Laboratórium apidológie and apiterapie
Oddelenie mikrobiálnej genetiky
Ústav molekulárnej biológie SAV
Dubravská cesta, 9 845 51 Bratislava

Analysis of antibacterial efficacy of honey samples

Name of customer: Rudkovsky Igor Gennadievich

Address of customer: Russia, Pskov region, Pechora district, Panikov parish, Shumilovo farm. Postal Code 181511

Year of production: 2019

Honey samples:

Sample No.	Type of honey
1	Apis mellifera carnica
2	Apis meliffera mellifera

Standardized honey (the base for medical grade honey): Manuka honey ORA UMF 15+ (Whakaari International, Whakatane, New Zealand)

Control honey solution: artificial honey (sugar solution where sugars are at same concentrations like in natural honey to maintain identical osmotic properties)

Laboratory analysis performed at: Laboratory of apidology and apitherapy, Institute of molecular biology, Slovak Academy of Sciences, Bratislava, Slovakia

Measured parameters and activities: a total honey antibacterial activity, overall protein profile of honey and quantification of hydrogen peroxide in diluted honey solution (40%) after 24 h of incubation at 37°C

RESULTS OF ANALYSIS AND DISCUSSION

Antibacterial activity of honey samples

Antibacterial activity of honey is depended on several different factors: high osmotic pressure (high sugar content), low pH (due to gluconic acid formation), the presence of antibacterial peptide defensin-1, production of hydrogen peroxide (generated by enzymatic reaction in the presence of glucose oxidase enzyme as well as by polyphenolic action), the presence of various phytochemicals and polyphenols. Antibacterial activity of some specific honeys such as manuka and honeydew honeys has different mechanisms of action due to presence of specific phytochemicals which are dominant antibacterial factors in these honeys.

In a case of manuka honey, methylglyoxal (MGO) plays a crucial role in antibacterial action. Honey antibacterial properties can be expressed as a minimal inhibitory concentration (MIC), *i.e.* the percentage of honey solution that is able to inhibit the bacterial growth. With decreasing value of the solution percentage increasing antibacterial efficacy of particular honey.

Analysis of two Russian honey samples and standardized manuka honey revealed that all tested honey samples were more effective against *Staphylococcus aureus* in compare to *Pseudomonas aeruginosa*. Both Russian samples showed to be more potent against both bacterial isolates when comparing to manuka honey. Russian samples were particularly effective against *Pseudomonas aeruginosa*, where both honey samples at concentration of 7% were able to inhibit growth of *Pseudomonas aeruginosa* in compare to manuka honey where bacterial growth inhibition was observed at concentration of 14%. Interestingly, antibacterial efficacy of both Russian samples was identical.

In a case of artificial honey, only high sugar concentration is able to inhibit bacterial growth. When the MIC values for honey sample are close to MIC values for artificial honey, antibacterial activity of honey samples will depend mainly on sugar content. It happens when honey was heated or prolonged storage.

