

Probiotic Research in Australia, New Zealand and the Asia-Pacific Region

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Abstract: Although the epicentres of probiotic research in the past decade have been Japan and Europe, researchers in the Asia-Pacific region have actively contributed to the growing understanding of the intestinal microbial ecosystem, and interactions between gut bacteria, diet and health of the human host. A number of new probiotic strains have been developed in the region that have been demonstrated to have beneficial impacts on health in animal and human trials, including improved protection against intestinal pathogens and modulation of the immune system. Probiotics targeted to animals, including aquaculture, feature heavily in many Asian countries. Developments in probiotic technologies have included microencapsulation techniques, antimicrobial production in fermented meats, and synbiotic combinations. In particular, the impact of resistant starch on the intestinal environment and fermentation by intestinal bacteria has been intensively studied and new probiotic strains selected specifically for synbiotic combinations with resistant starch. This paper provides an overview of probiotic research within Australia, New Zealand and a number of Asian countries, and lists scientists in the Asia-Pacific region involved in various aspects of probiotic research and development.

Key Words: Probiotic, prebiotic, lactobacilli, bifidobacteria, salmonella, intestine, dairy, resistant starch.

INTRODUCTION

Residing in the human gastrointestinal tract is a large and complex microbial ecosystem that develops through infancy and childhood to form a diverse, but relatively stable community in adults [1]. These autochthonous bacteria interact with the diet and the host, contributing to protection against intestinal pathogens through colonisation resistance and providing nutritional and colonic health benefits via their metabolic activities [2, 3]. It has become clear that these bacteria also interact with the host's immune system and are essential for the maturation and homeostasis of a healthy immune system [4]. Recognition of the importance of the intestinal microbiota to health has led to increasing interest in manipulating the composition and/or activity of the microbiota to improve both human and animal health.

Probiotics are live microbial food supplements that are consumed with the aim of providing a health benefit to the host by contributing to an improved microbial balance within the intestinal microbiota. Strains from the genera *Lactobacillus* and *Bifidobacterium*, both of which are indigenous to the human intestine, are the bacteria predominantly selected for use as probiotics. Fermented dairy products (yoghurts and drinks) and capsules with freeze-dried bacteria are the most popular vehicles for delivering these organisms to the gastrointestinal tract. Prebiotic ingredients represent a second strategy used to modulate the intestinal microbiota. In this case, the growth and/or activity of desirable populations of bacteria already resident in the gut are specifically stimulated through dietary intervention [5]. A number of non-digestible

oligosaccharides have been shown to selectively promote the proliferation of bifidobacteria in the colon and are recognised as prebiotics [6]. Additionally, some dietary fibre-like polysaccharides have shown promise as prebiotics [7, 8]. There is an obvious potential synergy between prebiotic and probiotic ingredients and foods that contain both are termed 'synbiotics' [6]. The main targets of pre-/probiotic intervention have been: (i) boosting host resistance to exogenous intestinal pathogens; (ii) controlling diseases where components of the intestinal microbiota have been implicated in aetiology; (iii) reducing putrefactive/toxicogenic microbial metabolism in the gut, and (iv) modulating the host immune system. To date, promising results have been observed in human clinical studies for rotavirus diarrhoea, inflammatory bowel disease (IBD), and the amelioration of atopic eczema in infants [9-11]. Not all probiotic strains are effective, and considerable strain-to-strain variation in properties relevant to probiotic efficacy is observed within bacterial species.

A range of research disciplines are required to select appropriate probiotic strains and prebiotics for specific applications, to test their impact on the intestinal microbiota, and to assess effects on the health of the host. These include manufacturing and food technologies; human and microbial physiology; intestinal microecology; immunology; and clinical medicine. The Framework Programmes of the European Commission have facilitated a co-ordinated, multidisciplinary, collaborative approach to probiotic research, an example of which is the "Food, GI-tract Functionality and Human Health Cluster", which has been reviewed by Saarela *et al.* [12]. Unfortunately, no such co-ordinated, transnational approach exists in the Asia-Pacific region. However, within this region there are a large number of research groups contributing to the understanding of microbial intestinal

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ecology, probiotic and probiotic technologies, and the benefits and safety of probiotic organisms. The current paper aims to provide an overview of recent prebiotic and probiotic research and development in Australia, New Zealand, and a number of countries in Asia.

RESEARCH IN AUSTRALIA

The growth in popularity of probiotic products within Australia has largely mirrored that seen in Europe and Japan. Indeed, the most prominent probiotic strains currently marketed in Australia in food products remain those from Europe and Japan, including Yakult's *Lactobacillus casei* Shirota, *Lactobacillus* GG (sold by Parmalat under licence), Nestle's *Lactobacillus johnsonii* La1 and Chr. Hansen's *Bifidobacterium lactis* Bb-12. However, the growth in commercial and consumer interest in probiotics in the 1990's spurred the emergence of a number of research groups and commercial interest in the development and testing of new probiotic strains, synbiotic combinations, probiotic food technologies, and investigations of health efficacy.

Probiotic Technologies in Australia

Research groups in Australia with a prominent interest in probiotics are listed in Table 1. A number of these groups have been involved in developing technologies to promote probiotic stability. In addition to the well-known parameters that effect probiotic survival in food products (pH, temperature, and oxygen), Shah and co-workers have investigated the influence of a range of food ingredients on various probiotic strains. The attributes of companion starter cultures [13], prebiotics [14], oxygen scavengers [15], water activity and sugar concentration [16] can all dramatically influence probiotic survival during product storage. Packaging materials can also impact on probiotic stability through variations in oxygen permeability [17]. Several groups within Australia have successfully trailed microencapsulation technologies using alginate and/or starch to promote probiotic survival during food product storage [18-20]. However, microencapsulation generally failed to promote survival during simulated gastric transit [19, 21] or during spray drying of probiotic bacteria [21].

Table 1. The Main Probiotic Research Groups in Australia

Researcher	Institution	Key Areas of Research
Michelle ADAMS	University of Newcastle, Newcastle, NSW	Intestinal adhesion; probiotic technologies
Jorma AHOKAS Diana DONAHUE	Royal Melbourne Institute of Technology (RMIT), Melbourne, VIC	Probiotic safety; sequestering food toxins using probiotics
Tony BIRD David TOPPING	CSIRO Health Sciences and Nutrition, Adelaide, SA	Prebiotics; resistant starch; colonisation; colon cancer; animal and human studies
Ross BUTLER	Women's and Children's Hospital, Adelaide	Probiotics in treatment of intestinal diseases; intestinal permeability
James CHIN	Elizabeth Macarthur Agricultural Institute, Camden, NSW	Immune effects of probiotics
Patricia CONWAY	VRI BioMedical and University of NSW, Sydney, NSW	Adhesion; pathogen inhibition; colonisation; animal and human studies
Ross CRITTENDEN	Food Science Australia, Melbourne, VIC	Dairy allergy; prebiotics; probiotic technologies; safety
Peter GIBSON Jane MUIR	Box Hill Hospital, Monash Medical School, Melbourne, VIC	Clinical trials; human studies; resistant starch
Anders HENRIKSSON	DSM Food Specialties, Sydney, NSW	Pathogen inhibition; colonisation; technologies Production technology
Kaila KAILASAPATHY	University of Western Sydney, Sydney, NSW	Microencapsulation, probiotic technologies
David J.W. MORIARTY	Acuabiotec LLC, Wellington Point, QLD 4160	Probiotics in aquaculture; prawns
Martin PLAYNE	Melbourne Biotechnology and RMIT, Melbourne, VIC	Prebiotics, probiotic technologies, health efficacy, safety
Susan PRESCOTT	The University of Western Australia, Perth, WA	Allergy; immuno-modulation; human clinical trials
Thomas V. RILEY	The University of Western Australia, Perth, WA	Probiotics and hospital related infections
Nagendra SHAH	Victoria University of Technology, Melbourne, VIC	Probiotic technologies; probiotic bioactives; soybean fermentation

The application of probiotic cultures to foods beyond fermented dairy products has been a commercial target for many culture manufacturers, and indeed many food matrices can provide protection for live cultures and enhance their survival when consumed. Although still within the dairy sector, Haynes and Playne [22] showed that low-fat ice-cream was a good vehicle for delivering viable probiotics. *Lactobacillus acidophilus*, *Lactobacillus paracasei* and *Bifidobacterium lactis* all maintained viable numbers above 10^6 CFU/g in ice-cream over a 12 month shelf-life. In soy-milk, enzymatic deglycosylation of isoflavone phytoestrogens was reported by Tsangalis *et al.* [23] for several strains of bifidobacteria, leading to increased bioactivity. The antimicrobial activities of a number of probiotic strains was exploited by Pidcock *et al.* [24] to strongly inhibit the pathogens *Escherichia coli* 0111 and *Listeria monocytogenes* in fermented meat.

