Chapter

PREBIOTIC AND PROBIOTIC PROPERTIES OF HONEY

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ABSTRACT

Laboratory studies have shown that honey contains olygosaccharides and low molecular weight polysaccharides exhibiting prebiotic properties. Like the well-known commercial prebiotics the honey oligosaccharides are not digested in the upper part of gastrointestinal tract but are fermented by beneficial microflora in large intestine of human and animals and stimulate its growth and vital activity. It is emphasized that prebiotic properties of honey depend on its plant origin. It is shown that fresh honey also contains probiotics - the microorganisms beneficial for human and animals that inhibit the growth and development of pathogenic and conditionally pathogenic microflora, and can also be a source of biologically active substances with antimicrobial activity. Bifidobacteria and lactobacilli inhabiting honey stomach can survive in honey within 2-3 months after its harvest. The microflora composition of honey stomach and fresh honey may depend on the botanical origin of honey, as well as habitat and subspecies of honey bees. The probiotic microorganisms are involved in the development of honey bee resistance to adverse environmental factors directly inhibiting the growth of pathogens, and stimulating components of the immune system. Antagonistic activity of probiotic bacteria against a broad spectrum of pathogenic microorganisms enable their application for prophylaxis and treatment of honey bee diseases, and in human and veterinary medicine.

Keywords: honey, prebiotics, probiotics, honey carbohydrates, lactic acid bacteria

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INTRODUCTION

In the past 20 years a surge of interest in medicinal honey properties is observed. The antimicrobial activity of honey against human pathogenic microorganisms is shown (Conway et al., 2010; Angela, 2012, Gomashe et al., 2014). This activity is caused by osmolality, acidity, hydrogen peroxide (White et al., 1963), flavonoids, phenolic acids (Taormina et al., 2001) and other antimicrobial components depending on honey type (Olofsson and Vásquez, 2008). Honey is also used for treatment of diseases of the respiratory system, gastrointestinal tract, cardiovascular system, etc. (Yaniv and Rudich, 1996). The scientific mechanism of honey therapeutic effect is currently not fully understood. However, the vast majority of scientific findings suggest that some of the honey beneficial properties are due to their probiotic and prebiotic components.

Probiotics are beneficial to human living microorganisms, which are normal inhabitants of healthy human intestine, inhibiting the growth and development of pathogenic and conditionally pathogenic flora. The most famous probiotic microorganismes are *Lactobacillus acidophilus*, *L.casei*, *L. delbrueckii subsp. bulgaricus*, *L. reuteri*, *L. brevis*, *L. cellobiosus*, *L. curvatus*, *L. fermentum*, *L. plantarum*, gram-positive cocci *Lactococcus lactis subsp. cremoris*, *Streptococcus salivarius subsp. thermophilus*, *Enterococcus faecium*, *S. diaacetylactis*, *S. intermedius*, *Bifidobacterium bifidum*, *B. adolescentis*, *B. animalis*, *B. infantis*, *B. longum*, and *B. thermophilum* (Kashirskaya, 2000). The mechanism of probiotic effect may be due to competition for adhesion receptors on the intestinal epithelium (Johnsson and Conway, 1992) and nutrients (Freter, 1992), the production of antibacterial substances (Sarkar and Banerjee, 1996) and the stimulation of the immune system (Nousiainen and Setala, 1993). Lactic acid bacteria in the intestine produce metabolites that inhibit the growth of pathogenic microorganisms and increase resistance of the host organism. Produced organic acids also decrease the intestinal pH, which can inhibit other bacterial pathogens.

Prebiotics are nondigestible food ingredients which promote the improvement of health by selectively stimulating the growth and/or metabolic activity of one or more groups of probiotic bacteria inhabiting the colon. Prebiotics are not hydrolyzed by the human digestive enzymes, not absorbed in the upper digestive tract and are selective substrates for growth and/or metabolic activation of one species or group of probiotic microorganisms, resulting in their ratio normalization (Van Loo et al., 1999). Most often prebiotics are different oligo- and polysaccharides in which molecule residues are connected by β-glycosidic linkages (Buchakhchyan et al., 2011). Human enzyme systems do not contain β-glycosidases, therefore prebiotics are hydrolyzed only by the normal intestinal microflora. The best-known prebiotics are inulin, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), soya-oligosaccharides, xylo-oligosaccharides, pyrodextrins, isomalto-oligosaccharides and lactulose (Conway et al., 2010).

A large number of oligosaccharides and low molecular weight polysaccharides in honey attract a research interest to the honey as a source of nutrients for colon microflora.

