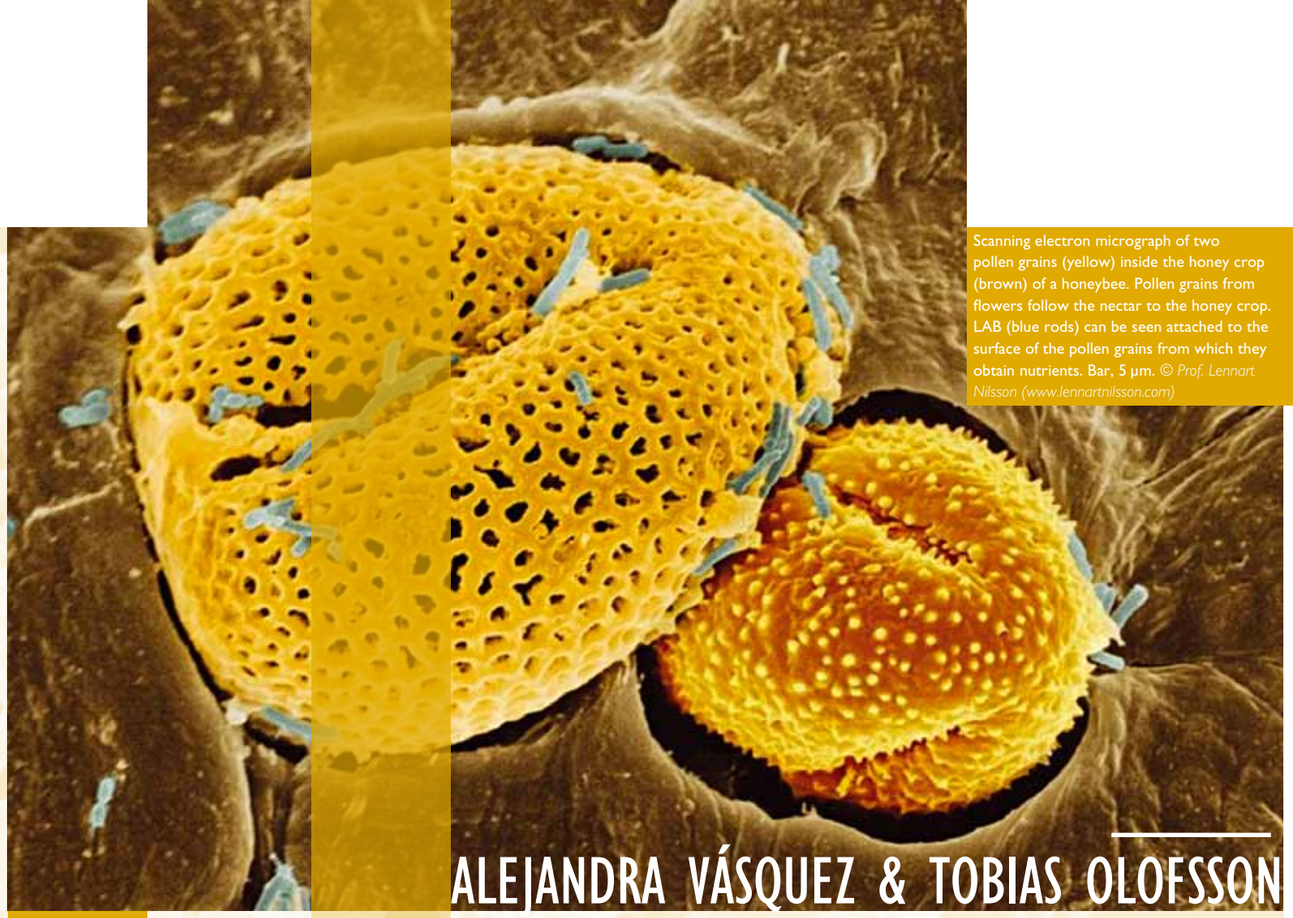
All of the work in this area started when Tobias Olofsson asked his grandfather Tage Kimblad, a beekeeper for 80 years, to participate in a research project. As a researcher in microbiology, Tobias became interested in investigating why honey has been regarded as a therapeutic agent by many different cultures independently throughout history, from the Maya in Mexico to the Pharaohs in Egypt. Together with his colleague Alejandra Vásquez, he discovered a large battery of beneficial bacteria inside the honey crop, a discovery that has opened up a new research field.



