# PROTEOMICS OF ROYAL JELLY PROTEINS- FROM EMPIRICISM TO SCIENTIFIC APPLICATIONS OF BEE PRODUCTS IN APITHERAPY.

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# THE ACTUAL MATTERS IN APITHERAPY

***FIGHT FOR THE BEST HONEY IN THE WORLD***

# *DISCUSSION*

***Reproduced from:*** *apitherapy-list@googlegroups.com*

### *E. mail Nr. 1*

***Dear Stefan and Apifriends,***

„All natural honey are good, a matter of using, applying and evaluating them appropriately.
We need more naturally produced honey without adulterations and over processing.“

*Siti Amrah, Malaysia*

### *E. mail Nr. 2*

***Dear Stefan,***

„I do believe that different honey's have different properties and that honey's such as Manuka may help with some disorders that other honey's don't.

But I certainly do believe that saying that it is the **"best' honey in the world“** or that it is worth the exorbitant prices that are being charged for it are **just very clever marketing.“**

Thank you Stephan (and all the others) who are keeping up the good fight.

*Wayne Myburgh., bee-keeper in Zululand in South Africa since 1992*

# *SUMMARY*

**Dear Siti and Apifriends,**

Yes, you are right. You and your team of researchers from Malaysia have brilliantly proved that natural honeys like Tualang or Gelang honey are also, besides excellent antimicrobial honeys, very good antioxidants, antitumoral, etc., etc.

I personally do not like the marketing part and the huge final prices of honeys like Manuka, Medihoney or now Surgihoney...

**The good news though, we must recognize that, is that these kind of honeys have open the "doors" of many hospitals and are impressing now many sceptical allopathic doctors.**

After some more years the pharma industry and the allopathic doctors will hopefully understand that you can **perfectly treat complicate wounds** ***with very cheap, but good quality honeys****...*

Best regards from Romania

*Dr Stefan Stangaciu*

## ***CONCLUSSION***

Empirical experiences of ***practical apitherapy*:**

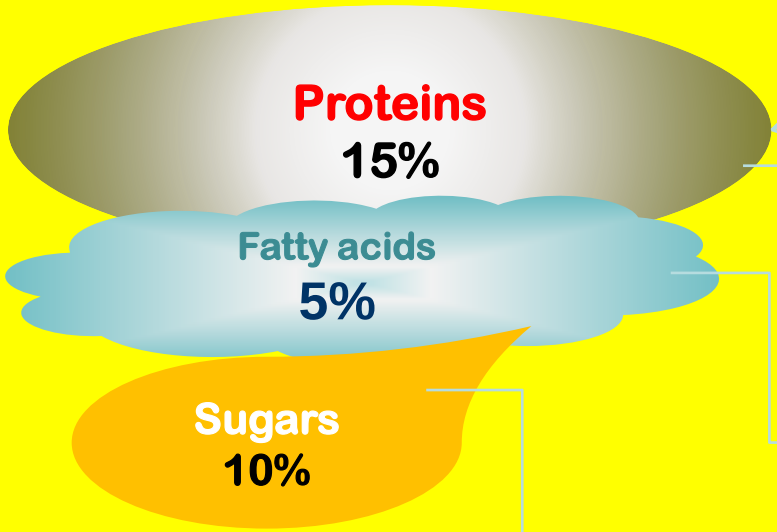
***All honeys are healing, if authentic***

Experimental evidences of ***molecular apidology:***

***Common denominator of authenticity of all honeys are proteins of royal jelly***

# Royal Jelly

RJ is a secretion product of the cephalic glands of nurse bees and is considered as a **unique nutrient developed in nature during evolution of animals**
Composition of RJ varies with seasonal and regional conditions.




APALBUMIN 1-9 : major RJ proteins--90%

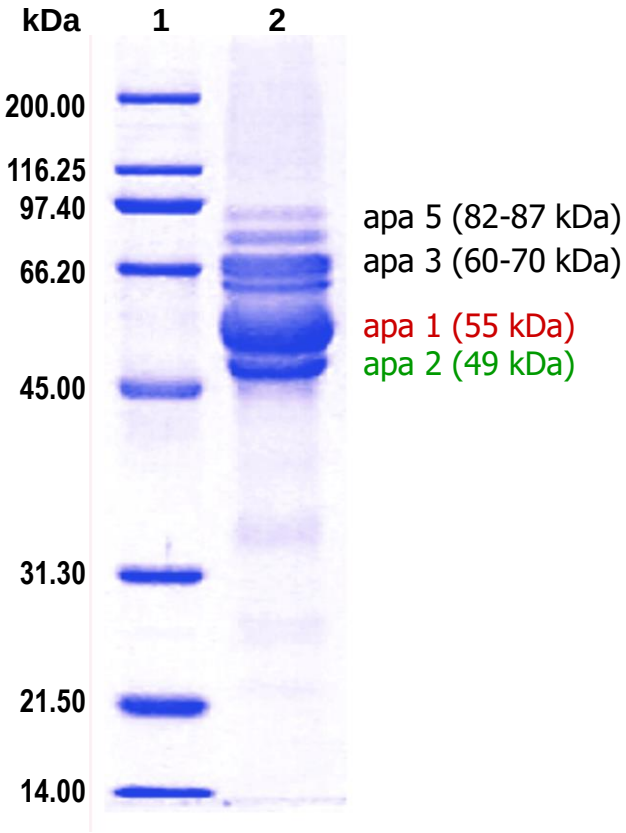
- Apalbumin1 ---------- 60%
- Apalbumin2-5 ---------- 34%
- Apalbumin 6-9 ---------- 1%
- Isoforms apalbumins 1-5 ---- 5%