Selection of Novel Probiotic Strains within Australia

Scientific and commercial interest in probiotics has been fuelled in recent years by an increased output of well-designed efficacy studies in animals and humans that demonstrate beneficial effects of probiotic cultures on various health conditions, including symptoms of atopic eczema and the severity and duration of GI infections, particularly rotavirus infections [25]. Recognition of considerable strain-to-strain variation in probiotic effects within bacterial species has driven research focused on discovery of new probiotic strains that can be applied to specific technological and health applications. A co-ordinated effort to identify new probiotic strains was undertaken within the Co-operative Research Centre (CRC) for Food Industry Innovation between 1994 and 2000. Within this program, two distinct approaches were used to identify novel probiotic organisms. The first was based on *in vitro* screening of isolated cultures from genera typically used as probiotics, such as lactobacilli, bifidobacteria, enterococci, and *Saccharomyces*. The second and novel approach, involved selecting mixed populations of bacteria from faeces of healthy donors with a low history of intestinal infections and screening for pathogen inhibition first *in vivo*, before isolating individual cultures and screening *in vitro* for technological properties. The two approaches used within the CRC for Food Industry Innovation are described below with examples of their outcomes.

In Vitro Culture Screening

In the first approach, over 100 cultures were screened using *in vitro* tests relevant to probiotic activity. The cultures screened included novel isolates from human donors, isolates from commercial culture collections, and prominent commercial probiotic strains that were used as benchmarks. More than 20 different screens were employed, covering four categories: (i) technological characteristics; (ii) intestinal transit; (iii) health functionality; and (iv) safety. Additionally, all of the strains were identified at the genus, species, and strain level using biochemical and molecular typing techniques. The result was a matrix database from which strains with the most promising attributes for specific technological and health applications could be selected for further animal or human studies to examine efficacy.

An example of the use of this matrix database to select new probiotic isolates for a specific application was the demand for *Bifidobacterium* and *Lactobacillus* strains to act synergistically with resistant starch in a synbiotic yoghurt. Of the bifidobacteria screened, only one strain, *Bifidobacterium lactis* LAFTI® B94, both hydrolysed resistant starch and possessed the required technological attributes to be manufactured and survive in the acidic environment of yoghurt [26]. The strain possessed suitable organoleptic properties and did not contain plasmids or unusual antibiotic resistances that might compromise safety. The *in vitro* screening results also provided indications of potential health benefits, including production of vitamin folate in yoghurt [27], and the inhibition of intestinal pathogens, including *Salmonella* Typhimurium. Subsequently, the strain was selected for *in vivo* examination of its ability to protect against *Salmonella* infection. Specific pathogen-free mice were fed for a week with either *B. lactis* LAFTI® B94, another common commercial *B. lactis*, or no probiotic as a control, and then challenged with a single dose of *Salmonella* Typhimurium [28, 29]. Even though the mice fed *B. lactis* LAFTI® B94 remained colonised with *Salmonella* to a similar degree as the control, the probiotic protected the animals against infection and the mice maintained body weight and condition. In contrast, the control mice and those fed the closely related strain of *B. lactis* were severely diseased and rapidly lost body weight (Fig. 1).

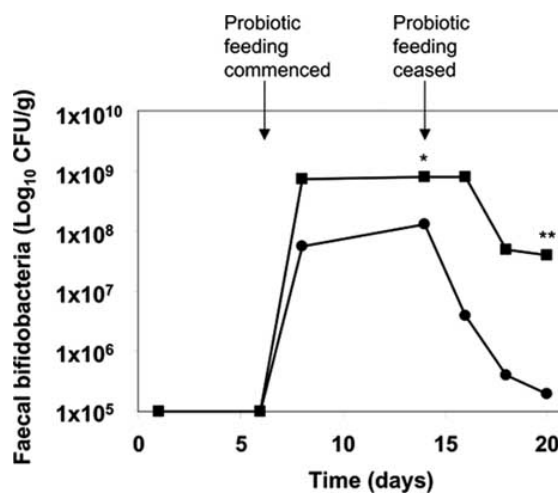


Fig. (1). Protection of mice against infection by *Salmonella* Typhimurium by probiotic *Bifidobacterium lactis* LAFTI® B94. Three groups of mice were fed for 7 days either *B. lactis* LAFTI® B94 (■); a common commercial *B. lactis* strain (○); or a control diet without probiotic supplementation (□), and then challenged with a single dose of *Salmonella*. Body weight was used as an index of health. (* $p < 0.05$).

To complete development of the new synbiotic system, a novel starch-utilising strain of *Lactobacillus* was required to complement the combination of *B. lactis* LAFTI® B94 and resistant starch. Few species of lactobacilli are able to utilize amylose as a carbon and energy source, but *Lactobacillus amyphilus* and *Lactobacillus amylovorus* are recognized as

starch-utilising species. Consequently, a number of strains of these species were collected and screened for probiotic properties, as well as for ability to utilize resistant starches. The strains of *L. amylovorus* grew faster, utilized starch better, and grew at higher temperatures than the strains of *L. amylophilus* that were tested. Furthermore, taxonomically, *L. amylovorus* is one of the six species formerly designated as *L. acidophilus*, which have GRAS status.

Most of the *L. amylovorus* strains were as tolerant to simulated gastric conditions as commercial *L. acidophilus* strains and grew well on industrial growth media. However, they varied considerably in their ability to grow in the presence of physiological concentrations of bile. The strains also varied in their *in vitro* adhesion to human intestinal cells (Caco-2) and human intestinal mucus (Crittenden R., Tuomola E., Salminen S., Playne M., unpublished data). Two strains of *L. amylovorus*, one of which produced an extracellular polysaccharide during fermentation, showed promise as potential probiotic strains to complement resistant starch.

In vitro screening trials represent only the first stage in establishing the effectiveness of potential probiotic strains. As an interim step before clinical trials, efficacy of potential probiotic cultures may be assessed in animal models. A number of novel strains developed within the CRC for Food Industry Innovation have been trialled in animal models to assess survival and colonization in the gastrointestinal tract [30], protection against infectious disease [28, 29], and anti-colon cancer effects [31]. Currently, a number of human clinical trials are underway to assess probiotic efficacy, particularly in immunomodulation.

In Vivo Culture Screening: Pathogen Inhibition

Selection of new probiotic strains using *in vitro* screens may be both expensive and time consuming. The costs involved are very much dependent on the selection methods used, and the type and number of strains that are being assessed. An alternative approach to selecting cultures with specific probiotic properties was used by Henriksson and Conway [28, 29] to identify new probiotic strains able to inhibit *Salmonella* Typhimurium. In this study, germ free animals were given faecal material from six different healthy human donors. After allowing the human flora to establish for one month, animals were challenged with a single dose of *Salmonella* Typhimurium. This resulted in colonisation of the pathogen in the intestinal tracts of some animals. As expected, the greatest faecal levels of pathogen were detected in control animals, which were not inoculated with the faecal flora. Animals inoculated with faecal material from any of the six donor subjects had reduced faecal levels of this pathogen. The greatest reductions were detected in animals given faecal flora from two particular human donors. Animals given flora from these subjects had faecal *Salmonella* in the order of 10^2 - 10^4 CFU/g, 3-4 magnitudes lower than that observed in the other groups. Interestingly, the faecal material from these two human donors contained a greater proportion of isolates that inhibited growth of *Salmonella* Typhimurium *in vitro* [28, 29]. Reduced signs of *Salmonella* infection were observed in animals inoculated with these inhibitory isolates.

It was demonstrated that mixtures of both Gram-positive and Gram-negative micro-organisms reduced faecal levels of *Salmonella* [28, 29]. Interestingly, the greatest reduction in the numbers of faecal salmonella was conferred by bifidobacteria. Animals fed a mixture of 10 salmonella-inhibiting *Bifidobacterium* isolates lost significantly less bodyweight following challenge with salmonella compared to animals given mixtures of other isolates. When tested individually, it was demonstrated that at least three of these 10 cultures protected against salmonella infection in mice. These bifidobacteria were identified as either *B. adolescentis* or *B. longum* and represent good candidate probiotic organisms. Other studies using the same animal model demonstrated that certain *Lactobacillus* strains could also inhibit salmonella *in vivo* [28, 29].

