Antibacterial activity of honey

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Abstract

The aim of this study was to monitor the antimicrobial activity of honey. The effects of 44 samples of honey (29 blossom honeys, 15 honeydew honeys) were tested using an agar dilution method on 65 bacterial strains (genera *Staphylococcus*, *Streptococcus*, *Listeria*, *Klebsiella*, *Escherichia* and *Paenibacillus*). All the samples of honey inhibited the growth of all tested strains of *L. monocytogenes*, *S. xylosus*, *P. larvae, P. alvei* and *Str. dysgalactiae* and one strain of methicillin-sensitive *S. aureus*. 54.5% of the honeys showed high antimicrobial activity (inhibiting the growth of all the bacterial strains). This activity was higher in honeydew honeys (73.3%) than in blossom honeys (44.8%). Acacia and sunflower honey inhibited the growth of all the bacteria. The lowest antibacterial activity of the samples of honey was seen on *Klebsiella* spp.

Agar dilution method, antibacterial effects, blossom honey, honeydew honey

Introduction

Honey has been an important foodstuff for millennia. It does not merely have a place in human nutrition, but is also known for its medicinal effects. It is used primarily in the treatment of superficial skin wounds, burns, ulcers, dermatitis, eye infections and gastroenteritis (Al-Waili et al. 2011). Further uses for honey are found in alternative medicine and the prevention of mastitis in cattle – a disease that causes considerable economic losses to breeders (Molan 1996).

Antibacterial factors of apian origin are present in honey that inhibit the growth of certain bacteria, e.g. *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Str. uberis*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Proteus vulgaris*, *Salmonella* spp., *Bacillus subtilis*, *B. cereus*, *Paenibacillus alvei*, *Pseudomonas* spp., *Vibrio cholerae*, and others (Molan et al. 1992; Vorlova et al. 2005; Moţ et al. 2016 and Cornara et al. 2017). Honey is also known for its antimycotic and antiparasitic effects (Cornara et al. 2017).

High osmolarity, low pH, the presence of hydrogen peroxide, lysozyme, derivatives of benzoic acid, methylglyoxal, oligosaccharides, phenolic acids and flavonoids, in particular, contribute to this antimicrobial activity. The mechanisms of these antimicrobial effects are extremely complicated and differ depending on the type of honey. The antibacterial effects of honey may be affected by the inappropriate treatment and storage of honey (Molan et al. 1992; Vorlova et al. 2005; Cornara et al. 2017 and Pita-Calvo & Vázquez 2017).

The aim of this study was to determine antimicrobial activity against selected bacteria in honeys produced in the Czech Republic and Slovakia.

Materials and Methods

Totally 44 samples of honey (29 blossom honeys, 15 honeydew honeys) obtained directly from beekeepers in the Czech Republic and Slovakia from the 2015 collection (n = 42) and 2016 collection (n = 2) were tested. Ten of the blossom honeys were monofloral honeys: lime (n = 6), acacia (n = 1), rapeseed (n = 1), sunflower (n = 1) and maple (n = 1). Before analysis, the samples were stored in a cool dark room in closable jars to prevent changes to their physiochemical parameters and the degradation of biologically active substances. Antimicrobial effects were analysed by an agar dilution method according to Vorlova et al. (2005) and

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Cooper et al. (1999) against 65 bacterial strains (Table 1). An inoculum of a concentration of 1.5×10^8 CFU ml^{-1} was made from a 24 – 48-hour culture and applied at an amount of 2.5 µl to agar prepared by mixing a 40% solution of honey in water and a double concentration of nutrient agar (at a ratio of 1:1). The growth of bacteria was controlled on agar with the addition of sterile distilled water in place of the honey sample. The osmotic effects of honey were compared by the cultivation of a set of bacteria on agar with a solution of saccharides in a composition and at a concentration imitating their proportions in honey (38.2% fructose, 31.3% glucose, 7.3% maltose, 1.3% saccharose) to substitute for a honey sample. Following incubation (conditions according to the optima for the species of bacteria) growths of bacterial cultures (- no growth, +/- isolated colonies, + weak growth with visible colonies, ++ medium growth, +++ extremely strong growth) were subtracted semi-quantitatively.

