Does synthetic biology have a role in developing strategies to control nematodes?

Keith G Davies

AAB Advances in Nematology
The Linnaean Society of London

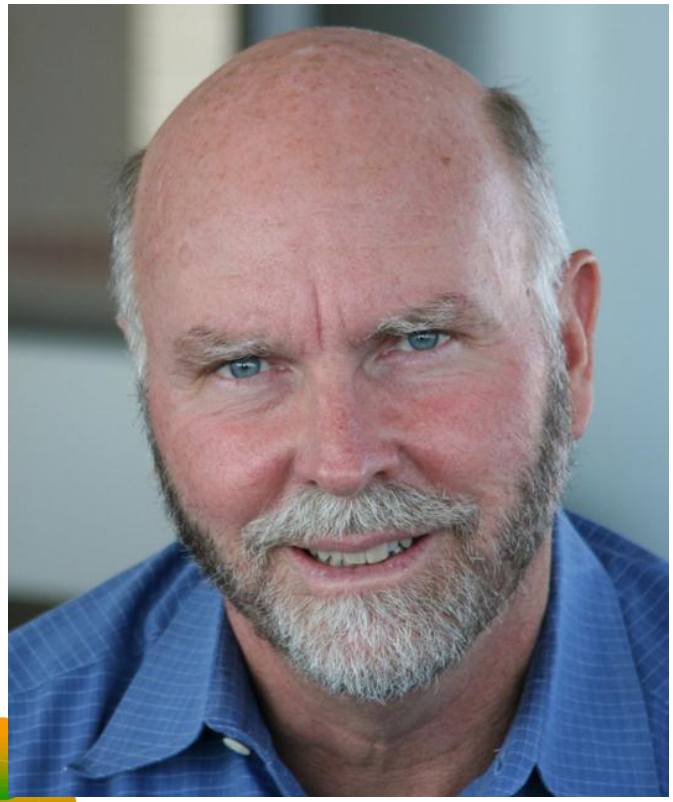
13th December 2011

Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Hertfordshire United Kingdom, AL5 2JQ

Synthetic biology comes of age

"… the real challenge will start when we enter the synthetic biology phase of research in our field. We will then devise new control elements and add these new modules to the existing genomes or build up wholly new genomes."

Szybalski, 1974



RESEARCH ARTICLE

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] Evgeniya A. Denisova,[1] Lei Young,[1] Zhi-Qing Qi,[1] Thomas H. Segall-Shapiro,[1] Christopher H. Calvey,[1] Prashanth P. Parmar,[1] Clyde A. Hutchison III,[2] Hamilton O. Smith,[2] J. Craig Venter[1,2]*

We report the design, synthesis, and assembly of the 1.08–mega–base pair *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *M. capricolum* recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome. The only DNA in the cells is the designed synthetic DNA sequence, including "watermark" sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication.

In 1977, Sanger and colleagues determined the complete genetic sequence of phage φX174 (*1*), the first DNA genome to be completely sequenced. Eighteen years later, in 1995, our team was able to read the first complete genetic sequence of a self-replicating bacterium, *Haemophilus influenzae* (*2*). Reading the genetic sequence of a wide range of species has increased exponentially from these early studies. The ability to rapidly digitize genomic information has increased by more than eight orders of magnitude over the past 25 years (*3*). Efforts to understand all this new genomic information have spawned numerous new computational and

We developed a strategy for assembling viral-sized pieces to produce large DNA molecules that enabled us to assemble a synthetic *M. genitalium* genome in four stages from chemically synthesized DNA cassettes averaging about 6 kb in size. This was accomplished through a combination of in vitro enzymatic methods and in vivo recombination in *Saccharomyces cerevisiae*. The whole synthetic genome [582,970 base pairs (bp)] was stably grown as a yeast centromeric plasmid (YCp) (*7*).

Several hurdles were overcome in transplanting and expressing a chemically synthesized chromosome in a recipient cell. We needed to improve

crude *M. mycoides* or *M. capricolum* extracts, or by simply disrupting the recipient cell's restriction system (*8*).

We now have combined all of our previously established procedures and report the synthesis, assembly, cloning, and successful transplantation of the 1.08-Mbp *M. mycoides* JCVI-syn1.0 genome, to create a new cell controlled by this synthetic genome.