Enzymes ----10%

Hydroxy decanoic acids

Honey

# APALBUMINS - major proteins of royal jelly (MRJPs)



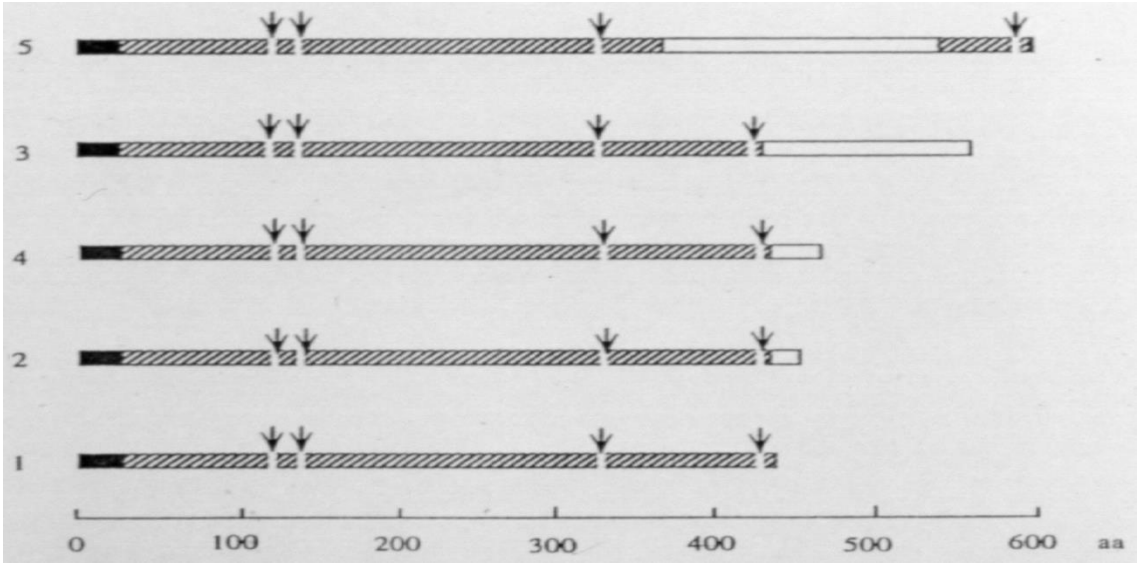

- dominant component of honeybee RJ proteins (90%)
- Mw 49-87 kDa
- although MRJPs are structurally closely related to each other, the expression profiles of MRJPs genes are not identical
- nutritional function
- multifunctional biological properties

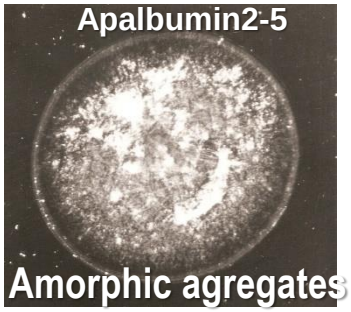
**Royal jelly proteins are honeybee specific,** they do not have any relatives in other insect or other metazoan species

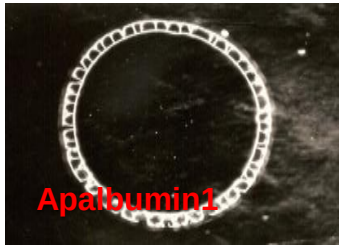
*Šimúth, J. and Bíliková K. as Members of Scientific Consortium: Insights into social insects from the genome of the honeybee Apis mellifera. Nature 443, 931-947 (2006).*

*Slovak Academy of Sciences*
*Bratislava, Slovakia*

# Maternal apalbumins-high degree of sequence homology-different function in honey bee colony



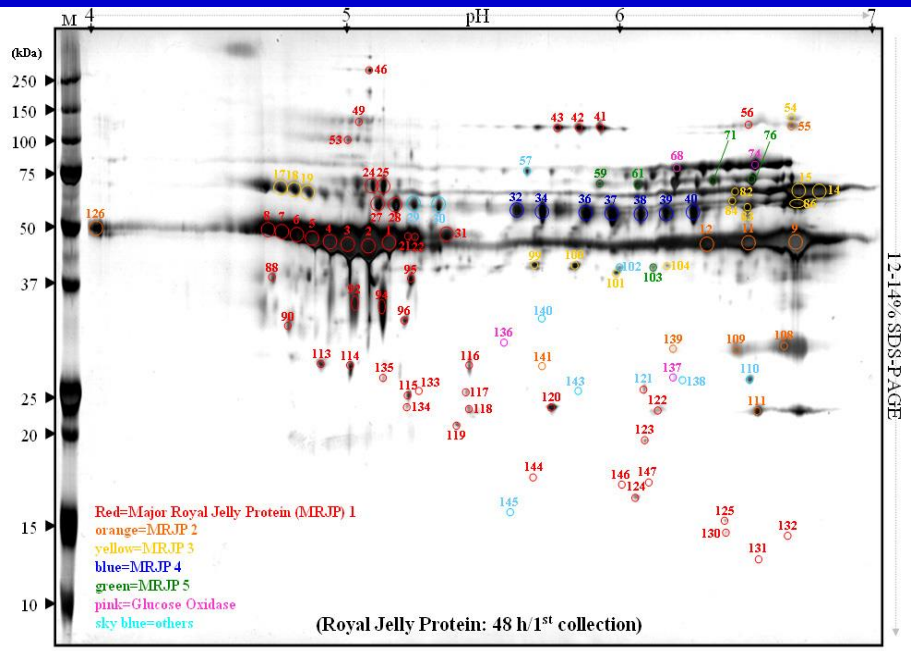




**Apalbumin2-5 (hydrophilic proteins)**
***amorphic water soluble agregates***

**Apalbumin1 (hydrophobic protein)-important properties for cosmetics application**
***regular selfassembled repeated structures***

