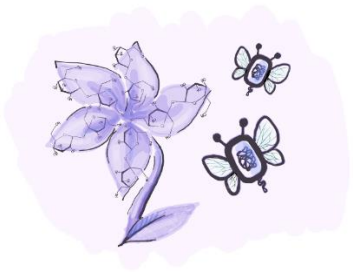
Case study made by Technion (Israel Institute of Technology) students: SBP capsules as a synthetic bee stomach



iGEM2019: A Report from Technion Team Project BeeFree honey (November 2019)

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Introduction: Honeybees produce honey to make the flower's nectar more digestible and well-preserved, using various enzymes secreted in their honey stomach. The honey possesses unique properties that make it highly attractive in fields such as medicine, cosmetics, and the food industry.

Our vision is to create a sustainable **"BeeFree"** honey using engineered bacteria, which will process a nectar-like solution using secreted enzymes that mimic the honey stomach environment. Ben Gurion University (BGU) has introduced us to Bio-Castle, a core element in our "synthetic bee stomach". The engineered bacteria will be separated from the final product using membrane-based capsules, providing the bacteria's favorable growth medium inside the capsule, while allowing enzyme secretion to the external "nectar" solution.

Industry Collaboration

To produce synthetic honey, we needed to maintain free diffusion of hydrogen peroxide as well as target proteins between the “unprocessed honey mixture” and the engineered bacteria, so that the "Honey circuit" could sense the solution and secrete enzymes in a controlled manner. Another requirement is to ensure that there are no bacteria left in the solution after the processing is done, making the product safe for consumption. Lastly, it is necessary to provide nutrition, such as found in LB medium, to allow proper bacterial growth and to enable the secretion of proteins. However, no traces of these nutrients should be found in the final product, for it to be considered honey.

To overcome these issues, we collaborated with an Israeli company named "Bio-Castle", and together we changed the use of one of their products, originally used for biodegradation of contaminants in water.

After a long discussion with the company representatives about their work, our project and iGEM in general, we decided to cooperate. We believe "Bio-Castle" provides a useful platform for many iGEM projects as a lab-scale model and even for future industrial-scale projects. Therefore, we would like to showcase our work using these capsules to expose more iGEM groups to this product. As mentioned before, we adjusted the product for our needs. While the original design was to allow for contaminants to enter the capsules and be degraded there by the bacteria inside, we used the capsules as a tool for protein secretion to the solution, while allowing hydrogen peroxide entry.

We hope other iGEM teams will take into consideration the use of such a capsule as a selective separation tool. We also hope our work will prove that the capsules meet the standards of biotech and food applications by growing the encapsulated bacteria and allow only the secreted enzymes to leave the capsule.

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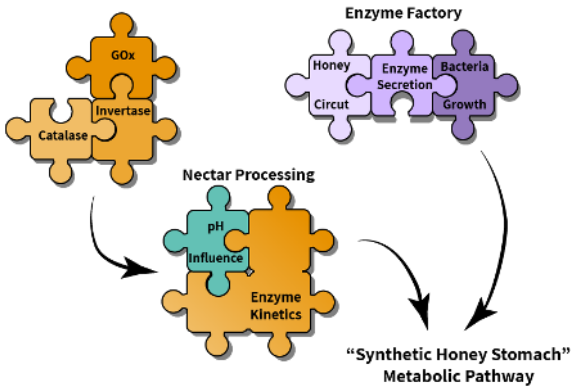
"Bio-Castle" Capsules' Suitability

Our "BeeFree" honey production is designed to generate inside the "BioCastle" capsules, to which our bacteria will be injected. The capsules are designed to prevent bacterial leakage, while at the same time enable protein secretion. Since those functions are crucial to our procedure, we have conducted an experiment aimed to demonstrate the capsules' ability to provide those two functions. The results indicate that, indeed, the capsules can secrete the desired enzymes in their functional form and prevent bacterial leakage. Thus, we have proven that these capsules can function well in our system.



Study outline design (Modeling) of bee stomach bio-mimic.

