香港中文大學醫學院
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Engineered Bacteria for Medical Use

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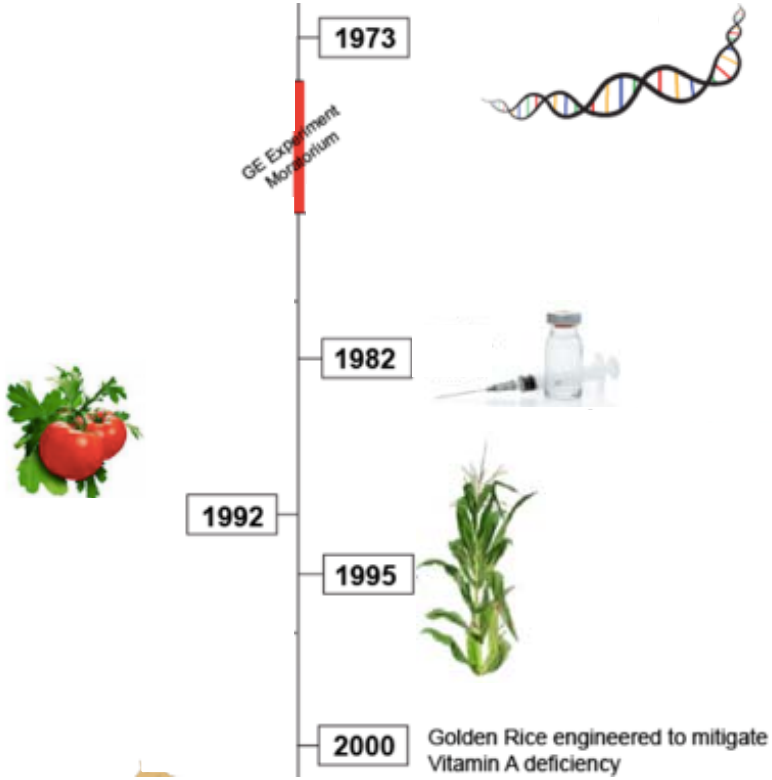
Microbiology

Genetically Modified Organisms (GMOs) emerged from gene engineering

- Definition: Organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally by meting and / or natural recombination.



Innovation: Using GMOs as therapeutics / diagnostics

From Corgis to Corn: A Brief Look at the Long History of GMO Technology. (2016, October 23). Retrieved from http://sitn.hms.harvard.edu/flash/2015/from-corgis-to-corn-a-brief-look-at-the-long-history-of-gmo-technology/

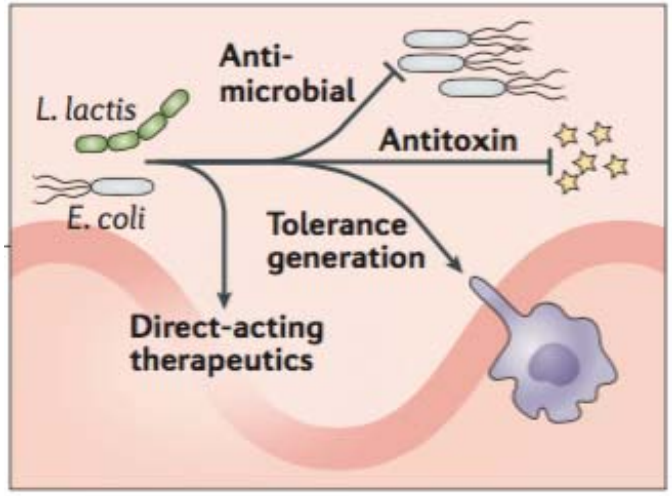
GMOs facilitate drug delivery

1. For drug molecules of short half-lives
   - The effector is synthesised after reaching the target site

2. Reaching target therapeutic sites directly
   - The treatment can be localised without severe systemic side-effect

Landry, B. P., & Tabor, J. J. (2017). Engineering Diagnostic and Therapeutic Gut Bacteria. Microbiology Spectrum, 5(5). doi:10.1128/microbiolspec.bad-0020-2017

