Bacto-Aid

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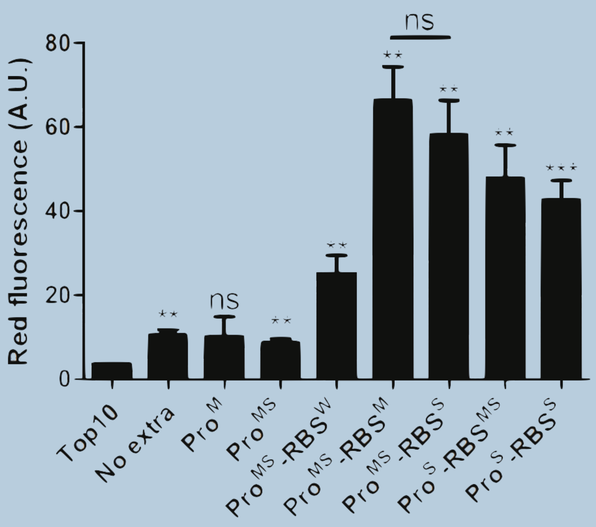
We set out to:

- Optimize the polyhydroxy-ß-butyrate (PHB) production
- Express bacteriocins in E. coli
- Create a functional hybrid bacteriocin
- Test bacteriocins on resistant pathogens
- Create a silk-bacteriocin hybrid

"Antibiotic resistance is one of the biggest threats to gobal health today, It can affect anyone, of any age, in any country"
-World Health Organization

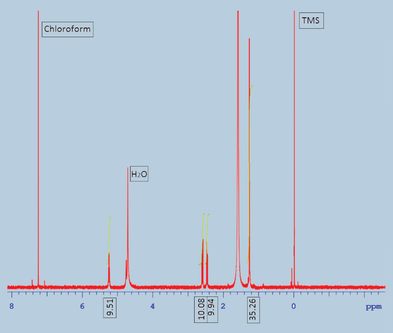
The strongest promoter does not produce the largest amount of plastic. This was determined by Flow Cytometry.



The figure displays the different *E. coli* strains on the x-axis. The promoters and ribosomal binding sites are marked with their corresponding affinity: "S" = strong, "W" = weak and "MS"=medium. The y-axis displays the average intensity on the flow cytometer detected by red fluorescence. The intensity of the red fluorescence is calculated from a mean value of the intensities detected in the flow cytometer.
* = p<0,05 and ** = p< 0,005, students t-test CI 95%.

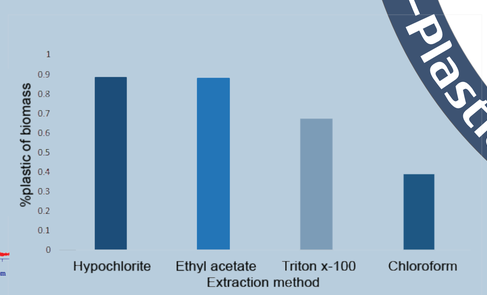
A high yield does not equal a high purity

H NMR for pure PHB plastic



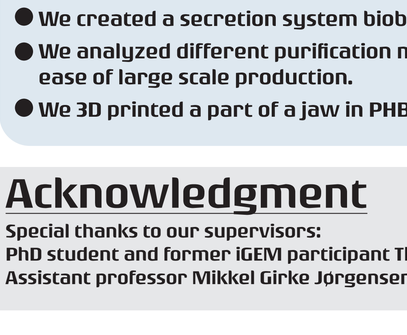
The figure illustrates the spectra of commercially bought pure PHB plastic, without any noise. This figure is used as a reference to determine the purity of the extractions methods.

Yield of extraction methods



This figure illustrates the percentages of PHB plastic extracted per biomass for the different methods of extraction used in this study. Based on this hypochlorite and ethyl acetate gave the best yield

H NMR for PHB plastic extracted with Chloroform



The figure illustrates an example of a spectra in this case the chloroform method of PHB plastic extraction. We have tested four types of methods: Chloroform, Ethyl acetate, Hypochlorite and Hypochlorite extraction with Triton X-100 pre-treatment.