Capsules Ability to secrete proteins

To test protein secretion, we compared the catalase enzyme's activity in three different types of samples, performed in triplicate. These samples were: Wild-type (WT) bacteria without a capsule, WT bacteria in capsules with distilled water as an outside solvent, and a capsule containing WT bacteria with a sugar solution as the outside solvent. The control sample contained a capsule injected solely with LB. The sugar solution samples were tested to determine whether the osmotic pressure resulting from the presence of sugars would affect protein secretion. The catalase enzyme was chosen to be our model enzyme due to its high activity (see activity results). After incubation of the capsules in the different solutions, we concentrated the solutions using Amicons (manufactured by Mercury). As catalase is a naturally produced enzymes in WT B. subtilis, there is an abundant amount of it in the supernatant, and therefore, additional concentration is not necessary. The average activity measured in the non-capsule samples was 12.27 U/ml, while the average activity measured in the capsule-DW samples was 8.54 U/ml, and the measured activity in the capsule-sugar solution samples was 1.7 U/ml. No activity was detected in the control sample. Fortunately, it appears that the capsules can secrete the proteins and keeping it in an active state. As to the sugar solution sample, it appears that protein secretion is interrupted under the condition of osmotic pressure, and while the viscosity and density resulted in decreased diffusion rate, it was not entirely eliminated. That crucial information could guide us in the future optimization of our system, utilizing a less concentrated solution that would result in decreased osmotic pressure, or using a device that would actively move the capsules, to increase diffusion rate. It is worth mentioning that the concentration of sugars found in our solution is higher than in real flower nectar, and therefore, we can predict that the proteins' secretion could be higher in real nectar solution. https://2019.igem.org/Team:Technion-Israel/Demonstrate

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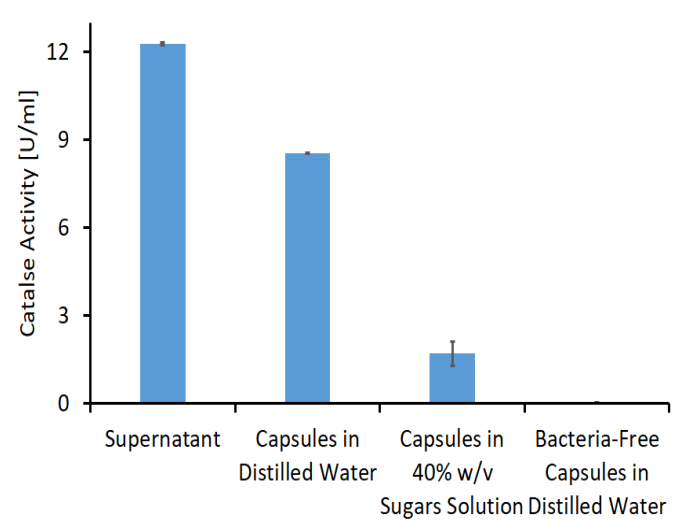
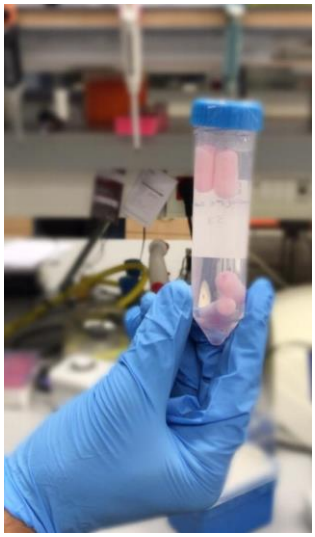