Engineered bacteria works by releasing effectors in specific regions



1. Releases molecules in the gut

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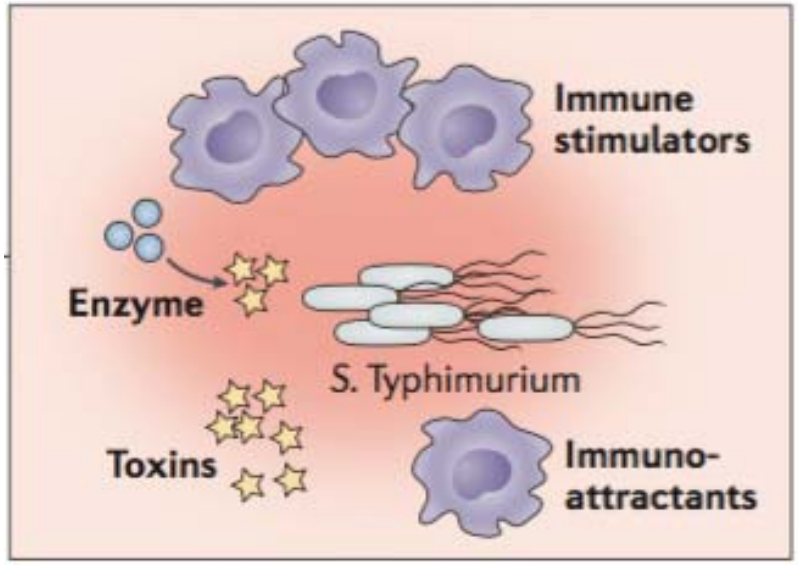
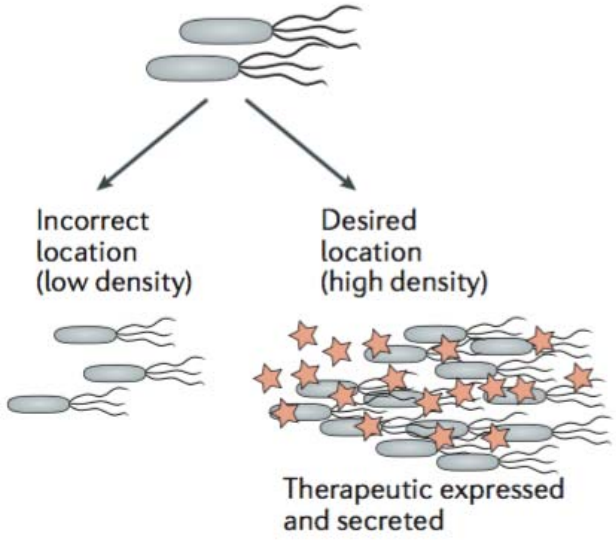


2. Releases toxin, triggers immunity against tumours



The specificity is achieved by coupling quorum-sensing with recombinant gene expressions

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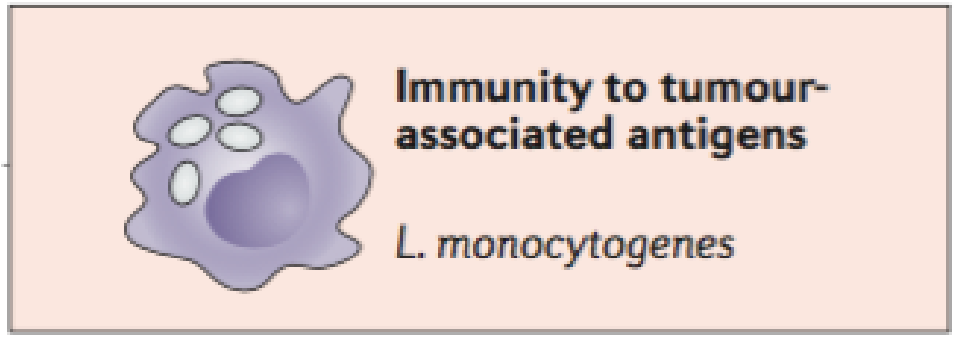
Engineered bacteria can stimulate immune response



3. Expresses antigens found in tumour

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Harmless bacteria to be selected

- Should be easy in genetic manipulation and can be grown rapidly
- Should cater to the target sites (the gut, mouth ……)
- Shouldn’t be colonised in humans (*L. lactis* eliminated from human body in approximately 3 days)
- For oral delivery to the gut: *Lactococcus lactis* and *Escherichia coli*

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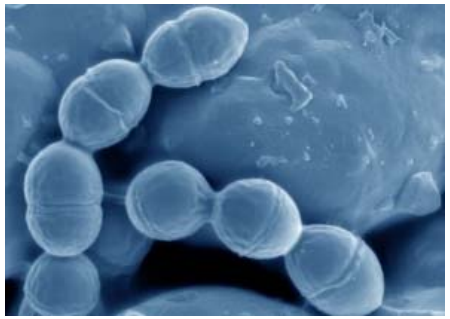
L. lactis as the first bacteria in practice

- *L. lactis* is well known as food bacteria in milk fermentation
- GRAS status (generally recognized as safe) by FDA



http://textbookofbacteriology.net/featured_microeb.html

Other factors making it suitable for genetic manipulation

- Well studied gene expression system
- Tendency to secret recombinant protein
- Only one constitutively expressed protease