# PROTEOMICS OF ROYAL JELLY PROTEINS




**2-D gel reference map of Royal Jelly proteins identified by nESI LC-MS/MS**

*Furusawa et al. (2008) Journal of Proteome Research 7, 3194-3229*

**Despite the fact that the honeybee genome contains a single copy of 9 apalbumins gene RJ icontains a large number of different isoforms of maternal apalbumins**

**Recent studies on proteomic complement of RJ showed on 2D gel at least 50 distinct proteins spots, that were identified by MS as apalbumins.**
***Jianke et al., 2007; Furusawa et al., 2008***

**Maternal aplbumins formed great number of minority isoforms with various posttranslational modifications and different physiological properties.**
***Bíliková et al., 2009***

**Isoforms of maternal apalbumins –**
minor proteins *manage*
**THE MAJOR PROCESSES in the honey bee colony**

Royalactin - isoform of apalbumin1: queen-differentiating factor.

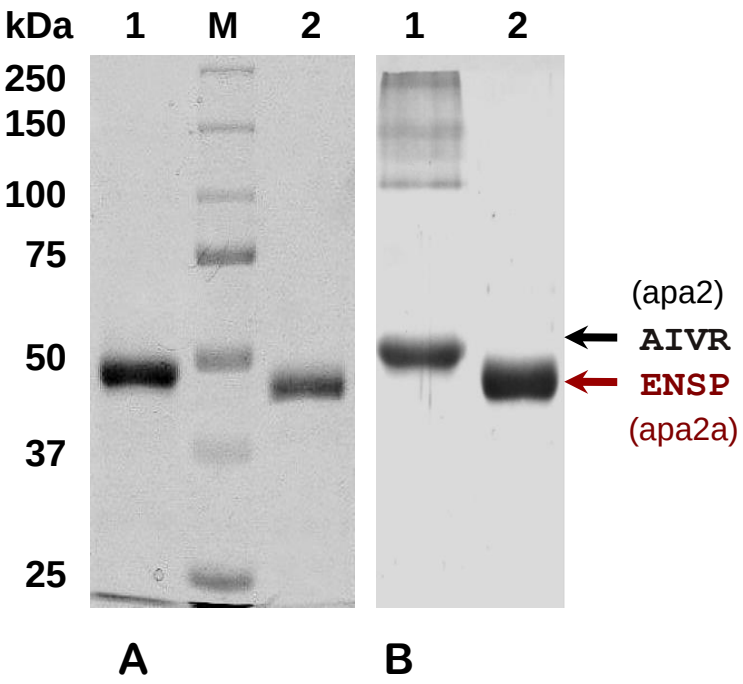
Masaki Kamakura shows that ***royalactin is*** responsible for the increased body size and ovary development and

shortened developmental time in of queen larvae

in comparisson to worker

*Kamakura, M. (2011) Royalactin induces queen differentiation in honeybees. Nature **473**, 478-483.*

# Apalbumin2a, isoform of apalbumin2 a new antimicrobial protein of RJ




**A . 10% SDS PAGE**
Coomassie blue staining

**B. Western blot analysis**
with polyclonal rabbit antibody against apalbumin2.

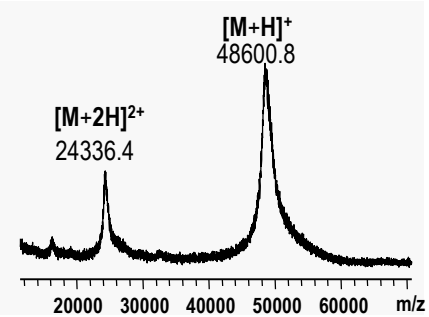
*Lane 1* (apa2) - apalbumin2
*lane 2* (apa2a) - apalbumin2a
*M* - protein molecular weight marker

*Bíliková K, Mirgorodskaya E, Bukovská G, Gobom J, Lehrach H & Šimúth J (2009) Proteomics, 9, 2131-2138.*

