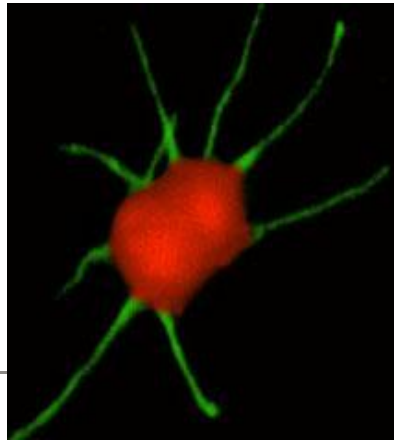
Antimicrobial activity of Finnish honeys against human pathogenic bacteria

Ruralia Institute

Adjunct Professor, Research Director

PhD Carina Tikkanen-Kaukanen

Antibiotic resistance



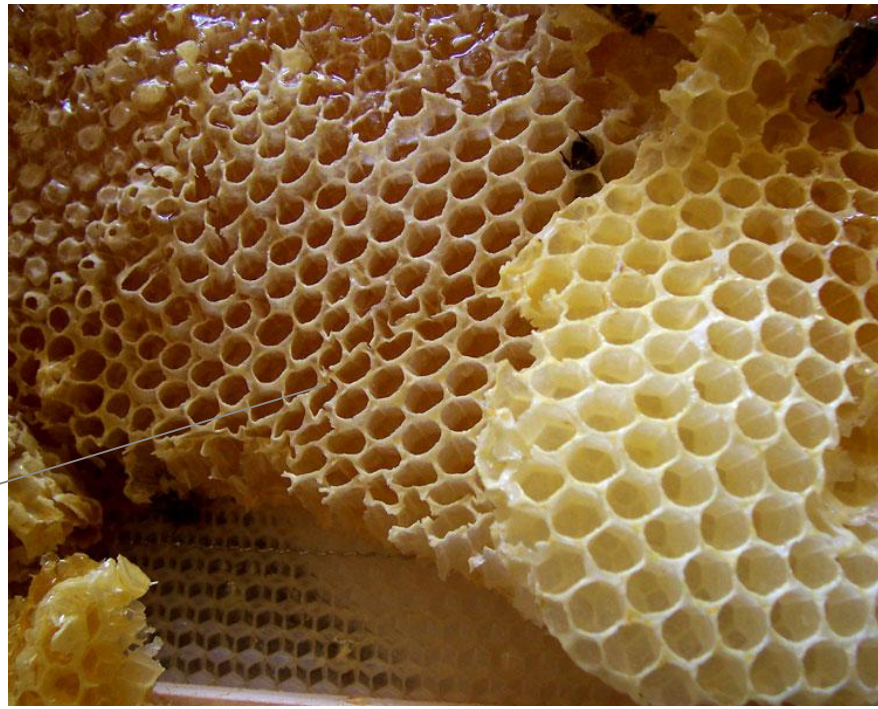
INSERM U570 Paris

Antibiotic resistance is a serious problem worldwide, and it has made the search for new antimicrobial compounds more important.

Honey has been used as a traditional medicine for centuries. Oldest information has been found from Egyptian pyramids. Already AD 50 Dioscorides recommended honey for wound healing.

Many *in vitro* studies have revealed antimicrobial activity of different honeys against a wide range of skin colonizing and food-borne bacterial species, including antibiotic resistant bacteria.

Modern beehive

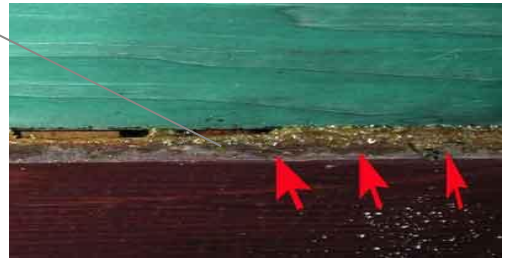


Beehive products

Beeswax (honeycombs)

Honey

Propolis



Bee pollen and bee bread

Royal jelly

Honey

Honey

European pharmacopeia 7. edition

HONEY*, Mel*

Definition

Honey is produced by bees (*Apis mellifera L*.) from the nectar of plants or from secretions of living parts of plants which bee collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature.