PREBIOTICS IN HONEY

The Carbohydrate Composition of Honey

Qualitative and quantitative carbohydrate composition of honey is variable and depends on the floral source of honey. In fact, the honey is a supersaturated sugar solution with about 17-20% water. 90% honey carbohydrates are glucose and fructose monosaccharides, and other carbohydrates include more than 30 different oligosaccharides (Anklam, 1998; Morales et al., 2007; Ruiz-Matute et al., 2010). Fructose is the predominant sugar with concentration range of 36-50%, glucose amounts to 28-36%. Disaccharides, trisaccharides, tetrasaccharides, hexasaccharides and other oligosaccharides are present in much smaller quantities than glucose and fructose (The National Honey Board, 2008). Honey disaccharides include sucrose, maltose, isomaltose, nigerose, turanose, maltulose, leucrose, kojibiose, neotrehalase, gentiobiose, laminaribiose isomaltulose, melibiose, palatinose, trehalose, and trehalulose (D'Arcy et al., 1999; Sanz et al., 2004; de la Fuente et al., 2007). 25 trisaccharides including planteose and a-30-glucosyl-isomaltose, which have been reported in honey for the first time, and 10 tetrasaccharides were identified in Spanish and New Zealand honeys (Ruiz-Matute et al., 2010). Honeydew honey is characterized by a high concentration of oligosaccharides, mainly the trisaccharides melezitose and raffinose, which usually are not found in blossom honeys (Bogdanov et al., 2004). New Zealand honeydew honeys contain tetrasaccharides maltotetraose, alpha-panasyl-D-fructofuranoside and alpha-maltosyl-D-fructofuranoside, two pentasaccharides and one hexasaccharide (Morales et al., 2007). Shin H.S. and Ustunol Z. (2005) have reported that bee alpha-D-glucosidase catalyzes the transfer of alpha-D-glucopyranosyl groups from sucrose to an acceptor carbohydrate resulting in the formation of FOS and various other oligosaccharides in different amounts.

Different grades of honey are found to contain specific oligosaccharides. For example, the New Zealand honey contains isomaltose and melezitose (Weston and Brocklebank, 1999), and the Italian honey contains raffinose (Oddo et al., 1995). Sugar composition and the ratio of particular carbohydrates are fairly reliable indicators for honey classification and authentication in the case of unifloral honeys with very high amount of dominating plant (Kaˇskoniene and Venskutonis, 2010). Morales and others (2007) found differences in the higher oligosaccharide compositions of ten different honeys by extraction of oligosaccharides with activated charcoal. It is also shown that sage, alfalfa and oxydendrum honey contains 3.8, 5.5 and 10.9% oligosaccharides respectively (Popa and Ustunol, 2011). The total content of kojibiose, maltose, nigerose, and turanose was highest in Spanish honey samples of rosemary, lavender, sunflower, eucalyptus, heather, and honeydew origin (Mateo and Bosch-Reig, 1997). Cotte and others (2004) determined the predominant disaccharides in some France honeys: maltose and turanose in acacia; maltulose and turanose in chestnut and linden; turanose and trehalose in fir; and sucrose and maltose in lavender origin honey. Maltose was the major disaccharide in 80 Brazilian honey samples (*Eucalyptus* spp., extra-floral, and multifloral honeys) (Da Costa Leite et al., 2000). Heather honey were characterized by the presence of erlose and nigerose; forest honey contained higher amounts of trehalose and

melezitose; spike lavender honey was specified by isomaltose; while French lavender and thyme honeys were noted for the panose presence (Nozal et al., 2005).

The variability of qualitative and quantitative carbohydrates composition of honey causes the differences in the glycemic index (GI) of different honeys and consequently in their dietary properties. Australian honeys Yellow Box, Stringybark, Red Gum, Ironbark, and Yapunyah have low values of GI (Arcot and Brand-Miller, 2005). In contrast, the average GI of four American honeys was 72.6 with no significant differences between the tested varieties (The National Honey Board, 2008). Obviously a low content of glucose and increase in share of oligo- and polysaccharides contributes to decrease in honey GI.

The Effect of Honey on the Probiotic Microorganisms

In vitro and *in vivo* investigations demonstrate a stimulating effect of honey and its carbohydrate components on the beneficial microorganisms inhabiting the lower sections of human and animal digestive tract (Table 1).

Bifidobacteria are quite fastidious organisms. Many researchers reported that bifidobacteria grow poorly in the milk and therefore require the addition of specific growth factors (Rybka and Fleet, 1997; Dave and Shah, 1998; Chick et al., 2001). It is assumed that the more preferred substrates for bifidobacteria are polysaccharides with a low degree of polymerization that are present in honey (Chick et al., 2001; Kajiwara et al., 2000).