Table 1. Overview of bacterial strains used to test the antimicrobial effects of honey and their origin

Bacterial species	Number of strains	Origin
Methicillin-resistant *S. aureus*	11	raw cow/goat/sheep milk – pooled samples, isolates from mastitis isolate
Methicillin-sensitive *S. aureus*	10	raw cow/goat/sheep milk – individual/pooled samples
Staphylococcus xylosus	1	isolate from mastitis
Streptococcus dysgalactiae	2	isolates from mastitis
Listeria monocytogenes	10	raw cow/goat milk
Klebsiella pneumoniae	3	goat colostrum
Klebsiella oxytoca	11	goat colostrum
Verotoxigenic *E. coli*	15	milk, rectal swab and carcass
Paenibacillus alvei	1	strain from the collection of the Veterinary Research Institute
Paenibacillus larvae	1	strain from the collection of the Veterinary Research Institute

Results and Discussion

The antibacterial effects of 44 samples of honey were tested using an agar dilution method. The results of bacterial growth on agar with samples of honey were subtracted in comparison with growths of cultures on control agar with the addition of sterile water.

All the samples of honeys inhibited the growth of all the tested strains of *L. monocytogenes* (n = 10), *S. xylosus* (n = 1), *P. alvei* (n = 1), *P. larvae* (n = 1) and *Str. dysgalactiae* (n = 2) and one strain of methicillin-sensitive *S. aureus* (isolate from cow's milk). The strains of *Str. dysgalactiae* did not, however, grow even on the control sugar solution (-). Partial inhibition (+/-) was also observed in strains of *P. alvei* and *P. larvae*. Strains of *L. monocytogenes* and *S. xylosus* showed weak growth (+). The growth of these strains may, therefore, have been affected by the osmolarity of the honeys itself. The other tested strains showed extremely strong growth on the medium with a sugar solution (+++).

Totally 54.5% of samples (n = 24) displayed high antibacterial activity and inhibited the growth of all the tested bacterial strains (n = 65). The inhibitory effects were greater on all bacterial strains in honeydew honeys (73.3%, n = 11) than in blossom honeys (44.8%, n = 13). The higher antimicrobial activity of honeydew honeys, thanks to their large content of peroxide components, is also confirmed by other authors (Molan et al. 1992; Vorlová et al. 2005; Moţ et al. 2016 and Pita-Calvo & Vázquez 2017). Growth of at least one bacterial strain of methicillin-resistant *S. aureus* (MRSA), methicillin-sensitive *S. aureus* (MSSA), *Kl. pneumoniae, Kl. oxytoca* or verotoxigenic *E. coli* was seen with other samples of honey (45.5%, n = 20). Bacterial cultures grew far more intensively with blossom honeys.

The samples of honey showed lowest antibacterial activity on *Klebsiella* bacteria (Table 2). Strains of *Kl. pneumoniae* and *Kl. oxytoca* grew on 40.9% of the honeys.

Table 2. Number of samples of honey inhibiting all the tested strains of a given species of bacteria

Bacterial species	Number of strains	Number of samples of honey without bacterial growth	[%]
Methicillin-resistant *S. aureus*	11	36	81.8
Methicillin-sensitive *S. aureus*	10	37	84.1
Staphylococcus xylosus	1	44	100
Streptococcus dysgalactiae	2	44	100
Listeria monocytogenes	10	44	100
Klebsiella pneumoniae	3	26	59.1
Klebsiella oxytoca	11	26	59.1
Verotoxigenic *E. coli*	15	33	75
Paenibacillus alvei	1	44	100
Paenibacillus larvae	1	44	100

The degree of sensitivity of microorganisms to the action of honey is strain-dependent. The antibacterial effects of honey also differ depending on the type of honey. Acacia and sunflower honeys inhibited the growth of all bacteria. Maple honey also showed high antibacterial activity and inhibited all the tested cultures with the exception of 5 strains of *Klebsiella*. Each honey has its own unique composition. The interaction of honey and bacteria is influenced by many factors, e.g. the type of vegetation on which the bees feed and the differing concentration of hydrogen peroxide. Geographical origin also plays an important role (Vorlová et al. 2005; Cornara et al. 2017 and Pita-Calvo & Vázquez 2017). The age of the honey did not have an effect on its antimicrobial activity.

Conclusions

Honeys produced in the Czech Republic and Slovakia were proven to have antibacterial effects and be capable of inhibiting the growth of significant pathogens to a certain degree. These effects differ depending on the type of honey. Honeydew honeys showed greater antibacterial activity. In view of the global problem of the increasing resistance of bacteria to antibiotics, honey and products made from honey represent an alternative with good prospects in the prevention and treatment of infectious diseases, particularly of the skin and the mucous membranes. Consideration must, however, be given to the possible differing action of various types of honey and to the strain-specific sensitivity of pathogens to these antibacterial constituents.

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