Encapsulation of *Bacillus subtilis.*

Interactions of *Bacillus subtilis* with the dipeptide FmocFF.

Prior to encapsulating *B. subtilis* in Peptidosomes, there existed the potential problem that the bacterial cells would be able to degrade Fmoc-FF, of which the Peptidosomes are comprised of. To test whether *B. subtilis* can utilize the Fmoc-FF as a nitrogen source nitrogen-free Jensen's medium was used in four different treatment methods:

1) Normal Jensen's medium.
2) Jensen's medium supplemented with 200 µL of Fe [III] ammonium citrate + 100 µL Potassium glutamate (as negative and positive controls respectively).
3) Jensen's medium mixed with 500 µL of Fmoc-FF solution in each plate.
4) Jensen's medium where 500 µL of Fmoc-FF were added on the dried plate.

After all the plates were dry, 5 µL of a day culture and 5 µL of an overnight culture (W168 strain) were added into each plate. The plates were then incubated at 37°C for 8 days.

During the production of the peptidosomes, *B. subtilis* must be transferred to an alkaline solution. To test the tolerance of the organism against the high pH, *B. subtilis* (W168) was cultured under four different treatments of varying pH. This was done at pH values of 7, 8, 9, and 10. The pH of each culture was adjusted after 1 hour of incubation in normal conditions (ph 7) with solutions of NaOH of different molarities (pH 8=136 mM, pH 9=408 mM, pH 10=910mM). The cultures were incubated at 37°C in a plate reader to follow the change of optical density (OD) at a wavelength of 600 nm, over a period of 16 hours. At the end of this time, the cultures were plated in normal LB plates and incubated overnight at 37°C.

Encapsulation of cells

The strains encapsulated are shown in the next table.

Strain	Description	Vector	Resistance
TMB4131	W168 *lacA*::*erm* P_{veg}_*sfGFP*	pBS2E	MLS (against Streptogramin B and Linkosamid)
TMB3090	W168 *sacA*::*cat* P_{veg}_*luxABCDE*	pBS3C*lux*	Cm5 (against Chloramphenicol)

From an overnight culture, a 1:500 day culture was prepared in LB broth and incubated at 37°C (shaking at 220 rpm) until an OD_{600} between 0.2 and 0.6. Subsequently, the required amount of bacterial culture was taken, centrifuged (5 min, 16000 G), and the pellet resuspended in 500 µL of Fmoc-FF solution to obtain an OD_{600} between 2.0 and 3.0.

Growth tests of *B. subtilis* in peptidosomes.

Peptidosomes with bacteria were generated and transferred to LB broth and preincubated for 1 hour at 37°C. Immediately afterwards, some peptidosome were plated to work as a Time=0 h reference. The rest of the peptidosomes were transferred to 200µL of LB broth and incubated at 37°C for 3.5 and 7 hours, and plated afterwards. The plates were incubated overnight at 37°C. The next day the number of colonies of each plate was counted.

In a different experiment, a washing step was added, which consisted in transferring the peptidosomes twice to fresh broth before the incubation. In a second treatment, before incubation, the peptidosomes were transferred into LB broth previously adjusted to a pH of 5; by using chlorhydric acid to adjust the pH. The whole supernatant where the peptidosomes were incubate was plated as well.