Song, A. A., In, L. L., Lim, S. H., & Rahim, R. A. (2017). Erratum to: A review on Lactococcus lactis: From food to factory. Microbial Cell Factories, 16(1). doi:10.1186/s12934-017-0754-1

Examples of *L. lactis* engineering

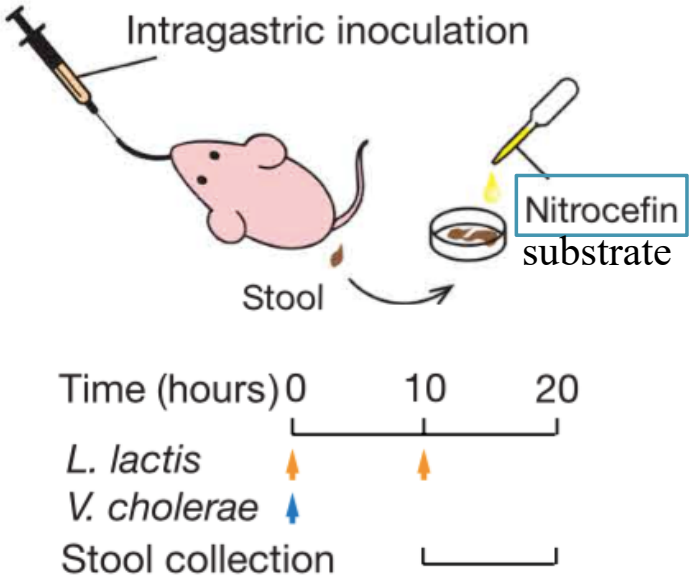
1. **Detection of cholera in mouse model**
2. Expression of IL-10 in mouse model with asthma

CAI-1 detection in mice infected with cholera

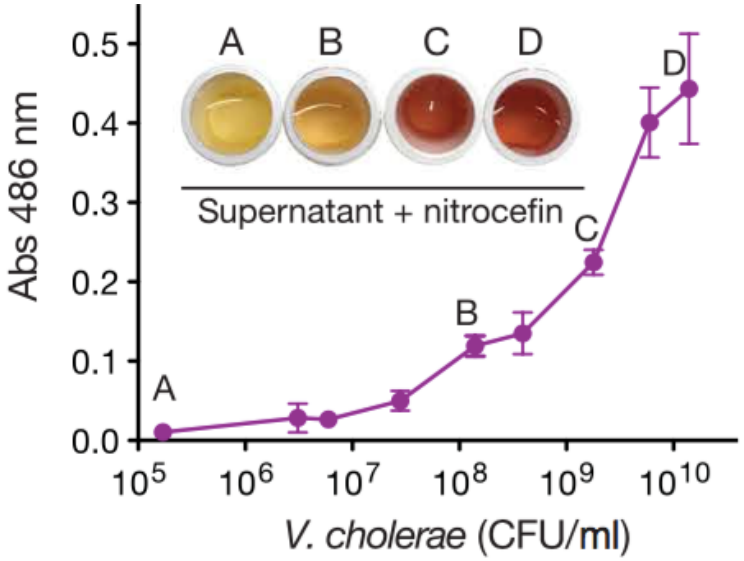
- Cholera is a disease mainly caused by poor water quality, resulting in severe dehydration and even death
- No *in vivo* cost-effective method in detection
- New detection assay developed based on GMOs
  - CAI-1: quorum-sensing molecules from *Vibrio cholerae*
  - *L. lactis* was modified to express hybrid receptor for CAI-1, binding of CAI-1 triggers reporter β-lactamase synthesis

Mao, N., Cubillos-Ruiz, A., Cameron, D. E., & Collins, J. J. (2018). Probiotic strains detect and suppress cholera in mice. Science Translational Medicine, 10(445). doi:10.1126/scitranslmed.aao2586

CAI-1 detection by β-lactamase reporter assay



Intragastric inoculation and collection of stool samples



Colour development within 30 mins

No positive signal from healthy individual and infection by mutant *V. cholera* : **CAI-1 specific**