# PROTE - *OMICS* characterization of apalbumin2a

**MS**

intact protein MW determination




Calculated MW = 50,111 Da
Observed MW = 48,600 Da

*digest*

tryptic peptides

**MALDI-TOF MS**

direct peptide mapping
protein identification

SwissPort database

**apalbumin2**

predicted peptide sequences of *MSMS*_mrjp2

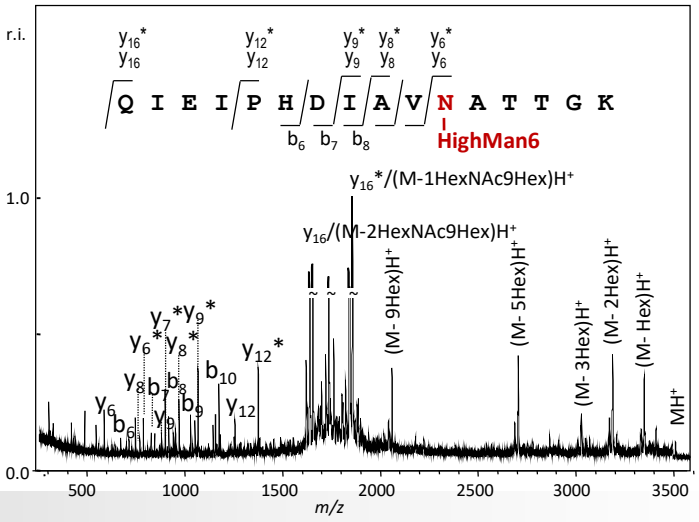
*compared*

- honeybee-EST clusters
- other MRJP
- honeybee-genome

**Nano LC**

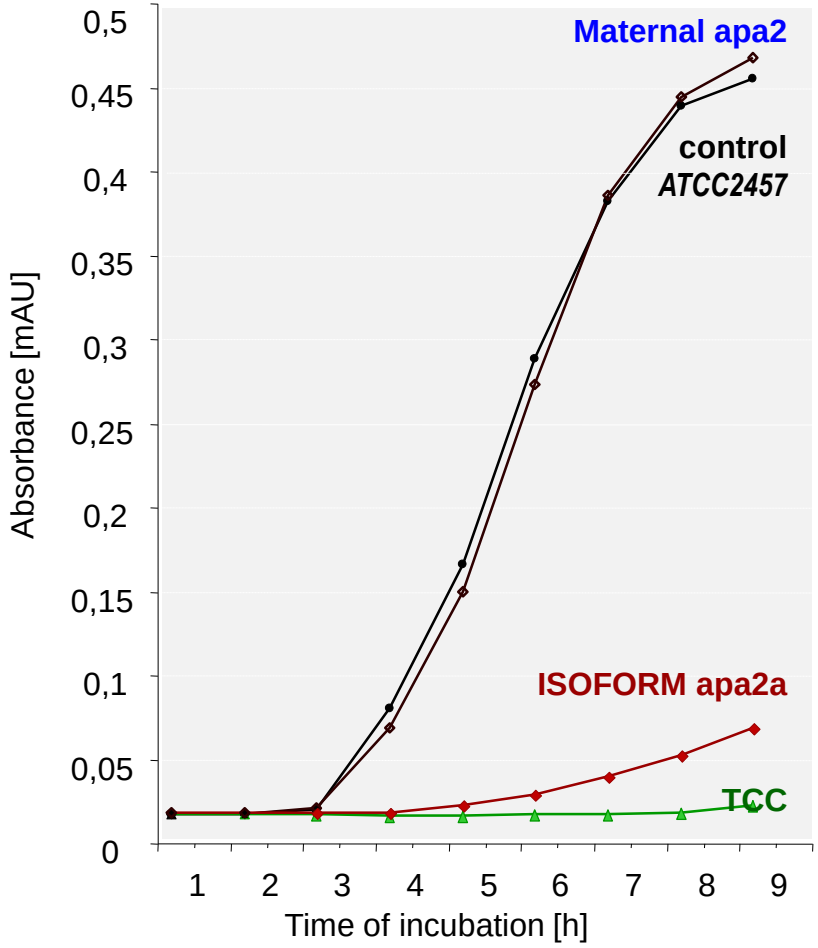
**MALDI-TOF/TOF MS**

improved sequence coverage and for protein characterization




*Bíliková K, et al (2009) Proteomics, 9, 2131-2138.*

# Antibacterial effect of apalbumin2a on the growth of *P. larvae*



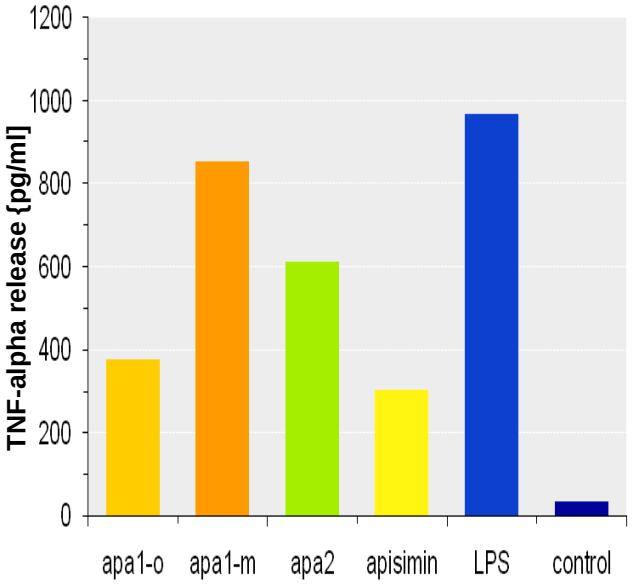

Growth of *Paenibacillus larvae* (ATCC 2547) in MYPGP medium, at 37°C, with addition of:

apa2 - apalbumin2 ($1.8 \times 10^{-4}$ M);
apa2a - with addition of apalbumin2a ($1.8 \times 10^{-4}$ M)
TCC - with addition of tetracycline hydrochloride ($1 \times 10^{-5}$ M) as a positive control.
control - *P. larvae* withouth of antimicrobial agent

Absorbance measured at 595 nm.

