

## Review

# Effect of honey in improving the gut microbial balance

Anand Mohan\*, Siew-Young Quek\*, Noemi Gutierrez-Maddox\*\*, Yihuai Gao\*\*\* and Quan Shu\*\*\*

\*School of Chemical Sciences, the University of Auckland, New Zealand, \*\*School of Applied Sciences, AUT University, Auckland, New Zealand and \*\*\*Bioactives Research New Zealand, Auckland, New Zealand

Correspondence to: Siew-Young Quek, School of Chemical Sciences, the University of Auckland, New Zealand. E-mail: [sy.quek@auckland.ac.nz](mailto:sy.quek@auckland.ac.nz)

Received 14 October 2016; Revised 5 March 2017; Editorial Decision 6 March 2017.

## Abstract

Increasing consumer emphasis on the health benefits of foods has enhanced the research focus in health promoting elements, such as probiotics, prebiotics, and synbiotics. Live probiotic bacterial strains, which are incorporated in various food systems, must survive unfavourable processing and gastric environments to confer the desired physiological responses in the human gut. Non-digestible oligosaccharides are provided as fermentable prebiotic substrates to selectively modulate the gut microbial balance in favour of probiotic lactobacilli and bifidobacteria, thus improving the host metabolic function. Honey contains oligosaccharides that can be utilized by the saccharolytic fermenters to yield beneficial metabolites that promote the prebiotic effect. There are numerous studies on the antimicrobial components and health effects of honey, and many have focused on the unique antibacterial activity of varieties such as Manuka. However, the possibility of the bactericidal and bacteriostatic factors in honey working synergistically with probiotics is yet to be adequately explored in the literature. The focus of this review is on the studies that have endeavoured to evaluate the prebiotic potential of honey, which has not been comprehensively assessed as the more established prebiotics. The results in most of the reported investigations are encouraging at optimal concentrations of honey, and further research is recommended as per the defined criteria of fermentation selectivity required for the endorsement of prebiotic functionality.

## Introduction

Functional food components, such as prebiotic carbohydrates and probiotic bacteria, have elicited a renewed research interest in the recent years, since they confer additional health benefits beyond basic nourishment by altering the gut microbial balance and its metabolic function (Salminen *et al.*, 1998; Roberfroid, 2000; Flint *et al.*, 2012). Gut microbiota is largely unknown and performs many health sustaining metabolic activities which influence some of the crucial aspects of the human physiological system, and also contributes nutrients and energy by the anaerobic fermentation process (Guarner and Malagelada, 2003; García-Elorriaga and del Rey-Pineda, 2013; Erejuwa *et al.*, 2014; Marchesi *et al.*, 2016). Several microorganisms, however, can be pathogenic or release antagonistic metabolites if allowed to proliferate (Flint *et al.*, 2012). Probiotic microbes predominantly are the lactic acid bacteria strains

from *Lactobacillus* and *Bifidobacterium* genera (Roberfroid, 2000; Reuter, 2001). The major bacterial genera inhabiting the human gastrointestinal (GI) tract are enlisted in Table 1. Strategies for improving the quality and balance of the microflora towards the more favourable species include providing the probiotic cells with growth factors such as prebiotic oligosaccharides, and microencapsulating them with a protective biopolymer coating for controlled release in the GI tract.

Several *in vivo* and *in vitro* studies on altering the composition of the gut microbiota, by increasing the growth of probiotic lactobacilli and bifidobacteria, have primarily focused on prebiotic fructo-oligosaccharides (FOS) such as inulin and oligofructose (Kolida *et al.*, 2002; Gibson *et al.*, 2004; Mussatto and Mancilha, 2007; Kellow *et al.*, 2014; Rastall and Gibson, 2015). Common sources of FOS include plant-based foods such as chicory, garlic, onion, jerusalem

**Table 1.** Microflora constituents of the human gastrointestinal tract. Total bacterial counts are estimated in colony-forming units (CFU) per millilitre.

Organ	Bacterial genera	Estimated counts, CFU/ml
Stomach	<i>Lactobacillus</i>	<1000
Small intestine	<i>Enterococcus</i>	10 <sup>2</sup> –10 <sup>9</sup>
Large intestine	<i>Lactobacillus</i>	10 <sup>4</sup> –10 <sup>12</sup>
	<i>Bifidobacterium</i>	
	<i>Enterococcus</i>	
	<i>Bacteroides</i>	
	<i>Clostridium</i>	
	<i>Eubacterium</i>	
	<i>Staphylococcus</i>	
	Coliforms	

Source: Savage (1977), Roccarina et al. (2010), and García-Elorriaga and del Rey-Pineda (2013).

artichoke, banana, wheat, asparagus, and leek (van Loo et al., 1995), and many emerging prebiotics are also being investigated due to their unique health and technological functionalities. Honey is also being recognized as a potential prebiotic, since it has oligosaccharides that can promote the growth of lactobacilli and bifidobacteria, in addition to antimicrobial components which can act synergistically with the probiotics against certain pathogens. A comparative study involving honey oligosaccharides has demonstrated a definite prebiotic potential, which however was not as prominent as FOS (Sanz et al., 2005). The aim of this review was to outline the studies that have utilized honey for promoting the growth and metabolic activity of probiotic bacteria, and to highlight further research aspects that may be necessary to incorporate honey as an effective prebiotic for modulating the gut microbiota.

### Chronological Events in Probiotics Research and Challenges for Incorporation in Food

The notion that colonic bacteria can have beneficial effects, through ingestion of fermented milk (Metchnikoff, 1908) or breastfeeding infants (Tissier, 1907), was hypothesized over a century earlier. Fuller (1989) redefined the term ‘probiotic’ to include only viable microorganisms which provide health benefits by improving the microbial balance in intestine of the host. In the year 2014, the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement ratified an earlier definition of probiotics outlined by Food and Agriculture Organization and World Health Organization (FAO/WHO, 2001), as ‘live microorganisms that, when administered in adequate amounts, confer a health benefit on the host’ (Guarner and Schaafsma, 1998; Hill et al., 2014). The expert panel concurred that the definition adequately covers the widely prevalent probiotic species that contribute to the core functions of sustaining healthy digestive and immune systems. Furthermore, there is accumulating scientific evidence to suggest their efficacy in prevention and management of gut diseases, such as inflammatory bowel disease and diarrhea, and potentially reducing the risk of colon cancer (Shu et al., 2001; Flint et al., 2012; Erejuwa et al., 2014). The probiotic effect against pathogenic species is attributed to factors such as production of acid and various other metabolites, competitive nutrient intake and adhesion on the gut epithelial lining, modulation of immune functions, and release of antibacterial agents (Collins and Gibson, 1999; Rolfe, 2000; Shamala et al., 2000; Shu et al., 2000; Gill et al., 2001).

Important prerequisites for probiotic bacteria, which mostly belong to *Lactobacillus* and *Bifidobacterium* genera, include their ability to withstand adverse heat and mechanical processing conditions and maintain viability in the product during storage (Fuller, 1989; Saarela et al., 2000; Guarner and Malagelada, 2003). The probiotic bacterial species are distinct from the common starter cultures in their ability to survive low pH in the stomach and high bile salt concentrations in the small intestine, and subsequently reach the large intestine to confer the desired health benefits. The novel microbiology technique flow cytometry (FCM) was applied by Chen et al. (2011, 2012) for *in vitro* validation of stress tolerance (thermal, acid, and bile salts) of probiotic strains. Probiotic mechanisms of specialized health benefits in the gut are strain specific (Hill et al., 2014), and particular strains of *Escherichia coli* (Marchesi et al., 2016) and *Bacillus cereus* (Hong et al., 2005), for example, are recognized as probiotics whereas several other strains are pathogenic (Shu and Gill, 2000, 2002; Marchesi et al., 2016). The safety and efficacy of the bacterial strain are mandated to be assessed and endorsed in peer-reviewed controlled studies for inclusion in the probiotic framework (Fuller, 1989; Shu et al., 1999; Saarela et al., 2000; Hill et al., 2014; Kumar et al., 2015).