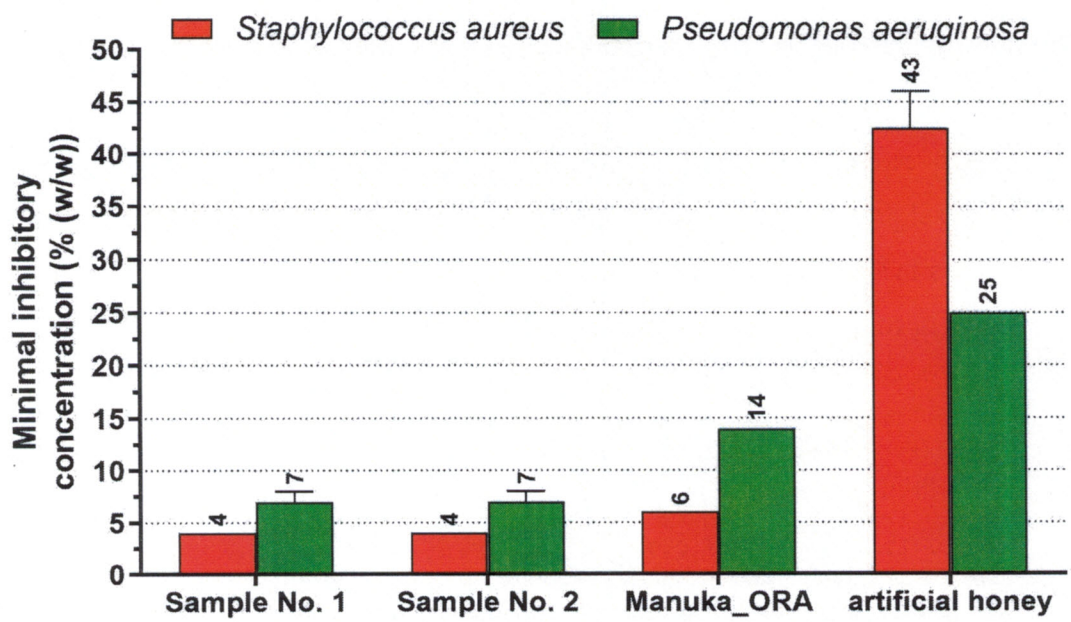

Figure 1. Antibacterial activity of honey samples determined by a minimal inhibitory concentration assay against two bacterial strain *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Electrical conductivity of honey samples

Blossom and honeydew honey are differentiated by electrical conductivity. According to Codex for honey standards (Codex, 2001), electrical conductivity less than 0.8 mS/cm indicates blossom and more than 0.8 mS/cm indicates honeydew honey with the exceptions of some types of honey including chestnut, linden, manuka or eucalyptus honey. The values of electrical conductivity for both Russian samples are shown in Table 1.

Table 1. Electrical conductivity of honey samples.

Sample No.	Type of honey	Electrical conductivity (mS/cm)
1	Apis mellifera carnica	0.362
2	Apis mellifera mellifera	0.389

Proteins' profile of tested honeys

We determined the presence of total protein content which should be mostly bee-derived. According to this analysis it might be revealed the adulterine honey (artificial honey or diluted honey with sugar solution). In the case of adulteration, very weak bands would be visible on Figure 2. Both Russian tested honeys (R1-R2) exhibited adequate protein amount; however, the protein profile is not identical with other honey samples from Slovakia (S1-S3, Figure 2). Both samples contained major royal jelly proteins (MRJPs) and the most intensive band should represent MRJP1, a dominant honey protein which are secreted into the nectar during its transformation into a honey. On the other hand, both tested samples showed additional proteins/peptides (green arrow) which, most likely, come from nectar or pollen. We are sure that they are not secreted from bees. A detailed proteomic approach could identify the origin of these unknown proteins/peptides.