Probiotic cultures may also protect against other type of infections. It was demonstrated that *Lactobacillus acidophilus* LAFTI® L10 inhibited *Listeria monocytogenes* in the mouse GI tract [32]. This observation was made in animals fed a fermented meat product containing *L. acidophilus* LAFTI® L10 and the pathogen. Animals of the control group did not receive the probiotic strain. The mechanism involved in protection against *Listeria* infection is not fully understood. However, it is known that *L. acidophilus* LAFTI® L10 exhibits strong antagonistic activity against *Listeria in vitro*. This suggests that compounds produced by *L. acidophilus* LAFTI® L10 may inhibit the growth of the pathogen *in vivo* and inhibit gastrointestinal colonization. This property may be used to control the pathogen in food products, and indeed, application of the *L. acidophilus* LAFTI® L10 in fermentation of sausages did significantly reduce levels of this pathogen during fermentation of the product, as compared to product inoculated only with a traditional meat starter culture [24]. Many other cultures have similar effects on this and other pathogens *in vitro*.

Anti-Cancer Effects

Anti-cancer effects are perhaps the most contentious of the health effects claimed for probiotics. However, a number of animal studies indicate that there is at least potential for beneficial effects. A study by McIntosh, Playne and Royle [31] compared the effect of Lactic Acid Bacteria (LAB) on development of intestinal cancer in rats challenged with a carcinogen (DMH). In this study, animals given *L. acidophilus* LAFTI® L10 developed fewer tumours than animals given other LAB. It is not yet known if *L. acidophilus* LAFTI® L10 could reduce cancer risk in humans. However, there are studies that indicate that at least one strain that protects against chemically induced tumours in rats also reduced formation of superficial bladder tumours in humans [33, 34]. It may be hypothesized that the same protective mechanisms are involved in reducing formation of both types of cancer.

Strain Origin

Many probiotic strains are of human intestinal origin. However, there are several examples of probiotic strains that originate from other sources, or, that at least are isolated from dairy products. Several studies discussed in this review have demonstrated that isolates of both human and dairy sources are efficacious by increasing resistance against

infection in animal models. Furthermore, strains of human vs. dairy origin have similar resistance to acid and bile [35], which suggests that some strains of dairy origin survive in the gastrointestinal tract. Probiotic strains of dairy origin are of great interest, since their impact on organoleptic properties of dairy products is usually well known. Probiotic cultures applied in yoghurt need to be particularly resistant to acidic conditions, since the pH of this type of products is at least 4.5, and often much lower. Freshly isolated bifidobacteria of human origin are often sensitive to acidic conditions [26]. In contrast, bifidobacteria that are currently used in fermented milk products are often more resistant to acidic conditions and, therefore, may be more suitable if they also display desirable effects on the host.

Australian Commercial Probiotic Strains

A number of novel probiotic strains developed in Australia have now been commercialised, including strains discussed in the previous section. *L. acidophilus* LAFTI® L10, *L. paracasei* LAFTI® L26 and *B. lactis* LAFTI® B94 are produced by DSM Food Specialties (Sydney) for use in fermented dairy products. VRI BioMedical (Perth) produce *L. fermentum* VRI-002 in a capsule form under the brands Progastrim® and ProBio PCC®.

Resistant Starches

Dietary carbohydrates that are indigestible or escape complete digestion in the small intestine may provide a carbon and energy source for bacteria residing within the large bowel. It has been demonstrated in numerous animal and human studies that a number of these carbohydrates can promote the relatively selective proliferation of bifidobacteria within the intestinal microbiota. Hence, these dietary components are termed prebiotics [5] and are produced commercially as functional food ingredients aimed at improving intestinal health [36]. Since many of the proposed health benefits attributed to dietary fibres stem from fermentation by the intestinal microbiota, there is increasing interest in understanding the relationships between intestinal bacteria and dietary polysaccharides arriving in the colon. In Australia, a considerable research effort has focused on the impact of resistant starch (RS) on the colonic microbiota and on the physiology and health of the host.

Supply of Resistant Starch to the Colon

That not all starch is digested and absorbed to completion in the human small bowel is a relatively recent realisation [37]. The detection of starch in ileal effluent [38] and faeces of volunteers [39] and experimental animals [40, 41] on starchy diets provides evidence that at least some starch actually reaches (and escapes) the large bowel. The fraction of ingested starch, and degradation products, that escape assimilation in the upper gastrointestinal tract of healthy individuals is termed resistant starch [42]. Although non-starch polysaccharides (NSP) are generally considered the main food source for the colonic flora of most humans (e.g., Australian average daily NSP intake for adults is 21 g [43]), it is claimed that RS may rival NSP in terms of the actual amount of material passing the terminal ileum each day [44]. Current estimates of daily per capita RS intake for industrialised nations range from 5-30 g [43], compared to

NSP which contribute between 8-18 g/d to total dry matter flux of the large bowel [45].

Fermentation of Resistant Starch in the Colon

The biochemical and microbial population changes that occur in the colonic lumen as a result of microbial fermentation of starch are considered important for bowel health. Short-chain fatty acids (SCFA), the principal non-gaseous end products of colonic RS fermentation, are generally regarded as the principal mediators of those benefits. Acetate, propionate and butyrate (the predominant SCFA) have a number of physiological properties in common, several of which have clinical significance. However, research has focussed on butyrate primarily because of its antineoplastic properties and its status as a preferred fuel for colonocytes [44]. Although the evidence that butyrate plays a critical role in the energy economy of colonocytes as well as regulating their growth and differentiation is compelling, it is based on *in vitro* and animal studies [44, 46]. The relevance of these findings for humans has yet to be established.

In general, fermentation of RSs increases the overall levels of SCFA in the large bowel, and that of butyrate in particular. Most studies in humans have shown an increase in faecal concentrations and/or excretion of butyrate in response to various dietary sources of RS [47-49]. These findings are confirmed and extended by research using experimental rodents [30] and pigs [8, 50, 51]. The propensity of RS for raising butyrate levels in the large bowel has fuelled interest in increasing RS consumption as a strategy to combat colorectal cancer [52]. Epidemiological studies lend weight to this hypothesis. Fermentation of RS also elicits other favourable metabolic changes in the colonic environment consistent with a reduction in risk of carcinogenesis and promotion of normal bowel function. For instance, faecal levels of potentially harmful toxic metabolites, such as phenols and cresols, and ammonia [53] are reduced in response to increased RS consumption. Luminal acidification, as reflected in lower faecal pH, is also another positive response to RS consumption [47].

Physiological Actions of RS in the Colon

Recent human studies have demonstrated that consumption of RS results in significant and favourable changes in bowel habit [54]. Stool mass is increased, albeit moderately, in subjects eating RS diets [47, 48] and defecation frequency is increased. These actions, which are a direct consequence of changes to the large bowel microbiota, suggest that RS may be of benefit in aiding laxation and also in reducing risk of diverticular disease and constipation.

Evidence of RS as a Prebiotic

Although the focus of much of the earlier research on RS related to its metabolic properties and butyrogenic capacity in particular, there is now growing interest in its potential to modulate the microecology of the large bowel. Evidence for this prospect is currently limited to animal studies. Nevertheless, RSs are showing considerable promise as dietary agents capable of increasing bacterial populations considered advantageous to host health or, alternatively, in suppressing those that may promote pathogenesis.

Largely because of the practical difficulties in measuring RS intake in humans, evidence of a role for it as a prebiotic derives from *in vitro* studies and *in vivo* experiments in animals. The pig is a well-established model for studying human large bowel metabolism of dietary fibre and interactions between diet and the enteric microflora [44, 55]. Studies with pigs fed various sources of starch have demonstrated that RS is a significant substrate for the large bowel microflora. Relatively high concentrations of starch have been detected in terminal ileal and large bowel digesta of pigs fed diets containing different starch sources [8, 50, 51]. In pig and rodent models, RSs have been shown to increase the population sizes of both lactobacilli and bifidobacteria in the colon [8, 51, 56]. Fructo-oligosaccharides (FOS), the most studied prebiotic, effectively raises faecal numbers of bifidobacteria even when ingested in small quantities. The comparative potency of RS to increase the size of the luminal bifidobacterial population remains to be determined. Some forms of RS appear to have the added advantage of raising counts of lactobacilli as well. The effect of RS on the population dynamics of a wide range of bacteria within the intestinal microbiota has yet to be assessed. The noted increase in proportions of butyrate and propionate in intestinal contents and faeces suggests that genera other than lactobacilli and bifidobacteria are also involved in fermenting RS in the colon, but elucidation of the organisms responsible has proved difficult [57].