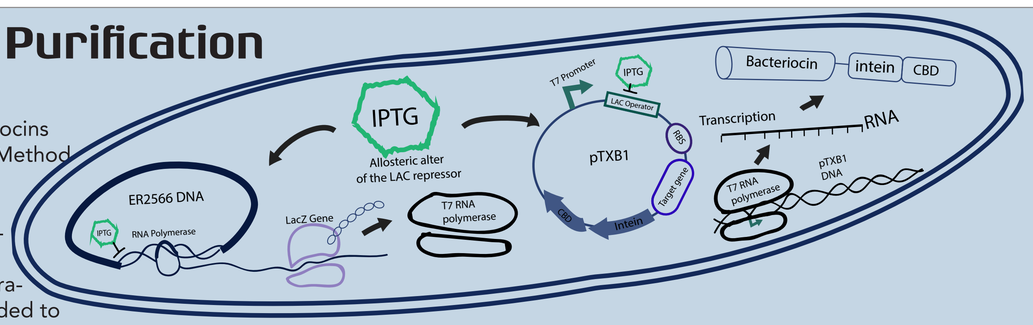
Sum up of extraction methods by H NMR and gas chomatography

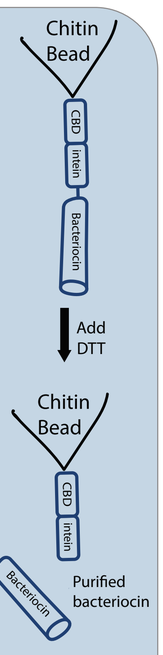
Extraction method	Yield	H NMR assessment	Soluble in chloroform
Hypochlorite	25.4 %	unpure	yes
Ethyl acetate	25.3 %	unpure	no
Triton X-100	19.3 %	pure	no
Chloroform	11.2 %	pure	yes

The table shows the determined properties of the different PHB extraction methods. The total amount of intracellular PHB is determined by gas chromatography and is used to calculate the yield of for extraction. The total intracellular amount was found to be 3.5 % of the cell mass. H NMR was used to determine purity of extracted plastic and since

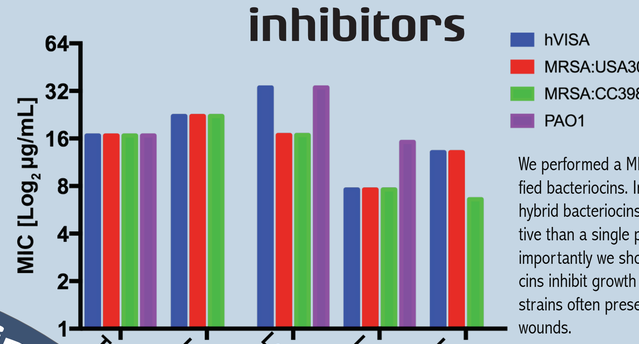
Intein Purification

We cloned and purified the bacteriocins using the IMPACT Method (Intein Mediated Purification with an Affinity Chitin-binding Tag). Isopropyl -D-1-thiogalactopyranoside (IPTG) is added to induce protein expression. The intein-bacteriocin protein is extracted by the use of French press. Cleavage between the intein tag and the bacteriocin is induced by a thiol agent (DTT), thus the native bacteriocin elutes. Final bacteriocin concentration is determined by using a Bradford Protein Assay with BSA.





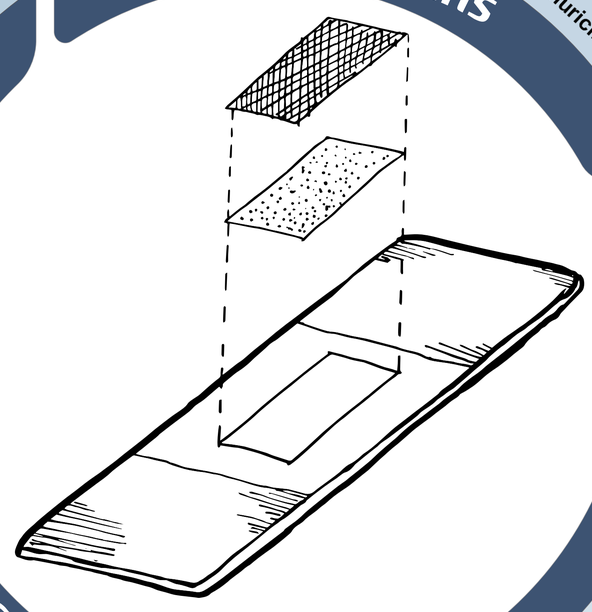
Hybrid Bacteriocins = Enhanced MRSA inhibitors



We performed a MIC test on the purified bacteriocins. In some cases the hybrid bacteriocins are more effective than a single protein. Most importantly we showed the bacteriocins inhibit growth of multi resistant strains often present in open wounds.