Thus, sourwood, alfalfa, and sage honeys are shown to have stimulating action on the growth and activity of *B. longum*, *B. adolescentis*, *B. breve*, *B. Bifidum*, and *B. infanti* inhabiting the human gut and are used in the production of fermented milk products (Shin and Ustunol, 2005). Presented honey effect was similar to that of the commercial oligosaccharides FOS, GOS, and inulin (Kajiwara et al., 2002; Ustunol, 2007). Moreover the native honey was more effective than the combination of its purified basic saccharide components. Based on these results, a synergistic effect of honey carbohydrate components to enhance the growth and activity of bifidobacteria has been proposed. However, it is not impossible that tested honey could contain additional unexplored saccharides that are more effective in enhancing the growth of bifidobacteria. Two types of monofloral honey, dark chestnut honey and light acacia honey, increased the enzyme activity and the number of viable cells of *B. lactis* (Bb-12) and *B. longum* (Bb-46) in soy milk (Slacanac et al., 2012). Furthermore, the addition of honey, especially chestnut honey, increased the inhibitory potential of the fermented soymilk against *Listeria monocytogenes*. Jordanian honeys enhanced the growth and increased short
chain fatty acids production of the two intestinal bacteria, *B. infantis* and *L. acidophilus* (Haddadin et al., 2007). In addition, different bifidobacteria strains specifically responded to the addition of this honey type in the medium. Australian honeys contributed to the growing of *B. lactis* and *L. plantarum* more than sucrose and inulin (Conway et al., 2010).

Differences in carbohydrate composition of various honeys suggest their diverse prebiotic effect. Thus, glucose-rich yellow box honey stimulated the growth of coliform bacteria, while fructose-rich banksia honey contributed to the growth of lactic acid bacteria (Conway et al., 2010). Comparison of sage, alfalfa, and oxydendrum honeys with sucrose, corn syrup with a high fructose content, and inulin was carried out by their ability to support the growth, activity and viability of bifidobacteria and lactic acid bacteria commonly used in the

production of yogurt (Popa and Ustunol, 2011). Alfalfa honey was more effective in enhancing the growth of *Streptococcus thermophilus* (St-133), sourwood honey - *L. delbrueckii subsp. bulgaricus* (Lr-78), and all three types of honey contributed to the growing of *B. bifidum* (Bf-1). Alfalfa and sourwood honeys were the most effective in stimulation of the lactic acid production of *L. delbrueckii subsp. bulgaricus* (Lr-78) and *L. acidophilus* (La-7). An important characteristic of bifidobacteria is the production of lactic and acetic acids as the final products of sugar fermentation. In an ideal synthetic medium fermentation of 2 moles of glucose by bifidobacteria leads to the formation of 3 moles of acetic acid and 2 moles of lactic acid (Scardovi and Trovatelli, 1965). In medium containing other substrates, including prebiotic oligosaccharides, this ratio is not supported. High acetate levels create a "vinegar" taste of foods. Therefore, an increase in the proportion of lactate and acetate production decline would be useful for dairy products manufacturing technology, since it would improve the organoleptic characteristics of the products. In this study, alfalfa honey increased in *B. bifidum* (Bf-1) production of lactic acid, and sourwood honey - acetic acid. Chic et al. (2001) also reported increases in lactic acid production to the level of acetic acid in the fermentation of milk by *B. bifidum* (Bf-1) in the presence of clover honey. Thus, applications as a sweetener of the types of honey, which alter lactate/acetate ratio in favor of lactate, can be recommended for the dairy production technology. Based on the results of these studies, such honeys as alfalfa and clover honeys stimulate the growth of bifidobacteria and lactic acid production.

Table 1. Monofloral honeys, stimulating the growth and activity of probiotic microorganisms