*Bíliková K, et al (2009) Proteomics, 9, 2131-2138.*

# Physiological activity of apalbumins
## as immunostimulator agents determined by production of TNF-alpha

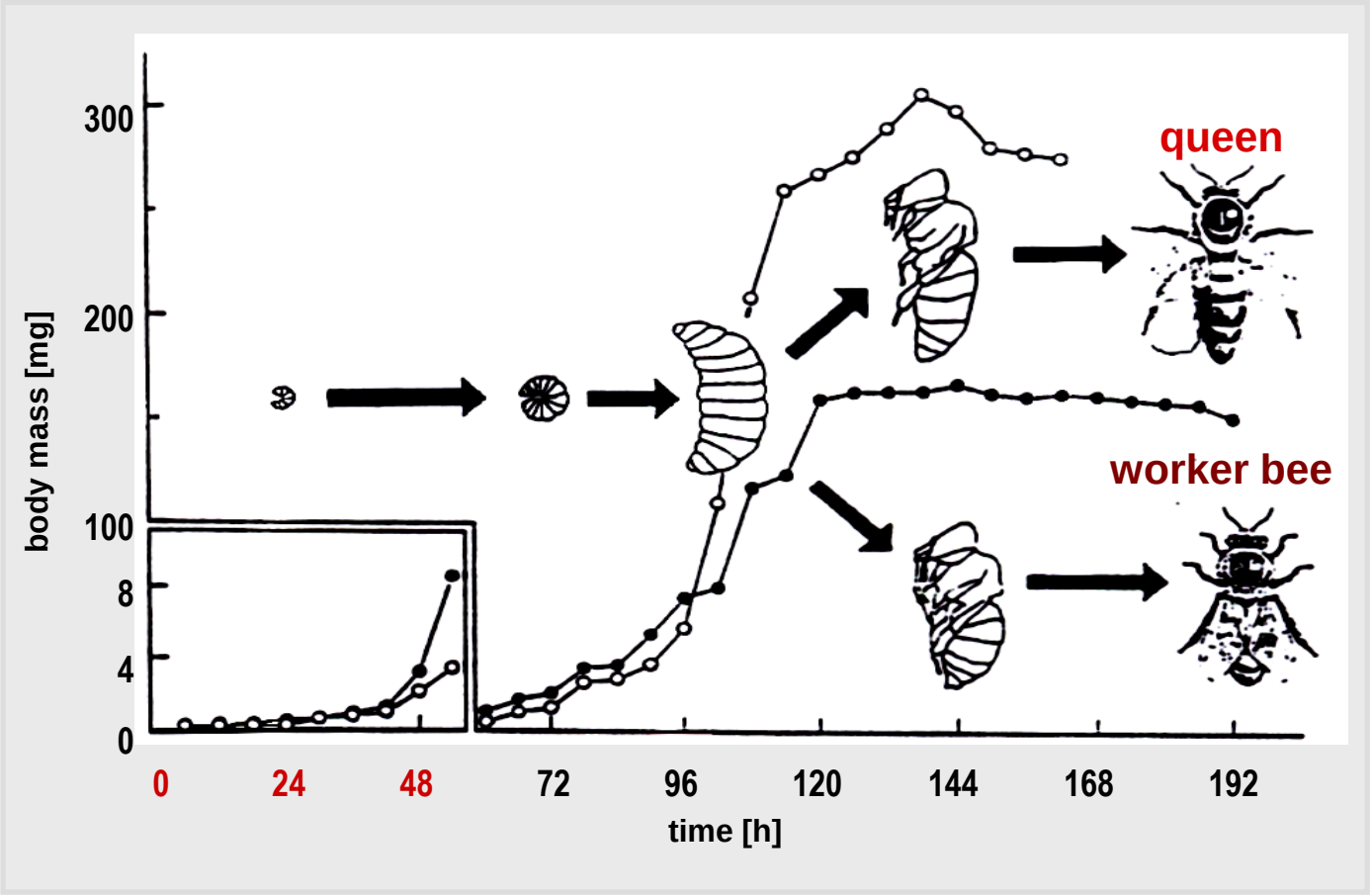



**TNF-alpha release from murine macrophages by royal jelly proteins-apalbuins (apa) after 6 h of incubation. Mouse macrophages, cell line: ATCC RAW 264.7 LPS - lipopolysaccharide of Salmonella typhimurium**.

*Šimúth J., Bíliková K., Kováčvá E., Kuzmová Z., Schroder W. (2004) J. Agric. Food Chem. 12154-12158.*

**The apalbumin1**
play a pivotal role
-in the nutrition, immune defense,
and cast determination of honeybee larvae
-have a wide range of pharmacological
and health-promoting functions
for humans as well

# Larval development of honeybee queen and worker bee is inluenced by by apalbumins




The phenomenon of long life span of honeybee queen made a basis for opinion that RJ might be the means for long-living of human beings.

# The paradoxy of queen honeybee longevity and aging

**The oxidative stress theory of aging proposes that accumulation of oxidative damage is the main proximate cause of aging and**

**that longevity is determined by the rate at which this damage occurs.**

## Paradoxy Nr.1

**Worker and queen honey bees (*Apis mellifera*) develop from the same genome, but queen lifespan is about 10 x longer.**

## Paradoxy Nr.2

**The expression of antioxidant genes generally decreased with age in queens, but not in workers.**

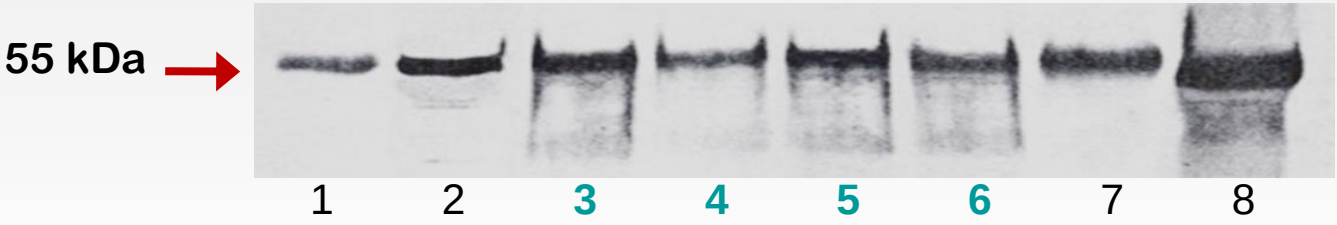
(Corona, M.et al.(2005) Gene expression patterns associated with queen honey bee longevity.*Mechanism of Agening and Development 126*, 1230-1238.)