"Bacteriocins are rather attractive as possible new antimicrobial compounds, as they are seldom developed resistance against"
- Frank Møller Aarestrup
Head of the reference laboratory for antimicrobial resistance in foodborne pathogens by the WHO and EU

Bacteriocins



Bio-Plastic

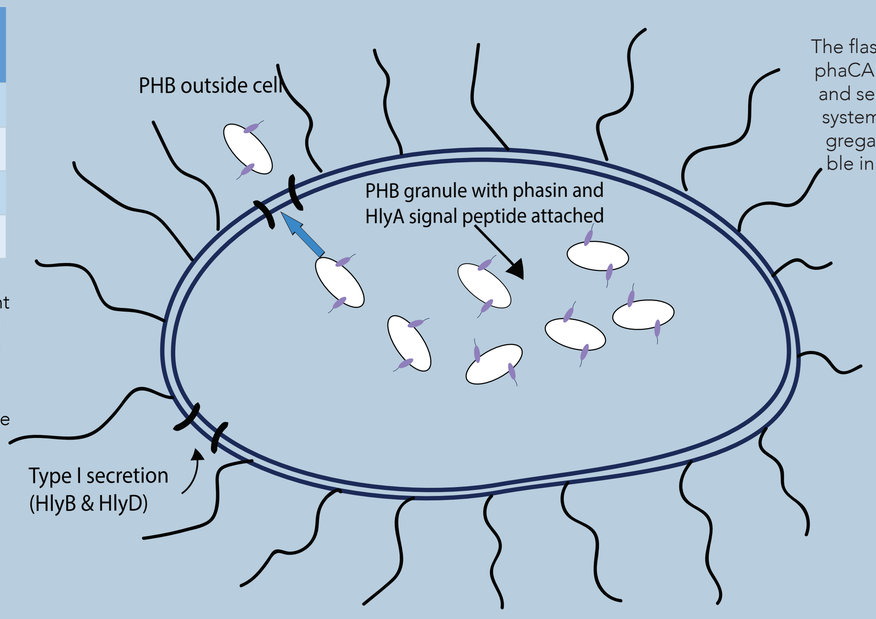
Silk

Why silk?

- Angiogenic properties
- Can be combined with bacteriocins
- Immunoneutral
- Proliferative effect on keratinocytes

We wanted to test the if the silk-bacteriocin hybrid would still be a functional bacterial inhibitor. Unfortunately the assembly method does NOT allow for components that expired 12 years ago. - Now we know...

The secretion system

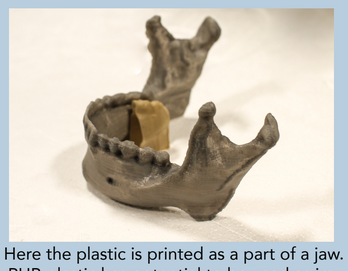


Non-secreting versus secretion system

The top left flask contains *E. coli* with phaCAB but without panK and secretion system. The flask on the right contains *E. coli* with phaCAB, panK and secretion system.

The flask contains phaCAB, panK and secretion system. PHB aggregates are visible in the flask

Our 3D printing is AWESOME!!!



Here the plastic is printed as a part of a jaw. PHB plastic has potential to be used as implants.

Conclusion

- We tested the bacteriocins and showed that the hybrids are more effective at inhibiting growth of multiresistant pathogens than a single bacteriocin.
- We created a PHB plastic promoter/RBS library, identifying the BioBrick producing the highest level of PHB.
- We created a secretion system biobrick for secretion of PHA plastics.
- We analyzed different purification methods with regards to purity, yield and ease of large scale production.
- We 3D printed a part of a jaw in PHB plastic.

Prospects

- Using the Iterative capped assembly (ICA) method we want to create a silk-bacteriocin hybrid.
- Create a new purification system for the PHB secreted in the media
- Create a scaffolding of PHB for our Bacto-Aid
- Test nanocoating of bacteriocins with PHB

Perspective

PHB is biocompatible and biodegradable. The hydrolysis of the PHB polymer produces a ketone body, commonly found in blood. These properties gives PHB the capability to become an implant that could be broken down slowly under the healing process of the bone.

Acknowledgment

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