Scanning electron micrograph of two pollen grains (yellow) inside the honey crop (brown) of a honeybee. Pollen grains from flowers follow the nectar to the honey crop. LAB (blue rods) can be seen attached to the surface of the pollen grains from which they obtain nutrients. Bar, 5 µm. © *Prof. Lennart Nilsson (www.lennartnilsson.com)*

ALEJANDRA VÁSQUEZ & TOBIAS OLOFSSON

The honey crop – the Holy Grail when antibiotics fail?

THE HONEY CROP of a honeybee is, as the name suggests, a special part of the insect's body used for the production of honey. Honeybees make honey by collecting nectars that are rich in sugars and high in water content. They suck the nectar up from the bottom of the flower using their proboscis and store it in the honey crop during flight. When the crop is full, the bee returns to the hive and the nectar is placed in a cell. Thousands of bees fill thousands of cells and it takes days for the bees to produce honey from this nectar by reducing the water content.

NECTAR ATTRACTION

Nectar is a rich source of sugars and therefore attracts many other insects besides bees, and other animals like humming birds and bats. Their beaks, feet, mouths, probosces and other body parts come into contact with the flower, leaving many kinds of micro-organisms (bacteria, yeasts and moulds), and even faecal residue, behind in the flower after their visit. These micro-organisms feed on the sugars in the nectar and start to multiply fast. Millions of them travel inside the honey crop of a bee back to the hive where the temperature is around 33–35 °C. This is an ideal temperature at which they could proliferate and it would be just a matter of hours before the nectar would be spoiled. Since it takes days for bees to make honey, some kind of protection needs to be in place.

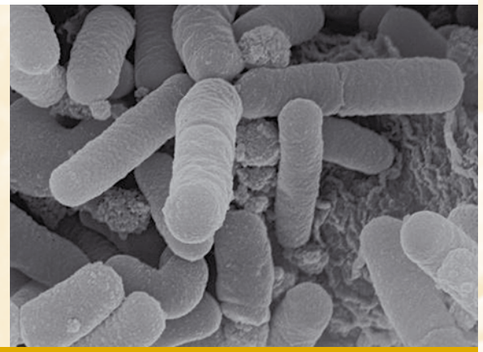
A full honey crop from a honeybee, containing nectar from flowers. The honey crop (a) is separated from the rest of the digestive tract at the proventriculus (b). *Reproduced with permission from Olofsson & Vásquez (2008) Curr Microbiol 57, 356–363.*

THE HOLY GRAIL

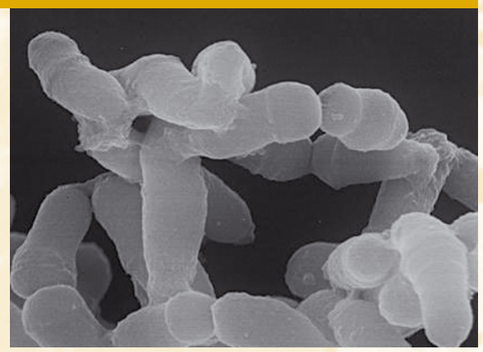
Recently, we discovered that a previously unknown group of 13 different beneficial bacteria reside inside the honey crop of honeybees. They are probably the reason why the nectar is not spoiled in the hive. Consisting of nine lactobacilli and four bifidobacteria, this group seems to be a Holy Grail of evolution, since our research indicates that these bacteria act as a barrier against unwanted micro-organisms. The evolved relationship between bees and beneficial honey crop bacteria gives the bacteria a special ecological place. In exchange for some of the nectar as food, the bacteria give the bees protection against spoiling micro-organisms and pathogens.

BENEFICIAL BACTERIA

Lactobacilli and bifidobacteria are included in a bacterial group called the lactic acid bacteria (LAB) as they produce lactic acid as their main end product. LAB are widespread in nature. In mammals, they are found along the gastro-intestinal tract and in the vagina. They are considered beneficial because they protect their host against unwanted microbes and produce important compounds, e.g. vitamins and antimicrobial substances.



Lactobacilli (above) and bifidobacteria (below) that reside in the honey crop. © *Prof. Lennart Nilsson (www.lennartnilsson.com)*



A honeybee with bee pollen attached to its legs. *Heidi & Hans-Jürgen Koch / Minden Pictures / FLPA*

LAB are commercially important for their use in the food and biotech industries as they are involved in processing foods like chocolate, sausages, olives, vanilla, vinegar, yoghurt and probiotics. In addition, LAB have been used by humans for thousands of years in the preservation of food, including meat, fish, fruits, vegetables and milk-based products. The main reason for these applications is the production of compounds that inhibit or kill other micro-organisms competing for food and space. One interesting aspect is that some of these bacterial compounds (e.g. organic acids) are already used in beekeeping today to help bees fight diseases. The beneficial honey crop bacteria we discovered constitute one of the largest bacterial groups ever found collaborating within one single organism.