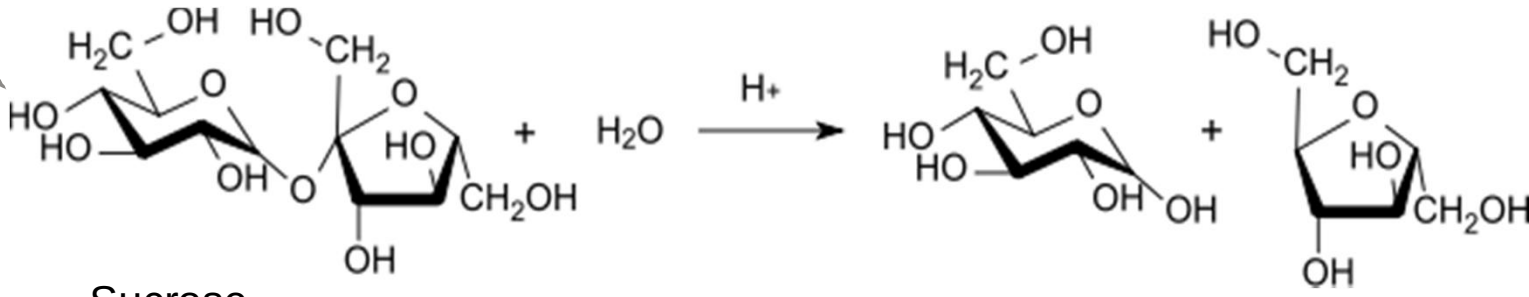
Honey

Characters

Appearance: viscous liquid which may be partly crystalline, almost white to dark brown

- The appearance may be a result from several factors, such as floral source, geographical origin or climate.
- Multifloral honeys, monofloral or unifloral honeys.

Composition of honey



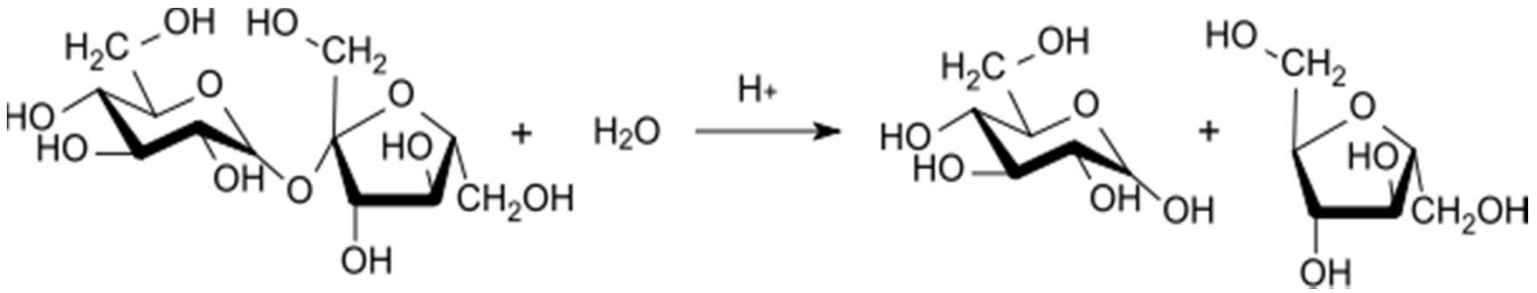
Sucrose

Invert sugar

- Honey is **70-80% of invert sugar**, which is a mixture of glucose and fructose. It contains small amount of sucrose (1%), maltose (7%) and higher sugars (1-2%).
- Honey contains **water** (17%), **proteins,** organic acids, acetylcoline, essential oils, mineral salts ja **phenolic compounds,** and when diluted, **hydrogen peroxide** is composed.
- Honey contains **aldonic acid,** which make **honey acidic** (pH 3,3-4,9)

Invert sugar

INVERSION



Sucrose Glucose Fructose

INVERT SUGAR

Change in optical rotation (invert) from left to right (+66.5º -39 º), when measured with polarmeter