Effects of Resistant Starch Type

Resistant starches are presently classified according to the nature of their resistance to amylolysis. Most research relating to physiological functionalities of RS has focussed on type II RS; especially high-amylose maize starches (HAMS). Topping *et al.* [58] were the first to show that HAMS has potential to promote the proliferation of purported beneficial intestinal bacterial populations *in vivo*. Using the pig model, they observed a marked increase in faecal bifidobacterial excretion in animals fed a commercial HAMS for 7 days. Potato starch (also RS2) had similar potential prebiotic properties in rats [59]. Categories of RS other than RS2 also promote intestinal bifidobacterial proliferation *in vivo*. Wang *et al.* [56] reported that carboxymethylated and acetylated amylo maize starches (both RS4) raised colonic bifidobacterial numbers in mice. However, these particular starches were not as effective as unmodified amylo maize.

Not all RS promote the growth of indigenous lactobacilli and bifidobacteria. When fed to young pigs, a diet containing cooked and cooled white rice (combination of RS1 and RS3) as the sole starch source did not elicit an increase in either lactobacilli or bifidobacterial numbers in the large bowel compared to controls on a diet containing highly digestible starch (Bird A., Jackson M., Rankin R., Topping D., unpublished data). Although it had no effect on these lactic acid bacteria, the high RS diet was particularly effective in suppressing populations of potentially pathogenic organisms, such as *E. coli*. This diet also had beneficial effects on the biochemical composition of colonic digesta. For instance, it reduced yields of intestinal phenols and cresols, which are suspected of causing cancer of the colon and bladder. Although information is limited, it is becoming apparent that

the current classification system for RS is a poor guide to the prebiotic potential of a given starch or its physiological properties.

Synbiotics with Resistant Starch

Australian research has demonstrated that RSs hold considerable promise as synbiotic agents. Brown and colleagues [8] showed that faecal bifidobacterial numbers in pigs dosed with freeze-dried *Bifidobacterium longum* (12.6 cfu/d; CSCC 1941) were increased significantly by feeding a HAMS compared to a low-amylose cornstarch diet. Faecal bifidobacteria could only be detected when the HAMS supplement was fed. In mice, the use of different types of amylo maize in a bifidobacterial probiotic preparation improved the efficacy of the probiotic in raising numbers of bifidobacteria in the colon [56]. The starch granules appear to protect probiotic organisms during storage and possibly intestinal transit [30]. Many bifidobacteria adhere strongly to a range of granular starches [30, 60, 61], which may both protect the cultures and facilitate competitive access to the starch substrate in the colon. Brown and colleagues [62] also showed that the loss of viability of probiotic cultures that normally occurs during storage of yoghurt was attenuated by inclusion of small amounts of a commercial HAMS preparation.

Synbiotic effects of HAMS were investigated in a pig model with the probiotic strain *B. longum* CSCC 1941. Faecal bifidobacterial numbers in pigs, supplemented with the probiotic *B. longum* CSCC 1941, were increased markedly in response to feeding either fructo-oligosaccharide (FOS) or HAMS. The HAMS proved just as effective as the FOS. However, co-administration of both prebiotic agents produced a further increase in the size of the faecal bifidobacteria population [58]. The microbial response to the prebiotics was additive, suggesting different modes of action for the probiotic and prebiotic. Also, after withdrawal of the probiotic from the food supply, continued supplementation of the diet with RS significantly extended the persistence of high faecal numbers of bifidobacteria (Fig. 2) (Topping D., Jackson M., Crittenden R., Hayakawa T., Playne M., Brown I., Bird A., unpublished results).

RESEARCH IN NEW ZEALAND

Historical Perspective

Lactic acid bacteria have long been a focus of research in New Zealand due to their importance to the dairy industry. Most of the early research on these bacteria concentrated on the traits that ensure consistency in the production of high quality cheese, and research into the health promoting potential of lactic acid bacteria did not receive much attention until the last decade. Current groups in New Zealand focused on research in lactic acid bacteria are listed in Table 2. A formal programme in probiotic research was established at what was then the New Zealand Dairy Research Institute (NZDRI) in late 1995. This program was set up in collaboration with what was the Milk and Health Research Centre at Massey University with the objective of developing proprietary probiotic strains with defined health benefits for the New Zealand dairy industry.

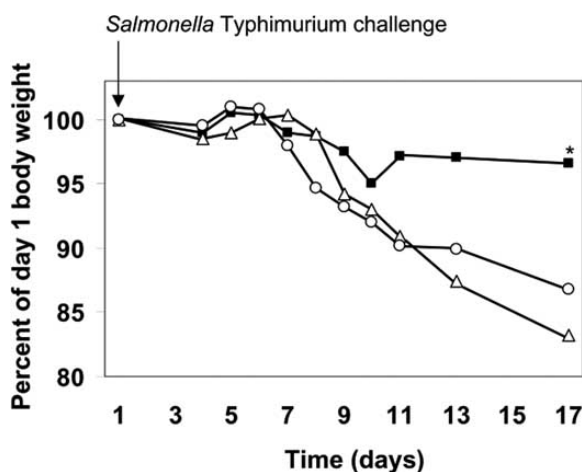


Fig. (2). Faecal *Bifidobacterium* numbers in pigs fed *Bifidobacterium longum* plus resistant starch (■), or *Bifidobacterium longum* plus digestible starch (●). Resistant starch promotes higher levels of intestinal bifidobacteria (* $p < 0.01$). Maintenance of dietary resistant starch following cessation of probiotic feeding promoted persistence of high numbers of intestinal bifidobacteria (** $p < 0.001$).

Selection of Probiotic Strains

NZDRI (now renamed Fonterra Research Centre) possesses a large collection of dairy lactic acid bacteria that provided an ideal source for potential probiotic strains. The culture collection of Prof. Gerald Tannock of Otago University, whose laboratory has a long history of research with lactobacilli and bifidobacteria of human origin, provided an additional source of potential probiotic strains. These two culture collections provided more than 2000 possible candidates for screening. A number of criteria are described in the scientific literature for selecting putative probiotic strains, including: (i) origin; (ii) survival and colonisation in the human gastrointestinal tract; (iii) safety for human consumption; and (iv) demonstrable efficacy. From a commercial view-point, issues for consideration during screening include the ability to grow on cheap nutrients, ease of processing (e.g., cell-concentration, freeze-drying) during commercial production, shelf-stability and the absence of any adverse effect on organoleptic properties when added to food or fermentation processes. The ability of

a putative probiotic strain to adhere and thus colonise the human gastro-intestinal tract is believed to be an important trait for probiotic bacteria and is screened for using *in vitro* adhesion assays. Gopal *et al.* [63] employed two independent methods using differentiated human intestinal cell lines, including Caco-2, HT-29 and HT-29 MTX, to demonstrate the adhesion ability of selected putative probiotic strains. The “adhesiveness” of strains was determined by measuring adhesion indices and, in the case of the best candidate strains, was found to be comparable with the adhesion indices of established commercial probiotic strains such as *Lactobacillus rhamnosus* GG and *Lactobacillus johnsonii* La-1.

Unequivocal characterisation of selected strains is very important, not only for protection of intellectual property, but also for proving the safety of strains. Prasad *et al.* [35] described the characterisation of four putative probiotic strains selected following stringent selection criteria. A polyphasic approach (phenotypic, genotypic and phylogenetic) was used for exhaustive characterisation of selected strains. Classical microbiological techniques and molecular methodologies including DNA-DNA homology, SDS-PAGE analysis of whole proteins, PFGE, species-specific probes and RAPD were employed. The strains were unequivocally identified as *Lactobacillus rhamnosus* HN001 also known as DR20™, *Bifidobacterium lactis* HN019 also known as DR10™, *Lactobacillus rhamnosus* HN064 and *Lactobacillus acidophilus* HN017.

Most of the research in the past five to six years has been focused on demonstrating the safety and efficacy of the selected strains. The following section summarises the strategies used and published research to demonstrate these two most important aspects of selected probiotic strains.