Probiotics are typically administered through yogurts and other traditional fermented food products, dried foods and tablets, and also immobilized in liquid suspensions or capsules as dietary supplements (Fuller, 1989; Gibson and Fuller, 2000). The key challenges to incorporate probiotics in food include ensuring the viability and stability of the bacterial counts at efficacious level until the end of its shelf-life (Mattila-Sandholm et al., 2002; Champagne et al., 2005). It is also important to ensure that the bacteria do not grow rapidly during storage since it may adversely affect the flavour, texture, or other sensory attributes. One of the promising methods for substantially improving the viability of bacterial cells in unfavourable processing and gastric conditions is microencapsulating the microbes in a polymer matrix that is not antimicrobial and affords an effective protection in highly acidic environments (Anal and Singh, 2007; Burgain et al., 2011; Zhao et al., 2011; Chen et al., 2012; Cook et al., 2012; Favarin et al., 2015; Atia et al., 2016). Other effective approaches involve administering potentially synergistic functional food elements with the probiotic bacteria in shelf-stable products (Bomba et al., 2002). In New Zealand, there are initiatives in this area as conducted by Bioactives Research New Zealand through their DrApac® range of products that include unique natural and value-added bioactive ingredients, such as colostrum (*Drpac Colostrum Milk Powder/Hi Cal. plus DHA*) and beneficial fermentation metabolites (*Drpac Probiotic PROF/Milk Protein/Ganoderma/Barley Grass/DrkiwiAMF 20+ Manuka Honey*), which have been innovatively combined to enhance the effectiveness of the probiotic component at the site of action in the GI tract (bioactives.co.nz, 2016; drpac.co.nz, 2016). In addition to the patented (Shu and Liu, 2008) live probiotic strains (*Lactobacillus reuteri* DPC16 in *Probiotics 6* and *Probiotic Colostrum*) delivered in the safe and efficacious quantities to confer the scientifically claimed health benefits, additional natural origin health ingredients that can also be synergistically incorporated with the probiotics or their fermentation metabolites include omega-3 fatty acids and squalene (Das, 2002; Eratte et al., 2015), berries (Kailasapathy et al., 2008; Jaksevic et al., 2011), essential oils (Bomba et al., 2002), minerals and vitamins (Winkler et al., 2005; Shah et al., 2010), antioxidants (Shah et al., 2010; López-Nicolás et al., 2014), herb products (Haitang et al., 2011), spirulina (Beheshtipour et al., 2013), propolis (Haddadin et al., 2008; Daneshmand et al., 2015), royal jelly (Metry and Owayss, 2009; Haddadin et al., 2012), and other honey products. Although the

nature and survivability of the probiotic strains are important factors for establishing a positive microbial balance in the gut, the availability of substrate is fundamental to the quality of metabolic output from the microbial population (Gibson and Roberfroid, 1995; Flint *et al.*, 2012).

### Role of Prebiotics in Altering the Gut Microflora and Fermentation Metabolites

Prebiotics are dietary ingredients that provide a fermentable carbohydrate substrate to selective probiotic genera, thus benefiting the host health by modulating the gut microbial balance (Gibson and Roberfroid, 1995; Gibson and Fuller, 2000; Cummings *et al.*, 2001; Kajiwara *et al.*, 2002; Gibson *et al.*, 2010). The prebiotic carbohydrates are essentially non-digestible by pancreatic amylases and brush-border enzymes such as dextrinases and glucoamylases. Prebiotics play an important role in favourably modifying the colonic microflora which may have lost the predominance of lactobacilli and bifidobacteria due to various factors such as food habits, drugs, chronic stress, and normal ageing. A good indicator of the effectiveness of prebiotics is their ability to restrain the growth and activity of the pathogens and other undesirable microflora. Numerous fermentable substrates from the diet are not digested and reach the opening of the large intestine (Collins and Gibson, 1999; Gibson *et al.*, 2010). These include carbohydrates such as dietary fibers, non-starch polysaccharides, resistant starch, oligosaccharides, polyols and other non-absorbable sugars, and to a lesser extent, proteins and amino acids. However, only those non-digestible substrates that are selectively fermented by the 'beneficial' bacteria are referred to as 'prebiotics', according to the definition coined by Gibson and Roberfroid (1995), and reiterated by the working group of ISAPP scientists (Gibson *et al.*, 2010). Lactobacilli and bifidobacteria, which ferment the non-digestible oligosaccharides, are regarded as beneficial bacteria since their metabolism is entirely saccharolytic without any proteolytic activity (Gibson and Roberfroid, 1995; Gibson *et al.*, 2010). In addition to the increase in the cell mass of bacteria and release of energy, end products of the anaerobic saccharolytic fermentation include gases and short-chain fatty acids (SCFA), which are anti-inflammatory, inhibit pathogens, control the appetite, and lower the risk of cancer and cardiovascular diseases (Roberfroid, 1993; Gibson and Fuller, 2000; Cummings *et al.*, 2001; Wong *et al.*, 2006; Roberfroid *et al.*, 2010; Flint *et al.*, 2012). In contrast, proteolytic fermentation of amino acids and proteins by the genus *Clostridia*, for example, releases toxic and potentially carcinogenic metabolites such as ammonia, amines, hydrogen sulfide, and phenolic and indolic compounds (Smith and Macfarlane, 1996; Kolida *et al.*, 2002; Gibson *et al.*, 2010; Windey *et al.*, 2012). Therefore, it is desirable to sustain prebiotic oligosaccharides as the dominant substrate in the colon for minimizing bacterial proteolytic activity.

Prebiotics also confer additional health benefits by improving the stool quality, stimulating the immune system, alleviating lactose intolerance, and reducing the risk of allergies and intestinal infections (Salminen *et al.*, 1998; Cummings *et al.*, 2001; Roberfroid *et al.*, 2010). The prebiotic effect of non-digestible oligosaccharides, particularly the FOS which withstand enzymatic degradation due to their inaccessible chemical structure, are well established but the mechanisms have not been fully understood. The glycosidic bonds in the oligosaccharides that resist human digestive enzymes are cleaved by hitherto unknown microbial enzymes in the large intestine (Gibson and Roberfroid, 1995; Gibson and Fuller, 2000; Kajiwara *et al.*, 2002; Kolida *et al.*, 2002; Rastall and Maitin, 2002; Dewulf

*et al.*, 2013; Kellow *et al.*, 2014). It is hypothesized that colonic bacteria have peculiar procedures to transport oligosaccharides with a specific degree of polymerization (DP) into the cell for enzymatic hydrolysis. Studies have shown that oligosaccharides with low DP were fermented by bifidobacteria, whereas those with high DP were depolymerized by bacteroides (Van Laere, 1997; Cummings *et al.*, 2001). This partly explains the predominance of low DP FOS such as inulin and oligofructose in the prebiotic landscape, and also elicits research interest in comparable oligosaccharide sources.

### Modulation of the Gut Microbiota by Honey

Honey, which possibly is the earliest sweetener known to mankind, was also being used for wound healing before the advent of modern antibiotics, and as a traditional medicine in many ancient cultures. Even in the present age, honey is regarded not only as a natural sweetener but also as a health food with medicinal properties (Shamala *et al.*, 2000; Wallace *et al.*, 2010), and has evoked a renewed interest with the reported upsurge in antibiotic resistance globally (Kwakman and Zaat, 2012). Worldwide, honey is categorized primarily as nectar or honeydew honey (Krauze and Zalewski, 1991; Anklam, 1998; Bogdanov and Martin, 2002; Kaškonienė and Venskutonis, 2010). Bees produce nectar honey from the floral nectar of various plants, and process honeydew honey from plant and insect secretions. The botanical source and geographical origin of honey are important trade factors, and the unifloral varieties command a high commercial value. The floral origin of honey being entirely unifloral is rare. However, the predominant floral source correlates with phytochemical markers such as the presence and distribution of volatile aroma compounds, phenolic and amino acids, oligosaccharides, and trace elements. There are numerous unifloral varieties of honey available in different regions of the world with distinct physiochemical characteristics of colour, aroma, and texture. Some of the popular unifloral honeys include Acacia, Chestnut, Clover, Manuka, Rape, and Wild thyme. Each produce of the polyfloral types, however, has different composition and organoleptic properties.