Microorganism	The botanical origin of honey	Reference
Lactobacillus acidophilus	*Eucalyptus sideroxylon*, *Eucryphia lucida*	Conway et al., 2010
L. delbrueckii subsp.bulgaricus	*Oxydendrum arboreum*, *Medicago sp.*	Popa and Ustunol, 2011 Conway et al., 2010
L. plantarum	*E. sideroxylon*, *Banksia sp.*, *E. melliodora*, *E. paniculata*, *E. longifolia*, *E. triantha*	Conway et al., 2010
L. ramnosus	*E. sideroxylon*, *E.lucida*	Conway et al., 2010
L. paracasei	*E.lucida*	Popa and Ustunol, 2011
Streptococcus thermophilus	*Medicago sp.*	Popa and Ustunol, 2011;
Bifidobacterium bifidum	*O. arboreum*, *Medicago sp.* *Salvia sp.*, *Trifolium sp.*	Chick et al., 2001 Popa and Ustunol, 2011
B. adolescentis	*O. arboreum*, *Medicago sp.* *Salvia sp.*	Popa and Ustunol, 2011 Popa and Ustunol, 2011;
B. infantis	*O. arboreum*, *Medicago sp.* *Salvia sp.*	Slacanac et al., 2012 Popa and Ustunol, 2011
B. longum	*O. arboreum*, *Medicago sp.* *Salvia sp.*, *Castanea sp.*, *Acacia sp.*,	Popa and Ustunol, 2011; Slacanac et al., 2012
B. breve	*O. arboreum*, *Medicago sp.* *Salvia sp.*	
B. lactis	*Castanea sp.*, *Acacia sp.*, *E. sideroxylon*, *E. melliodora*, *E. paniculata*, *E. longifolia*, *E. triantha*	

In a detailed study of Australian honeys carried out by Conway P.L. et al. (2010), mugga honey showed prebiotic effect for four probiotic cultures: *B. lactis*, *L. rhamnosus*, *L. plantarum*, and *L. acidophilus*. Tasmanian leatherwood honey demonstrated good prebiotic effects on 3 probiotic cultures: *L. rhamnosus*, *L. acidophilus*, and *L. paracasei*. Banksia honey, woolly butt honey, grey ironbark honey and yellow stringybark honey had a prebiotic effect on at least one probiotic culture. In comparison with inulin and sucrose all tested honey showed higher values of prebiotic index (the PI), measured by changes in the number of four bacterial groups (bifidobacteria, lactobacilli, clostridia and bacteroides): from about 130 in creek woolybutt honey up to 420 in mugga ironbark honey against 46 in inulin. The authors assumed that this result reflects the synergistic effect of simple and complex sugars that are present in honey, and assessed the prebiotic potential of oligosaccharides isolated from the investigated honeys. These oligosaccharides are also largely contributed to the growth of *L. acidophilus.* At the same time, PI values of honey oligosaccharides were much lower than in honeys, but almost the same as in inulin.

The situation in which honey monosaccharides are digested in the upper human intestinal tract and oligosaccharides pass into the colon, where indigenous bacteria are living, was modeled (Sanz et al., 2005). For this, monosaccharides have been removed from honey and oligosaccharide fraction was studied in comparison with FOS on prebiotic activity in relation to fecal bifidobacteria, lactobacilli, and eubacteria. According to the study, honey oligosaccharides have a potential probiotic activity (PI values between 3.38 and 4.24) increasing the population of bifidobacteria and lactobacilli, although not to the level of FOS (PI 6, 89). In another study the prebiotic potential of honey was evaluated in comparison with inulin and gum acacia towards lactobacilli isolated from human feces (Tejpal, Goyal, 2009). The highest specific rate of growth of lactobacilli was observed in the presence of honey.

According to the studies conducted by Shamala et al. (2000) *in vitro*, the amount of *L. acidophilus* and *L. plantarum* increased by 10 - 100 times in the presence of honey compared with sucrose. *In vivo* study found that the number of viable lactic acid bacteria from the intestine of rats was significantly higher when feeding with honey than with sucrose. From these results the authors concluded that a known curative effect of honey on the liver, cardiovascular system and gastrointestinal tract can be associated with changes in the microbial profile, with a significant increase in the amount of lactic acid bacteria, which in turn affect the physiology and health of the host. In experiments carried out *in vivo* by El-Arab et al. (2006) the addition of honey in mice diet has been shown to increase the amount of bifidobacteria and lactobacilli in the mice intestine too, and also reduce the histopathological and genotoxic effects of mycotoxins.

PREBIOTICS IN HONEY

Probiotic Lactic Acid Bacteria in Honey

Species Content of Lactic Acid Bacteria in Honey

At present we know that fresh honey contains a large number of lactic acid bacteria (LAB), derived from honey bee stomachs, that possess a wide spectrum of antimicrobial

activity against various microorganisms, pathogenic to bees and humans (Olofsson et al., 2014).

LAB are a clade of Gram-positive microaerophilic microorganisms functionally associated by their ability to ferment carbohydrates in homo- or heterofermentative metabolism with lactic acid formation (Salminen et al., 2004). Traditionally LAB include immobile, catalase negative, asporous, either rod- or cocci-shaped representatives of *Lactobacillales*. *Bifidobacterium* are distant relatives of *Lactobacillales*. It is genus of Gram-positive, anaerobic, catalase negative, asporogenous, rod-shaped bacteria, by definition, is not a "true"' member of the LAB. However *Bifidobacterium* are generally regarded to LAB group due to their lactic acid production, use in the manufacture of dairy products and well-known beneficial effects on the flora of the gastrointestinal tract of humans and animals (Coenye and Vandamme, 2003).