Proving Efficacy: Primary Criterion

The focus of health benefits arising from probiotic strains in New Zealand has been immune enhancement. The immune system is a key indicator of good health and is responsible for protection against most diseases including infectious illnesses and malignancy. Strains were screened for their ability to impact on the *in vitro* and *in vivo* indices of natural and acquired immunity in healthy mice [64]. Mice were fed daily, with the strains being screened at a dose of 10^9 CFU and their immune function was assessed on day 10 or day 28. The indices of immune function measured included: (i) phagocytic activity of peripheral blood leucocytes

Table 2. The Main Probiotic Research Groups in New Zealand

Researcher	Institution	Key Areas of Research
Gerald TANNOCK	Microbiology Department University of Otago, Dunedin	Intestinal microecology; molecular methods of microbiota analysis
Harsharn GILL*	Institute of Food and Human Nutrition, Massey University Palmerston North	Immunology of probiotic interaction; anti-infection effects of probiotics in animal models
Mark LUBBERS	Fonterra Research Centre Palmerston North	Genomics of <i>Lactobacillus rhamnosus</i> HN001
Pramod GOPAL	Fonterra Research Centre Palmerston North	Impact of probiotics and prebiotics (GOS) on human microbiota; anti-infection properties; technology

*Now located at the Victorian Department of Primary Industries, Werribee, Australia

and peritoneal macrophages; (ii) proliferative response of spleen cells to concanavalin A (a T cell mitogen) and lipopolysaccharide (a B cell mitogen); (iii) serum antibody response to orally- and systemically-administered antigens; and (iv) production of cytokines including interleukin-4 and interferon-gamma [64]. *B. lactis* HN019, *L. rhamnosus* HN001, *L. rhamnosus* HN064 and *L. acidophilus* HN017 were selected on the basis of these assays.

Anti-Infection Studies

A practical and readily measurable outcome of immune enhancement is protection against infection. The efficacy of the selected strains was tested in several anti-infection animal models, including protection against *Salmonella*, *E. coli* and rotavirus. The ability of *B. lactis* HN019 to confer protection against *Salmonella* Typhimurium was demonstrated in a BALB/c model. Shu *et al.* [65] showed that feeding mice with *B. lactis* HN019 conferred a significant degree of protection against single or multiple oral challenges with *S. Typhimurium*. The protection included a ten-fold increase in survival rate; significantly higher post-challenge feed intake; weight gain; and reduced pathogen translocation to visceral tissues. Furthermore, the degree of pathogen translocation showed a significant inverse correlation with splenic lymphocyte proliferative response to mitogens, blood and peritoneal cell phagocytic activity and intestinal mucosal anti-*Salmonella* Typhimurium antibody titres in infected mice. These results clearly suggest that dietary consumption of *B. lactis* HN019 provide a significant degree of protection against *Salmonella* infection by enhancing various parameters of immune function that are relevant to immunological control of salmonellosis.

Similarly, the efficacy of *B. lactis* HN019 in reducing the severity of *E. coli* O157:H7 infection in mice was demonstrated in another study [66]. Mice were fed a diet supplemented with *B. lactis* HN019 for 7 days prior to and following oral challenge with the pathogen. Behavioural parameters (morbidity, feed intake) were measured for 7 days following the challenge, while immunological responses (phagocytosis, antibody titre) and pathogen translocation were measured in a sub-sample of ostensibly healthy animals one week post-challenge. The results showed that HN019-fed mice maintained significantly higher post-challenge feed intake and exhibited a lower cumulative morbidity rate than the control mice.

A piglet model was used to demonstrate the efficacy of *B. lactis* HN019 against reduction of rotavirus-induced weaning diarrhoea [67]. Seventeen piglets were allocated into two groups (test and control) balanced for live weight and litter origin. Compared with the controls, piglets that received *B. lactis* HN019 had lower severity of weaning diarrhoea and maintained greater feed conversion efficiency during weaning. The protective effect of probiotic feeding was associated with a lower concentration of faecal rotavirus and *E. coli*, higher blood leukocyte phagocytic and T-lymphocyte proliferative responses and higher gastrointestinal tract pathogen-specific antibody titres. The study again provides good evidence that *B. lactis* HN019 is effective in reducing severity of rotavirus-associated diarrhoea, possibly via a mechanism of enhanced immune-mediated protection.

Since the piglet model is the closest to the human situation, results from this study can be extrapolated to suggest that this strain may be effective in preventing or limiting rotavirus-associated diarrhoea in the human infant.

Several mechanisms have been proposed to explain the enhanced resistance to gastro-intestinal tract pathogens conferred by probiotic micro-organisms. These include: (i) inter-microbial competition with the pathogens for intestinal attachment sites; (ii) the production of substances that are directly microbiocidal for pathogens; (iii) enhancement of protective immune responses; and (iv) reduction in intestinal permeability of hosts. However, the theories are not mutually exclusive, and the probiotic strains may be wholly or partially reliant on one or more of the different mechanisms for conferring enhanced protection against gastrointestinal tract pathogens such as rotavirus and *E. coli*.

Human Dietary Intervention/Clinical Trials

To carry out meaningful human clinical trials with probiotics, there are certain key criteria that must be fulfilled. They include use of well defined strains, well defined products, subjects from well defined study populations, randomised, double blind, and placebo controlled trial designs and subject numbers calculated statistically so that clinically significant outcomes are achieved. Finally the results must be subjected to peer review and published in reputable scientific journals. Over the past several years, NZDRI have followed these principles and performed a number of clinical trials to demonstrate the impact of the consumption of *B. lactis* HN019 and/or *L. rhamnosus* HN001 on indices of the immune system in humans. A summary of results from some of these trials is presented here.

In a randomised, double blind, placebo-controlled clinical trial, Arunachalam *et al.* [68] demonstrated that the dietary consumption of *B. lactis* HN019 could impart measurable benefits on the natural immune function among healthy elderly (median age 69 years) subjects. Indices of immunity measured in this study included interferon production, phagocytic capacity and phagocyte-mediated bactericidal activity. In another trial (double blind, three-stage before and after intervention), fifty healthy Taiwanese were allocated to two groups. In stage one, all subjects consumed low fat milk for three weeks. In stage two, subjects consumed low fat milk with *B. lactis* HN019 for three weeks. In stage three, all subjects returned to non-supplemented low fat milk for a further three weeks. The innate immune function of two different leucocyte types, polynuclear (PMN) cells and natural killer (NK) cells, were assessed at four time points via *in vitro* analysis of peripheral blood samples. While consumption of low fat milk alone had no significant effect on immune responses, stage 2 results indicated significantly enhanced PMN cell phagocytosis and NK cell tumour killing activity following consumption of milk with *B. lactis* HN019 [69].

The impact on aspects of cellular immunity through consumption of *B. lactis* HN019 in humans was demonstrated in another clinical trial [70]. In this study, thirty subjects participated in a three stage clinical trial. During stage one, subjects consumed low fat milk as base-diet control for three

weeks. During stage two, they consumed milk supplemented with *B. lactis* HN019 in a typical dose (5×10^{10} organism/day) or a low dose (5×10^9 organism/day) for three weeks. During the final stage (wash-out), they returned to consumption of low fat milk. Increases in the proportions of total, helper (CD4) and activated (CD25) T lymphocytes and natural killer cells were measured in subjects' blood after the consumption of *B. lactis* HN019. The *ex vivo* phagocytic capacity of mononuclear and polynuclear phagocytes and the tumoricidal activity of natural killer cells were elevated after *B. lactis* HN019 consumption. The greatest changes in immunity were found in subjects who, prior to treatment, already had poor immune responses.

Similar effects on immune parameters were observed when studies were completed with *L. rhamnosus* HN001. The effects on cellular immunity of *L. rhamnosus* HN001 consumption in low fat milk or hydrolysed lactose low fat milk were studied in fifty-two healthy volunteers. The study design was a three-stage, pre-post intervention trial spanning nine weeks. During stage one, subjects consumed low fat milk twice daily for three weeks, followed by an intervention stage when subjects consumed milk supplemented with *L. rhamnosus* HN001 (10^9 CFU/day) for three weeks. Stage 3 was a wash out period of three weeks when subjects consumed low fat milk alone. *In vitro* phagocytic activity of peripheral blood polymorphonuclear (PMN) leukocytes and *in vitro* tumoricidal activity of natural killer (NK) leukocytes were measured at week 0, 3, 6, and 9. Results showed significantly increased activity in both parameters following the consumption period [71]. Several other studies have been performed with *L. rhamnosus* HN001, with similar results [72, 73]. These clinical trials demonstrate that both *B. lactis* HN019 and *L. rhamnosus* HN001 may enhance components of the innate and acquired immune systems in humans. These effects may translate into enhanced immune protection, however, further studies are required.