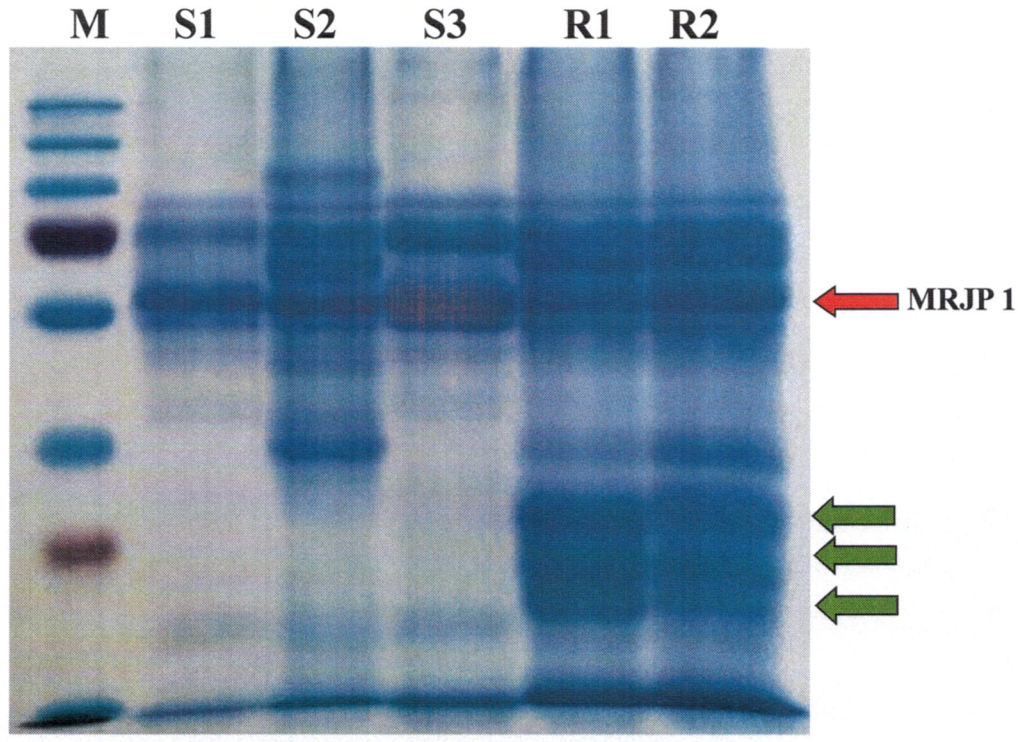

Figure 2. Protein profile of various honey samples. S1-S3 – represent Slovak honey samples collected in 2019, R1 and R2 are Russian tested honey samples No. 1 and No. 2, respectively. M – Protein marker (a mixture of pre-stained proteins with exact molecular weight). Red arrow represents the position of dominant protein (MRJP1) in honey. Green arrows represent unknown proteins/peptides in honey samples.

Hydrogen peroxide production in honey samples

As it has been shown in previous literature, a strong correlation between the antibacterial activity and the level of hydrogen peroxide was documented. Therefore, the level of hydrogen peroxide after incubation of honey samples could be linked with overall antibacterial efficacy of honey samples. Honeydew honeys together with blossom polyfloral honeys produce the higher levels of hydrogen peroxide in compare to monofloral blossom honey types.

Both two tested honey samples were capable to accumulate hydrogen peroxide after 24 hours incubation at 37°C. Honey sample No. 2 was superior to honey sample No. 1 with average value of hydrogen peroxide concentration of 2116 μM and 1088 μM, respectively (Figure 3). Although, honey samples No. 2 produced two-times more hydrogen peroxide, the

overall antibacterial activity of both samples was identical.

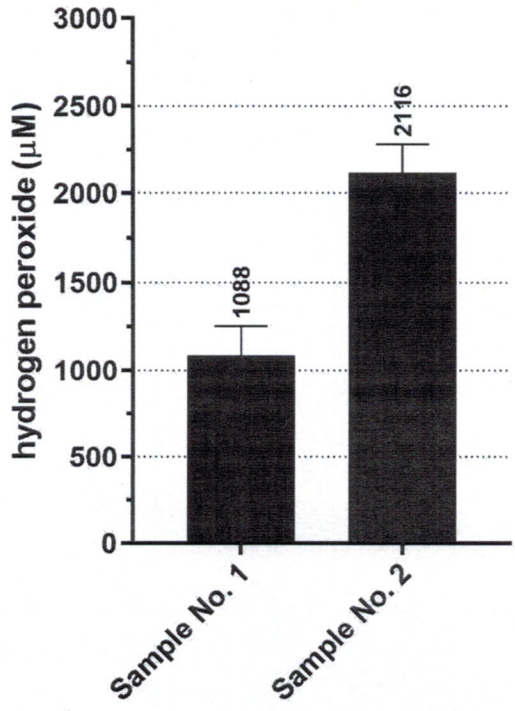

Figure 3. Hydrogen peroxide production in honey samples. Russian tested honey samples No. 1 and No. 2, were diluted at concentration of 40% (w/w) at pH value of 7.0 and hydrogen peroxide was measured after 24-h incubation of honey solutions using a commercially available kit. Data represent mean value with standard deviations.

Conclusions

Obtained results from analysis of two Russian honey samples suggest that both honey samples exhibited a strong antibacterial activity against two wound pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Antibacterial activity of both samples was superior to activity of manuka honey, which represents the most expensive honey in the market. Interestingly, the protein profile of both samples was identical but different to European honey samples. Russian honey samples contained the proteins/polypeptides with unknown origin, most likely are nectar/pollen-derived proteins. Based on data from the electrical conductivity assay we can conclude that both honey samples are clearly blossom honeys without the honeydew honey.

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From our ongoing and long-lasting research in apimedical science, we can conclude that polyfloral honey samples and mixture of blossom and honeydew honeys as well as honeydew honeys accumulated the higher levels of hydrogen peroxide and thus, representing the ideal source for medical-grade honey or health-care cosmetics.

Ústav molekulárnej biológie
Slovenskej akadémie vied
Dúbravská cesta 21
845 51 Bratislava
IČO 00166634 DIČ 2020894689

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Ing. Juraj Majtan, DSc.
Head of Laboratory of apidology and apitherapy
Institute of Molecular Biology SAS