Synthetic genome design. Design of the *M. mycoides* JCVI-syn1.0 genome was based on the highly accurate finished genome sequences of two laboratory strains of *M. mycoides* subspecies *capri* GM12 (*8*, *9*, *11*). One was the genome donor used by Lartigue *et al.* [GenBank accession CP001621] (*10*). The other was a strain created by transplantation of a genome that had been cloned and engineered in yeast, YCpMmyc1.1-Δ*typeIIres* [GenBank accession CP001668] (*8*). This project was critically dependent on the accuracy of these sequences. Although we believe that both finished *M. mycoides* genome sequences are reliable, there are 95 sites at which they differ. We began to design the synthetic genome before both sequences were finished. Consequently, most of the cassettes were designed and synthesized based on the CP001621 sequence (*11*). When it was finished, we chose the sequence of the genome successfully transplanted from yeast (CP001668) as our design reference (except that we kept the intact *typeIIres* gene). All differences that appeared biologically significant between CP001668 and previously synthesized cassettes were corrected to match it exactly (*11*). Sequence differences between our synthetic cassettes and CP001668 that occurred at 19 sites appeared harmless and so were not corrected. These provide 19 polymorphic

Gibson *et al.*, 2010 Science 329: 52-56

Within the synthetic genome *Mycoplasma mycoides* there were ‘watermarks’

CRAIGVENTER coded as:
TTAACTAGCTAA**TGTCGTGCAATTGGAGTAGAGAACACAGAACGA**TTAACTAGCTAA

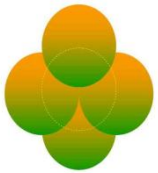
VENTERINSTITVTE coded as:
TTAACTAGCTAA**GTAGAAAACACCGAACGAATTAATTCTACGATTACCGTGACTGAG**TTAACTAGCTAA

“**WHAT I CANNOT BUILD, I CANNOT UNDERSTAND**.”

– attributed to Richard Feynman

“**TO LIVE, TO ERR, TO FALL, TO TRIUMPH, TO RECREATE LIFE OUT OF LIFE**.”

– from James Joyce’s



CONTEXT

European legislation regarding the use of pesticides

New legislation to replace Directive 91/414

A) New Registration regulation Directive 1107/2009
B) Sustainable use Directive 2009/128/EC

Came into effect 14 June 2011

Also water framework directive

Priority to be given to non-chemical methods

Need for alternative nematode control methods

Life-cycle disruption

(a) Hatch and migration
- nematicides
- root diffusates
- rhizosphere microorganisms

(b) Feeding, maturation and fecundity
- nematicides
- host plant susceptibility
- rhizosphere and endophytic micro-organisms

(c) Mate finding and reproduction
- nematicides
- mating disruption

Biological control perspective

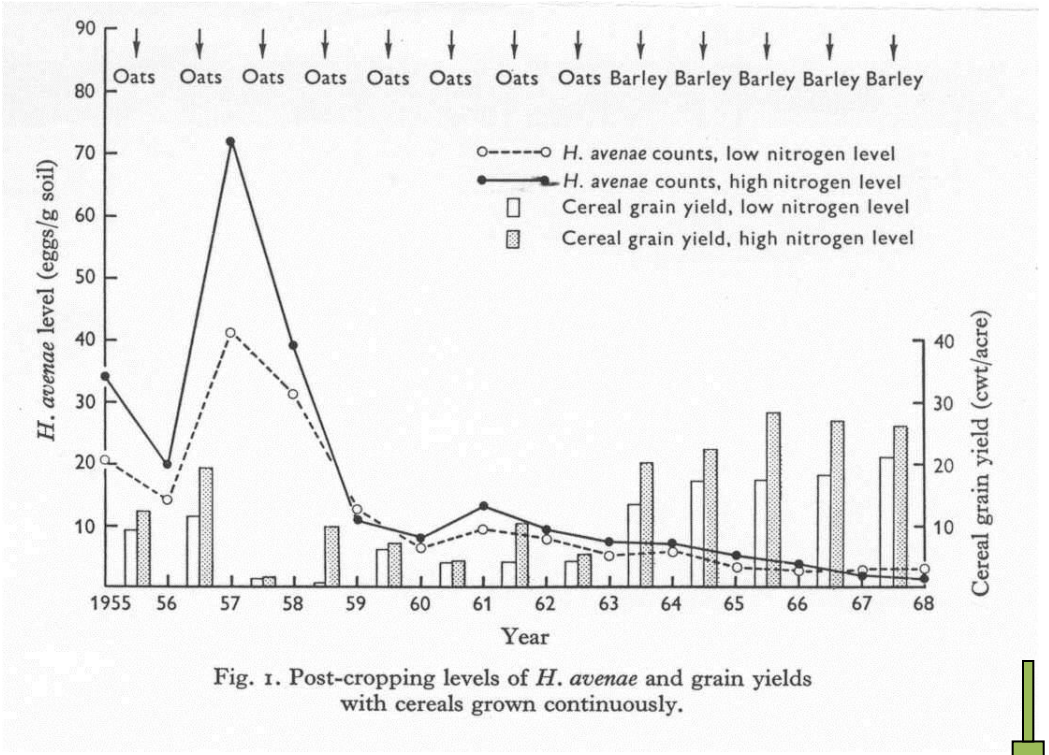
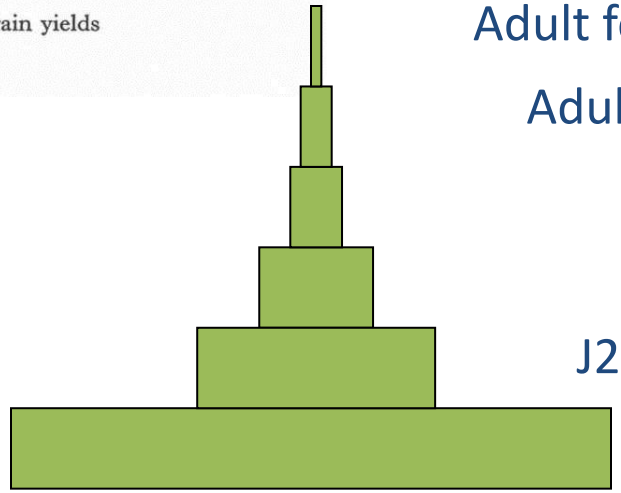