→

Reported antimicrobial activity of honey

- *E. coli*
- *Staphylococcus aureus*
- MRSA
- *Staphylococcus epidermidis*
- Campylobacter
- Salmonella
- *Pseudomonas aeruginosa*
- *Klebsiella pneumoniae*
- *Helicobacter pylori*

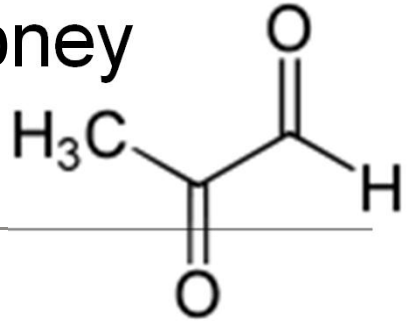
Why honey kills bacteria ?

- High osmolarity caused by high sugar concentration
- Low pH prevents the growth of bacteria
- Hydrogen peroxide, fenolic compounds, organic acids
- Effects on immune system
- Honey contains microbes that can be antimicrobial

  Bacteria which forms spores; yeasts

yests

Antimicrobial compounds in honey



- Antibacterial **methylglyoxal** has been found from Manuka honey from New Zealand
- (100-1000 mg/kg, conventional honeys 1-10 mg/kg)(Adams et al 2008, Carbohydrate Res; Mavric et al 2008, Mol Nutr Food Res).

- Bee defensin 1 protein from Revamil medical grade –honey in the Netherlands (Kwakman et al 2010, FASEB J)
  - Previously was found from bee royal jelly (Fujiwara et al 1990, J Biol Chem).

Manuka honey

Manuka tree (*Leptospermum polygalifolium*)

Organic honey

Production of organic honey is regulated by EU-directive.

- In Finland it is controlled by Evira (Finnish Food Authority): 18220/3, Organic Production 4.

- Main difference compared to non-organic honeys is purity: authenticity and chemical purity.

- Regulations concern mainly treatments of beehives and bees, feeding of bees and enviroment, where the necter is collected.

Our own research

I Finnish monofloral honeys

- Finnish Beekeepers Association
- Willow herb-, heather-, cloudberry-, lingonberry- and buckwheat honeys
- Sanna Huttunen, Kaisu Riihinen, Jussi Kauhanen & Carina Tikkanen-Kaukanen. Antimicrobial activity of different Finnish monofloral honeys against human pathogenic bacteria. 2013, 121:827-234;
- Funding: Finnish Agency for Technology and Innovation ja European Regional Fund,,Finnish Beekeepers Association, VipJuicemaker (Refresco Scandinavia LTD), Fazer-Makeiset Oy, Kiantama Oy (Foodfiles Oy), Academy of Finland

II Certified organic honeys

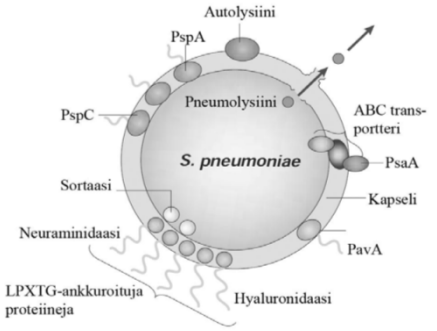
- Local beekeepers / shops
- Multifloral honeys (6)
- Djamila Zacarias, Ulrike Lyhs, Marjatta Lehesvaara & Carina Tikkanen-Kaukanen. Antimicrobial activity of diffferent organic honeys against *Clostridium perfringens.* Manuscript submitted
- Funding/supported: Mikkeli University Consortium, Evira

Own research

Finnish monofloral honeys

- Finnish Beekeepers Association
- Willow herb-, heather-, cloudberry-, lingonberry-
- and buckwheat honeys