Colonisation and Passage Through the Human Gastro-Intestinal Tract

Research into the composition and role of human gut microflora is fundamental to our understanding of the concept of probiotics. Professor Gerald Tannock, based at the Microbiology Department at University of Otago, is a leader in this field and has made significant contributions to our understanding of human microbiota. Current research interests of his group include analysis of the gut microflora of humans in health and disease, the study of host-microflora interactions and impact of probiotics and prebiotics on microbial ecology of the gut. In a collaborative study, Tannock examined the impact of long-term consumption of *L. rhamnosus* HN001 on the micro-ecology of human subjects [74]. This dietary intervention study monitored the faecal microflora of 10 healthy human subjects before (six month control period), during (six month test period) and after (three month wash-out period) the consumption of a probiotic product containing *L. rhamnosus* HN001 (daily dose of 1.6×10^9 CFU). Monthly faecal samples were examined by a variety of methods, including bacteriological culture analysis; fluorescent *in situ* hybridisation with group-specific DNA probes; denaturing gradient gel electrophoresis

of V2-V3 region of 16S rRNA genes amplified with PCR; gas liquid chromatography; and bacterial enzyme analysis. The fate of the ingested strain of *L. rhamnosus* HN001 was followed by pulse-field gel electrophoresis of the DNA digest of the faecal bacteria. The presence of *L. rhamnosus* HN001 was detected in the faeces of all subjects during the test period, but at differing frequencies. The study concluded that the consumption of *L. rhamnosus* HN001 transiently altered the *Lactobacillus* and enterococcal populations in the majority of consumers. In another dietary intervention study with *B. lactis* HN019, with and without galactooligosaccharides, Gopal *et al.* [75] showed that the consumption of *B. lactis* HN019 altered not only the population of bifidobacteria in human subjects, but also that of lactobacilli. This confirmed that it is possible to have a demonstrable effect on the population sizes of various groups of bacteria in the human gastrointestinal tract through the dietary consumption of probiotic bacteria.

Safety

The best proof of safety of a probiotic strain comes from its history (e.g., demonstrable presence in the food chain for a long period of time), hence one of the reasons for choosing strains of food origin. Even so, in addition to the unequivocal characterisation of strains that provides identity of the micro-organism, data on important toxicological parameters are required for assessing the safety of new probiotic strains. Acute oral toxicity and bacterial translocation studies on the four probiotic strains described earlier demonstrated no adverse effects on BALB/c mice [76]. The mice were fed a high dose (10^{11} CFU/mouse/day) of the newly-described probiotic strains and acute oral toxicity was assessed by measuring their effect on general health status, feed intake, and intestinal mucosal morphology. No viable bacteria were recovered from blood and tissue samples. The oral LD₅₀ of these strains was calculated to be more than 50g/kg/day for mice. This can be extrapolated to acceptable daily intake (ADI) value of 35 g of pure dry bacteria per day for a 70 kg person.

In another safety study, the four probiotic strains were fed to BALB/c mice for 4 weeks at three doses (2.5×10^9 , 5×10^{10} and 2.5×10^{12} CFU/kg/day). Throughout the study period, the feed intake, water intake, general health and live weight gain were monitored. At the end of the 4-week period, samples of blood, liver, kidney, mesenteric lymph nodes and gut tissue were examined for haematological, histological parameters and bacterial translocation. The results demonstrated that none of the four strains had any adverse effects at any dose and were non-toxic to mice. They were, therefore, considered likely to be safe for human consumption [76, 77].

The mucus layer coating the surface of the gastrointestinal tract plays an important role in the mucosal barrier system. It is believed that any damage or disturbance to the mucin layer will compromise the mucosal defence function in the host. It is, therefore, important to ascertain that probiotic strains do not degrade the mucin layer through the lytic activity of secreted enzymes. Zhou *et al.* [78] showed that the four selected strains did not degrade gastric mucin.

Probiotic Technologies in New Zealand

The development of probiotic technologies in New Zealand has focused on enhancing the shelf-stability of two specific strains, namely *B. lactis* HN019 and *L. rhamnosus* HN001. Pre-stressing *L. rhamnosus* HN001 with either heat (50°C) or salt (0.6 M NaCl) showed significant ($P < 0.05$) improvement in viability compared with non-stressed control cultures when stored at 30°C in a freeze-dried form. Using 2D-gel electrophoresis and N-terminal sequencing, interesting differences were observed between stressed and non-stressed cultures [79]. In addition, Fonterra have studied the effect of storage temperature, moisture content, oxygen content and various other additives on the shelf-stability of two strains of probiotics. Fonterra have been successful in enhancing the shelf-life of *B. lactis* HN019 to two years without significant loss in cell numbers when stored in dry blends at temperatures of up to 30°C. This technology is subject to patent application. Technology options such as micro-encapsulation are also the subject of active research in New Zealand.

Genomic Approach

Genomics encompasses the systematic study of the structure, content and evolution of complete genomes. Investigation of the gene content of probiotic strains holds great promise for both the discovery of important genes and understanding the mechanism through which probiotic bacteria elicit beneficial activities. At Fonterra Research Centre (formerly NZDRI), the objective of the probiotic genomics project is to develop new high value functional foods through 'mining' for components that can be used commercially to deliver bioactive components. The genome sequence of *L. rhamnosus* HN001 has been determined and is being used by Dr Mark Lubbers's group to define the molecular basis of bioactive effects associated with probiotic cultures and to identify the bioactive components of these bacteria. Two parallel approaches are being pursued: firstly, the activity of whole cells and fractionated components of cell are being tested for impacts in relevant assays such as adhesion to gastrointestinal cells. Secondly, the genome sequence is 'mined' for genes that might be involved in these activities, followed by direct testing of specific gene mutants and purified gene products. These approaches will lead to an

understanding of the correlation of genes and gene networks to phenotypic behaviours, and analysis of gene expression will be critically important in unravelling the functional properties and behaviour of probiotic strains.

Commercialisation of Probiotic Strains Developed in New Zealand

The main objective of the probiotic programme at NZDRI was to develop credible probiotic strains based on highest quality science. The approach was integrated and involved collaboration between the NZDRI and Universities and Research Institutions outside New Zealand. This resulted not only in achievement of the main objective of the project (i.e., development of commercial probiotic strains backed by good science), but also enhanced impetus of further research within this ever developing area. The strain *B. lactis* HN019 was trademarked as DR10™, and the strain *L. rhamnosus* HN001 as DR20™. These strains have been successfully commercialised in products such as milk powder and cheese (the latter sold in Australia under the brand name "Inner Balance") and in dietary supplements.

RESEARCH IN ASIA

Research and Industrial Activities in Asia (Excluding Japan)

The consumption of functional foods has a long tradition in Asia, and the concept of probiotics was readily accepted in Asia in the 1930's when it was first introduced. Research and development activities in Asia also have a long history, but remain largely aimed at national and regional levels. Many scientific publications are targeted to local markets and communities, and they are not written in English. In this section, the main probiotic research activities in a number of Asian countries are briefly summarised.

China

Major research activities in pro- and prebiotics are concentrated at a few research centres (Table 3), where the focus is predominantly on applied research in product development, and their commercial applications. Currently, there are about 100 types of probiotic organisms produced for the Chinese market, mostly sold as functional food

Table 3. The Main Probiotic Research Groups in China

Researcher	Institution	Key Areas of Research
Xinh Hua GUO	Institute of Microbiology, Chinese Academy of Sciences	Ecology of intestinal microflora; probiotic mechanisms
Bai KANG	Medical University, Dalian	Probiotic for man
Yuanho MA	Institute of Microbiology, Chinese Academy of Sciences	Prebiotics
ChinYun Yang	Medical College, Kiamoshi University, Hailongxian	Chines medicinal herbs as prebiotics
LuHong Mei	Chinese Agriculture University, Beijing	Probiotics for crops
MinChin He	Agriculture University, Shichuan	Probiotics for animals
ChernPin Chiang	Agriculture University, Nanjing	Probiotics for animals

Information provided by Guo XH, Institute of Microbiology, Chinese Academy of Sciences

ingredients. Clinical trials have been conducted for about 10% of the probiotic products, and these are approved by the Chinese regulatory authority to be marketed as pharmaceutical products (Table 4). Most of the pharmaceutical-style probiotic products target gastrointestinal disorders. In addition to the pharmaceutical-style probiotic product manufacturers there are about 300 fermented milk manufacturers in China.

