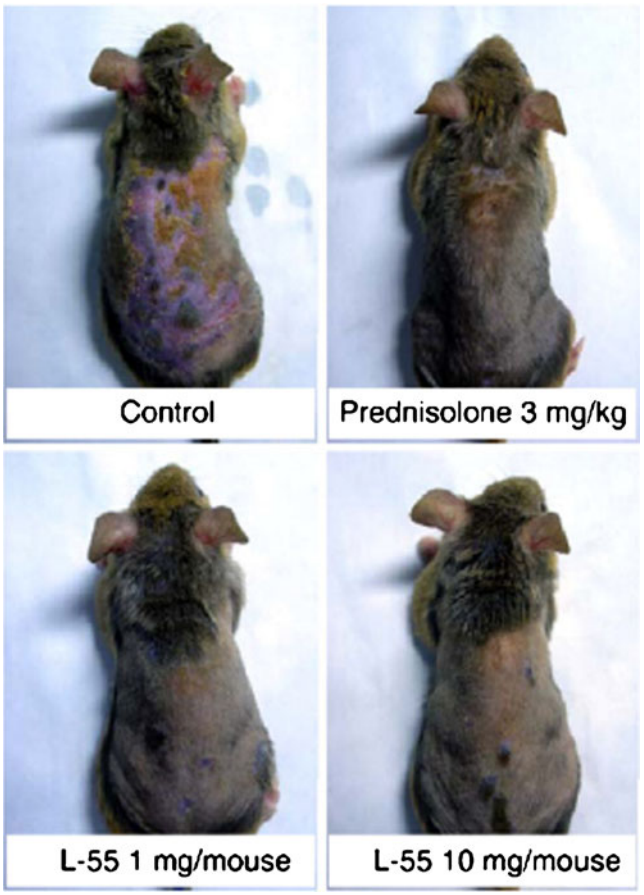

Fig. 4. Images of mice predisposed to atopic dermatitis treated with prednisolone and *Lactobacillus acidophilus* strain L-55 showing significant improvement in the treatment of this condition using the probiotic bacteria when compared to the control group. [Image reproduced with permission from Sunada *et al.* (17) © 2008 Elsevier B.V.]

determine the effectiveness of the systemic administration of both pathogenic and non-pathogenic bacteria for the possible site-specific protein treatment in cancer patients bearing tumors. The mechanism of delivery of drugs using bacteria is that bacteria agglomerate within cancer tumors and agglomerate significantly less in organs such as the spleen and liver resulting in less toxicity expected with systemic administration of bacteria (55). The use of probiotic bacteria, which by definition are non-pathogenic, and would have significantly less systemic side effects, has been researched with *E. coli* Nissle 1917. Results of this study showed a large number of bacteria cells agglomerating in tumors within mice, proposing probiotic bacteria as a possible tumor-targeting delivery system that can be used in both immuno-competent and immuno-compromised patients, which in the treatment of cancer patients is vital (55). Probiotic bacteria have also been found to be therapeutic in patients suffering from high cholesterol levels as well as in patients diagnosed with obesity (56,57). Furthermore, a study conducted by Guerra *et al.* (58) has also shown that probiotic bacteria can also be used as an alternative growth enhancer in pigs, a commonly used *in vivo* test model for correlation with human patients.

Concomitant Antibiotic Use and Probiotics

Antibiotics have been proven to disturb intestinal flora balance by killing susceptible intestinal flora bacteria. Probiotic supplementation traditionally seeks to increase the numbers of intestinal flora after antibiotic administration, preventing commonly seen side effects of antibiotic therapy. A study conducted by Madden *et al.* (59) have indicated that in patients receiving multiple forms of antibiotics, probiotics are effective in restoring intestinal flora removed by antibiotic therapy. Commonly used probiotic bacteria (*L. acidophilus* and *Bifidobacterium bifidum*), showed an increase in colony numbers after the therapeutic treatment of antibiotics and indicated that the possibility of the use of these supplementary bacteria are vital in preventing any permanent changes in intestinal flora after treatment with antibiotics. This study also showed that the susceptibility of intestinal flora varies from species to species, with potentially pathogenic bacteria such as *Enterobacteriaceae* and *Staphylococci*, increasing in numbers after 14 days compared to other species of naturally occurring intestinal flora. The ideal probiotic formulation may therefore need to be resistant to the effects of the administered antibiotic so that it may protect the patient from potentially pathogenic bacteria such as *Staphylococci* but may not, by definition, pass on this resistance to other bacteria which may potentially have life-threatening consequences. With this issue of antibiotics removing functional bacteria that protect the human body, it has been shown that probiotic bacteria may be a substitute for antibiotics for certain gastrointestinal conditions. Furthermore, with the ability of probiotic bacteria to compete for adhesion sites, species of pathogenic bacteria have been shown to decrease upon administration of probiotics with up to 40% decrease in pathogenic bacteria dependent on the species of bacteria causing the infection (4). *Lactobacillus* has been shown to produce an antimicrobial agent that is effective against a variety of potentially pathogenic bacteria species such as *Clostridium*, *Bacteroides*, *Enterobacteriaceae*, *Pseudomonas*, *Staphylococcus*, and *Streptococcus* spp. that commonly infect the intestinal system. The produced antimicrobial agent, being effective against a relatively wide spectrum of bacteria did not, however, affect the viability of *Lactobacillus* bacteria (60).