Finnish monofloral honeys



Studied bacterial strains

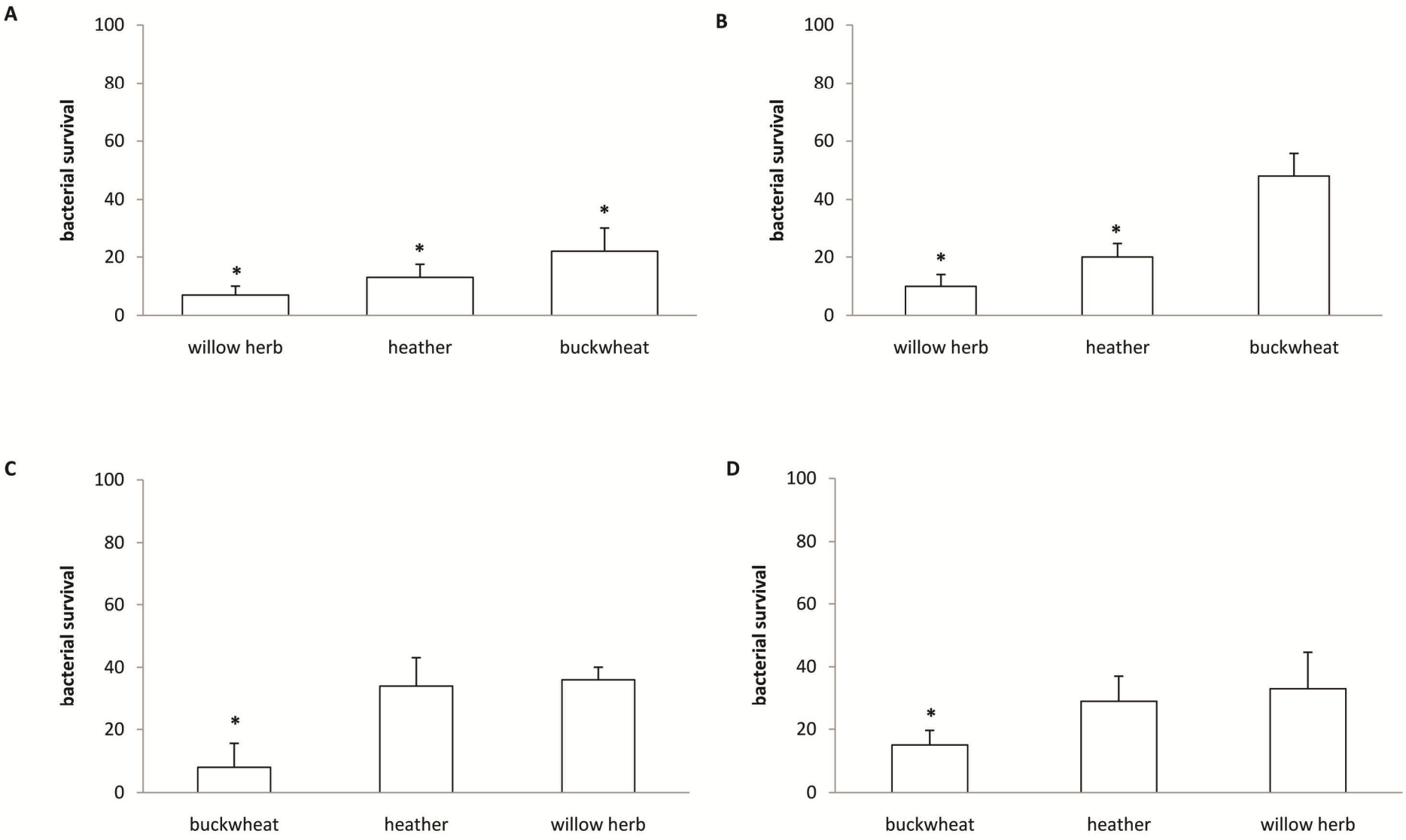
- *Streptococcus pneumoniae* (pneumococcus) (respiratory infections: pneumonia, sinusitis, otitis media
- *Streptococcus pyogenes (*pharyngitis)
- *Staphylococcus aureus* and MRSA (hospital infections, wound infections)

Finnish monofloral honeys

- Significant antimicrobial activity against all the studied bacteria (*S. pneumoniae, S.pyogenes S. aureus,* MRSA) was found in the dilution of 40%
- Willow herb, buckwheat and heather honeys showed the highest activity (even after heating for 15 min, at 67 C (60% honeys)