As in many Asian countries, there is a strong research and commercial interest in animal probiotics in China. There are about 400 commercial manufacturers of probiotic products for animals, and the total annual production amounts to 30, 000 metric tons [80]. *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Bacillus*, photosynthetic bacteria, *Bdellovibrio bacteriovorus*, *Aspergillus* and *Candida* are all used as probiotics. The probiotics are fed to pigs; dogs; cats; broiler and egg-laying chickens; ducks; dairy cattle; rabbits; fish; and prawns. Feeding of probiotics have been reported to enhance weight gain (7.1-14.2%); increase feed conversion efficiency (4.2-11.8%); lower the incidence of disease (14.1-22.9%); lower mortality rates (3.8-14.6%) and increase egg/milk production (6.3-7.3%). Interestingly, the Chinese have extended the probiotic concept to biocontrol of plant microbial pathogens. *Bacillus cereus* and *Bacillus licheniformis* are used on a wide range of crops including, rice; wheat; corn; potato; cotton; soy; peanut;

vegetables; ginseng; pasture; apples; oranges; water melon; sugar cane; tea and tobacco. Use of the probiotics has been reported to increase productivities (6.4-21.0%) and boost disease resistance (43.0-86.4% reduction) [81].

The annual production of prebiotic carbohydrates in China, in the form of oligosaccharides, amounts to approximately 20, 000 metric tons [82]. A major producer is the Tianyuan Group at Yunnan. It is worth noting that a number of Chinese medicinal herbs (*Panax ginseng*, *Codonopsis pilosula*, *Ganoderma lucidum*, *Eguus asinus*, *Polyporus umbellatus*, *Lycium chinense*, *Cordyceps sinensis*, *Patrinia villosa*, *Ligustrum lucidum*) have been found to promote the growth of intestinal commensal bacteria [83].

Indonesia

The level of probiotic research in Indonesia is relatively modest, and investigations of probiotic effects centre largely on local, traditional fermented foods (Table 5). An example is the study of the potential probiotic attributes among lactic acid bacteria in Dadih, a traditional fermented buffalo milk particular to Indonesia.

Korea

In Korea, basic probiotic research is hosted by both academic institutions (Table 6) and companies (Table 7).

Table 4. Probiotics Marketed as Pharmaceutical Products in China

Probiotic bacteria	Functions	Company
<i>Bifidobacterium adolescentis</i>	Modulate intestinal microflora	Lichu Drug House
<i>Bifidobacterium longum</i> <i>Lactobacillus acidophilus</i> <i>Enterococcus faecalis</i>	Modulate intestinal microflora	Shanghai Sinyi Drug Pte. Ltd. Shansi Haishi Drug Pte. Ltd.
<i>Bifidobacterium longum</i> <i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i> <i>Streptococcus thermophilus</i>	Modulate intestinal microflora	Mongolian Shuanchi Drug Co. Ltd.
<i>Bacillus cereus</i>	Modulate intestinal microflora	Dalian Medical University Tayue Drug Co. Chendu Bioproduct Institute Anyang Yuanshou Biodrug Pte. Ltd.
<i>Bacillus licheniformis</i>	Modulate intestinal microflora	Shenyang First Drug House
<i>Bifidobacterium bifidum</i> <i>Lactobacillus acidophilus</i> <i>Enterococcus faecalis</i> <i>Bacillus cereus</i>	Modulate intestinal microflora	Jilin Weite Group
<i>Clostridium tyrobutyricum</i>	Modulate intestinal microflora	Chongqing Taipin Drug Co. Ltd.
<i>Bifidobacterium infantis</i> <i>Clostridium tyrobutyricum</i>	Modulate intestinal microflora	Shantong Kesing Biodrug Pte Ltd.
<i>Bacillus subtilis</i> <i>Enterococcus faecalis</i>	Modulate intestinal microflora	Beijing Hanbei Drug Co.
<i>Lactobacillus debrueckii</i> subsp. <i>delbrueckii</i>	Treatment of bacteria vagina infection	Dalian Medical University
<i>Lactobacillus acidophilus</i> <i>Enterococcus</i>	Modulate intestinal microflora	Jianshu Taizhou Drug House
<i>Bacillus subtilis</i>	Treatment of infection of burn and cut	Harbin
<i>Lactobacillus plantarum</i>	Promote general health	Shanghai Jiaotong University
<i>Bifidobacterium</i> <i>Lactobacillus</i> <i>Enterococcus faecalis</i>	Promote general health	Shantong Jinan Sanzhu Co.
Heat inactivated <i>Lactobacillus acidophilus</i>	Not indicated	Harbin Taige Co.

Based on Yuan [82].

Table 5. The Main Probiotic Research Groups in Indonesia

Researcher	Institution	Key Areas of Research
Ingrid SURONO	Center for the Assessment of Biotechnology,	Antimutagenic properties of Dadih LAB
Usman PATO	Faculty of Agriculture, Riau University	Hypocholesterolemic properties of Dadih LAB
Endang S. RAHAYU	Faculty of Agricultural Technology, Gajah Mada University	Indonesian fermented foods as probiotic carrier
Rina AGUSTINA	Seameo, University of Indonesia	Clinical trials in LAB prevention & treatment of acute diarrhoea

Table 6. The Main Probiotic Research Groups in Academic Institutions in Korea

Researcher	Institution	Key Areas of Research
Kook Hee KANG	Department of Food & Life Science, Sungkyunkwan University	Application of LAB as probiotics
Dong Hyun KIM	College of Pharmacy, Kyung Hee University	Anti-rotaviral activity of locally isolated bifidobacteria
Jae Seong SO	Department of Biological Engineering, Inha University	Bacteriocin production by vaginal lactobacilli
Hyonh Joo LEE	Department of Food Science & Technology, Seoul National University	Gene expression systems in lactococcus
Nam Ju KIM	Department of Food Science & Nutrition, Seoul National University	Immunomodulation by bifidobacteria
Jong Hwa LEE	School of Bioresources Science, Andong National University	Immunomodulation by LAB of Kimchi
Keum Il JANG	Department of Food Science & Technology, Chungbuk National University	Microencapsulation of LAB as poultry probiotic
Seung Bae LEE	Applied Animal Science Division, Sangji University	16s rDNA based identification of bifidobacteria
Keun KIM	University of Suwon	<i>H. pylori</i> antagonistic LAB
Yung Ho YOON	Department of Animal Science, Chung Ang University	<i>H. pylori</i> antagonistic LAB
Min Ho Choi	Department of Molecular Science & Technology, Ajou University	Nisin resistant probiotic LAB
Yoo Beom LIM	Department of Food Science & Technology, Hankyong University	LAB & <i>Enterococcus</i> as fish probiotics
Young Hyo CHANG	Korea Research Institute of Bioscience & Biotechnology	LAB as pig probiotics
Ji Eon KIM	Department of Food Science & Technology, Seoul National University	Antitumour activity of LAB
Geon Eog JI	Department of Food & Nutrition, Seoul National University	Bifidobacteria as probiotics
Yun Hee PARK	Department of Molecular Science & Technology, Ajou University	LAB functions in Kimchi
Tae Kwang OH	Korea Research Institute of Bioscience & Biotechnology	Bifidobacteria in Kimchi production
Yong Ha PARK	Gene Bank, Korea Research Institute of Bioscience & Biotechnology	Isolation & identification of novel probiotic strains for animals and fishes

Information provided by Kang K.H. of the Department of Food & Life Science, Sungkyunkwan University.

Many of the probiotics studied were originally isolated from Kimchi (a traditional Korean fermented cabbage). There are strong interests in the identification of novel bacteriocins and the development of encapsulation technology. It should be mentioned that a cloning vector for food grade *Bifidobacterium* has been constructed by cloning both a 4960 bp plasmid (pKJ50) from *B. longum* and a chloramphenicol resistance gene into pBR322 [84]. A bifidobacterial surface

protein was isolated and used as a mediator for the surface display of foreign proteins.