Recently for the first time the symbiotic flora from honey stomachs and fresh honey of Swedish honey bees has been detected and associated with many healing honey properties (Olofsson and Vásquez, 2008). Approximately 40 LAB strains with 13 taxonomically identified species of *Lactobacillus* (9 spp.) and *Bifidobacterium* (4 spp) has been found in the new microbiota: *L. kunkeei* Fhon2, *L. apinorum* Fhon13, *L. mellis* Hon2, *L. mellifer* Bin4, *L. kullabergensis* Biut2, *L. kimbladii* Hma2, *L. helsingborgensis* Bma5, *L. melliventris* Hma8, *L. apis* Hma11, *B. coryneforme* Bma6, *B. asteroides* Bin2, *B.* sp Bin7, and *B.* sp Hma3 (Butler et al., 2014). Most of these LAB symbionts are the species described for the first time. It is shown that the discovered LAB symbionts present in honey stomachs of honey bees of all species, as well as stingless bees, and in the corresponding fresh honey on all continents of the world (Vasquez et al., 2009; 2012; Olofsson et al., 2011; Tajabadi et al., 2011; 2013). In addition to the unique honey bee LAB flora honey may also contain other LAB strains, for example, *L. acidophilus* contributing to its antibacterial activity (Angela, 2012; Aween et al., 2012.). The maximum registered number of viable LAB in fresh honey is 10^8 LAB/g of honey (Vasquez et al., 2012). In process of honey dehydration number of viable LAB decreases and becomes zero at a water content of less than 20% (Olofsson et al., 2014).

These LAB are not collected by bees from flowers, but are symbiotic organisms that inhabit honey bee stomachs. The number and species composition of honey stomach LAB microflora depends on the season, the source and amount of nectar, the health of bees, and the presence of other microorganisms in the collected nectar (Olofsson and Vásquez, 2008; Vasquez et al., 2009; 2012; Tajabadi et al., 2011; Butler et al., 2013; Forsgren et al., 2010). Thus, the number of LAB flora is lowest in early spring, when bees start collecting pollen and nectar after winter, and increases with foraging activity. Transient microbes collected from flowers, provoke the growth of LAB microbiota in honey bees and the production of anti-microbial proteins (Butler et al., 2013).

Value of LAB in the Honeybee Organism

Each member of the discovered honey bee LAB microbiota ferments nectar, excretes strain-specific spectrum of metabolites and thus participates in the process of conversion of nectar to honey (Olofsson et al., 2014). The substances produced by LAB are present in fresh honey and stored in the mature honey. In addition, these LAB are assumed to play a key role in the production of bee bread from a pollen (Vasquez and Olofsson, 2009).

Environment of honey stomach is characterized by the micro aerobic condition, the presence of nectar sugars and sufficiently optimal temperature (35°C), independent from the

outside air temperature (Jones et al., 2004), and is the optimal niche for LAB. Based on the received data, Olofsson T. and Vásquez A. (2008) suggested that bees and LAB flora developed in mutual dependence on each other: LAB received a niche in which nutrients were available, and the bees obtained a protection from harmful. Thus, it is known that certain types of LAB can produce bioactive compounds, such as organic acids, free fatty acid, ethanol, benzoate, enzymes, hydrogen peroxide, bacteriocins, and antimicrobial peptides (De Vuyst and Leroy, 2008). At the same time, there is a distinct difference in the production of antimicrobial substances and other useful properties between various species and genera in LAB (Pfeiler and Klaenhammer, 2007). Different extracellular LAB metabolites possess bactericidal or bacteriostatic properties, and have different mechanisms of action, such as violation of cell membrane permeability and the DNA synthesis or changing of the growth conditions, for example by reducing the pH (Butler et al., 2014). These qualities together result in a broad inhibiting spectrum against pathogens: 55 species of bacteria and 5 species of yeast which are found in flowers (; Forsgren et al., 2010; Vasquez et al., 2012).

Genomic analysis of LAB flora in honey stomach has shown that the genome sizes of these microorganisms vary within 1,5 - 2,2 mega-base pairs (Mbps), which indicates a clear distinction in produced proteins and the specialized functions of bee microbiota and its adaptation to the host (Butler et al., 2013).