- *S. pneumoniae*: all the studied honeys were active
- *S. pyogenes* ja *S.aureus*: willow herb, buckwheat, heather and lingonberry honeys were active
- MRSA: willow herb, buckwheat and heather honeys were active
  - This is the first report on antimicrobial activity of Finnish honeys.
  - This is also the first report on the antimicrobial effect of honey against *S. pneumoniae.*

Figure 2


A
bacterial survival
100
80
60
40
20
0
*
*
*
willow herb
heather
buckwheat
B
bacterial survival
100
80
60
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C
bacterial survival
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D
bacterial survival
100
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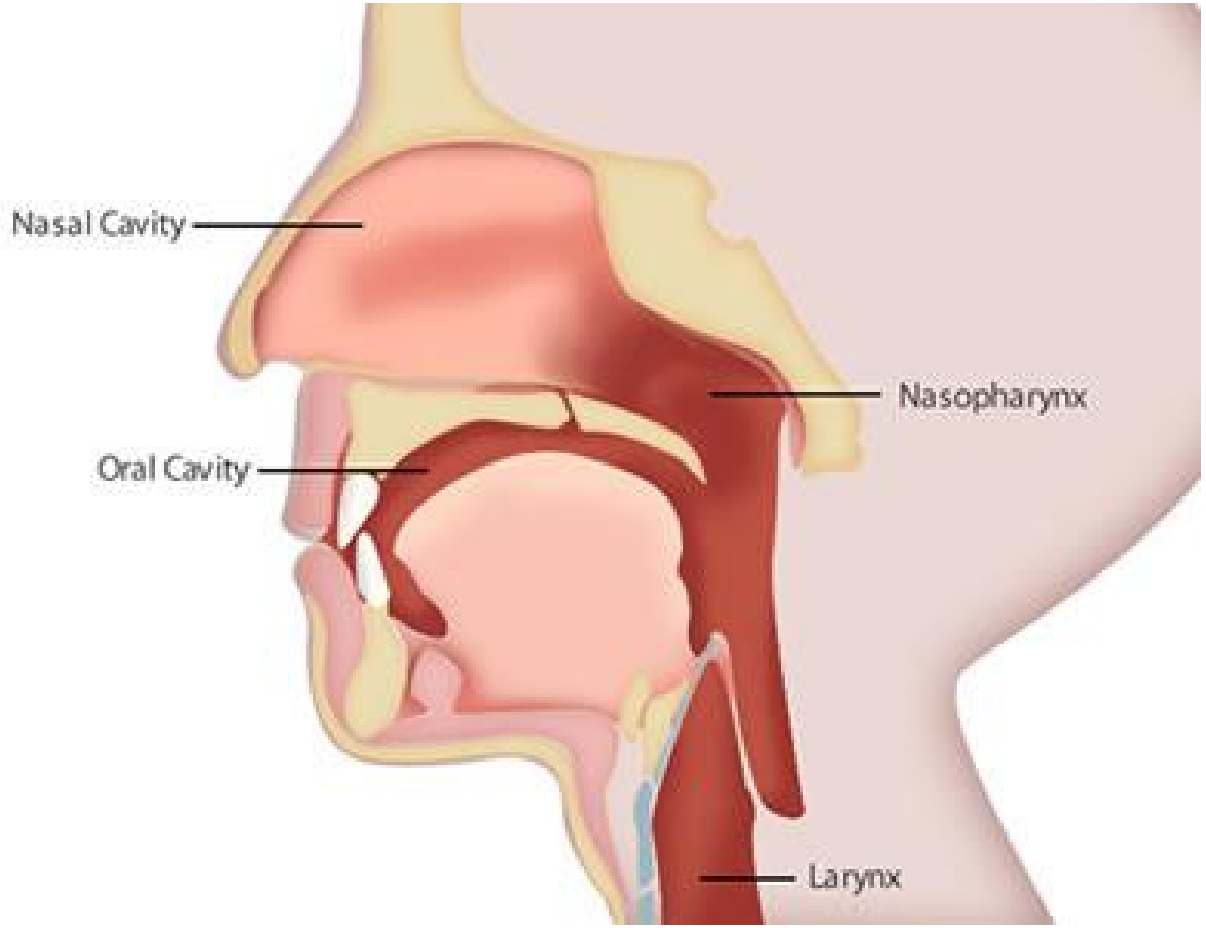
*
buckwheat
heather
willow herb