Other Bacterial Species That Provide Health Benefits

Bacterial species not strictly classified as probiotics have also been shown to provide health benefits to patients. One such example is *Enterococcus faecium*, which have been shown to provide functional benefits in the treatment of *Salmonella* infections (49). A comparison on the functions of the various species of probiotic bacteria and some of their physiological functions can be found in Table I with a brief summarization on the physiological functions of intestinal flora found in Table II.

THE ABILITY OF CONVENTIONAL PHARMACEUTICAL AND NON-CONVENTIONAL FORMULATIONS TO DELIVER PROBIOTICS

Delivery of Viable Functional Probiotic Bacteria

With the large number of probiotic formulations available, a large variability in the viability and thus the effectiveness of the delivery systems exists. Due to the need to deliver probiotic bacteria to the small intestine and colon, the most common and most effective delivery route is orally, with the main chosen delivery systems being tablets or capsules, both with or without chemical and structural modification. Tablets have been shown to be good delivery systems for the delivery of probiotics with the manipulation of tablet excipients drastically improving the number of viable cells reaching the human intestine. With the addition of gastro-resistant polymers, adhesion enhancers and controlled release enhancers, the manipulation of formulation excipients have overcome many challenges facing effective probiotic delivery. Furthermore, with tablets and capsules being easier to administer to patients and due to their increased storage stability over liquid preparations, capsules and tablets are currently the preferred dosage forms for the delivery of probiotics to the human body (65). The concern with tablets, however, is the heat production that occurs during compression with temperatures reaching up to 60°C, which can destroy bacteria that are most viable at their optimal temperatures (52,66). Other conventional delivery systems for probiotics include microcapsules and beads, which have been shown to drastically increase the number of viable bacteria surviving the formulation process, storage period, and subsequent delivery to the intestinal system (32,47). The degree of bacterial survival in these formulations was however dependent on the polymer combinations used, the formulation processes undertaken, and the size of particulates formed. The survivability of the probiotic bacteria however was ultimately shown to be substantially more in these formulations when compared to the unmodified probiotic bacteria.

With commercial food products, cheeses have been commonly used as carriers for probiotic delivery. Ong *et al.* (67) showed the ability of three batches of cheddar cheese to deliver a variety of probiotic bacteria. Results showed that the Cheddar cheeses can be an effective vehicle for delivery of the *Lactobacillus* and *Bifidobacterium* strains tested. Fresh cheese was also found to be a suitable vehicle for the oral administration of *B. bifidum*, *Streptococcus thermophilus*,

Table I. Comparative Analysis of Probiotic Bacteria Genera and Their Functional Health Benefits

Genus (Probiotic spp. included)	Functional benefit (of genus)	Reference
Lactobacillus	Prevention of vaginosis	
L. acidophilus	Antibiotic-associated diarrhea	(61)
L. fermentum	Infant diarrhea	(62)
L. helveticus	Atopic dermatitis	(62)
L. paracasei	Promotion of vitamin	(62)
L. rhamnosus	production	(63)
L. salivarius	Digestion	(63)
Bifidobacterium	Irritable bowel disease	(62)
B. bifidum	Gut transit time control	(62)
B. breve	Immune support	(63)
B. longum	Antimutagens	(63)
	Anticholesterol agents	(63)
	Digestion	(63)
Enterococci		
E. faecium	Treatment of gastroenteritis and *Salmonella* infections	(49)
Escherichia		
E. coli Nissle 1917	Anti-tumor	(55)
	Vaccine delivery	(51)

Lactococcus lactis, *L. acidophilus*, and *Lactobacillus paracasei* (68). A study conducted on the ability of cheese to deliver *L. acidophilus* and *L. paracasei* was done by influencing the stage during the cheese making process at which the probiotic was added. All tests conducted showed an increase of intestinal flora after ingestion of the probiotic cheese (69). Similar studies were conducted by Phillips *et al.* (70) to deliver probiotics through cheddar cheese with results showing significant increases in all species tested except for *L. acidophilus*; Bergamini *et al.* (71) and Ong and Shah (72) also showed the ability of Argentinean and cheddar cheese respectively to deliver probiotic bacteria to the human GIT.

Milk has also been documented for the delivery of probiotics. The delivery of *Lactobacillus* species showed an increase in colony numbers after administration through milk (69). The use of milk was further studied by the possible use of Caseinomacropeptide in milk to deliver *Bifidobacterium lactis* (15). Caseinomacropeptide is a glycopeptides that contains nitrogen and amino-sugars that act as a growth substrate for *Bifidobacteria*. Results showed an increase in microbial growth with a variation dependent on the concentration of Caseinomacropeptide used. The use of yogurts in the delivery of probiotics has also been widely documented (8). Hemsworth *et al.* (73) showed the ability of yogurts to deliver probiotics. *Lactobacillus rhamnosus* cell counts increased after administration of the yogurt which included the probiotic, milk and macronutrients. A study conducted by Marafon *et al.* (74) showed the ability to optimize the use of yogurts as delivery systems for probiotics. In the study, a starter culture blend that consisted of *S. thermophilus*, *Lactobacillus bulgaricus*, and *B. lactis* was used. Results showed that the addition of milk proteins of *L. bulgaricus* only decreased significantly leaving the possibility of optimization of the other strains of bacteria. A review conducted by Lourens-Hattingh and Viljoen (8) on the use of yogurts to deliver probiotics showed that with an increase in the use of yogurts to supplement intestinal flora by the general public required a deeper investigation into the effectiveness of these yogurts. Also the ability of yogurts to deliver probiotic bacteria was described to be low in comparison to other food sources due to factors such as acidity and oxygen content which do not favor the growth of naturally occurring bacteria such as anaerobic *Bifidobacterium*.