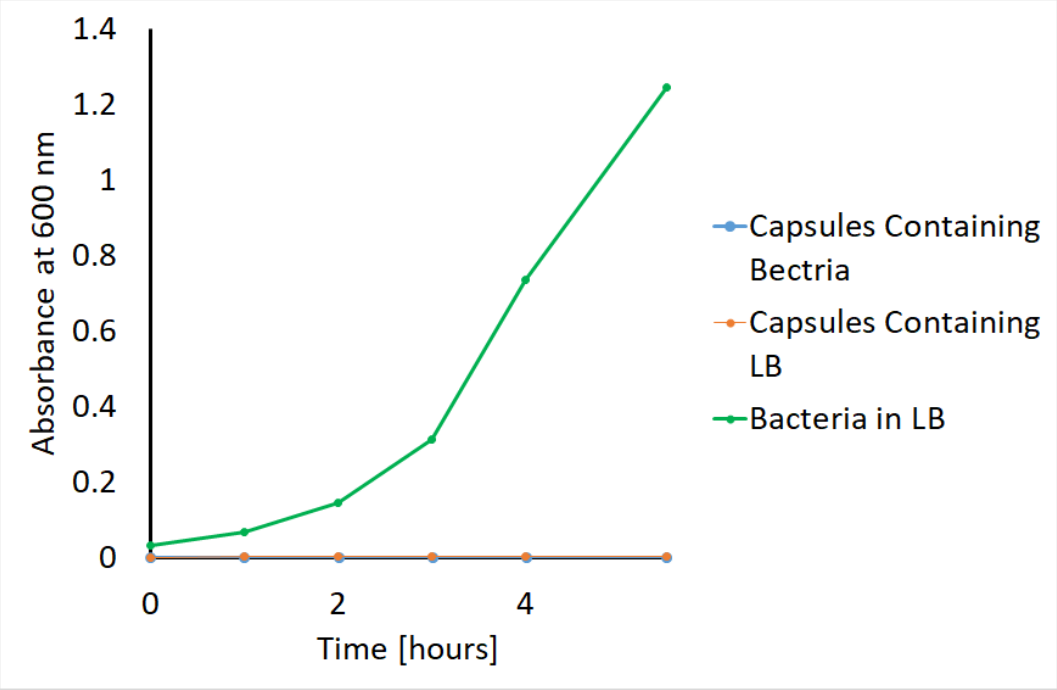
Capsule's ability to prevent bacterial leakage

In this part, we aimed to show the capsules' capacity to prevent bacterial leakage. We followed the bacterial presence and growth using absorbance measurements at 600 nm in three different types of samples (in triplicate), as followed:

i. A capsule injected with LB solution only, placed in LB (LB-Containing capsules).
ii. A capsuled injected with WT bacteria, placed in LB (Bacteria-containing capsules).
iii. WT bacteria placed in LB, without capsules (Bacteria-LB).

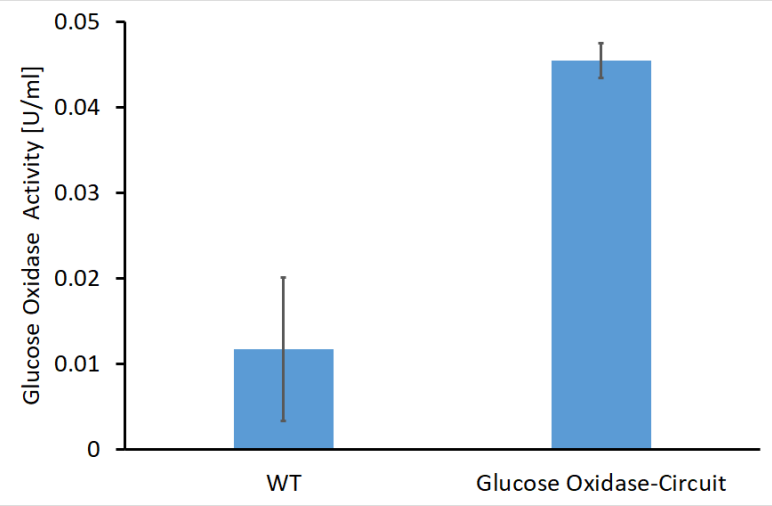
The samples were incubated for 5.5 hours at 37°C and were shaken at 210 rpm. The absorbance at 600 nm was measured to test for bacterial presence. The absorbance measured both in the bacteria-containing capsule and the LB-containing capsules samples remained at zero throughout the test, while the LB-Bacteria sample (without capsules) was gradually increasing. Therefore, one can claim that indeed, the capsules do prevent bacterial leakage. As can be seen in the figure below, the bacteria did not grow in neither the LB-capsules sample nor the bacterial capsules sample, while they grew in the bacteria-LB samples, proving the capsules' ability to prevent bacterial leakage.

https://2019.igem.org/Team:Technion-Israel/Demonstrate



Bacterial growth outside capsules measured by absorbance at 600 nm of various solutions: capsules containing bacteria, capsules containing LB, LB and bacteria without capsules, incubated for 5.5 hours at 37°C.