Honey is essentially a supersaturated solution of sugars, primarily fructose and glucose, and has numerous other minor components (Viuda-Martos *et al.*, 2008). Disaccharides, such as sucrose and maltose, and several higher oligosaccharides, containing 3–10 monosaccharide units, constitute between 5 and 10% of honey, depending on the variety (Siddiqui and Furgala, 1967, 1968; Astwood *et al.*, 1998; Weston and Brocklebank, 1999; Sanz *et al.*, 2004; Bogdanov *et al.*, 2008; Viuda-Martos *et al.*, 2008). The reported average sugar content of nectar and honeydew honey is depicted in Table 2. The nutritional composition of honey, which greatly influences its significant physiological effects, is also dependent on various considerations such as pollen sources, processing, storage, and environmental conditions. Furthermore, the oligosaccharides are less sweet than the mono- and disaccharides, but being mostly non-digestible, are desirable for their potentially prebiotic physiological functions ascribed to the production of metabolites and growth enhancement of probiotics.

Honey also possesses natural antibacterial activity due to factors such as high sugar content, acidity, and hydrogen peroxide which is formed by glucose oxidation during the ripening of honey. The activity attributable to hydrogen peroxide is somewhat sensitive to both heat and light, which denature the endogenous glucose oxidase (White *et al.*, 1963; Weston *et al.*, 1998; Bogdanov *et al.*, 2008; Wallace *et al.*, 2010; Kwakman and Zaat, 2012). The non-peroxide

**Table 2.** The average sugar composition of honey (%).

	Nectar honey	Honeydew honey
Monosaccharides		
Fructose	38.2	31.8
Glucose	31.3	26.1
Disaccharides		
Sucrose	0.7–1.31	0.5–0.8
Maltose and others	5–7.31	4–8.8
Trisaccharides		
Melezitose	<0.1	4.0
Erllose and others	1–1.5	4–4.7
Undetermined		
Oligosaccharides	3.1	10.1
Total sugars	79.7	80.5

Source: Doner (1977) and Bogdanov et al. (2008).

antimicrobial activity, which varies significantly with the floral source of the nectar, however, remains unchanged during prolonged storage periods (Molan and Russell, 1988). The non-peroxide antibacterial effect of the unifloral New Zealand Manuka (*Leptospermum scoparium*) honey against the pathogen *Helicobacter pylori*, which is of great commercial significance since it commands a premium over the other varieties, has been attributed to high levels of methylglyoxal (Allen et al., 1991; Al Somal et al., 1994; Snow and Manley-Harris, 2004; Mavric et al., 2008; Daglia et al., 2013), leptosin which is a novel glycoside of methyl syringate (Kato et al., 2012; Mannina et al., 2016), and other bioactive components and mechanisms that are not yet fully identified (Rosendale, 2009; Wallace et al., 2010; Carter et al., 2016). The bactericidal activity of honey is represented by Unique Manuka Factor (UMF), which is equivalent to the concentration (% w/v) of phenol solution that yields a comparable zone of growth inhibition in *Staphylococcus aureus* radical diffusion assay (Allen et al., 1991; Kwakman and Zaat, 2012). UMF is considered to be an industry standard in the Oceania (Australia and New Zealand) for grading the characteristic non-peroxide antibacterial activity of Manuka honey.

Appropriate synbiotic combinations, however, can be more effective in benefiting the host than individually administering probiotic or prebiotic. In synbiotic food systems, the probiotic strain is co-administered with specific prebiotic carbohydrates so that a substrate is adequately available for its proliferation (Gmeiner et al., 2000; Rastall and Maitin, 2002; Nagpal and Kaur, 2011; Adebola et al., 2014). Honey contains potentially prebiotic oligosaccharides and antibacterial components, both of which can synergistically enhance the probiotic efficacy against pathogens. In addition to increasing the viable cell count, other reported benefits include enhanced probiotic persistence in the GI tract, elevated levels of SCFA, and increased resistance to pathogens (Gmeiner et al., 2000; Asahara et al., 2001; Rastall and Maitin, 2002). Tian et al. (2010b) provided a good illustration of synergy between probiotics and bovine lactoferrin in enhancing the antibacterial activity against select pathogens. A beneficial synergistic effect of Manuka honey (UMF 20+) in improving the growth of probiotics (*Lactobacillus reuteri*, *Lactobacillus rhamnosus*, and *Bifidobacterium lactis*) and inhibiting the pathogens (*Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus*) was demonstrated by Rosendale et al. (2008). In this context, it is interesting to note that strains of *Lactobacillus reuteri*, which produce the antibacterial reuterin in hosts, have revealed a superior probiotic capability in several studies over the recent decades (Talarico et al., 1988; Casas and Dobrogosz,

2000; Reuter, 2001; Lee et al., 2008; Montiel et al., 2014), including against *Helicobacter pylori* pathogenicity (Valeur et al., 2004; Dore et al., 2014, 2015; Khoder et al., 2016). The broad-spectrum bactericidal nature of reuterin (Axelsson et al., 1989; Casas and Dobrogosz, 2000) was also validated in doctoral research by Lu (2007) through agar diffusion assay of diverse foodborne pathogens. *Lactobacillus reuteri* DPC16, which was isolated and patented in New Zealand by Shu and Liu (2008), has shown promising antimicrobial activity against select Gram-negative and Gram-positive pathogens by producing organic acids, SCFA, and reuterin (Bian et al., 2011; Chen et al., 2012), survivability during passage through simulated GI tract (Chen et al., 2011; Zhao et al., 2012), and also other critical probiotic functionality, such as continued *in vitro* growth and production of beneficial metabolites, adhering to but simultaneously inhibiting the adhesion of pathogens to epithelial cells (Bian et al., 2011; Zhao et al., 2012). A recent doctoral dissertation by Tian (2013) postulated the protective effect of DPC16 cells against toxicological damage to DNA in the intestinal cells, and an *in vitro* antigenotoxicity in combination with bovine lactoferrin was demonstrated in immune and colon epithelial cell models (Tian et al., 2010a).

Besides the aforementioned functionalities, including that against the pathogens, the growth and stability challenges of probiotic species can be addressed to a large extent by prebiotic carbohydrate supplementations (Gibson and Roberfroid, 1995; Gibson et al., 2010), and the research incorporating different varieties of honey is compiled in Table 3.

In most of the studies reported in Table 3, honey has shown to support the growth of the probiotics when incubated in optimum conditions with milk (including reconstituted or fermented) or selective growth media. Furthermore, inhibitory action was demonstrated against the pathogens and other intestinal microbes (Shin and Ustunol, 2005; Lucan et al., 2009; Saran et al., 2011). This does provide some evidence for the selectivity of honey as a prebiotic substrate for the lactic acid bacteria belonging to *Lactobacillus* and *Bifidobacterium* genera over other undesirable microorganisms. However, as noted by Ustunol and Gandhi (2001), it is highly likely that some of the lactic acid production can be ascribed to the utilization of fructose and glucose, instead of the oligosaccharide component. A prebiotic effect has been attributed to honey in many of these studies, but the evaluation criteria outlined by Gibson et al. (2004) also includes resistance to enzymatic digestion and fermentation profile studies with batch or continuous culture systems. Sanz et al. (2005) did study the batch fermentation of the isolated honey oligosaccharides by faecal bacteria, but hardly any of the studies compiled in Table 3 have evaluated the resistance to acidic and enzymatic hydrolysis in simulated GI conditions. Moreover, demonstrating that the oligosaccharide substrate is metabolized selectively by the probiotic(s) can be more challenging because of the likely interactions with other dominant gut bacteria (Gibson et al., 2004) as mentioned in Table 1. This list of bacterial species is also not exhaustive since it is estimated that only half of the colonic microflora has been identified to date (García-Elorriaga and del Rey-Pineda, 2013). Many of the experiments reported in this review have utilized a single strain or only a few pure cultures in selective media(s), which can be valuable for preliminary studies in establishing that the experimental prebiotic being evaluated has fermentation selectivity for bifidobacteria and lactobacilli over other undesirable bacteria. Faecal samples utilized by Sanz et al. (2005) and Narayanan and Subramonian (2015), however, are more representative of human colonic microflora. Moreover, as reviewed by Flint et al. (2012), even for the established FOS prebiotics, only very limited number