The use of other food products has also been identified for the delivery of probiotics. A study conducted by Possemiers *et al.* (75) showed the possibility of chocolate

Table II. Biological Health Effects of Functional Probiotic Bacteria. [Adapted with Permission from Scheinbach (64) © 1998 Elsevier Science Inc. and Penner *et al.* (16) © 2005 Elsevier Ltd.]

Biological health effects			
Stimulation of the immune system	Epithelial barrier protection	Anti-microbial effects	Other health benefits
Enhanced antibody production	Enhanced tight junction protein phosphorylation	Production of acids, peroxides or bacteriocins bactericidal to groups that negatively impact health	Alleviation of lactose intolerance Cholesterol reduction
Enhanced natural killer cell activity	Upregulation of mucous production	Stimulation of defensin secretion	Tumor targeting
Modulation of dendritic cell phenotype and function	Enhanced epithelial cell glycosylation	Secretion of anti-microbial peptides	
Modulation of NF-kB and AP-1 pathway	Increased sIgA production	Inhibition of pathogenic bacterial invasion	
Altered cytokine release	Competition with pathogens for mucosal binding sites	Blockade of bacterial adhesion to epithelial cells	
Induction of regulatory T- cells	Competition for substrates	Release of nitric oxide	
Induction of PPAR-g			
Modulation of apoptosis			
Inhibition of proteasome activity			

coatings for the protection and delivery of *Lactobacillus helveticus* and *Bifidobacterium longum*. Results showed significant survival of bacterial cells leading to the belief that chocolate can be used to protect and deliver probiotics as well as other gastro-sensitive products (75). The use of other chocolate-based products such as chocolate mousse has also been used to deliver probiotics (76). Chocolate mousse was also supplemented with *L. paracasei* with a high degree of survival of the bacteria upon administration of the chocolate mousse (76). The use of ice cream in the delivery of probiotics have also been explored with ice creams showing greater protection of bacteria in simulated gastric conditions compared to milk and yogurts (77). This was hypothesized to be due to the higher fat content in ice cream (approximately 10%), which may have provided a greater degree of protection against gastric acid and bile salts compared to the yogurt which had a proximate fat content of 5%. The ingredients in ice cream, such as cocoa powder and stabilizers, were also believed to provide protection against the harmful effects of the gastric acid and bile. This further explains the protective effects of chocolate-based products as cocoa is a significant part of chocolate itself. Probiotics have also been delivered through maple sap which was supplemented with *B. lactis* and *L. rhamnosus* with and without inulin (76,78). The inulin significantly enhanced the survival of the bacteria through simulated gastric and intestinal conditions (78).

Freeze–Dried Formulations and Advancements with Cryo-Protection

Lyophilized probiotic bacteria have been widely used as the chosen form of delivery of probiotic bacteria to the human GIT. The reason for this trend is that lyophilization of these bacterial cells preserves their viability due to their low water activity as well as improves the stability and viability of the formulation itself (79). Freeze–drying also been shown to not have an effect on the ability of probiotic bacteria to protect the intestinal system from pathogenic bacteria, with a test pathogen of *Shigella sonnei* co-incubated with probiotic bacteria after lyophilization showing a significant decrease in *S. sonnei* levels after the test period (80). Lyophilization on its own, however, has been shown to be detrimental to the survival of bacteria and therefore protection against the harsh effects of freeze–drying is most often needed (81). Cryo-protection seems to be the answer to the issue of freeze–drying on cell viability. This leads to a better treatment regimen due to greater numbers of viable bacteria reaching the intestinal system, as well as a decrease in the cost of manufacture as a result of the decreased loss in functional bacteria using relatively cheap cryo-protectants. The type of cryo-protectant used, however, also determines the degree of viability during lyophilization as well as during shelf-life and storage. Traditionally milk based products such as lactose and skimmed milk have been used as cryo-protectants for the protection of bacteria during lyophilization but this protection is short-lived during the shelf-life of the product, with bacteria viability decreasing only after a few months of storage (81,82). Other excipients such as ascorbic acid have been shown to be more effective in the protection of viability of bacteria during shelf-life with a possible combination of milk and ascorbic acid in lyophilization of probiotic bacteria being the most effective (81). A study conducted by Savini *et al.* (83) further showed the cryo-protective properties of polyalcohols glycerine, sorbitol, mannitol and prebiotic oligosaccharides inulin, Crystalean® starch and dextrin. Results of this analysis determined that all the tested cryo-protectants were effective in the protection of probiotic bacteria during freeze–drying with little change seen in bacterial cell counts when the bacteria were stored at 4°C for 5 months. A difference however was seen when the cultured probiotic were exposed to room temperature for the tested time period. A significant decrease in bacterial cell counts was seen over time with glycerine showing the best protective properties during storage as compared to other compounds tested. This was determined to be attributed to the penetrating effects of glycerine over the tested oligosaccharides and being more active than the other polyalcohols analyzed.

