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Review
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Review

Effect of honey in improving the gut microbial balance

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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	*Lactobacillus*	<1000
Small intestine	*Enterococcus*	10^2–10^9
	Lactobacillus	
Large intestine	*Lactobacillus*	10^4–10^{12}
	Bifidobacterium	
	Enterococcus	
	Bacteroides	
	Clostridium	
	Eubacterium	
	Staphylococcus	
	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 (*Drapac Colostrum Milk Powder/Hi Cal. plus DHA*) and beneficial fermentation metabolites (*Drapac 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; drapac.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; Jakesevic *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 glyosidic bonds in the oligosaccharides that resists 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; Kaškoniene 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
Erlose 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 lactoferrrin 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
Lactococcus lactis	Locally sourced (India)	*In vitro* incubation and storage studies	Growth and survivability during refrigerated storage of dahi* prepared with honey increased significantly	Manhar *et al.* (2016)
L. helveticus Streptococcus thermophillus	Locally sourced (India)	*In vitro* 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 *et al.* (2016)
Faecal *Bifidobacterium* spp.	Locally sourced (India)	*In vitro* incubation	Total viable count of the isolated strains significantly increased with addition of honey (3%) to the whey medium	Narayanan and Subramonian (2015)
L. acidophilus, *B. animalis* ssp., *Lactis* and *Streptococcus thermophillus*	Black Locust (Hungary)	*In vitro* 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 *et al.* (2014)
Yogurt starters** plus *B. bifidum* or *L. rhamnosus* or *L. reuteri*	Locally sourced (Saudi Arabia)	*In vitro* 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 *Lactobacillus* spp.	Locally sourced (India)	*In vitro* 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)
L. acidophilus	Locally sourced (India)	*In vitro* 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 *Escherichia coli*.	Saran *et al.* (2011)
Yogurt starters,** *L. acidophilus* and *B. bifidum*	Three unifloral (USA)	*In vitro* 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)
L. casei Lc-01	Chestnut and acacia (Croatia)	*In vitro* fermentation	Growth and activity was stimulated by both the honeys, faster in goat milk than in cow milk.	Slacanac *et al.* (2011)
B. longum spp BB536	Tulang and Tapah (Malaysia)	*In vitro* incubation after removing sugars	All the honey types (wild and commercial, 5%) supported the growth and acid production in RSM.	Jan Mei *et al.* (2010)
B. lactis Bb-12	Chestnut and acacia (Croatia)	*In vitro* incubation, and agar diffusion assay for pathogen inhibition	Growth and LA production was enhanced in both cow and goat milk, and inhibitory potential against Listeria monocytogenes was demonstrated.	Lucan *et al.* (2009)
Yogurt starters**	Polyfloral (Algeria), Unifloral (France)	*In vitro* 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)
Lactobacillus and *Bifidobacterium* spp.	Apis mellifera (Brazil)	*In vitro* 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 *et al.* (2008)
B. infantis and *L. acidophilus*	Three regions (Jordan)	*In vitro* incubation	Growth and the production of SCFA and LA increased significantly in both RSM and skim milk.	Haddadin *et al.* (2007)
Faecal bifidobacteria and lactobacillii	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 *et al.* (2005))
Mixed culture of 5 bifidobacteria and 5 other gut bacteria	Three unifloral (USA)	*In vitro* growth and co-culturing studies	All the honeys (5% w/v) with distinct oligosaccharide contents enhanced the Bifidobacterium growth in the media, and selectively inhibited Clostridium and Eubacterium sp.	Shin and Ustunol (2005)
Five bifidobacteria spp.	Clover (USA)	*In vitro* incubation	Honey (5% w/v) also supported the growth and LA production by the bacteria in RCM, comparable to FOS, GOS and inulin.	Kajiwara *et al.* (2002)
Two bifido-bacteria spp.	Clover (USA)	*In vitro* incubation and storage studies	Growth, LA production and refrigerated storage survivability upto14 days was higher with the addition of honey (5% w/v) in RSM than the mono- and disaccharides.	Ustunol and Gandhi (2001)
Yogurt starters,** *L. acidophilus* *B. bifidum*	Clover (USA)	*In vitro* incubation	Honey (5% w/v) was not inhibitory to the growth of all the four microbes in RSM, and the Bifidobacterium spp. significantly enhanced LA production.	Chick *et al.* (2001)
L. acidophilus *L. plantarum*	Locally sourced (India)	*In vitro* incubation, and *in vivo* studies on rats	*In vitro* growth of LAB in the agar media was enhanced more by honey addition than sucrose (1% sugar), and this was corroborated in *in vivo* trials.	Shamala *et al.* (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 *Streptococus 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 physiochemical 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 emphases 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.

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