L. kunkeei is a dominant species in honey bee stomach microbiota (Olofsson and Vásquez, 2008, Vasquez et al., 2012). It is remarkable that this microorganism was first isolated during spoilage of wine, as a strain strongly inhibiting alcoholic fermentation of yeasts *Saccharomyces bayanus* and *S. cerevisiae* (Huang et al., 1996; Edwards et al., 1998). It was also shown that namely the bees distribute *L. kunker* among the damaged grapes with which this microorganism gets into the wine raw (Bae et al., 2006). Therefore, the value of *L. kunkeei* for bees may be to inhibit the process of fermentation of immature honey by yeast *Saccharomyces* causing honey spoilage (Snowdon and Cliver, 1996; Madras-Majewska et al., 2016).

Paenibacillus larvae infection of bee colonies was associated with the presence in bee organisms of bacterial phylotypes closely associated with genera *Actinobacillus* and *Phocoenobacter* of the family *Pasteurellaceae* (Olofsson and Vásquez, 2008). The authors have suggested that the interaction of *P. larvae*, *Pasteurellaceae* phylotypes and LAB flora of honey stomach affects the number of pathogens. In another study, in all bee colonies *Bifidobacterium* activity negatively correlated with the activity of pathogenic microbes (Mattila et al., 2012). Forsgren et al. (2010) have demonstrated strong inhibitory effects of combined LAB flora of honey stomach on the growth of *P. larvae in vitro*. LAB addition to honey bee young larvaes contaminated with *P. larvae* spores reduced the proportion of larvae infected with the causative agent of American foulbrood. It is remarkable that individual phylotypes of LAB inhibited *P. larvae* strains differently. Thus, the dominant strain *L. kunkeei* Fhon2 showed only partial inhibition of *P. larvae*, whereas *L. apis* Hma11 and *L. kullabergensis* Biut2 possessed strong inhibitory activity on the growth of *P. larvae*. Based on these data, it was concluded by the authors that the whole LAB flora may act synergistically against *P. larvae* and possibly other harmful microorganisms.

Field observations indicate that *P. larvae* infection may be present in bee colonies without causing clinical symptoms and can be naturally reduced to undetectable levels (Fries et al., 2006). This phenomenon may be due to the action of LAB flora, the activity of which can be changed with the switching of bees to another nectar source. For example, in colonies

infected with *P. larvae*, the number of the pathogen grown when collecting of oil-seed rape and wild raspberry nectars, but subsequently decreased until the disappearance of American foulbrood pathogen when collecting of linden nectar (Olofsson, Vásquez, 2008).

Organic acids such as formic acid, which is produced by bifidobacteria (Van der Meulen et al., 2006), lactic and acetic acids, which are produced by LAB, are antimicrobial agents and may be important in the protecting of bees against pathogens. Thus *in vitro* experiments have shown that lactic acid produced by *L. johnsonii* inhibit *P. larvae* (Audisio et al., 2011), and metabolites produced by bifidobacteria exhibit antagonistic effects on the growth of the pathogen of European foulbrood *Melissococcus plutonius* (Wu et al., 2013). It is indicative that these acids are widely used by beekeepers for protection of bees from *Varroa destructor* and *Nosema apis*. In conditions of microbial stress (under the action of the representatives of *Pseudomonas*, *Enterobacteriaceae*, *Bacillus* and *Candida* from the flowers and the environment) LAB symbionts of honey bees produce extracellular proteins: enzymes, DNA-chaperones, S-layer proteins, bacteriocins, lysozyme and a number of new proteins with presumably antimicrobial function (Vasquez et al., 2012; Butler et al., 2013).

Currently, immunotropic and immune stimulatory mechanisms of probiotics action on human oragnizm are clinically and experimentally proven (De Vrese and Schrezenmeir, 2008; Ng et al., 2009). Indirectly LAB action on pathogens by activating of the immune system is also shown in the organism of honey bee. So, the addition of *Lactobacillus* and *Bifidobacterium* to the bee feed caused increase of the gene expression level of abaecin (Evans, Lopez, 2004) which is specific to *Hymenoptera* antibacterial peptide having bactericidal activity against Gram-positive and Gram-negative bacteria (Rahnamaeian et al., 2015). Per os treatment of larvae and adult bees with LAB also caused increasing of gene transcription levels of antimicrobial peptides abaecin, defensin and hymenoptecin (Yoshiyama et al., 2013).

These results show that LAB stimulate the innate immune response in honey bees, and may be useful for the prevention of bee infectious diseases. Preventive methods which enhance the bee LAB flora or additional food with LAB, can promote sustainability of honey bee, which is especially relevant in light of the current problems of the colony collapse disorder (Vasquez et al., 2012). Thus, it is shown that the stimulating fertilizers containing probiotic additives based on LAB, improve intestinal microbiocenosis of bees, increase their strength, winter hardiness, productivity of bee colonies, and reproductive performance of queens (Mishukovskaya, 2015).