**Table 3.** Studies reporting the prebiotic potential of honey for the probiotic lactobacilli and bifidobacteria.

Probiotic	Honey	Methods	Key findings	References
<i>Lactococcus lactis</i>	Locally sourced (India)	<i>In vitro</i> incubation and storage studies	Growth and survivability during refrigerated storage of dahi* prepared with honey increased significantly	Manhar <i>et al.</i> (2016)
<i>L. helveticus</i> <i>Streptococcus thermophilus</i>	Locally sourced (India)	<i>In vitro</i> incubation and storage studies	Viable counts of the probiotic were optimally maintained for 3 weeks in lassi* with the addition of honey (5% w/v)	Sharma <i>et al.</i> (2016)
Faecal <i>Bifidobacterium</i> spp.	Locally sourced (India)	<i>In vitro</i> incubation	Total viable count of the isolated strains significantly increased with addition of honey (3%) to the whey medium	Narayanan and Subramonian (2015)
<i>L. acidophilus</i> , <i>B. animalis</i> ssp., <i>Lactis</i> and <i>Streptococcus thermophilus</i>	Black Locust (Hungary)	<i>In vitro</i> incubation and storage studies	Honey (5% w/v) was not inhibitory to the starter culture in cow and camel milk. Viability in refrigerated storage for upto 5 weeks was reported higher for bifidobacteria than lactobacilli	Varga <i>et al.</i> (2014)
Yogurt starters** plus <i>B. bifidum</i> or <i>L. rhamnosus</i> or <i>L. reuteri</i>	Locally sourced (Saudi Arabia)	<i>In vitro</i> incubation and storage studies	One of the two honey types (3% w/v) added was more effective than even inulin in improving the growth and viability of the LAB in cow milk.	Rayes (2012)
Five <i>Lactobacillus</i> spp.	Locally sourced (India)	<i>In vitro</i> incubation and storage studies	Growth and viability during refrigerated storage in RSM with honey (5% w/v) was enhanced but not as prominently as FOS, and the activity was strain specific.	Nagpal and Kaur (2011)
<i>L. acidophilus</i>	Locally sourced (India)	<i>In vitro</i> incubation and aggregation assays	Auto-aggregation and cell surface hydrophobicity was improved in presence of inulin, and honey was more effective for co-aggregation with <i>Escherichia coli</i> .	Saran <i>et al.</i> (2011)
Yogurt starters,** <i>L. acidophilus</i> and <i>B. bifidum</i>	Three unifloral (USA)	<i>In vitro</i> incubation and growth studies	All the honeys varieties (5% w/v) supported the growth and activity of the four bacterial species in RSM, and the effect was comparable to sucrose, HFCS and inulin.	Popa and Ustunol (2011)
<i>L. casei</i> Lc-01	Chestnut and acacia (Croatia)	<i>In vitro</i> fermentation	Growth and activity was stimulated by both the honeys, faster in goat milk than in cow milk.	Slacanac <i>et al.</i> (2011)
<i>B. longum</i> spp BB536	Tulang and Tapah (Malaysia)	<i>In vitro</i> incubation after removing sugars	All the honey types (wild and commercial, 5%) supported the growth and acid production in RSM.	Jan Mei <i>et al.</i> (2010)
<i>B. lactis</i> Bb-12	Chestnut and acacia (Croatia)	<i>In vitro</i> incubation, and agar diffusion assay for pathogen inhibition	Growth and LA production was enhanced in both cow and goat milk, and inhibitory potential against <i>Listeria monocytogenes</i> was demonstrated.	Lucan <i>et al.</i> (2009)
Yogurt starters**	Polyfloral (Algeria), Unifloral (France)	<i>In vitro</i> incubation and storage studies	Both the honeys were not inhibitory to the starter cultures at the optimum concentration (5% w/v) during 28 days refrigerated storage of yogurt.	Riazi and Ziar (2008)
<i>Lactobacillus</i> and <i>Bifidobacterium</i> spp.	Apis mellifera (Brazil)	<i>In vitro</i> incubation and storage studies	Growth and viability, in refrigerated storage for upto 46 days in fermented RSM with honey (3% w/v) addition, was enhanced more for bifidobacteria than lactobacilli.	Macedo <i>et al.</i> (2008)
<i>B. infantis</i> and <i>L. acidophilus</i>	Three regions (Jordan)	<i>In vitro</i> incubation	Growth and the production of SCFA and LA increased significantly in both RSM and skim milk.	Haddadin <i>et al.</i> (2007)
Faecal bifidobacteria and lactobacilli	Artisanal honeydew (Spain)	Batch fermentation after removing sugars	Prebiotic activity of the extracted honey oligosaccharides was promising but not equivalent to that of the FOS.	Sanz <i>et al.</i> (2005)
Mixed culture of 5 bifidobacteria and 5 other gut bacteria	Three unifloral (USA)	<i>In vitro</i> growth and co-culturing studies	All the honeys (5% w/v) with distinct oligosaccharide contents enhanced the <i>Bifidobacterium</i> growth in the media, and selectively inhibited <i>Clostridium</i> and <i>Eubacterium</i> sp.	Shin and Ustunol (2005)
Five bifidobacteria spp.	Clover (USA)	<i>In vitro</i> incubation	Honey (5% w/v) also supported the growth and LA production by the bacteria in RCM, comparable to FOS, GOS and inulin.	Kajiwara <i>et al.</i> (2002)
Two bifido-bacteria spp.	Clover (USA)	<i>In vitro</i> incubation and storage studies	Growth, LA production and refrigerated storage survivability upto 14 days was higher with the addition of honey (5% w/v) in RSM than the mono- and disaccharides.	Ustunol and Gandhi (2001)
Yogurt starters,** <i>L. acidophilus</i> <i>B. bifidum</i>	Clover (USA)	<i>In vitro</i> incubation	Honey (5% w/v) was not inhibitory to the growth of all the four microbes in RSM, and the <i>Bifidobacterium</i> spp. significantly enhanced LA production.	Chick <i>et al.</i> (2001)
<i>L. acidophilus</i> <i>L. plantarum</i>	Locally sourced (India)	<i>In vitro</i> incubation, and <i>in vivo</i> studies on rats	<i>In vitro</i> growth of LAB in the agar media was enhanced more by honey addition than sucrose (1% sugar), and this was corroborated in <i>in vivo</i> trials.	Shamala <i>et al.</i> (2000)

LA(B), lactic acid (bacteria); FOS, fructo-oligosaccharides; RSM, reconstituted skim milk; HFCS, high fructose corn syrup; SCFA, short chain fatty acid; GOS, galacto-oligosaccharide; RCM, reinforced clostridial medium.

\*Traditional Indian fermented milk products.

\*\*Yogurt starters (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*).

of studies have attempted to examine the holistic changes in the gut microbiota on dietary supplementation of the carbohydrate source. A clearer picture of the changes in the bacterial population during fermentation of colonic microflora can be obtained with the application of advanced molecular techniques such as fluorescence *in situ* hybridization, PCR, direct community analysis, denaturing and temperature-gradient gel electrophoresis, and FCM (Gibson et al., 2004). Shelf-life studies conducted by Sharma et al. (2016) on physicochemical characteristics and total probiotic viable counts needs to be reaffirmed in further research for different varieties and dosages of honey.

A very recent study by Favarin et al. (2015) reported that addition of honey as an encapsulant improved the survivability of two probiotic *Bifidobacterium* strains in simulated GI conditions, and the protective effect was comparable to sodium alginate microencapsulation. The confirmation of a potential prebiotic effect, however, needs to be obtained by *in vivo* animal studies and human clinical trials once supporting evidence is established by rigorous *in vitro* trials. Shamala et al. (2000) were able to report significant increase in the counts of lactic acid bacteria in the intestines of rats fed with honey, possibly indicating its role in altering the gut microbiota. Furthermore, according to the criterion outlined by Gibson et al. (2004) for establishing the prebiotic effect, recovery of the non-digestible oligosaccharides in faeces also needs to be demonstrated. Promising synbiotic combinations, such as those including strains of *Lactobacillus reuteri* and Manuka honey that are effective against *Helicobacter pylori* infections causing stomach ulcers, can also be explored. In this perspective, the structural similarity between the two antibacterial components, reuterin (3-hydroxypropionaldehyde) and methylglyoxal, is noteworthy.