An issue seen with many freeze–dried probiotic formulations is when they are added to liquid preparations prior to storage (seen in many yogurts and other food-based products), the result is that ultimately rehydrating the freeze–dried bacteria decreases the stability of the probiotics affecting their viability through storage (84). This issue was investigated by Weinbreck *et al.* (85) which showed that dried encapsulated *Lactobacillus* bacteria when exposed to water over a 2-week period significantly decreased the viability of the encapsulated bacteria further proving that even when encapsulated bacteria will have to remain dry to the point of delivery to have the viability needed to exert the required health benefits. This decrease was further explained by Vesterlund *et al.* (86) who showed that when dried foods containing probiotic bacteria was exposed to or contained water, the viability of probiotic bacteria during the shelf-life of the product decreased considerably. Over-drying of probiotic bacteria however can be detrimental in the bacterial survival rate over time (87). This was due to the biological nature of bacterial cells, where a 0.0% moisture content revealed a bacterial viability decrease of 44% within 1 week of storage when compared to bacteria containing a moisture content of 2.8%. It was therefore determined that an ideal moisture content for the probiotic bacteria analyzed, *Lactobacillus salivarius*, was between 2.8% to 5.6% where a moisture content of 8.8% and over resulted in a large decrease in bacterial viability over time. These values were however specific to the bacteria tested and would vary from species to species.

An alternative to cryo-protection is the use of microencapsulation, which is also used to protect probiotic bacteria during freeze–drying. Microencapsulation using polysaccharide or protein based systems has been shown to be far more effective in the protection of bacteria during freeze–drying and storage as compared to traditional cryo-protection. Combined with the effect of polysaccharides, some of which are used as prebiotics, this allows for a suitable delivery system that protects the delivered probiotic bacteria and has the added effect of producing a synbiotic formulation (84). Prebiotics by definition, provide growth enhancers and nutrients that assist in the growth of probiotic bacteria when delivered to the small intestine. Synbiotics are defined as a "combination of pre- and probiotics." The most commonly used prebiotics in Europe are fructo-oligosaccharides [FOS], which are naturally found in a variety of vegetables such as asparagus, leeks, artichokes, onions, and garlic (88).

The parameters of the freeze–drying process have also been shown to have a large effect on bacterial viability. This effect has been shown to be strain specific with certain species of probiotic bacteria being capable of surviving lower temperatures when compared to other bacterial species. An example of a bacterium that is unstable at low temperatures is *Lactobacillus delbrueckii*, a probiotic whose numbers decrease drastically at temperatures below 0°C (89). In comparison, *L. paracasei*, has been shown to survive at much lower temperatures, commonly associated with freeze–drying, with a significantly larger proportion of bacterial cells surviving the formulation process. This difference was shown to be attributed to the membrane structure of the respective bacterial cells affecting the resistance of the bacteria against low temperatures. Low temperature vacuum drying (LTVD) is therefore proposed as an alternative to freeze–drying due to the lower temperature ranges utilized and higher viable bacteria yields seen in cryo-labile bacteria such as *L. delbrueckii*.

Storage conditions of probiotics before and after formulation processes have also been shown to be an important factor in the viability of the delivered probiotic bacteria. Probiotics have been shown to survive in greater numbers when stored at −70°C prior to the formulation process compared to when stored at 7°C in a refrigerator. This was due to the cryo-protectants used such as glycerol, milk, etc. that prevented intracellular formation of ice within the bacteria, thus preventing a decrease in their viability when frozen (90). The issue that arises, however, from storing probiotic bacteria at frozen temperatures is the problem of transportation and cold storage across great distances. This can be solved by transportation of cultures to the site of culturing and processing and maintaining a cold chain from production to the patient (90). It was further shown that the presence of other bacteria in the formulation, oxygen content, the amount of acid-producing bacteria as well as the temperature affected the viability of probiotic bacteria in liquid or semi-solid food based product such as yogurts (91).

Protection of Probiotics Against Harsh Gastric Conditions

A major issue in probiotic therapy is the ability of formulations to protect probiotic bacteria from the harsh gastric environment with as much as 60% of probiotic bacteria being killed in the gastric environment prior to reaching the intestine where the bacteria will exert their health benefits. This number depends on the species of bacteria delivered with some species showing more protection in the gastric environment compared to others. Gastro-resistant polymers and coatings have been shown to supply protection against the harsh gastric environment. These coatings included enteric coated tablets and capsules that site-specifically deliver the administered probiotic bacteria to the intestinal system. These enteric coats are often pH selective and allow for protection against the harsh gastric conditions and subsequently dissolve in the alkali media of the intestinal system. Hydroxypropyl methylcellulose phthalate has been used to deliver *Lactobacillus fermentum* under simulated human gastric and intestinal conditions (92). It was found that the hydroxypropyl methylcellulose phthalate not only protected the probiotic bacteria but also contributed to the development of hard tablets with a high tensile strength with bacterial viability still intact (92). Other excipients that have gastro-resistant properties and have been used for probiotic delivery is carboxymethyl high amylose starch. Starch is a commonly used excipient as a filler and binder in tablet manufacturing and is polysaccharide in nature. However when chemically substituted, it yields a starch derivative capable of protecting bacteria in the gastric environment. It has been used previously to deliver *E. coli* as a vaccine against neonatal and post-weaning diarrhea in pigs (93). Being polysaccharide in nature, the high amylose starch was quickly dissolved by enzymatic hydrolysis upon reaching the small intestine and safely delivered the *E.coli* bacteria into the intestine for an immunological response to take place. Tests done on the formulation showed that a high survival of viable bacterial cells reaching the small intestine after being exposed to gastric acid leading to carboxymethyl high amylose starch being an effective carrier for gastric sensitive products for intestinal delivery.