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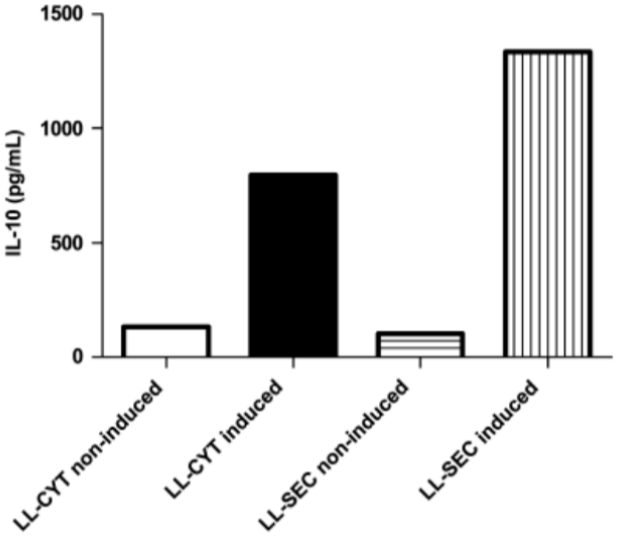
Delivery of IL-10 in mice with asthma

- Asthma is characterised by acute inflammation in lungs, constriction of airways
  - 300 million people suffer from it
- Eosinophil is an essential modulator in lung inflammation
- IL-10: cytokine synthesis inhibitory factor, potent inhibitor of eosinophil
- *L. lactis* expressing IL-10 was made and intranasal administration was done in mice with ovalbumin-induced acute airway inflammation

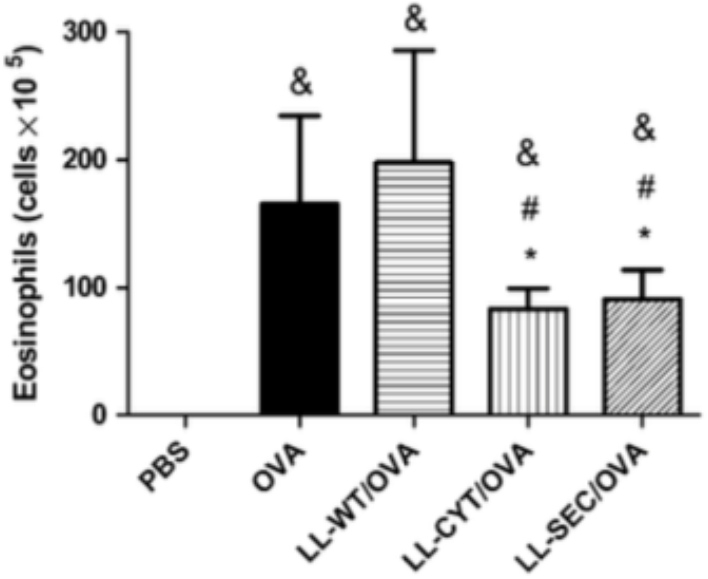
Marinho, F. A., Pacífico, L. G., Miyoshi, A., Azevedo, V., Loir, Y. L., Guimarães, V. D., . . . Oliveira, S. C. (2010). An intranasal administration of Lactococcus lactis strains expressing recombinant interleukin-10 modulates acute allergic airway inflammation in a murine model. *Clinical & Experimental Allergy, 40*(10), 1541-1551. doi:10.1111/j.1365-2222.2010.03502.x

Recombinant IL-10 was successfully synthesised and suppressed eosinophil level



Amount of IL-10 in control and samples

LL-CYT: IL-10 in cytoplasmic form
LL-SEC: IL-10 in secreted form



Eosinophil amount

OVA: asthma induction

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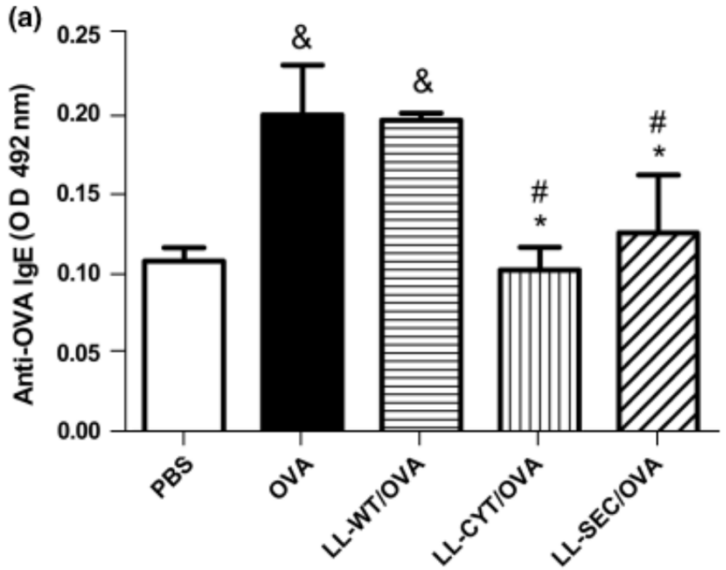
Recombinant IL-10 reduced OVA-specific IgE level