Human milk oligosaccharides are bifidogenic (Roberfroid et al., 2010), and the above studies support the potential of honey to exhibit a similar prebiotic effect by altering the composition of the gut microflora. The holistic physiological benefits, however, will be distinct for different types of honey, and needs to be established in *in vivo* trials for the functional health claims. It is interesting to note that the potential prebiotic effect has been reported more often for bifidobacteria than the lactobacilli probiotics, and this trend was also stated earlier by Kolida et al. (2002) for FOS. This can be attributed to a greater fermentation selectivity of prebiotic oligosaccharides for the *Bifidobacterium* than the *Lactobacillus* genera. Bindels et al. (2015), however, are advocating a more comprehensive definition which emphasizes more on the metabolic health benefits of prebiotics in humans (Kellow et al., 2014), rather than the fermentation specificity towards the recognized probiotic species.

## Conclusions and Future Prospects

Our understanding on the role of intestinal microflora in the maintaining host health and nutrition has vastly improved in the recent times, driven largely by the advancements in novel analytical techniques and global research initiatives on the gut microbiome. Dietary application of probiotic strains and non-digestible oligosaccharides aim to achieve a positive microbial balance towards a more favourable bacterial community. Furthermore, effective synbiotic combinations can potentially enhance the discrete health benefits of prebiotic carbohydrate and probiotic microorganisms, and also present development opportunities for innovative functional foods. The unique oligosaccharide components and antibacterial mechanisms of honey are of a great research interest for the physiological effects. A more

rigorous evaluation of the potential prebiotic effect of honey on probiotic lactobacilli and bifidobacteria, and the action mechanisms involved, however, may be necessary to incorporate the functional ingredient with scientifically substantiated health claims. *In vitro* models of the human gut can be employed to test digestibility and fermentation selectivity of honey oligosaccharides, followed by *in vivo* animal studies and randomized control trials in human subjects. Although the selectivity of honey as a substrate for the probiotic bacteria is an important aspect of the prebiotic effect, the holistic metabolic benefits of gut microbiota modulation must also be adequately considered.

Conflict of interest statement. None declared.

## References

- Adebola, O. O., Corcoran, O., Morgan, W. A. (2014). Synbiotics: the impact of potential prebiotics inulin, lactulose and lactobionic acid on the survival and growth of lactobacilli probiotics. *Journal of Functional Foods*, 10: 75–84.
- Al Somal, N., Coley, K. E., Molan, P. C., Hancock, B. M. (1994). Susceptibility of *Helicobacter pylori* to the antibacterial activity of manuka honey. *Journal of the Royal Society of Medicine*, 87: 9–12.
- Allen, K. L., Molan, P. C., Reid, G. M. (1991). A survey of the antibacterial activity of some New Zealand honeys. *Journal of Pharmacy and Pharmacology*, 43: 817–822.
- Anal, A. K., Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science and Technology*, 18: 240–251.
- Anklam, E. (1998). A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry*, 63: 549–562.
- Asahara, T., Nomoto, K., Shimizu, K., Watanuki, M., Tanaka, R. (2001). Increased resistance of mice to salmonella enterica serovar typhimurium infection by synbiotic administration of bifidobacteria and transgalactosylated oligosaccharides. *Journal of Applied Microbiology*, 91: 985–996.
- Astwood, K., Lee, B., Manley-Harris, M. (1998). Oligosaccharides in New Zealand honeydew honey. *Journal of Agricultural and Food Chemistry*, 46: 4958–4962.
- Atia, A., Gomaa, A., Fliss, I., Beyssac, E., Garrait, G., Subirade, M. (2016). A prebiotic matrix for encapsulation of probiotics: physicochemical and microbiological study. *Journal of Microencapsulation*, 33: 89–101.
- Axelsson, L. T., Chung, T. C., Dobrogosz, W. J., Lindgren, S. E. (1989). Production of a broad spectrum antimicrobial substance by *Lactobacillus reuteri*. *Microbial Ecology in Health and Disease*, 2: 131–136.
- Beheshtipour, H., Mortazavian, A. M., Mohammadi, R., Sohrabvandi, S., Khosravi-Darani, K. (2013). Supplementation of *Spirulina platensis* and *Chlorella vulgaris* algae into probiotic fermented milks. *Comprehensive Reviews in Food Science and Food Safety*, 12: 144–154.
- Bian, L., Molan, A., Maddox, I., Shu, Q. (2011). Antimicrobial activity of *Lactobacillus reuteri* DPC16 supernatants against selected food borne pathogens. *World Journal of Microbiology and Biotechnology*, 27: 991–998.
- Bindels, L. B., Delzenne, N. M., Cani, P. D., Walter, J. (2015). Opinion: towards a more comprehensive concept for prebiotics. *Nature Reviews Gastroenterology and Hepatology*, 12: 303–310.
- bioactives.co.nz. (2016). Bioactives Research New Zealand. Available at: <http://www.bioactives.co.nz/> (accessed 15 September 2016).
- Bogdanov, S., Martin, P. (2002). Honey authenticity. *Mitteilungen Aus Lebensmitteluntersuchung Und Hygiene*, 93: 232–254.
- Bogdanov, S., Jurendic, T., Sieber, R., Gallmann, P. (2008). Honey for nutrition and health: a review. *Journal of the American College of Nutrition*, 27: 677–689.
- Bomba, A., Nemcová, R., Mudronová, D., Guba, P. (2002). The possibilities of potentiating the efficacy of probiotics. *Trends in Food Science and Technology*, 13: 121–126.
- Burgain, J., Gaiani, C., Linder, M., Scher, J. (2011). Encapsulation of probiotic living cells: from laboratory scale to industrial applications. *Journal of Food Engineering*, 104: 467–483.