## Paradoxy Nr.3

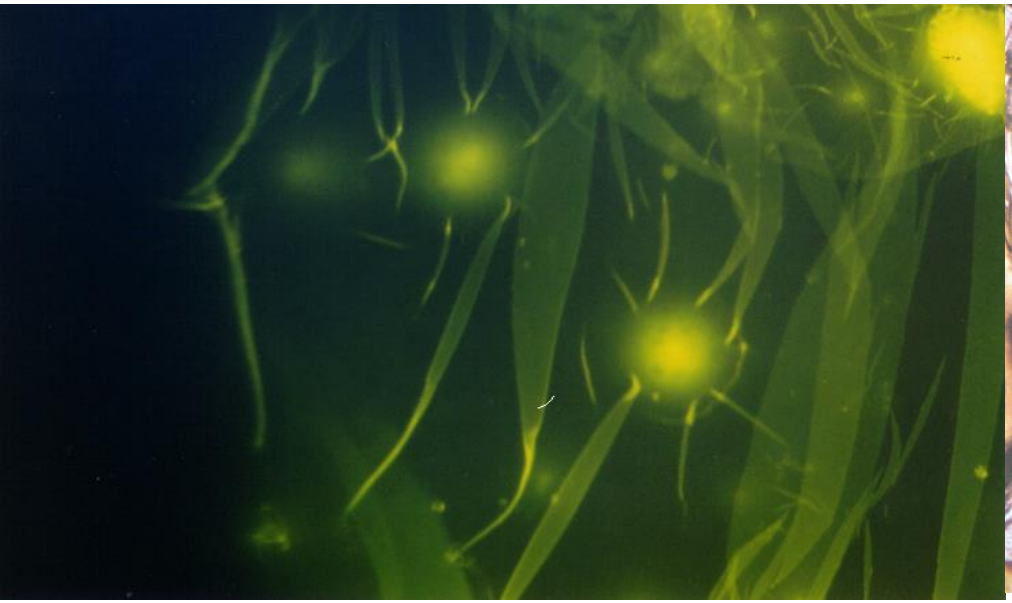
***Queen honey bee longevity*** **appears to have evolved** ***on base of royal jelly proteins*** **via** ***mechanisms other than increased antioxidant gene expression***.

**The APALBUMIN 1 is regular component of honey and forms nanostructural membrane that provides an improved surface for evaporation of water from nectar**

*Light microscopy observation*

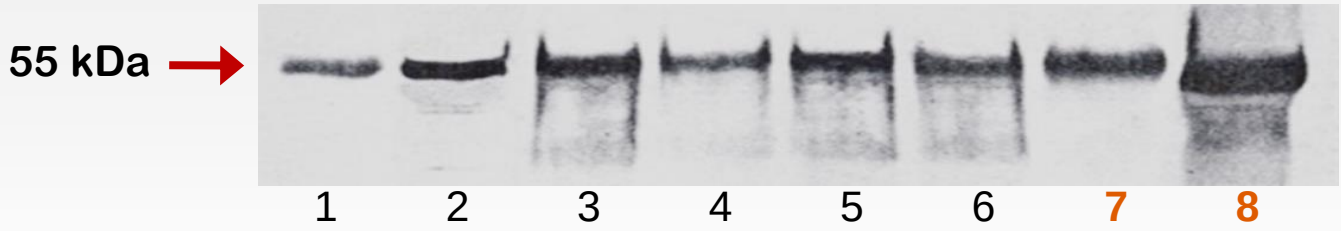



**1 - Apalbumin1**; 2 - RJ; **3 - Aacacia; 4 - Apple tree; 5 - Raspberry; 6 - Sunflower**
7 - pollen pellet; 8 - pollen bread



# Immunochemical detection of apalbumin1 in honey and bee pollen

*Šimúth J., Bilikova K. et al.(2004) J. Agric. Food Chem. 52, 2154-215*

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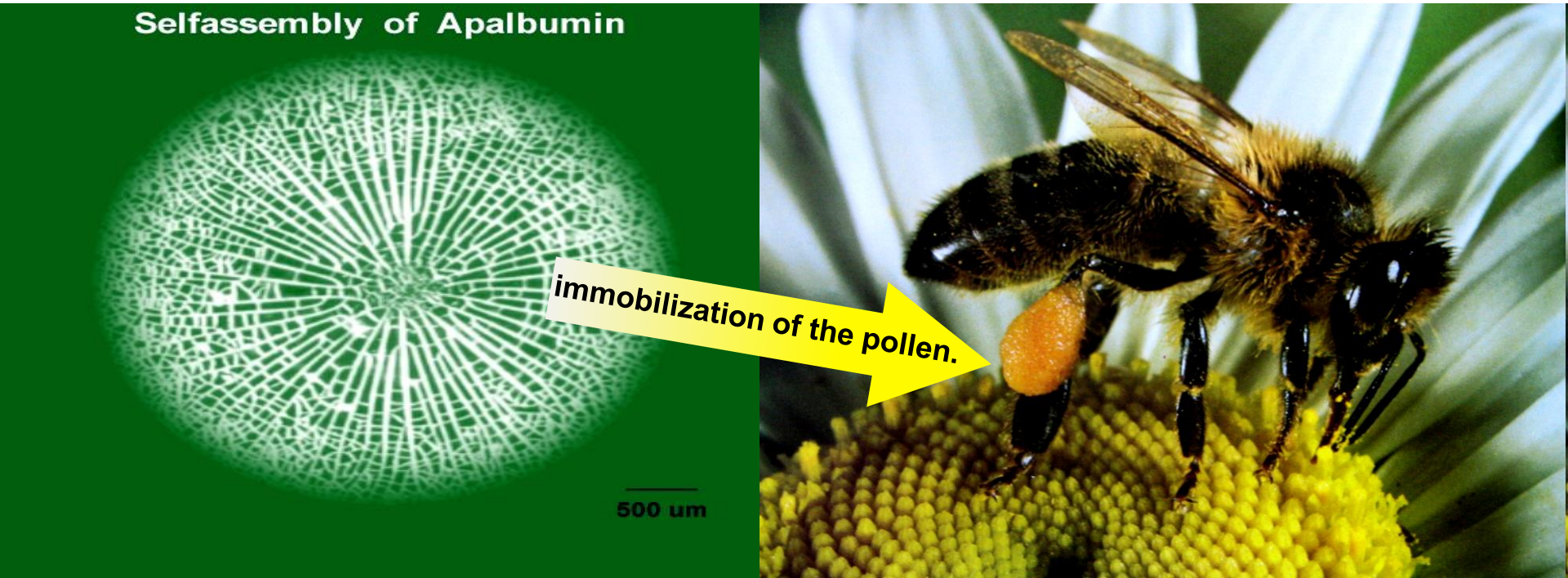


# Apalbumin1: criterion of authenticity and quality of honey bee products

Until now, the properties of honey have been defined based exclusively on the content of plant components in the nectar of a given plant.

