BARE CILLUS

TEAM Newcastle

iGEM 2013

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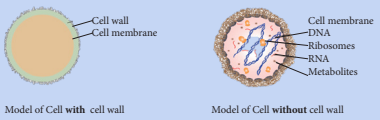
L-FORMS: NAKED BACTERIA

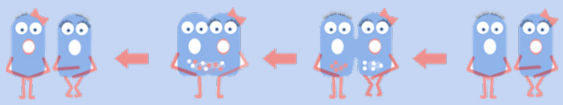
This novel chassis is based on *Bacillus subtilis* but with one major improvement: The cell wall of this chassis can be switched on or off, on demand. Amazingly these bacteria are still able to grow and divide. The technical name for these organisms is L-forms.



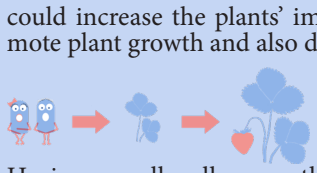
Model of Cell **with** cell wall — Model of Cell **without** cell wall

Without the cell wall, many things are made easier.

Bacteria without cell walls can be fused together easily. The genomes of fusants can recombine to encourage genome evolution (see the GENOME SHUFFLING section).

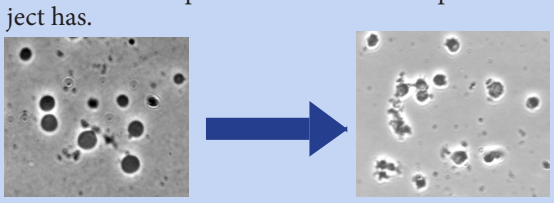


We have inserted naked bacteria into plants where they could increase the plants' immunity to pathogens, promote plant growth and also deliver useful compounds.



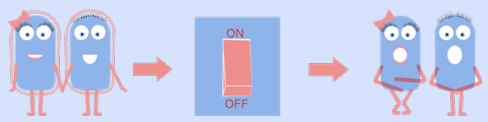
Having no cell wall means that they are able to adopt unusual shapes. We explored whether we can use microfluidics to force naked bacteria to form different shapes.

The survival of L-forms is entirely dependent on osmotically permissible environments. This means that if they were to escape into an unprotected environment they would burst and die.
Have a read over the blue boxes to see what we achieved with L-forms and the pink box to see what implications our project has.

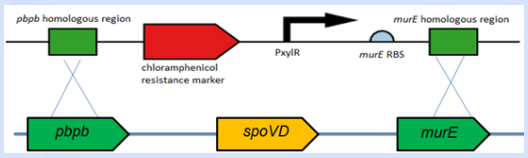


What will you do with L-forms?

We created a BioBrick that allows the *Bacillus subtilis* cell wall to be switched on or off.



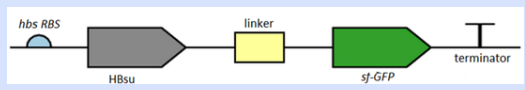
This BioBrick allows control over the expression of the *murE* operon, involved in the biosynthesis of peptidoglycan, part of the cell wall. *murE* is placed under the control of *PxylR*, a xylose-controlled promoter. When xylose is present, *murE* is expressed and the cell wall forms. This brick is inserted into the genome of *B. subtilis* with the *PxylR* promoter replacing the native *murE* promoter and the *spoVD* gene disrupted.

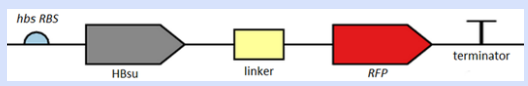


We have shown that wild-type *B. subtilis* cells (rods) convert to L-forms (round) following transformation with this BioBrick.

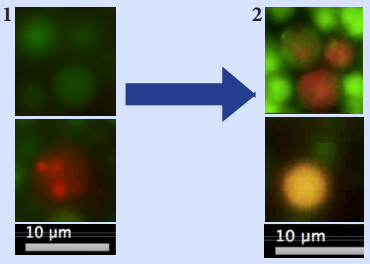
L-forms can be used to speed up evolution using a process called genome shuffling.

L-forms can be fused together easily and their genomes then come into close proximity. Similar sections of the genomes can be swapped via homologous recombination. Some of the resulting cells will contain a combination of each original cell's DNA. By applying artificial selection and further rounds of genome shuffling, bacteria with desired characteristics can be created.