- Carter, D. A., et al. (2016). Therapeutic manuka honey: no longer so alternative. *Frontiers in Microbiology*, 7: 1–11. doi:10.3389/fmicb.2016.00569.
- Casas, I. A., Dobrogosz, W. J. (2000). Validation of the probiotic concept: *Lactobacillus reuteri* confers broad-spectrum protection against disease in humans and animals. *Microbial Ecology in Health and Disease*, 12: 247–285.
- Champagne, C. P., Gardner, N. J., Roy, D. (2005). Challenges in the addition of probiotic cultures to foods. *Critical Reviews in Food Science and Nutrition*, 45: 61–84.
- Chen, S., Cao, Y., Ferguson, L. R., Shu, Q., Garg, S. (2012). The effect of immobilization of probiotic *Lactobacillus reuteri* DPC16 in sub-100 µm microcapsule on food-borne pathogens. *World Journal of Microbiology and Biotechnology*, 28: 2447–2452.
- Chen, S., Cao, Y., Ferguson, L. R., Shu, Q., Garg, S. (2012). Flow cytometric assessment of the protectants for enhanced in vitro survival of probiotic lactic acid bacteria through simulated human gastro-intestinal stresses. *Applied Microbiology and Biotechnology*, 95: 345–356.
- Chen, S., Ferguson, L. R., Shu, Q., Garg, S. (2011). The application of flow cytometry to the characterisation of a probiotic strain *Lactobacillus reuteri* DPC16 and the evaluation of sugar preservatives for its lyophilization. *LWT—Food Science and Technology*, 44: 1873–1879.
- Chen, S., Zhao, Q., Ferguson, L. R., Shu, Q., Weir, I., Garg, S. (2012). Development of a novel probiotic delivery system based on microencapsulation with protectants. *Applied Microbiology and Biotechnology*, 93: 1447–1457.
- Chick, H., Shin, H. S., Ustunol, Z. (2001). Growth and acid production by lactic acid bacteria and bifidobacteria grown in skim milk containing honey. *Journal of Food Science*, 66: 478–481.
- Collins, M. D., Gibson, G. R. (1999). Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *American Journal of Clinical Nutrition*, 69: 1052S–1057S.
- Cook, M. T., Tzortzis, G., Charalampopoulos, D., Khutoryanskiy, V. V. (2012). Microencapsulation of probiotics for gastrointestinal delivery. *Journal of Controlled Release*, 162: 56–67.
- Cummings, J. H., Macfarlane, G. T., Englyst, H. N. (2001). Prebiotic digestion and fermentation. *American Journal of Clinical Nutrition*, 73(2 Supp): 415S–420S.
- Daglia, M., Ferrari, D., Collina, S., Curti, V. (2013). Influence of in vitro simulated gastroduodenal digestion on methylglyoxal concentration of manuka (*Lectospermum scoparium*) honey. *Journal of Agricultural and Food Chemistry*, 61: 2140–2145.
- Daneshmand, A., Sadeghi, G. H., Karimi, A., Vaziry, A., Ibrahim, S. A. (2015). Evaluating complementary effects of ethanol extract of propolis with the probiotic on growth performance, immune response and serum metabolites in male broiler chickens. *Livestock Science*, 178, 195–201.
- Das, U. N. (2002). Essential fatty acids as possible enhancers of the beneficial actions of probiotics. *Nutrition*, 18: 786–789.
- Dewulf, E. M., et al. (2013). Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut*, 62: 1112–1121.
- Doner, L. W. (1977). The sugars of honey—a review. *Journal of the Science of Food and Agriculture*, 28: 443–456.
- Dore, M. P., Cuccu, M., Pes, G. M., Manca, A., Graham, D. Y. (2014). *Lactobacillus reuteri* in the treatment of *Helicobacter pylori* infection. *Internal and Emergency Medicine*, 9: 649–654.
- Dore, M. P., Goni, E., Di Mario, F. (2015). Is there a role for probiotics in *Helicobacter pylori* therapy? *Gastroenterology Clinics of North America*, 44: 565–575.
- drapac.co.nz. (2016). Drapac. Available at: <http://www.drapac.co.nz/> (accessed 15 September 2016).
- Eratte, D., McKnight, S., Gengenbach, T. R., Dowling, K., Barrow, C. J., Adhikari, B. P. (2015). Co-encapsulation and characterisation of omega-3 fatty acids and probiotic bacteria in whey protein isolate-gum Arabic complex coacervates. *Journal of Functional Foods*, 19, 882–892.
- Erejuwa, O. O., Sulaiman, S. A., Ab Wahab, M. S. (2014). Modulation of gut microbiota in the management of metabolic disorders: the prospects and challenges. *International Journal of Molecular Sciences*, 15: 4158–4188.
- Favarin, L., Laureano-Melo, R., Luchese, R. H. (2015). Survival of free and microencapsulated *Bifidobacterium*: effect of honey addition. *Journal of Microencapsulation*, 32: 329–335.
- Flint, H. J., Scott, K. P., Louis, P., Duncan, S. H. (2012). The role of the gut microbiota in nutrition and health. *Nature Reviews Gastroenterology and Hepatology*, 9: 577–589.
- Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology*, 66: 365–378.
- García-Elorriaga, G., del Rey-Pineda, G. (2013). Nutrition and intestinal microflora. *Journal of Nutritional Therapeutics*, 2: 112–121.
- Gibson, G. R., et al. (2010). Dietary prebiotics: current status and new definition. *The Food Science and Technology Bulletin: Functional Foods*, 7, 1–19.
- Gibson, G. R., Fuller, R. (2000). Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and prebiotics for human use. *Journal of Nutrition*, 130(2 Suppl.), 391S–395S.
- Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews*, 17: 259–275.
- Gibson, G. R., Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition*, 125: 1401–1412.
- Gill, H. S., Shu, Q., Lin, H., Rutherford, K. J., Cross, M. L. (2001). Protection against translocating *Salmonella typhimurium* infection in mice by feeding the immuno-enhancing probiotic *Lactobacillus rhamnosus* strain HN001. *Medical Microbiology and Immunology*, 190: 97–104.
- Gmeiner, M., et al. (2000). Influence of a synbiotic mixture consisting of *Lactobacillus acidophilus* 74-2 and a fructooligosaccharide preparation on the microbial ecology sustained in a simulation of the human intestinal microbial ecosystem (SHIME reactor). *Applied Microbiology and Biotechnology*, 53: 219–223.
- Guarner, F., Malagelada, J. (2003). Gut flora in health and disease. *Lancet*, 361: 512–519.
- Guarner, F., Schaafsma, G. J. (1998). Probiotics. *International Journal of Food Microbiology*, 39: 237–238.
- Haddadin, M. S. Y., Haddadin, J., Benguiar, R. (2012). The effect of royal jelly on growth and short-chain fatty acid production of probiotic bacteria and activity of bacterial procarcinogenic enzymes in rat faeces. *Polish Journal of Food and Nutrition Sciences*, 62: 251–258.
- Haddadin, M. S. Y., Nazer, I., Raddad, J. A., Robinson, R. K. (2007). Effect of honey on the growth and metabolism of two bacterial species of intestinal origin. *Pakistan Journal of Nutrition*, 6: 693–697.
- Haddadin, M. S. Y., Nazer, I., Raddad, S. J. A., Robinson, R. K. (2008). Effect of propolis on two bacterial species with probiotic potential. *Pakistan Journal of Nutrition*, 7: 391–394.
- Haitang, Z., Yanrong, W., Ziliang, W. (2011). Comparative experiment on the effects of Chinese herb, probiotic and antibiotics on growth performance and immune function in growing pigs. *China Feed*, 15: 010.
- Hill, C., et al. (2014). Expert consensus document: The International Scientific Association for Probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology*, 11: 506–514.
- Hong, H. A., Le, H. D., Cutting, S. M. (2005). The use of bacterial spore formers as probiotics. *FEMS Microbiology Reviews*, 29: 813–835.
- Jaksevic, M., Aaby, K., Borge, G. A., Jeppsson, B., Ahrné, S., Molin, G. (2011). Antioxidative protection of dietary bilberry, chokeberry and *Lactobacillus plantarum* HEAL19 in mice subjected to intestinal oxidative stress by ischemia-reperfusion. *BMC Complementary and Alternative Medicine*, 11: 1.
- Jan Mei, S., Mohd Nordin, M. S., Norrakiah, A. S. (2010). Fructooligosaccharides in honey and effects of honey on growth of *Bifidobacterium longum* BB 536. *International Food Research Journal*, 17: 557–561.
- Kailasapathy, K., Harmstorf, I., Phillips, M. (2008). Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. lactis in stirred fruit yogurts. *LWT—Food Science and Technology*, 41: 1317–1322.
- Kajiwarra, S., Gandhi, H., Ustunol, Z. (2002). Effect of honey on the growth of and acid production by human intestinal *Bifidobacterium* spp.: an in vitro