BEES ARE BAKERS

Bees do not only collect nectar from flowers; they collect pollen as well, which is mixed with honey from the honey crop. The resulting sticky ball called 'bee pollen' attaches to specialized structures on their legs for transportation back to the hive. In the hive, the bee fills cells with pollen and then covers the pollen-filled cells with a drop of honey. It is known that a fermentation process starts in this mixture in the hive due to the presence of micro-organisms, but the exact identity of the microbes involved has been a subject for research. During this fermentation process, which takes 2 weeks, the bee pollen changes to 'bee bread' that is loaded with nutrients from the pollen and serves as an essential food, not only for the bees and their larvae, but also for the honey crop bacteria.

The fermentation process makes the nutrients contained in the pollen available and preserves it from spoilage. Our research has identified the bacteria involved and revealed that bees, in producing bee bread, add all the beneficial LAB to the pollen when they collect it at the site of the flower.

Bee bread in varying colours. *W. Treat Davidson / Science Photo Library*

BEE HEALTH

Honeybees are our most important pollinator and their health has come into focus during the last few years because of as yet unexplained conditions and diseases threatening this essential insect. Honey crop bacteria could potentially be of crucial importance for the well-being of honeybees, their pollination potential, and for their production of honey and bee bread. These bacteria have already been shown to inhibit the bee disease American foulbrood (see the article by Forsgren & Genersch on p. 238). With further studies, we hope to understand more about the importance of these bacteria and their impact on the honeybees' immune system and larval defences, and on bee foods. We are currently investigating how some of the drugs fed to bees affect the bacteria and how this may impact both the honeybees' defence against diseases and their food production.

"No microbe yet examined has been able to withstand the myriad of compounds produced by honeybee LAB."

AN INTERESTING PARALLEL

Sir Alexander Fleming received the Nobel Prize after his discovery of penicillin, a potent antibacterial substance produced by the mould *Penicillium*. Penicillin and the huge range of antibiotics subsequently developed have saved many lives, but our overuse of antibiotics has caused worldwide concern, and is linked to increasing bacterial resistance. We are in desperate need of alternative tools to solve this worldwide problem. The group of 13 LAB species discovered in the honeybee have evolved together in the honey crop and each species of bacterium can produce several different antimicrobial substances, resulting in a myriad of compounds. Working with a large arsenal of antimicrobial substances seems like a good approach to withstand development of resistance by other micro-organisms, a strategy already implemented by bees.

FINAL COMMENTS

Mature honey (with a water content of less than 20%) sold in shops does not contain any viable, beneficial honey crop bacteria. The novel lactic acid bacteria are only present and active in fresh or wild honey and only for a couple of weeks. This may be one reason why honeys differ in their antimicrobial properties. Fresh honey from wild bee colonies in trees and cliffs contains viable, beneficial bacteria and may reflect the historical use of honey as a therapeutic agent, consumed and applied with a high LAB content. The results of our research may explain why humans have used honey as a cure, e.g. for sore throats and wound healing (see the article by Cooper on p. 234). Millions of bacteria of each of the 13 species of LAB found in the honey crop, in combination with their secondary metabolites, end up in fresh honey during its production.

The LAB that have evolved with the honeybee have been a potent weapon used by bees to defend themselves against microbes found in their environment. In our ongoing research, no microbe yet examined has been able to withstand the myriad of compounds produced by honeybee LAB. The important core of honey as a folk medicine has probably been revealed and may be the source of a natural antibiotic alternative not only for bees but also for humans.

ALEJANDRA VÁSQUEZ & TOBIAS OLOFSSON work as researchers at the Department of Medical Microbiology, Lund University, Lund, Sweden. Both Alejandra (email alejandra.vasquez@med.lu.se) and Tobias (email tobias.olofsson@med.lu.se) completed their PhD studies in food technology at Lund University and started up their research group in applied microbiology. Research into the LAB from honeybees has been their focus since 2006. They have patents on technical applications of these lactic acid bacteria. Since their discovery, Lund University has helped them to protect their findings and start up a biotech company which they run in parallel with their research group. BBSRC (UK) supports their research in bee health. Along with many others, the authors believe that they have opened the Pandora's Box of the old folk medicine – honey – and of the honeybee's well-being. More results of interest to a range of fields will soon be published.

FURTHER READING

Research group homepage: www.med.lu.se/labmedlund/medical_microbiology/research
Research innovations: www.doctorhoney.com

Honeybee foraging on a pumpkin flower. *Konrad Wothe / MindenPictures / FLPA*