The Antibacterial Activity of Honey LAB against Human and Animal Pathogens

The antagonistic effect of LAB isolated from honey stomach and honey against a broad spectrum of microorganisms creates a perspective of their application in the fight against human and animal pathogens, including those resistant to modern antibiotics. Thus, it is shown that LAB symbionts individually and together have a strong antimicrobial activity against a wide range of human pathogens, including antibiotic resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Olofsson et al., 2014). Cells and metabolites of *L. acidophilus* strains isolated from Malaysian honeys inhibited the growth of human pathogens with multiple antibiotic resistance: *S. aureus*, *S. epidermis*, and *B. subtilis* (Aween et al., 2012).

In vivo experiments proved the effectiveness of dressings based on honey enriched with the bee LAB for the treatment of wounds infected with various pathogens (Butler et al.,

2014;. Olofsson et al., 2014). In these studies, the strain-dependence of the LAB properties and substances produced by them were taken into account. So, *L. mellifer* Bin4 was shown to inhibit all investigated wound pathogens and produce benzene, which is a toxic volatile compound that increases the rate of wound closure and epithelialization. *L. kunkeei* Fhon2 produced a wide variety of extracellular proteins in response to microbial stress and three different 3-OH fatty acid as well as had the most potent activity against human wound pathogens, particularly against members of the *Pseudomonas spp*. (Butler et al., 2013). *Pseudomonas* is widely distributed on plants and at the same time is one of the therapeutically-resistant pathogens in human chronic wounds due to biofilm formation, drug resistance, and interactions with other microbes in the wound environment (Scales and Huffnagle, 2013). Honey in combination with LAB also inhibited the growth of bovine mastitis pathogens *Staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli*, and *Klebsiella pneumoniae* including those exhibiting the antimicrobial resistance to one or more antibacterial compounds (Piccart et al., 2016).

Spore-Forming Probiotic Bacteria in Honey

In addition to the LAB flora honey also contains spores of aerobic spore-forming bacteria of the genus *Bacillus* spp. which are collected by bees from plants during foraging (Madras-Majewska et al., 2016). Physico-chemical properties of honey do not allow these bacteria spores to pass into the vegetative form. However, the intestine of honey bee and human represents a suitable habitat for their germination and reproduction, where some strains of *Bacillus* spp. manifest themselves as probiotics. Thus, strains isolated from honey *B. cereus* (m363, mv86, mv81, mv75), *B. circulans* (Fr231, m448b), *B. megaterium* (m435), *B. pumilus* (m354), *B. subtilis* (m329) и *Paenibacillus alvei* (m321) showed antagonistic effect against honey bee fungal pathogen *Ascosphaera apis* (Reynaldi et al., 2004), and the strains *B. subtilis* (m351), *B. pumilus* (M350), *B. licheniformis* (m347), *B. cereus* (mv33), *B. cereus* (m387), *B. cereus* (m6c), *B. megaterium* (m404), *Brevibacillus laterosporus* (BLAT169), *B. laterosporus* (BLAT170) и *B. laterosporus* (BLAT171) had the antagonistic activity to *P. larvae* (Alippi and Reynaldi, 2006). Evans J.D. and Armstrong T.N. (2006) also demonstrated antagonistic effects of bee symbionts *Bacillus spp*. against *P. larvae.*

We have shown the enhancement of honey bee humoral defense as a result of the probiotic impact based on *B. subtilis* (Gaifullina et al., 2016). We have registered the activity increase of phenoloxidase, which is an integral link of the insect immune reactions involved in almost all cellular and humoral immune responses (Theopold et al., 2004), and antioxidant enzymes that are functionally associated with phenoloxidase and reduce the oxidative damage of cells and tissues (Dubovskii et al., 2010). Furthermore, in our experiments *B. subtilis* has caused an increase of abaecin and vitellogenin gene expression levels of honey bees. We have mentioned above about the immune functions of abaecin. Levels of this peptide increase with the penetration of different pathogens into bee organism and can serve as an immunocompetence criterion of separate honey bee colonies (Evans and Lopes, 2004). Vitellogenin is a protein carrying out many functions in honey bee organism, among which the antioxidant (Havukainen et al., 2013) and the immune (Zhang et al., 2011) properties are especially remarkable in this context. Vitellogenin participates in the immunological

recognition of Gram-negative bacterium *E. coli* and a Gram-positive bacterium *P. larvae*, as well as pathogen-associated molecular patterns (Salmela et al., 2015).