- comparison with commercial oligosaccharides and inulin. *Journal of Food Protection*, 65: 214–218.
- Kaskoniene, V., Venskutonis, P. R. (2010). Floral markers in honey of various botanical and geographic origins: a review. *Comprehensive Reviews in Food Science and Food Safety*, 9: 620–634.
- Kato, Y., Umeda, N., Maeda, A., Matsumoto, D., Kitamoto, N., Kikuzaki, H. (2012). Identification of a novel glycoside, leptosin, as a chemical marker of manuka honey. *Journal of Agricultural and Food Chemistry*, 60: 3418–3423.
- Kellow, N. J., Coughlan, M. T., Reid, C. M. (2014). Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials. *British Journal of Nutrition*, 111: 1147–1161.
- Khoder, G., Al-Menhali, A. A., Al-Yassir, F., Karam, S. M. (2016). Potential role of probiotics in the management of gastric ulcer. *Experimental and Therapeutic Medicine*, 12: 3–17.
- Kolida, S., Tuohy, K., Gibson, G. R. (2002). Prebiotic effects of inulin and oligofructose. *British Journal of Nutrition*, 87(SUPPL. 2), S193–S197.
- Krauze, A., Zalewski, R. I. (1991). Classification of honeys by principal component analysis on the basis of chemical and physical parameters. *Zeitschrift Für Lebensmittel-Untersuchung Und-Forschung*, 192: 19–23.
- Kumar, H., et al. (2015). Novel probiotics and prebiotics: road to the market. *Current Opinion in Biotechnology*, 32, 99–103.
- Kwakman, P. H. S., Zaat, S. A. J. (2012). Antibacterial components of honey. *IUBMB Life*, 64: 48–55.
- Lee, K., Lee, H., Choi, Y. (2008). Proteomic analysis of the effect of bile salts on the intestinal and probiotic bacterium *Lactobacillus reuteri*. *Journal of Biotechnology*, 137(1–4), 14–19.
- López-Nicolás, R., González-Bermúdez, C. A., Ros-Berruazo, G., Frontela-Saseta, C. (2014). Influence of in vitro gastrointestinal digestion of fruit juices enriched with pine bark extract on intestinal microflora. *Food Chemistry*, 157, 14–19.
- Lu, G. (2007). *A novel approach for controlling foodborne pathogens using modified atmosphere and Lactobacillus reuteri DPC16* (Doctoral dissertation), Massey University, NZ.
- Lucan, M., et al. (2009). Inhibitory effect of honey-sweetened goat and cow milk fermented with bifidobacterium lactis Bb-12 on the growth of listeria monocytogenes. *Mljekarstvo*, 59: 96–106.
- Macedo, L. N., Luchese, R. H., Guerra, A. F., Barbosa, C. G. (2008). Prebiotic effect of honey on growth and viability of *Bifidobacterium* spp. and *Lactobacillus* spp. in milk. [Feito prebiótico do mel sobre o crescimento e viabilidade de *Bifidobacterium* spp. e *Lactobacillus* spp. em leite.] *Ciencia E Tecnologia De Alimentos*, 28: 935–942.
- Manhar, A. K., et al. (2016). Assessment of goat milk-derived potential probiotic *L. lactis* AMD17 and its application for preparation of dahi using honey. *Annals of Microbiology*, 1–12.
- Mannina, L., et al. (2016). Antistaphylococcal activity and metabolite profiling of manuka honey (*Leptospermum scoparium* L.) after in vitro simulated digestion. *Food and Function*, 7: 1664–1670.
- Marchesi, J. R., et al. (2016). The gut microbiota and host health: a new clinical frontier. *Gut*, 65: 330–339.
- Mattila-Sandholm, T., Myllärinen, P., Crittenden, R., Mogensen, G., Fondén, R., Saarela, M. (2002). Technological challenges for future probiotic foods. *International Dairy Journal*, 12(2–3), 173–182.
- Mavric, E., Wittmann, S., Barth, G., Henle, T. (2008). Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. *Molecular Nutrition and Food Research*, 52: 483–489.
- Metchnikoff, E. (1908). *The prolongation of life*. New York, London: G.P. Putnam's Sons.
- Metry, W. A., Owayss, A. (2009). Influence of incorporating honey and royal jelly on the quality of yoghurt during storage. *Egyptian Journal of Food Science*, 37, 115–131.
- Molan, P. C., Russell, K. M. (1988). Non-peroxide antibacterial activity in some New Zealand honeys. *Journal of Apicultural Research*, 27: 62–67.
- Montiel, R., et al. (2014). Antimicrobial activity of reuterin produced by *Lactobacillus reuteri* on *Listeria monocytogenes* in cold-smoked salmon. *Food Microbiology*, 44, 1–5.
- Mussatto, S. I., Mancilha, I. M. (2007). Non-digestible oligosaccharides: a review. *Carbohydrate Polymers*, 68: 587–597.
- Nagpal, R., Kaur, A. (2011). Synbiotic effect of various prebiotics on in vitro activities of probiotic lactobacilli. *Ecology of Food and Nutrition*, 50: 63–68.
- Narayanan, R., Subramonian, B. S. (2015). Effect of prebiotics on bifidobacterial species isolated from infant faeces. *Indian Journal of Traditional Knowledge*, 14: 285–289.
- Popa, D., Ustunol, Z. (2011). Influence of sucrose, high fructose corn syrup and honey from different floral sources on growth and acid production by lactic acid bacteria and bifidobacteria. *International Journal of Dairy Technology*, 64: 247–253.
- Rastall, R. A., Gibson, G. R. (2015). Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. *Current Opinion in Biotechnology*, 32, 42–46.
- Rastall, R. A., Maitin, V. (2002). Prebiotics and synbiotics: towards the next generation. *Current Opinion in Biotechnology*, 13: 490–496.
- Rayes, A. A. H. (2012). Enhancement of probiotic bioactivity by some prebiotics to produce bio-fermented milk. *Life Science Journal*, 9: 2246–2253.
- Reuter, G. (2001). The *Lactobacillus* and *Bifidobacterium* microflora of the human intestine: composition and succession. *Current Issues in Intestinal Microbiology*, 2: 43–53.
- Riazi, A., Ziar, H. (2008). Growth and viability of yogurt starter organisms in honey-sweetened skimmed milk. *African Journal of Biotechnology*, 7: 2055–2063.
- Roberfroid, M. (1993). Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects. *Critical Reviews in Food Science and Nutrition*, 33: 103–148.
- Roberfroid, M., et al. (2010). Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition*, 104(Suppl 2), S1–S63.
- Roberfroid, M. B. (2000). Prebiotics and probiotics: are they functional foods? *American Journal of Clinical Nutrition*, 71(6 Suppl), 1682S–1690S.
- Roccarina, D., Lauritano, E. C., Gabrielli, M., Franceschi, F., Ojetti, V., Gasbarrini, A. (2010). The role of methane in intestinal diseases. *American Journal of Gastroenterology*, 2010, 105: 1250–1256.
- Rolfe, R. D. (2000). The role of probiotic cultures in the control of gastrointestinal health. *Journal of Nutrition*, 130(2 Suppl), 396S–402S.
- Rosendale, D. I. (2009). *Antimicrobial Activity of Functional Food Ingredients Focusing on Manuka Honey Action Against Escherichia Coli* (Doctoral dissertation), Massey University, New Zealand.
- Rosendale, D. I., Maddox, I. S., Miles, M. C., Rodier, M., Skinner, M., Sutherland, J. (2008). High-throughput microbial bioassays to screen potential New Zealand functional food ingredients intended to manage the growth of probiotic and pathogenic gut bacteria. *International Journal of Food Science and Technology*, 43: 2257–2267.
- Savage, D. C. (1977). Microbial ecology of the gastrointestinal tract. *Annual Reviews in Microbiology*, 1977, 31: 107–133.
- Saarela, M., Mogensen, G., Fondén, R., Mättö, J., Mattila-Sandholm, T. (2000). Probiotic bacteria: safety, functional and technological properties. *Journal of Biotechnology*, 84: 197–215.
- Salminen, S., et al. (1998). Functional food science and gastrointestinal physiology and function. *British Journal of Nutrition*, 80(Suppl 1), S147–S171.
- Sanz, M. L., González, M., De Lorenzo, C., Sanz, J., Martínez-Castro, I. (2004). Carbohydrate composition and physico chemical properties of artisanal honeys from Madrid (Spain): occurrence of *Echium* sp honey. *Journal of the Science of Food and Agriculture*, 84: 1577–1584.
- Sanz, M. L., et al. (2005). In vitro investigation into the potential prebiotic activity of honey oligosaccharides. *Journal of Agricultural and Food Chemistry*, 53: 2914–2921.
- Saran, S., Singh, K., Bisht, M. S., Teotia, U. V. S., Dobriyal, A. K. (2011). Comparison of prebiotics for the functional attributes of an indigenous isolate of *Lactobacillus acidophilus*. *International Journal of Probiotics and Prebiotics*, 6: 173–178.
- Shah, N., Ding, W., Fallourd, M., Leyer, G. (2010). Improving the stability of probiotic bacteria in model fruit juices using vitamins and antioxidants. *Journal of Food Science*, 75: M278–M282.