Mongolia

There is an interest in developing LAB isolated from traditional Mongolian fermented milk products as probiotics for man and animals (Table 8). Probiotic products containing locally-isolated *L. plantarum* and *L. acidophilus* are effective

Table 7. The Main Probiotic Research Groups in Commercial Companies in Korea

Researcher	Institution	Key Areas of Research
Sun Young KIM	Kimchi Research Center, Doosan Corporation	Microbiology & functional properties of Kimchi
Myung Jun CHUNG	R & D Center of Cell Biotech Co. Ltd.	LAB bacteriocins (SAFELAC) against food pathogens; micro-encapsulated LAB (PROLAC, DUOLAC) stable at ambient temperature
Myeong Soo PARK	Research Center, BIFIDO Co. Ltd.	Foreign gene expression system in bifidobacteria; isolation & selection of probiotic bifidobacteria
Seung Chun BAICK	Seoul Dairy Cooperative	Microencapsulation of LAB
Ki Tae KIM	Seoulin Bioscience Institute, Seoulin Bioscience Co. Ltd.	PCR based LAB identification kit
Young Jin BAEK	R & D Center, Korea Yakult Co. Ltd	<i>H. pylori</i> antagonistic LAB
Yong Ha PARK	ProBionic Co.	Isolation & identification of novel probiotic strains for animals and fishes

Information provided by Kang K.H. of the Department of Food & Life Science, Sungkyunkwan University.

Table 8. The Main Probiotic Research Groups in Mongolia

Researcher	Institution	Key Areas of Research
Shirchingiin DEMBEREL	Laboratory of Pathology & Physiology of young Animals, Mongolian Veterinary Institute Department of Biochemistry & Microbiology, National University of Mongolia	Microbiology of Mongolian yoghurt, Koumiss, undaa milk drink & clabber hoormog; probiotic properties of locally isolated LAB; animal probiotics

Information provided by Demberel S.H. of the Mongolian Veterinary Institute

Table 9. The Main Probiotic Research Groups in The Philippines

Researcher	Institution	Key Areas of Research
Julie D. TAN	Leyte State University	Bacteriocin, fermented root crop & coconut food products
Charina B. BANAAAY	Philippine National Collection of Micro-organisms, University of the Philippines, Los Banos	Bacteriocin production by LAB in indigenous foods
Francisco ELEDAGO	BIOTECH, University of the Philippines, Los Banos	Bacteriocin production by LAB
Erlinda DIZON	Institute of Food Science and Technology, University of the Philippines, Los Banos	Fermented food products from indigenous materials

Information provided by Tan JD of the Leyte State University.

against bacterial (in particular *E. coli* and *Salmonella*) diarrhoea in young animals [85].

The Philippines

The identification of novel bacteriocins produced by LAB in traditional fermented foods is the current focus of probiotic-related research in the Philippines. The main research groups involved in probiotic research in the Philippines are listed in Table 9.

Singapore

The probiotic research focus in Singapore is biomedical (Table 10). Areas of research include investigation of the

molecular cross-talk between the intestinal bacteria and the host; modulation of the immune system and intestinal microbial ecology; and roles of LAB in the development and treatments of asthma and cancers. It has been demonstrated that LAB species show higher cytotoxicity to human bladder cancer cells than *Mycobacterium bovis* (*Bacillus Calmette-Guerin* - BCG), suggesting that selected LAB species could replace BCG as intravesical agents for treating bladder cancers [86].

Thailand

Research and development activities in Thailand focus on probiotics and prebiotics for animal applications. In

Table 10. The Main Probiotic Research Groups in Singapore

Researcher	Institution	Key Areas of Research
Yuan Kun LEE	Department of Microbiology, National University of Singapore	Adhesion of LAB to intestinal mucosa; microbe-host cross talk in the intestinal tract; LAB as foreign protein and gene delivery systems
Boon Huat BAY	Department of Anatomy, National University of Singapore	LAB in prevention & treatment of colon and breast cancers
Mahendran RATHA	Department of Surgery, National University of Singapore	LAB in prevention & treatment of bladder cancer
Bee Wah LEE	Department of Paediatrics, National University of Singapore	Intestinal bacteria and asthma in infant

Information provide by Lee Y. K., of the National University of Singapore.

Table 11. The Main Probiotic Research Groups in Thailand

Researcher	Institution	Key Areas of Research
Sunee NITISINPRASERTOrpin BHUMIBHAMORN Vichien LEELAWACHALAMAS Mungkorn RODPRAPAKORN Sarote SIRISANSANEEYAKUL Penkhae WANCHAITHANAWONG Suttipan KAEWSOMPONG	Dept. of Biotechnology, Faculty of Agro-Industry, Kasetsart University	Probiotic and prebiotic in food and feed; molecular studies; probiotics for chickens, pigs, and aquiculture (shrimp); production technologies
Vichai LEELAWACHALAMAS	Dept. of Biotechnology, Khonkaen University	Probiotics for aquiculture - shrimp (screening, characterization and production)
Sirirat RENGPIPAT	Dept. of Microbiology, Faculty of Veterinary Science, Chulalongkorn University	Probiotics for chicken and shrimp
Suriya SASSANARAKKIT	Biotechnology Laboratory, Thailand Institute of Scientific and Technological Research	Probiotics for shrimp (Screening, characterization and production)

Information provided by Nitisinprasert S., of the Kasetsart University.

Table 12. Probiotic and Prebiotic Products Developed in Thailand

Product	Producer	Distributor
Rocket (probiotics for shrimp)	Dept. of Microbiology, Fac. of Veterinary, Chulalongkorn University	FRESH AGRO Co.
Zoolack (probiotic for shrimp)	KMP BioTECH	FREASH AGRO Co.

Information provided by Nitisinprasert S., of the Kasetsart University.

particular, research and commercial activity has centred on probiotics for use in aquiculture (Tables 11 & 12).

Malaysia

There are two key centres for probiotic research in Malaysia (Table 13), both of which are investigating probiotic bacteria and prebiotic carbohydrates for both humans and animals. Dr Yazid's research group at the Universiti Putra Malaysia has concentrated on studies on bifidobacteria in relation to bile tolerance [87]; antimicrobial susceptibility [88, 89]; reductions in serum cholesterol levels [90]; and adherence to HT29 cells [91]. Survival of strains in milk has also been studied [92]. More recent publications have been

concerned with the characterization of fructose-6-phosphate phosphoketolase enzyme from *Bifidobacterium asteroides* [93]; the cloning and sequencing of the bile salt hydrolase gene from *Bif. longum* [94]; and molecular techniques to fingerprint isolates of *Bifidobacterium* and other probiotic bacteria [95-96].

CONCLUSIONS

This review demonstrates the breadth of research and development on probiotic cultures and prebiotic carbohydrates in the countries of the Asia-Pacific region. The extensive work carried out on strain selection in Australia and New Zealand using different approaches, and the targeted approach to health efficacy, contrasts with strain selection

Table 13. The Main Probiotic Research Groups in Malaysia

Researcher	Institution	Key Areas of Research
Mohd YAZID Manap	Universiti Putra Malaysia, Faculty of Food Science and Biotechnology.	Bifidobacteria and human health; effects on cholesterol; molecular biology; probiotics for poultry.
YEOH Quee Lan WONG Hee Kum	MARDI Biotechnology Research Centre and Livestock Research Centre, Kuala Lumpur.	Human and animal probiotics.

Information provided by Dr. Mohd Yazid Manap, Universiti Putra Malaysia, Faculty of Food Science and Biotechnology, and by Ms Yeoh Quee Lan, MARDI.

undertaken elsewhere. Australian prebiotic research has concentrated on the use of resistant starch and complementary cultures for synbiotic applications. The New Zealand research effort has looked more strongly at effects of probiotics on immune function, and this has been supplemented by the leading edge work of the Otago researchers on indigenous bifidobacteria and lactobacilli in the human gut, and on human-human variation that can be found in the distribution of strains of these two genera.

In Asian countries, most emphasis has been on the development of cultures for use in traditional fermented foods, and in determining the nature of the cultures found in those traditional foods. Interestingly, a far wider range of microbial species are commonly used as probiotics in Asia (e.g., *Bacillus* species, *Clostridium* species). Applications of bacteria extend into wider areas than in Europe. Great emphasis is placed in China on the use of animal probiotics, but they are also used to protect plants from disease. China is also a major producer of prebiotic oligosaccharides. In Thailand, emphasis is on the role of probiotics in fish and crustacean production. In Singapore, a much greater emphasis has been placed on fundamental understanding of the role of probiotics in the health of the host (e.g., the mathematics of adhesion of microbial cells to epithelia).

The Asia-Pacific region is characterised by the diversity of approaches to research and development being undertaken. The strength of this diversity will be best realised if researchers in the region develop strong and enduring collaborative links.

ACKNOWLEDGEMENTS

The authors of this paper are indebted to the many researchers in the region who have provided invaluable information on activities in their countries. It is inevitable in reviews of this type that important research work and research groups will be inadvertently omitted. We apologise to those so affected.

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