This concept was also again seen in the use of carboxymethyl high amylose starch for the delivery of *L. rhamnosus* to the colon. Colon drug delivery requires that the triggering mechanism of the delivery system has to respond to the physiological conditions particular to the colon in order to prevent release in the stomach or intestine (94). The carboxymethyl high amylose starch was combined with chitosan for delivery of the probiotic to the colon. The resultant chitosan, carboxymethyl high amylose starch swelled upon reaching the small intestine due to chitosan and thereafter released the probiotic into the colon. It therefore became possible to site specifically deliver probiotics to the human intestine and colon. Other studies also confirm that different polymers with different properties, or similar polymers with different properties, can be used, depending on the site of intended delivery to deliver an active ingredient, in this case probiotic, to different areas of the GIT (94). The effectiveness of carboxymethyl high amylose starch has also been documented to deliver F4 fimbriae, another gastro-sensitive product to the intestinal system (95). A schematic derived from Yang *et al.* (94) can be found in Fig. 5 and shows the mechanism of release of a commercial colon-targeting dosage form as it passes through

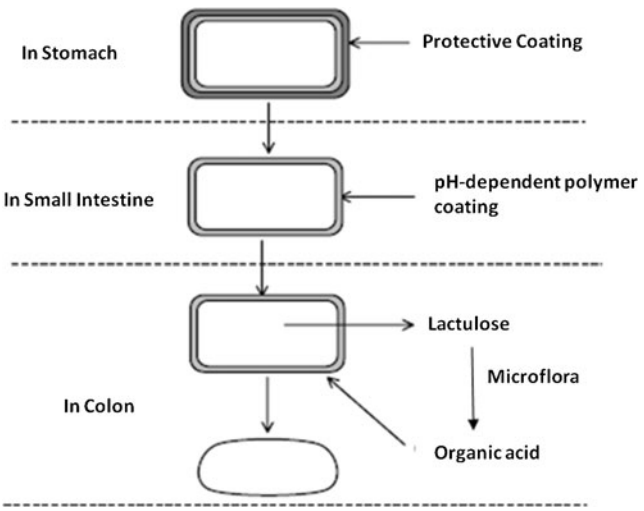

Fig. 5. A schematic depicting the use of colon microflora for site-specific drug delivery. [Adapted with permission from Yang *et al.* (91) © 2002 Elsevier Science B.V.]

the gastrointestinal system in which colon microflora is utilized for site-specific drug delivery.

Compression coatings have also been effective in the protection of probiotic bacteria from gastric conditions. A compression of sodium alginate, a gelling agent that erodes over time in gastro-intestinal conditions has been used for the protection of probiotic bacteria from the harsh gastric conditions. Results of a study conducted by Chan and Zhang (96) showed a significant increase in the survival rates of bacteria when exposed to gastric conditions with the resultant dosage form able to deliver the probiotic bacteria late in the small intestine and in the early colon. More recent studies have been attempting to deliver probiotics through the use of biopolymers such as succinylated β-lactoglobulin, a protein commonly found in dairy products. This β-lactoglobulin protein was found to possess gastric-resistant properties once modified and through a compressed tablet, was able to deliver viable *B. longum* bacterial cells to the intestine (97). Commercial food-based products such as proteins and carbohydrates have also been proven to be effective in the gastric protection of probiotic bacteria. Whey protein isolate, a by-product of cheese manufacture and commonly found in other food products has been shown to be effective in the protection of *Lactobacillus* bacteria in simulated gastric conditions. The whey protein isolate beads also tended to be more stable as compared to alginate beads that tend to have limited stability once formulated (98). Further studies into the use of commercial food based products for encapsulation was conducted using milk with encapsulation done using enzyme-induced gellation. As with the other studies, survival rates of bacteria drastically increased after incubation in gastric conditions when compared to the non-encapsulated bacteria (99). The proposed method of protection is that proteins have a buffering capacity, allowing little change in pH concentrations within the protein structure preventing the lower gastric pH affecting encapsulated probiotic bacteria within the protein system (99).

Enhanced Coatings for Stability and Physiological Protection of Probiotics

Delivery systems used for the delivery of probiotics include the use of coated beads, capsules and tablets. A study conducted by Krasaekoopt *et al.* (47) showed the effectiveness of alginate beads in the delivery of both *Lactobacillus* and *Bifidobacterium* bacteria. The formulated beads were composed of sodium alginate and chitosan which were both coated and uncoated. Results showed that after exposure to simulated human gastric and intestinal fluid with or without bile salt, the chitosan coated beads showed the highest survival rate of *Lactobacillus* bacteria and that the *Bifidobacterium* bacteria did not survive exposure to the gastric media, even when coated. Iannitti and Palmieri (12) also stated that *Lactobacillus* bacteria do possess gastric-protective properties compared to other species of bacteria which would result in the survival of *Lactobacillus* bacteria and not the *Bifidobacterium* bacteria. Kaushal and Shao (100) stated that *Lactobacillus sp* have the ability to survive exposure to gastric acid. A similar study was conducted by Brachkova *et al.* (101), which showed the possibility of delivering different probiotic bacteria species through alginate beads. This study also showed that alginate beads can be used to deliver probiotics effectively to the human intestine while providing protection for the bacteria against the harsh gastric environment.