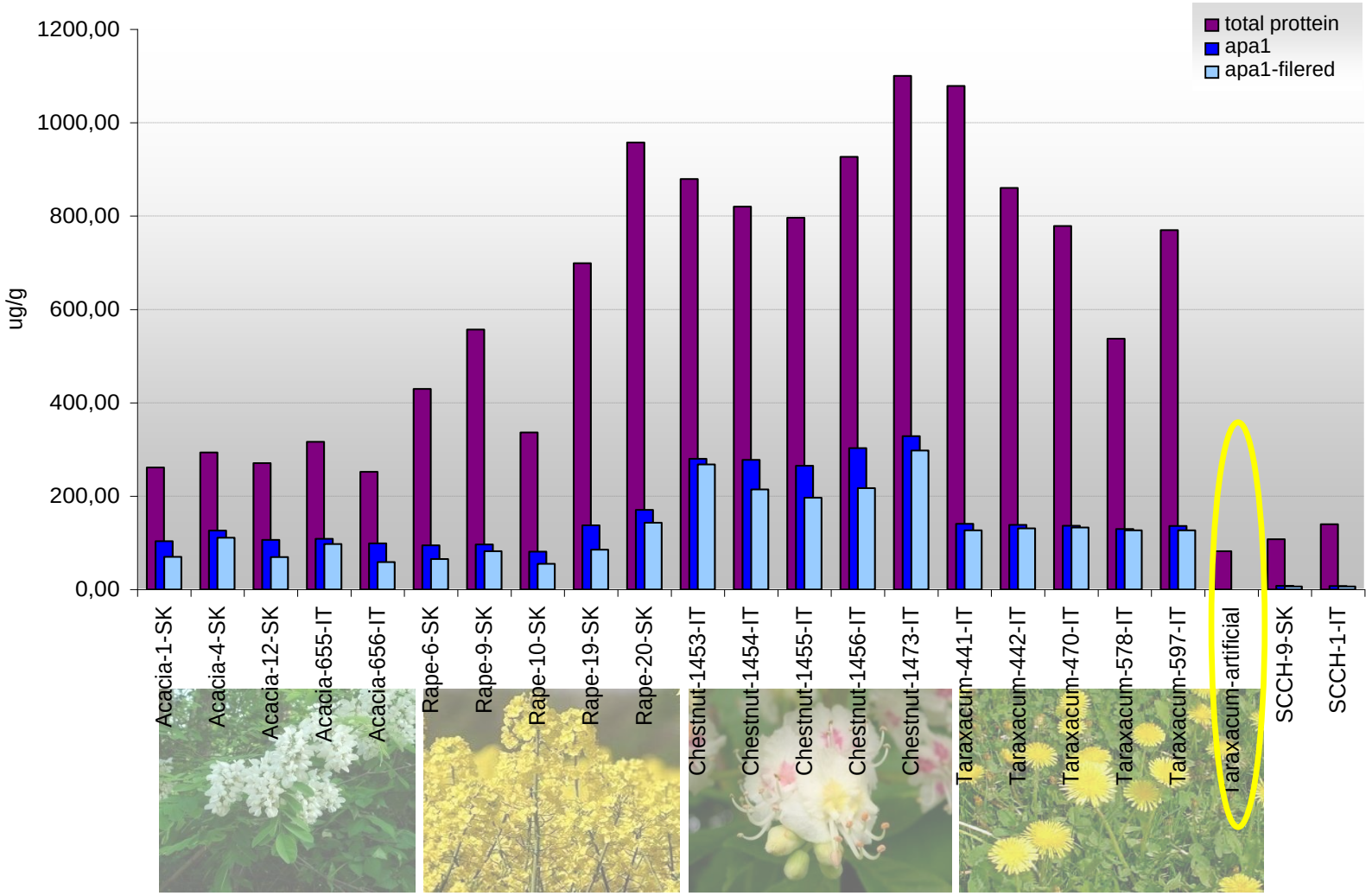
A new, rapid, highly specific, simple, reproducible, and sensitive enzyme-linked immunoassay (ELISA) of quantitative determination of predominate of honeybee royal jelly (RJ) protein apalbumin1 in honey and royal jelly has been developed.

Bíliková K., Šimúth J. (2010) New criterion for evaluation of honey: quantification of royal jelly protein apalbumin 1 in honey by ELISA. J. Agric. Food Chem. 58, 8776-8781

# Amount of Apalbumin1 versus total protein content in honey samples

***Apalbumin1 represent of the most physiologically***

active components of honey.



# Screening of Europen Floral honeys on production of cytokines

(Final Report of BEESHOP Projekt 6RP EU -15.5.2009)

1. The tested honeys of BEE SHOP samples
   and their proteins stimulated production TNF – alfa
   in range from 300 to 1 200 pg cytokine per ml.