Possible effect on nasopharyngeal carriage state (pneumococcus)



Own research

II Certified organic honeys

- From local beekeepers/ shops
- Multifloral (6), refereed here as A, B, C, D, E, F

II Certified organic honeys

Studied bacterial strain

Clostridium perfringens

- *C. perfringens* type A is known to cause a broad spectrum of human and animal diseases.
- Main source: meat products, especially poultry meat
- One of the most common causes of foodborne illness in Europe, Japan and the United States .
- Antibiotic resistance of *C. perfringens* strains are becoming a major health concern.
- Copious use of antibiotics in agriculture is promoting a large antibiotic resistance problem in foodborne pathogens, including *C. perfringens.*

Method

- Antibacterial activity of honey was analyzed using **a disc diffusion assay** according to Bauer *et al.* (1966) with adaptation by Taormina *et al.* (2001) by using a **sterile paper disks (Whatman - type 3) with the diameter of 5 mm.**
- **Negative control: artificial honey (sugar)**
- **Positive control: antibiotic**

Own results

II Certified organic honeys

Organic Finnish honeys with antimicrobial activity (4)

- Honey F had the highest antimicrobial activity. The main floral source was willow herb.
- Honey E had the second best activity after honey F. In honey E the main floral source was clover.
- The main floral sources in honeys B and C were wild raspberry, willow herb, lingonberry and bilberry and in honeys B and C the antibacterial activity was quite equal.

Negative honeys

- Honey D was negative Finnish organic honey. The main floral sources were wild raspberry and lingonberry. Honey had been treated by heating (50 C, for few sec).
- Honey A from Argentina and Hungary was negative. The floral source of honey A was not reported. Honey A was untreated.

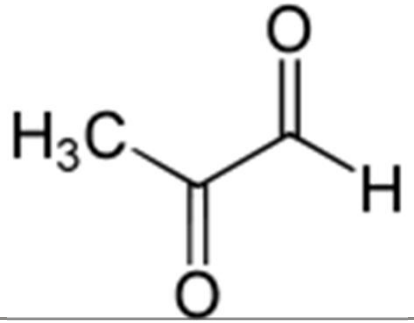
Conclusions

- Globally important: the first report on the antimicrobial effect of honey against *S. pneumoniae.*
- We also show for the first time antibacterial activity of organic honeys, and antibacterial activity of honey against *C. perfringens*.
- We found significant antimicrobial activity in Finnish honeys against hospital and wound infections causing by MRSA and *S. aureus* and against respiratory pathogen *S.pyogenes.*
- *The antimicrobil components in honeys remain unknown*

Applications of Finnish honeys

- As a food product to respiratory tract and gastrointestinal tract (eg. in drink, porridge, yougurt).
- In wound healing and to nasophanrynx area topically administrated (medical grade honey or eg. as a spray, respectively).
- In (organic) food products against food poisoning.
- Caution ! Honey is not allowed to give for children under 1-year-old, because it may contain botulinium spores.

Future tasks



- Characterization of the antimicrobial components in Finnish honeys.
- Authenticity of honey !

- Antimicrobial activity of African honeys against human gastrointestinal and respiratory pathogens
- Jackie Obey, University of Eastern Africa, Baraton, Kenia

Thank you for your attention !