Advancements Through Encapsulation for Increased Probiotic Viability

Studies have been conducted on the use of protecting probiotic bacteria using capsules by the encapsulating process. The use of calcium alginate is common in the encapsulation of probiotics as it is non-toxic to the bacteria. The study conducted by Chandramouli *et al.* (102) on *L. acidophilus* involved encapsulated bacteria subjected to human gastric conditions. The encapsulation of the bacteria was done using alginate at various concentrations. The survival of these bacteria was found to be dependent on the concentration of the alginate coat with the higher concentration leading to a larger amount of viable bacteria surviving (102). Albertini *et al.* (103) conducted a study on various polymers microcapsules and beads. Similar results were observed with large numbers of probiotic bacteria surviving incubation under simulated gastric conditions.

Delivery of Probiotics Outside the Gastro-Intestinal System

Newer studies have also been focusing on areas of probiotic delivery other than to the gastro-intestinal system. A pilot study conducted by Santiago *et al.* (61) showed the possibility of probiotic delivery to the human vagina to treat or prevent vaginal bacterial and fungal infections. Naturally colonizing bacteria such as *Lactobacillus* bacteria are found in the uro-genital tract of females and assist, as with intestinal flora, in the prevention of colonization of potentially pathogenic bacteria and yeasts (104). The chosen delivery system was fast integrating starch pellets that were found to be acceptable means of delivery probiotic bacteria to the human vagina. Further studies into the use of probiotic bacteria for the treatment of bacterial vaginosis through the use of a vaginal capsule have also shown positive results for this treatment and due to its site-specific delivery, it avoids the issue of gastric conditions and bile exposure (105). With traditional probiotic formulations focusing mostly on the delivery of probiotic bacteria to the intestine and colon, very few studies have shown the positive benefit of delivery probiotics to the oral cavity for the prevention of oral bacterial and fungal conditions. A study conducted by Bosch *et al.* (5) showed the potential of probiotic bacteria for the prevention of oral conditions such as gingivitis and peridontitis with positive results showing that probiotic bacteria can exert health benefits outside the intestine and colon and prevent the occurrence of orally experienced conditions and due to it site-specific delivery, the issue of cell viability due to physiological conditions is not experienced. The possibility of rectally administering probiotic formulations which avoids the gastric environment exists, with the ability of delivering a large variety of bacterial species to the colon. The risk of invasion, however, of pathogenic bacteria to the small intestine is increased due to the large amount of bacteria in the colon and in fecal matter (63).

EFFECTIVENESS OF CONVENTIONAL PHARMACEUTICAL FORMULATIONS *VS.* NON-CONVENTIONAL COMMERCIAL PRODUCTS

While many studies have determined the unreliable variation among non-conventional commercial food products, many agree that conventional pharmaceutically based delivery systems are still the most reliable in the delivery of probiotics. It is for that reason for the inclusion of many pharmaceutically carriers suspended in commercial food products. Examples of this are the encapsulation of powdered bacteria to be delivered in yogurts (106). This study showed a significant increase in viable bacteria delivered to the intestine compared to the unencapsulated bacteria. Studies have also been conducted to determine the difference in viable numbers of bacteria when delivered as a capsule, in cheese or in milk. Saxelin *et al*. (107) showed the comparison of cheese, capsules, and yogurts for the delivery of probiotics by determining the amount of bacteria found in the feces of the participants after ingestion of the respective delivery systems. It was also determined that the longer the bacteria took to appear in the fecal samples, the higher the degree of adhesion of the bacteria to the intestinal walls. Results from the study showed that the highest fecal bacterial count came from the yogurt product with a close comparison found between the yogurt and the capsules. The cheese product was found to have the lowest number of fecal bacteria and was determined to be the poorest delivery system of the three tested. The use of commercial food products can, in pharmaceutical products, be found to be beneficial for the delivery of probiotics. Capsules of probiotics were formulated using calcium alginate and Hi-Maize starch, which improved the encapsulation of viable bacteria compared to when the bacteria were encapsulated without the starch (108). The encapsulated bacteria in this study, however, did not demonstrate a significant increase in survival when subjected to simulated gastric and intestinal conditions.

A preliminary study was conducted in order to monitor the effects of encapsulation on the survival of *L. acidophilus* and *Bifidobacterium* in yoghurt over a period of eight weeks (108). This study showed that the survival of encapsulated cultures of *L. acidophilus* and *Bifidobacterium* spp. showed a smaller decline in viable counts of about 0.5 log over the test period, while free cells had a decline of about 1 log in cultures stating that encapsulation within the yogurt might prove beneficial to ensure survival of the bacteria in the product until the end of its shelf life (108). From the studies that have been discussed, it can be seen that in the delivery of probiotic bacteria, due to the effectiveness and reproducibility of pharmaceutical formulations and the nutritional and microbiological value of commercial food-based pharmaceutical products, the ideal probiotic formulation may exists in the combination of these two broad groups of probiotic formulations.