2. Deproteinized honeys didnt stimulated production of TNF-alfa

3. **Apalbumins determine imunostimulatory properties of honey**

# Physiological activity of apalbumins

## Nutrition

Donor of proteinous nutritution (80%) in larval diet. Essential proteinous component of honey.

*Šimúth et al., 2004, J. Agric. Food Chem. 52: 2154-2158*

*Šimúth J., Bíliková K., 2004, Honeybee Science 25: 53-62*

## Antibiotic activity - antibacterial, antifungal, antiyeast

*Fujiwara S.; Imai J. et. al, 1990, J. Biol. Chem. 265:11333-11337*

*Bilikova K.; Gusui W.; Simuth J. Apidologie, 2001, 32: 275-283*

*Bíliková K.; Hanes J.; Šimúth J. et al.,2002 FEBS Letters, 528: 125-129*

*Fontana et al. Peptide, 2004; 25: 919-928*

*Bilikova et.al, Proteomics, 2009, 9: 2131-21388*

## Immunostimulation

N-terminal protein domain of apalbumin1 is most effective stimulators of TNF-alpha release and it is not affected by posttranslationaln modifications.

*Šimúth J., Bíliková K., Kováčvá E., et al., 2004, J.Agric. Food Chem. 12154-2158*

*Majtán J., Kováčová E., Bíliková K., Šimúth J., 2006, International Immunopharmacology, 6: 269-278*

## Antihypertensive activity

Peptides derived from apalbumin1 possess potent antihypertensive activity.

*Matsui et al., J. Nutr. Biochem. 2002; 13: 80-86*

## Proliferation

*Kamakura et al., Bioch. Bioph. Res.Comm., 2001; 282: 865-874*

*Kimura, M.; Kimura, Y.; Tsumura, et. al., 2003 Biosci. Biotechnol. Biochem., 67: 2055-2058*

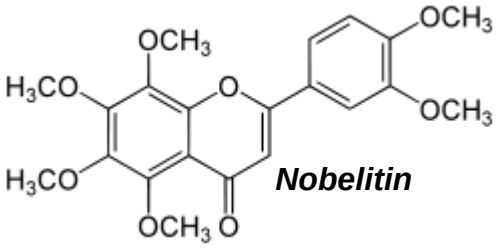
## Suppression of allergic reaction

*Okamoto et al., Life Science 2003; 73: 2029-20045*

# NEW TREND INAPITHERAPY - Extending the therapeutic effect of bee products by adding flavonoids

## Nobiletin

citrus flavonoids isolated from citrus peels hydrophobic character, a bitter taste

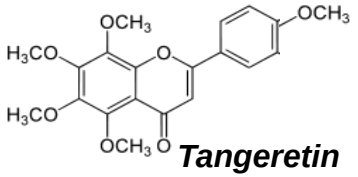


***Nobelitin***

**anti-inflammatory and anti-tumor invasion, proliferation, and metastasis** *in vitro* and *in vivo*
Nobiletin was also found to potentially inhibit
**cartilage degradation**, can be used to treat arthritis

2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxychromen-4-one
Mw 402.4, $C_{12}H_{22}O_8$

*Henrotin, Y. Et al, (2013), Osteoarthritis and Cartilage **19** (1): 1-21doi:10,1016/joca,2010,10,10.17*



***Tangeretin***

**Japan Royal Jelly Co. Ltd. Tokyo, Japan have developed a drug containing RJ and Nobiletin to prevent or delay neurodegeneration in the brains of patients with Alzheimer's disease**

*Hironori Fujiwara, et all (2011) J Pharmacol. Sci. 116, 384 – 387.*

5,6,7,8-tetramethoxy-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one
Mw 372.37 $C_{20}H_{20}O_7$

# Postgenomics in Apidology and APITHERAPY

Bee genomics → Bee proteomics → APITHERAPY

The sequencing of honeybee genome opens a new territory of honeybee proteomics in pharmacy

*for research of* **physilogicaly active proteins and antimicrobial peptides**

*with the aim* of their application as:

**immunomodulators**
**suppressors of allergic reactions**
**anti-hypertensive agents**
**alternative of classic antibiotics**

# *CONCLUSSION*

**The RJ proteins**
**are potential protein markers**
**for preliminary immunochemical testing**
**of adulteration of honey and foods claimed honeybee products**

**and**

**a tool for exact physiological evaluation**
**of honey, RJ and bee pollen**
**for practical applications**
**in apitherapy**

# *RESONANCE*

**on**

***I. CONGRESS, EXPO AND WORKSHOPS OF THE INTERNATIONAL FEDERATION OF APITHERAPY AND VII. ROMANIAN APITHERAPY CONGRESS***

**in Brasov, Romania**

## IN HONOR

***OF THE I. CONGRESS, EXPO AND WORKSHOPS OF THE INTERNATIONAL FEDERATION OF APITHERAPY AND VII. ROMANIAN APITHERAPY CONGRESS***

we offer an assessment of quality honeys by quantification stable, physiologically active proteins, apalbumi1 in honeys samples of all continents.

**Participitants of these CONGRESSES can send samples of honeys to the following address:**

RNDr. Katarina Bilikova, PhD.
head of Dpt. of Molecular Apidology
Institute of Forest Ecology,
Slovak Academy of Sciences
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http://www.savzv.sk

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# Acknowledgment

**6.FP EU-022568 - BEESHOP**

Bees in Europe and Sustainable Honey Production.

**6.FP EU-037592 - CONCO**

Applied Venomics for Production of Inovative Biomedical Drugs.

**Partner Group Project**

Institute of Molecular Biology Slovak Academy of Sciences, Bratislava, Slovakia
Max-Planck Institute of Molecular Genetics, Berlin, Germany

Functional genomics of physiological active proteins and peptides of honeybee (*Apis mellifera* L.).