- Shamala, T. R., Shri Jyothi, Y., Saibaba, P. (2000). Stimulatory effect of honey on multiplication of lactic acid bacteria under in vitro and in vivo conditions. *Letters in Applied Microbiology*, 30: 453–455.
- Sharma, S., Sreeja, V., Prajapati, J. B. (2016). Development of synbiotic lassi containing honey: studies on probiotic viability, product characteristics and shelf life. *Indian Journal of Dairy Science*, 69: 148–153.
- Shin, H., Ustunol, Z. (2005). Carbohydrate composition of honey from different floral sources and their influence on growth of selected intestinal bacteria: an in vitro comparison. *Food Research International*, 38: 721–728.
- Shu, Q., Gill, H. S. (2000). A dietary probiotic (*Bifidobacterium lactis* HN019) reduces the severity of *Escherichia coli* O157:H7 infection in mice. *Medical Microbiology and Immunology*, 189: 147–152.
- Shu, Q., Gill, H. S. (2002). Immune protection mediated by the probiotic *Lactobacillus rhamnosus* HN001 (DR20™) against *Escherichia coli* O157:H7 infection in mice. *FEMS Immunology and Medical Microbiology*, 34: 59–64.
- Shu, Q., et al. (2000). Dietary *Bifidobacterium lactis* (HN019) enhances resistance to oral *Salmonella typhimurium*, infection in mice. *Microbiology and Immunology*, 44: 213–222.
- Shu, Liu. (2008). *Novel Probiotic Strain and Compositions*, 526544th ed. New Zealand Patent.
- Shu, Q., Qu, F., Gill, H. S. (2001). Probiotic treatment using *Bifidobacterium lactis* HN019 reduces weanling diarrhea associated with rotavirus and *Escherichia coli* infection in a piglet model. *Journal of Pediatric Gastroenterology and Nutrition*, 33: 171–177.
- Shu, Q., et al. (1999). Probiotic lactic acid bacteria (*Lactobacillus acidophilus* HN017, *Lactobacillus rhamnosus* HN001 and *Bifidobacterium lactis* HN019) have no adverse effects on the health of mice. *International Dairy Journal*, 9: 831–836.
- Siddiqui, I. R., Furgala, B. (1967). Isolation and characterization of oligosaccharides from honey. Part I. Disaccharides. *Journal of Apicultural Research*, 6: 139–145.
- Siddiqui, I. R., Furgala, B. (1968). Isolation and characterization of oligosaccharides from honey. Part II. Trisaccharides. *Journal of Apicultural Research*, 7: 51–59.
- Slacanac, V., Hardi, J., Lucan, M., Kun, S., Havas, P., Krstanovic, V. (2011). Effect of honey addition on fermentation activity of *Lactobacillus casei* Lc-01 in cow's and goat's milk: a kinetic study. *Acta Alimentaria*, 40: 270–281.
- Smith, E. A., Macfarlane, G. T. (1996). Enumeration of human colonic bacteria producing phenolic and indolic compounds: effects of pH, carbohydrate availability and retention time on dissimilatory aromatic amino acid metabolism. *Journal of Applied Bacteriology*, 81: 288–302.
- Snow, M. J., Manley-Harris, M. (2004). On the nature of non-peroxide antibacterial activity in New Zealand manuka honey. *Food Chemistry*, 84: 145–147.
- Talarico, T. L., Casas, I. A., Chung, T. C., Dobrogosz, W. J. (1988). Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*. *Antimicrobial Agents and Chemotherapy*, 32: 1854–1858.
- Tian, H. (2013). *The biotherapeutic potential of Lactobacillus reuteri DPC16 and bovine lactoferrin in controlling some pathogens, genotoxicity and inflammation in the gut*. (Doctoral dissertation), Massey University, New Zealand.
- Tian, H., Maddox, I. S., Ferguson, L. R., Shu, Q. (2010a). Evaluation of the cytoprotective effects of bovine lactoferrin against intestinal toxins using cellular model systems. *BioMetals*, 23: 589–592.
- Tian, H., Maddox, I. S., Ferguson, L. R., Shu, Q. (2010b). Influence of bovine lactoferrin on selected probiotic bacteria and intestinal pathogens. *BioMetals*, 23: 593–596.
- Tissier, H. (1907). Traitement des infections intestinales par la méthode de transformation de la flore bactérienne de l'intestin. [The treatment of intestinal infections by the method of transforming bacterial flora of the intestines.] *Comptes Rendus Des Seances De La Societe De Biologie Et De Ses Filiales*, 60, 359–361.
- Ustunol, Z., Gandhi, H. (2001). Growth and viability of commercial *Bifidobacterium* spp. in honey-sweetened skim milk. *Journal of Food Protection*, 64: 1775–1779.
- Valeur, N., Engel, P., Carbajal, N., Connolly, E., Ladefoged, K. (2004). Colonization and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract. *Applied and Environmental Microbiology*, 70: 1176–1181.
- Van Laere, K. (1997). Fermentative degradation of plant cell wall derived oligosaccharides by intestinal bacteria. Non-digestible oligosaccharides: healthy food for the colon? *Proceedings of the International Symposium*, Wageningen Graduate School VLAG, Wageningen, the Netherlands, 37–46.
- van Loo, J., Coussement, P., de Leenheer, L., Hoebregs, H., Smits, G. (1995). On the presence of inulin and oligofructose as natural ingredients in the western diet. *Critical Reviews in Food Science and Nutrition*, 35: 525–552.
- Varga, L., Süle, J., Nagy, P. (2014). Short communication: viability of culture organisms in honey-enriched acidophilus-bifidus-thermophilus (ABT)-type fermented camel milk. *Journal of Dairy Science*, 97: 6814–6818.
- Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., Pérez-Álvarez, J. A. (2008). Functional properties of honey, propolis, and royal jelly. *Journal of Food Science*, 73: R117–R124.
- Wallace, A., et al. (2010). Demonstrating the safety of manuka honey UMF@20+ in a human clinical trial with healthy individuals. *British Journal of Nutrition*, 103: 1023–1028.
- Weston, R. J., Brocklebank, L. K. (1999). The oligosaccharide composition of some New Zealand honeys. *Food Chemistry*, 64: 33–37.
- Weston, R. J., Mitchell, K. R., Allen, K. L. (1998). Antibacterial phenolic components of New Zealand Manuka honey. *Food Chemistry*, 64: 33–37.
- White Jr, J. W., Subers, M. H., Schepartz, A. I. (1963). The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. *BBA—Biochimica Et Biophysica Acta*, 73: 57–70.
- Windey, K., de Preter, V., Verbeke, K. (2012). Relevance of protein fermentation to gut health. *Molecular Nutrition and Food Research*, 56: 184–196.
- Winkler, P., De Vrese, M., Laue, C., Schrezenmeir, J. (2005). Effect of a dietary supplement containing probiotic bacteria plus vitamins and minerals on common cold infections and cellular immune parameters. *International Journal of Clinical Pharmacology & Therapeutics*, 43: 318–326.
- Wong, J. M. W., De Souza, R., Kendall, C. W. C., Emam, A., Jenkins, D. J. A. (2006). Colonic health: fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology*, 40: 235–243.
- Zhao, Q., Lee, S. J., Mutukumira, A. N., Maddox, I., Shu, Q. (2011). Viability and delivery of immobilised *Lactobacillus reuteri* DPC16 within calcium alginate gel systems during sequential passage through simulated gastrointestinal fluids. *Beneficial Microbes*, 2: 129–138.
- Zhao, Q., Maddox, I. S., Mutukumira, A., Lee, S. J., Shu, Q. (2012). The effect of cell immobilization on the antibacterial activity of *Lactobacillus reuteri* DPC16 cells during passage through a simulated gastrointestinal tract system. *World Journal of Microbiology and Biotechnology*, 28: 3025–3037.
- Zhao, Q., Mutukumira, A., Lee, S. J., Maddox, I., Shu, Q. (2012). Functional properties of free and encapsulated *Lactobacillus reuteri* DPC16 during and after passage through a simulated gastrointestinal tract. *World Journal of Microbiology and Biotechnology*, 28: 61–70.