CURRENT FACTORS AFFECTING PROBIOTIC DELIVERY AND FUTURE RECOMMENDATIONS

With the issues already highlighted in this review and the necessary changes which have been taken by research to overcome these problems, certain issues still surround the effectiveness and safety of probiotic formulations. While recently, some researchers have questioned the effectiveness of probiotics in general to exert health benefits, the general consensus is that probiotics do supplement intestinal and urogenital flora which by themselves exert functional health benefits. The safety of probiotic formulations have also been questioned with studies finding probiotic bacteria in some formulations harboring antibiotic resistance, which can further precipitate life-threatening pathogenic conditions (7). A further issue raised has been that the large numbers of probiotic bacteria in formulations are so high that it may inadvertently prevent the detection of contaminants. A study performed by Joosten *et al.* (36) which showed that current methodology for the detection of *Salmonella*, a potentially serious pathogenic bacteria, was ineffective in infant formulas due to the high numbers of probiotic bacteria in the infant formulas. This study highlighting that the tested infant formulas can potentially contain a variety of pathogenic bacteria and a false-negative result of non-contamination seen during microbial tests. There is also no guarantee that natural products including probiotics are free of contaminants with cases of pathogenic bacteria, toxins and heavy metals being found in natural products (16). There is therefore a need for more stringent quality control in the preparation of probiotic formulations. *Enterococci* are a species of bacteria that have been linked to many pathological conditions in humans with many having the ability to transfer antibiotic resistance onto other bacteria, allowing for non-effective treatment in many patients. However, some species of *Enterococci* have been found to treat gastroenteritis as well as not harboring virulent genes and not being resistant to vancomycin, the chosen treatment for methicillin-resistant *Staphylococcus* infections (49). While it has been shown that probiotic and health benefits may differ between species of the same class of bacteria, many meat and dairy products containing *Enterococci* probiotics have been shown to harbor antibiotic resistance, placing the patient at a higher risk of serious infections while on probiotic treatment. With this issue of safety, microorganisms in food now have to undergo screening tests to be regarded as safe for consumption by humans (109). Further studies will also need to be conducted to distinguish between potentially pathogenic and beneficial species of bacteria to increase the number of functional probiotics on the market.

Probiotic Formulation Efficacy

Issues that have been further raised in probiotic therapy are the effectiveness of many probiotic formulations on the market. Many formulations tend to deliver multiple strains of bacteria with many of them not effective as probiotics and do not comply with the requirements of probiotics. Multiple strains in a single formulation have been shown to be ineffective in the supplementation of probiotic bacteria and have been found to be a major issue in probiotic therapy (56). Another issue is the misconception that all bacteria of the same class have probiotic effects. Many formulations display that probiotic bacteria are contained within the product, but deliver ineffective bacteria for the supplementation of intestinal flora, with others delivering pathogenic bacteria capable of inducing antibiotic resistance as well as other pathological conditions in the patient it is being administered to (104). Natural intestinal flora also varies from person to person, from

race or ethnicity as well as within age groups. Children tend to have higher levels of *Bifidobacterium* bacteria in their intestinal flora compared to older patients with many *in vitro* studies not taking into account host-specific factors that may affect the efficacy of the delivered probiotic bacteria (110). For this reason, it is important to know the intestinal flora of the patient being administered to ensure maximum health benefits for the patient (57). It is therefore recommended that more formulations be specific to the type of patient being treated. Most feasible would be by age group, which would have maximum health benefits for the patient.

Safety

Lack of industry standardization as well safety issues have plagued the use of probiotics with many having a negative view on probiotics despite their health benefits (6). New legislation and regulations in many countries are, however, now requiring research and validity of bacterial probiotic cultures prior to administration as probiotics, which will lead to increased safety and effectiveness for all patients being administered with probiotic supplementation (56,110). FDA regulations do not control the premarket approval of food or supplementary products including that of probiotics. Few products do show the effectiveness of their products; state the health benefits of their products, with even fewer accurately proving the effectiveness of the formulation in human trials (62). It is thus up to the patient to read labels, do extensive research and absolve from any misinformation that may be exposed to them. This is extremely difficult considering the trust that the common public give to medical formulations that are presumed to provide health benefits. Further education of prescribing healthcare professionals will also prove to be beneficial as they can provide vital information to patients about the effectiveness, misinterpretations, and risks of taking formulations that have not been proven or tested.

Viability

One other issue in probiotic therapy is the use of unviable, inactivated bacteria in many probiotic formulations. With this increase in formulations delivering unviable bacteria, a few studies have been conducted to determine the effectiveness of these bacteria as probiotics. While a certain degree of functionality has been found for non-viable bacteria to provide health benefits to patients, the common agreement is that they are no substitute and are unlikely to be as effective as viable bacteria for the supplementation of intestinal flora. More research is therefore required into these types of bacteria to determine their effectiveness as probiotic formulations (56).

Autoimmune Induction

More research is also needed into the possible effect of probiotics on the induction of autoimmune conditions due to the immune-modulating effects of probiotic bacteria (111). A study into the inflammatory effects of probiotic supplementation in mice genetically predisposed to the development of autoimmune conditions showed no significant increase in intestinal inflammation of mice supplemented with probiotics in their diet *versus* mice that did not, furthermore leading to the belief that probiotic bacteria can be used for its immune-modulating health benefits without the issue of side effects in patients with or predisposed to autoimmune conditions (111). While this *in vivo* study has shown that probiotic bacteria have little or no effect on immune-induction, the severity of the condition warrants more research to prove this phenomenon does not occur in humans.