Activity of the honey circuit placed in capsules



In our attempt to demonstrate the functionality of our engineered parts as a complete system, we have conducted a test, which we believe can serve as a **suitable prototype for our system**. We have tested the GOx activity, secreted by bacteria containing the "Honey-Circuit" and placed in capsules with WT bacterial capsules as control. The samples were placed in distilled water and incubated overnight (12 hours) at 210 rpm, 37°C. Afterwards, they were concentrated using Amicons (Mecrury) and tested for the activity of glucose oxidase (to see method, please see results). It appears there were low, yet **detectable secretion and activity**. To conclude, due to the functionality and suitability of each component, we believe that we are not far from assembling a functional system.

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More about the system

To ensure the "Honey Circuit" could sense molecule concentration in the honey production process, we need to enable the **transfer of molecules**, such as hydrogen peroxide and selected proteins, between the "unprocessed honey mixture" and the engineered bacteria membranes. Another requirement is to **separate the bacteria** at the end of the process. To overcome these obstacles, we designed a **unique capsule** that entraps the bacteria inside a membrane, which acts as a **selective barrier** separating the bacteria from the processed honey mixture. We collaborated with an Israeli company named **Bio-castle**, which developed a small membrane capsule suited for growing and caging microorganisms for pollutes degradation. Together, we modified one of the company products to obtain our designed capsule.

The capsule entraps the microorganisms under optimal growing conditions and coated with a **semi-permeable membrane** allowing particles less than 0.78µm in diameter pass across the membrane. That way, the microorganisms in the honey process are maintained in a distinct volume, while the secreted enzymes are defused into the external solution, and small molecules, such as hydrogen peroxide, can enter the capsule. Therefore, the bacteria can "sense" the mixture and activate the "Honey Circuit" we have designed to secrete enzymes according to the mixture status. At the end of the enzymatic process, we can pull out the capsules from the sugar mixture to get a bacteria-free solution that is ready for the final step: drying.

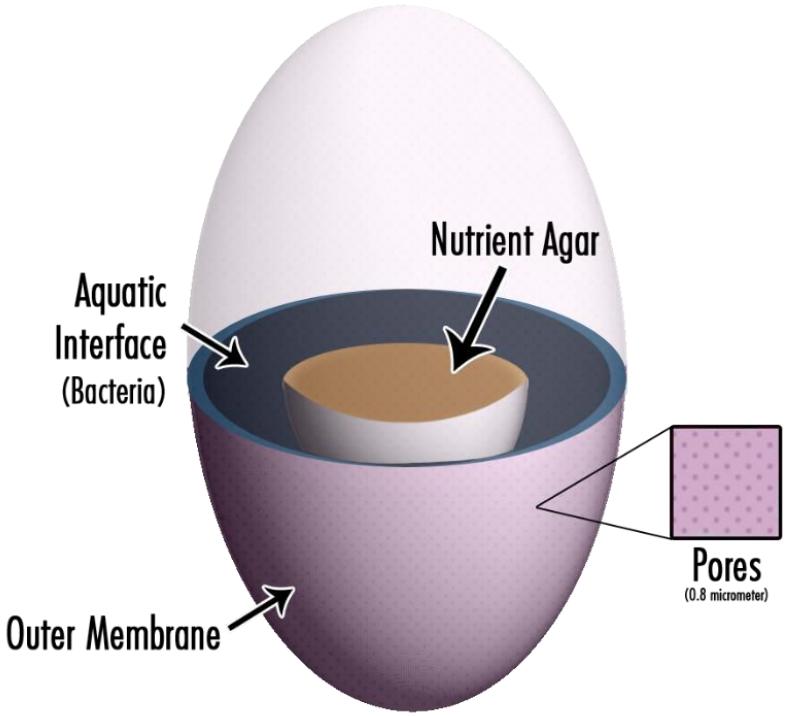

Illustration of BioCastle capsule

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Scale-up of our system can be achieved by evaluation of the enzymatic activity and enzyme secretion in each capsule, per volume. Following that, we can adjust the capsules number needed for each volume to achieve the desired composition in the external solution.

After the enzymatic degradation, our mixture contains about 70% of water. Naturally, in this phase, honeybees swing their wings and using the air for drying, which consequently decreases the water content to a desired 17%, as stated in the description chapter.

Our team chose to mimic the natural process, using pharmaceutical tray dryer. Applying this drying technique, we can dry our mixture without changing the temperature or affecting the sugar's properties.