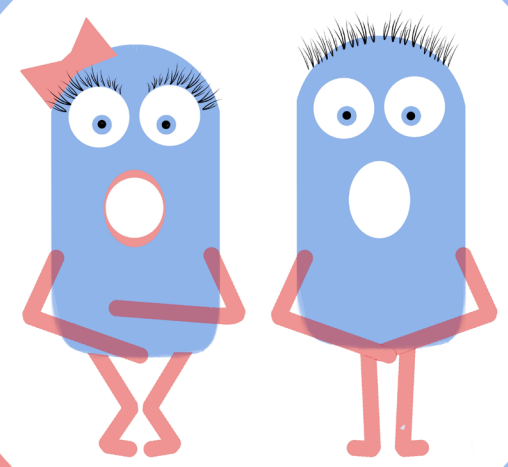




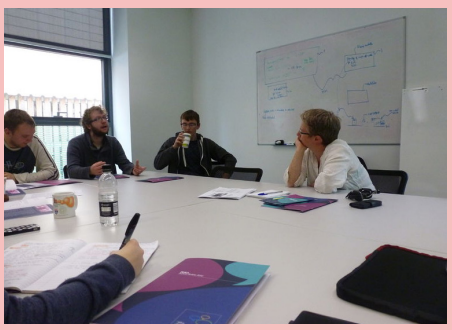
To do the fusion of L-forms we created BioBricks that encoded DNA-tagging HBsu-fluorescent proteins. The HBsu protein ensures that the fluorescent proteins will bind to DNA allowing us to detect true genomic recombination. The BioBricks were inserted into the genomes of *B. subtilis* cells containing our L-form switch. Once these cells were fused we were able to see cells co-expressing GFP and RFP bound to their DNA.



1) Cells showing GFP (top) and RFP (bottom)-bound DNA.
2) Cells showing DNA-bound GFP and RFP simultaneously.



Modelling is a key component of the Synthetic Biology process. With this in mind, we held a workshop outlining mathematical modelling, using the rule-based modelling language BioNetGen. This workshop was delivered at the YSB1.0 – UK iGEM meet-up. We believe the material we developed is a great resource for those new to biological modelling.



The team discussing our projects safety with Professor Janice McLaughlin from PEALS

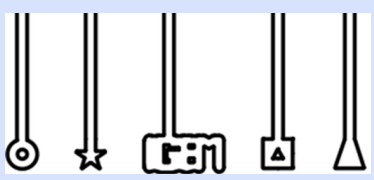
We also studied the laws on experimenting with genetically modified organisms (GMOs). Though legislation is generally strict on the definition of GMOs, it appears that, in some jurisdictions, cells that have undergone genomic fusion may be exempt from many of the regulations that other genetically modified organisms are subject to. Bacteria generated by genome shuffling may not be technically classed as GMOs.

In collaboration with our resident architect, we also explored the relationship that exists between Synthetic Biology and Architecture. We looked into the possibility of using Synthetic Biology to inspire architectural design and to produce products that could be applied as building materials. The similarities in the engineering process of both disciplines were also compared. The need for extensive design and modelling in both areas is clear.

For more information see our wiki:

http://2013.igem.org/Team:Newcastle/Outreach/Workshop

When *B. subtilis* loses its cell wall anchoring proteins the cell loses its support and becomes spherical. L-form *B. subtilis* can grow to large sizes before they divide. L-forms could be used to fit into cracks or squeeze into hard to reach places such as intercellular spaces. We intended to trap L-forms in chambers which were of atypical shapes via microfluidics. We could then observe whether the cell adopts the shape of the chamber once the cell wall was switched back on. Microfluidic chips for silicon wafer master moulds were designed using autoCAD.



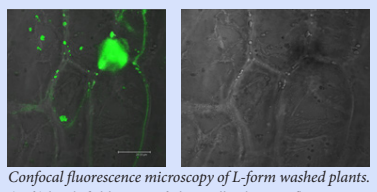
The design of the chambers into which L-forms would be forced. The L-forms should adopt the shape of the terminal part of the chambers once the cell wall had been restored.

We also constructed a model of the predicted cell shape as it grows inside a square shaped chamber.

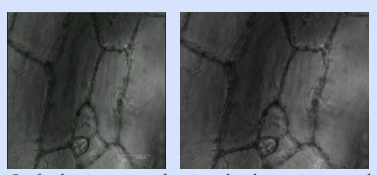
We inoculated L-forms into Chinese cabbage to try to confirm that it is possible for L-forms and plants to have a symbiotic relationship.

The seedlings were washed in a solution of GFP labelled L-forms.

We conducted light and confocal microscopy on samples four days after inoculation. L-form washed seedlings, unlike unwashed controls, showed green fluorescent bodies consistent with the size of L-forms (2-6µm).



Confocal fluorescence microscopy of L-form washed plants. (Left) brightfield image of plant cells, showing fluorescence. (Right) the original brightfield image of plant cells inoculated with L-forms.



Confocal microscopy of uninoculated negative control plants.
(Left) a brightfield image of plant cells, with an image of the green fluorescence overlaid on top.
(Right) the original brightfield image of plant cells.

Since L-forms are osmotically sensitive, they lyse if they escape into an unprotected environment. Therefore L-forms could be used to:

- Supply nutrients, plant hormones, nitrates and other substances to aid plant growth and thereby increasing crop yield

-Produce antifungal compounds to protect the host from infection

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Ferguson, C.M.J., Booth, N.A. and Allan, E.J. (2000) An ELISA for the detection of *Bacillus subtilis* L-form bacteria confirms their symbiosis in strawberry. *Letters in Applied Microbiology.* 31(5), p. 390-394.
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Leaver M., Dominguez-CuevasP., Coxhead J.M., Daniel R.A. and Errington J. (2009) Life without a wall or division machine in *Bacillus subtilis*. *Nature, 457, 849-853.*