Research Outputs

With the large number of probiotic publications currently in existence, it has been documented that one of the greatest

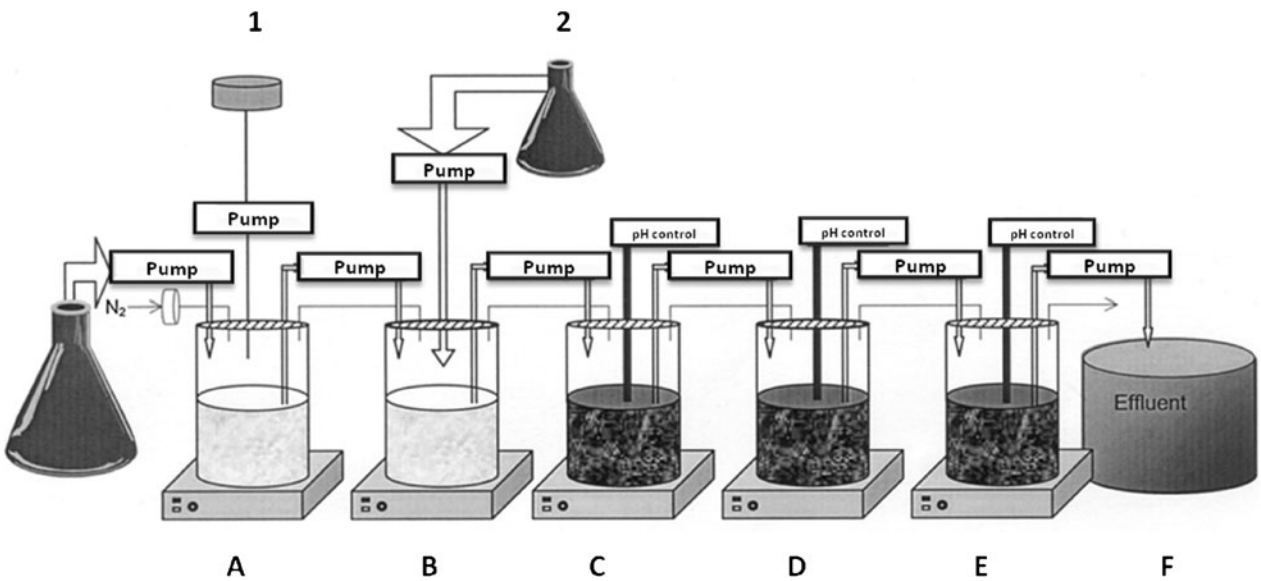

Fig. 6. A schematic of an SHIME unit. Flasks 1–2 depict supplementary media for the system with vessels A–E depicting various areas of the intestinal system and colon. These vessels are pH controlled using control units. Vessel F contains the effluent of the system. [Image adapted with permission from De Boever *et al.* (111) © 2000 American Society for Nutritional Sciences]

issues facing probiotic delivery is the lack of original research. Stevenson and Blaauw (9) have shown that using a common publication search engine that approximately 26% of all probiotic publications are review articles, concluding that probiotic research has a large number of review articles and therefore suffers from a deficiency in original data as opposed to other research fields. With the many unanswered questions with regards to probiotic effectiveness and safety as well as the constant finding of more safe and effective probiotic bacteria, probiotic research still remains a largely untapped field to be undertaken.

Alternative Analyses for the Determination of Effectiveness for the Delivery of Probiotics

With the inability of simple dissolution analysis to accurately determine the effectiveness of probiotic bacteria to adhere to the intestinal wall and to simulate competition with the natural intestinal flora of the human intestinal system, models have been developed for the accurate simulation *in vitro* on the ability of probiotic bacteria to merge into the intestinal environment (112). Such an example of a model is the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) which looks for the competitive issue between administered probiotic with the current natural flora in the intestinal system (113). The SHIME is basically a series of vessels that each contains the microbial content of a different part of the human gastro-intestinal system that has been set up to simulate the conditions, fluids, oxygen content and microbial content of the respective part of the gastro-intestinal system (112–114). A schematic of the SHIME can be found in Fig. 6. Adhesion of delivered probiotic bacteria to the intestinal wall is also a vital issue if the bacteria delivered has to provide functional health benefits for the patient. Unadhered bacterial cells would travel through the intestinal system and would subsequently be eliminated from the body before it could provide any health benefits to the patient. Many *in vitro* studies do not test the adhesion properties of the delivery system and many *in vivo* studies test fecal matter that contain probiotic bacteria that may have not adhered to the intestinal wall. However, ways have been developed to determine the adhesion ability of delivered probiotic bacteria. One such option is to use fluorescent markers to test the ability of bacteria to adhere to the intestinal wall. A study conducted by Mare *et al*. (115) showed that adhesion by *Lactobacillus* can be determined using fluorescence *in situ* hybridization which picked up and quantified the ability of *Lactobacillus plantarum* and *L. salivarius* to adhere to the intestinal wall of pigs. Other methods for adhesion quantification of delivered probiotic bacteria include intestinal cell lines, DNA probes, and growths specific media; however, each of these require specialized equipment that may not be available to all researchers (115).

CONCLUSIONS

The delivery of probiotics has been a topic of interest for years with many different ways and delivery systems being developed for the delivery of these bacteria to the intestinal system. These systems are often pharmaceutically or naturally occurring food delivery systems each having the capability of delivering adequate amounts of bacteria to ensure a health benefit for the patient. However, with all the factors affecting probiotic therapy, it has been shown to be beneficial to have probiotic formulations that incorporate both pharmaceutical and commercial food based ingredients together to have an effective dosage form for the delivery of probiotics to not only the intestinal system but as well as the uro-genital system. It is therefore hypothesized that formulation enhancement through the addition of energy sources, prebiotics, and vitamins, all of which is present in commercial food-based probiotic formulations combined with pharmaceutical products that provide among others, gastric protection, cryo-protection, and formulation protection, that the ideal probiotic formulation does not exist in the development of both fields of probiotic delivery but in a possible combination of the two that will provide functional, viable bacteria that will provide benefits to the patients that they have been administered to.

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