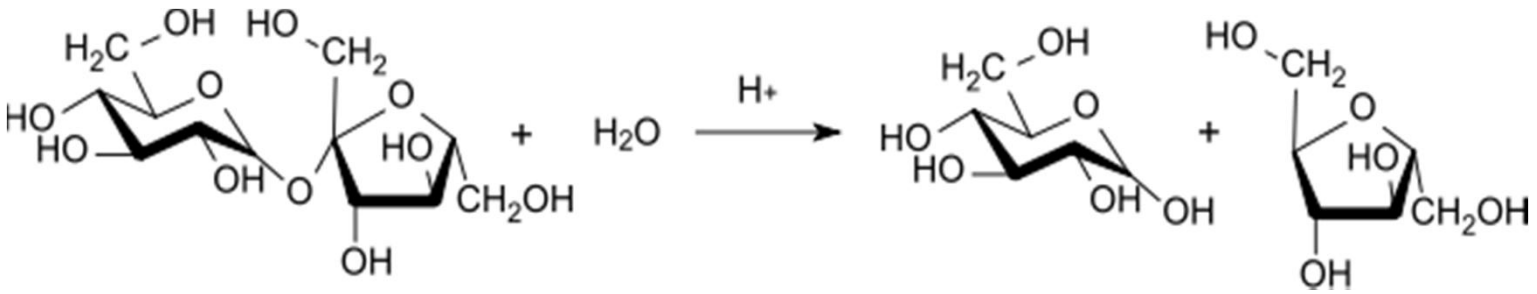
Sample	Zone of inhibition (diameter in mm) mean ± SD)		*n*	
A	5 ± 0		3	
B	8.3 ± 2		3	
C	7.5 ± 0.7		2	
D	5 ± 0		2	
E	11 ± 2		3	
F	14.3 ± 0.6		3	
SS	6.1 ±1.5		6	
P	30.8 ± 1.4		8	
N	5.2 ± 0.5		7	

Lääkkeellinen Manuka-hunajavoide

- Lääkkeellinen hunajavoide sairaalakäytössä Suomessa: Medical grade honey, Manuka-hunaja (Activon tube).
- Käyttö: monenlaiset haavat (mm. sääri-, leikkaushaavat, pahanhajuiset haavat, kuivat ja nekroottiset haavat) palovammat, hiertymät, ihonotto- ja siirrekohdat.
- Myös yksityiseen käyttöön.

Inverttisokeri

INVERSIO



Sakkaroosi hydrolysoituu

Glukoosi Fruktoosi

INVERTTISOKERI

Tasopolarisoituneen valon optinen rotaatio muuttuu (invert) reaktiossa oikealta vasemmalle (+66.5^{o} -39 ^{o}) mitattaessa polarimetrillä

Clostridium perfringensin aiheuttamat ruokamyrkytykset

C. perfringens produces a large range of potent toxins and enzymes that are responsible for severe diseases in humans. It has been recognized as a cause of foodborne illness since the late 1940s*. C. perfringens* type A food poisoning is ranked as the third most commonly reported agent of human food-borne diseases. The most common food vehicles for *C. perfringens* type A foodborne illness are meats (notably beef and poultry and also meat-containing products)

Clostridium perfringens food poisoning

C. perfringens produces a large range of potent toxins and enzymes that are responsible for severe diseases in humans. It has been recognized as a cause of foodborne illness since the late 1940s*. C. perfringens* type A food poisoning is ranked as the third most commonly reported agent of human food-borne diseases. The most common food vehicles for *C. perfringens* type A foodborne illness are meats (notably beef and poultry and also meat-containing products (e.g., gravies and stews and Mexican foods). The symptoms of *C. perfringens* are characterized by acute abdominal pain and diarrhea; nausea, fever, and vomiting are rare, this symptoms are generally developed about 8 to 16 h after ingestion of contaminated food(food containing > $10^{\wedge 6}$ to $10^{\wedge 7}$ vegetative cells per gram of food). These symptoms associated with *C. perfringens* type A food poisoning are caused by the *C. perfringens* enterotoxin (CPE). If the food are improperly prepared or cooked, spores of *C. perfringens* are able to survive and germinate in the nutrient rich environment, leading to the proliferation of large number of *C. perfringens* cells. Once the contaminated food is ingested, the majority of the bacteria might be killed by the acidic condition of the stomach; however, a small number of the population will survive and enter into the intestine.

The intestine is an environment that favors *C. perfringens* to multiply and then sporulate. CPE is expressed during the sporulation cells in the small intestines, after CPE binds to intestinal epithelial and cause damage to intestinal cells, which is clinically manifest as diarrhea (Doyle, 2005).