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Concerns about the biological safety

1. Spreading outside lab

2. Unknown interaction between microbes
   - e.g. DNA sharing between microbes
     - The recombinant proteins may be overproduced by modified bacteria and residential gut microbes

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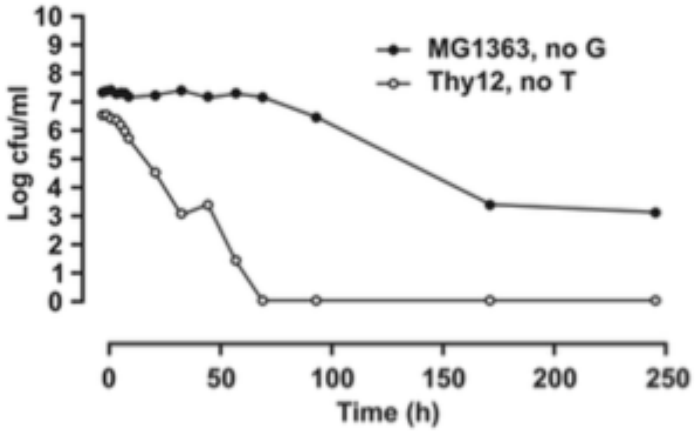
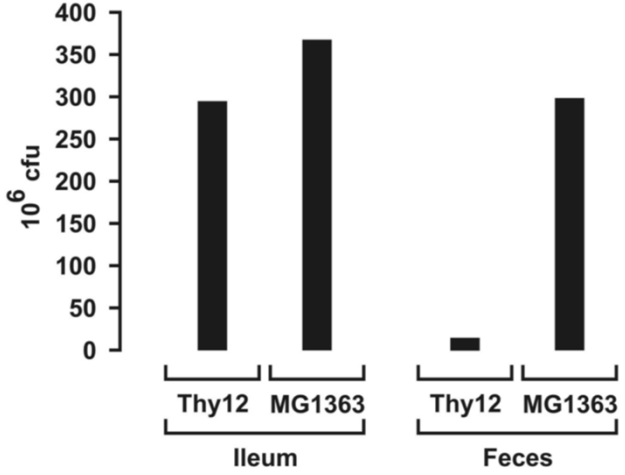
Cell death by *thyA* gene deletion

- In modified *L. lactis*, the thymidylate synthase gene *thyA* was replaced with recombinant genes



Cell culture in thymidine-free medium



L. lactis detection from pig

MG1363: Wild type
Thy12: *L. lactis* without *thyA* gene

Steidler, L., Neirynck, S., Huyghebaert, N., Snoeck, V., Vermeire, A., Goddeeris, B., . . . Remaut, E. (2003). Biological containment of genetically modified Lactococcus lactis for intestinal delivery of human interleukin 10. Nature Biotechnology, 21(7), 785-789. doi:10.1038/nbt840

No acquisition of foreign *thyA* gene observed

- The research teams tried mixing modified and wild type *L. lactis* during cell growth
  - No isolation of modified *L. lactis* showing thymidine-independent growth
  - Possible reason: diversity in *thyA* gene sequences

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The functional stability may not be high

- Longer duration of bacteria in body, higher possibility for mutations to happen
  - The insertion of foreign genes reduces growth rate
  - The microenvironment in body may be competitive (less oxygen / nutrient)
  - Provides selective pressure (point mutation / loss of function)

- Resulted in 50% loss of cells carrying desired plasmids in some studies

Danino, T., Prindle, A., Hasty, J., & Bhatia, S. (2013). Measuring Growth and Gene Expression Dynamics of Tumor-Targeted S. Typhimurium Bacteria. *Journal of Visualized Experiments,* (77). doi:10.3791/50540

The expression condition is not optimal in practice

- *In vitro* protein synthesis always done in optimal conditions (full of nutrients, rich in oxygen)

- Reality: The nutrient and oxygen are limited, lowering expression level and ultimately limiting cell growth

Ceroni, F., Furini, S., Gorochowski, T. E., Boo, A., Borkowski, O., Ladak, Y. N., . . . Ellis, T. (2017). Burden-driven feedback control of gene expression. *bioRxiv* . doi:10.1101/177030

Summary

- GMOs can be useful in treating disease
- Aside from drug delivery, GMOs can also be used as diagnostic tools
- Stability and safety problems have to be addressed before application in real-world settings

Thank you very much for your kind attention

References

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Reardon, S. (2018). Genetically modified bacteria enlisted in fight against disease. Nature, 558(7711), 497-498. doi:10.1038/d41586-018-05476-4

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