Like LAB, various strains of *Bacillus spp*. exhibit antagonistic activity against pathogens of human and vertebrates, and also stimulate the immune system of mammals, in connection with which find application as probiotics in medicine and veterinary (Lazovskaya et al., 2013).

CONCLUSION

Taking into account all the facts and conclusions presented here, it can be summarized that honey is a fermented food product which is a nectar, partially digested by enzymes of bees and their LAB symbionts. The presence of prebiotic substances and probiotic microorganisms in fresh honey defines it as a synbiotic, the physiologically functional food ingredient, which is a combination of probiotics and prebiotics in which probiotics and prebiotics have a synergistic effect on the host organism (Figure 1).

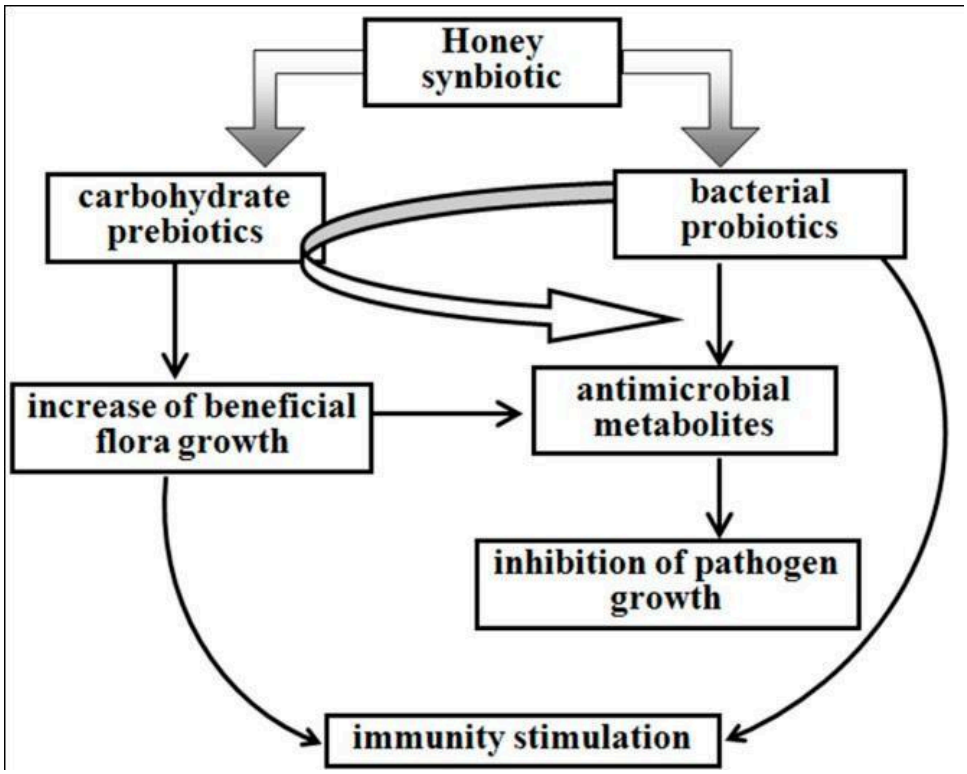

Figure 1. Honey as a synbiotic food product.

Using as a substrate honey polysaccharides and oligosaccharides, honey bee LAB symbionts during their vital activity produce metabolites that are involved in the formation of

honey organoleptic characteristics (taste, flavor, texture) and the spectrum of its therapeutic properties. Qualitative and quantitative composition of honey prebiotic sugars and LAB flora, and consequently bacterial metabolites depends on many factors, such as geographical and botanical origin of honey, taxonomic affiliation and health state of bees, which explains the various medicinal properties of different honey types. Synergistic direct antimicrobial effect of all microorganisms of beneficial bee microbiota and indirect immunostimulation effect determines the important role of probiotic bacteria in honeybee pathology and, consequently, in the manufacture of high-quality apiculture products. This fact defines the opportunity for development of adaptogenic supplements for bees based on probiotic bacteria, that is particularly relevant taking into consideration the massive loss of bee colonies around the world. The high level of prebiotic activity of honey oligo- and polysaccharides in relation to human beneficial intestinal flora, and antagonistic action of bacteria in fresh honey on human pathogens, makes the honey an attractive source of components for new prebiotic, probiotic and synbiotic supplements for human. In honey and honey stomach microbiota the detection of bacterial strains with high levels of antimicrobial activity against pathogens, that are resistant to antibiotics, opens up the possibility for development of new alternative tools to overcome the therapeutically resistant infectious agents.

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