Fig. 1. Post-cropping levels of *H. avenae* and grain yields with cereals grown continuously.

(Gair *et al.*, 1969)



Adult females 3 %

Adult males 5%

J4 8%

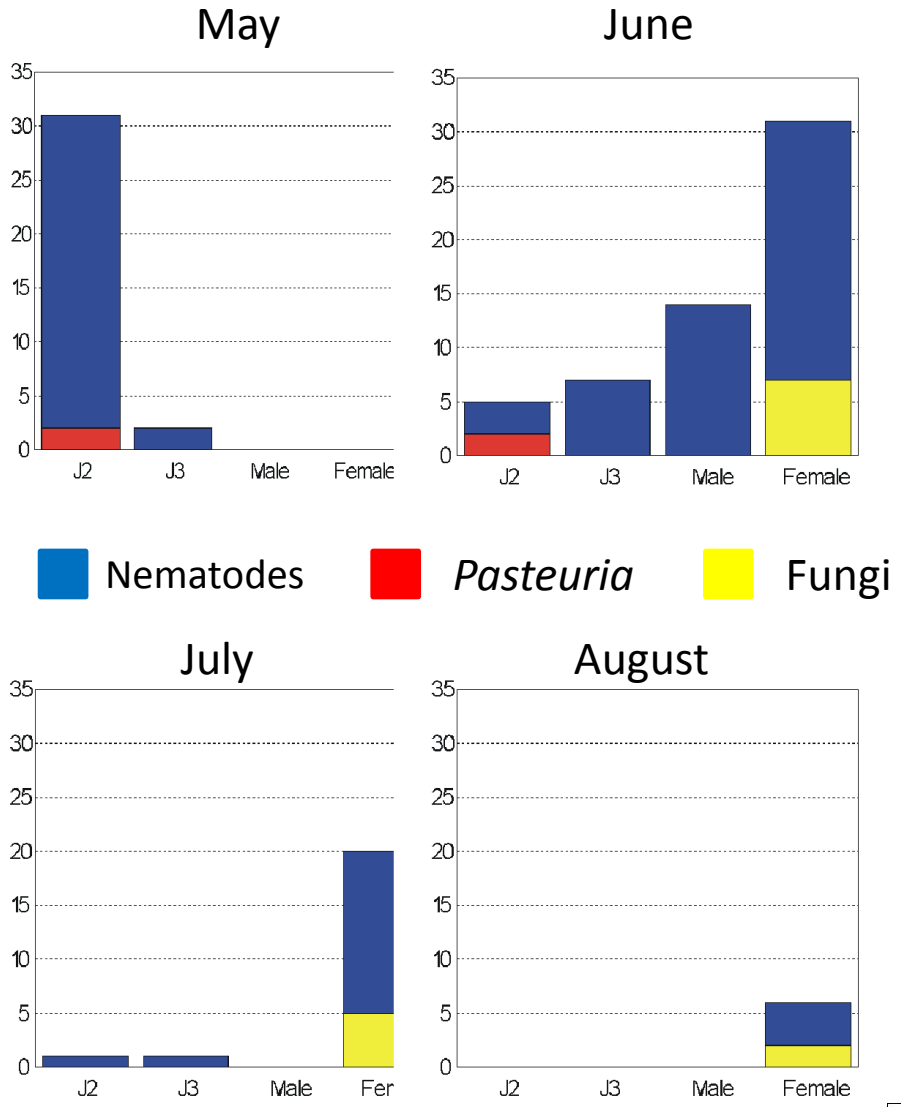
J3 11%

J2 roots 25 %

Eggs 100 %

Cereal Cyst Nematode decline phenomenon

Suppressive soils are usually the product of more than one organism



Davies *et al*., 1990

Biological control organisms

1) Fungal parasites

Pochonia chlamydosporia
Paecilomyces lilacinus
Arthrobotrys oligospora
Trichoderma spp.

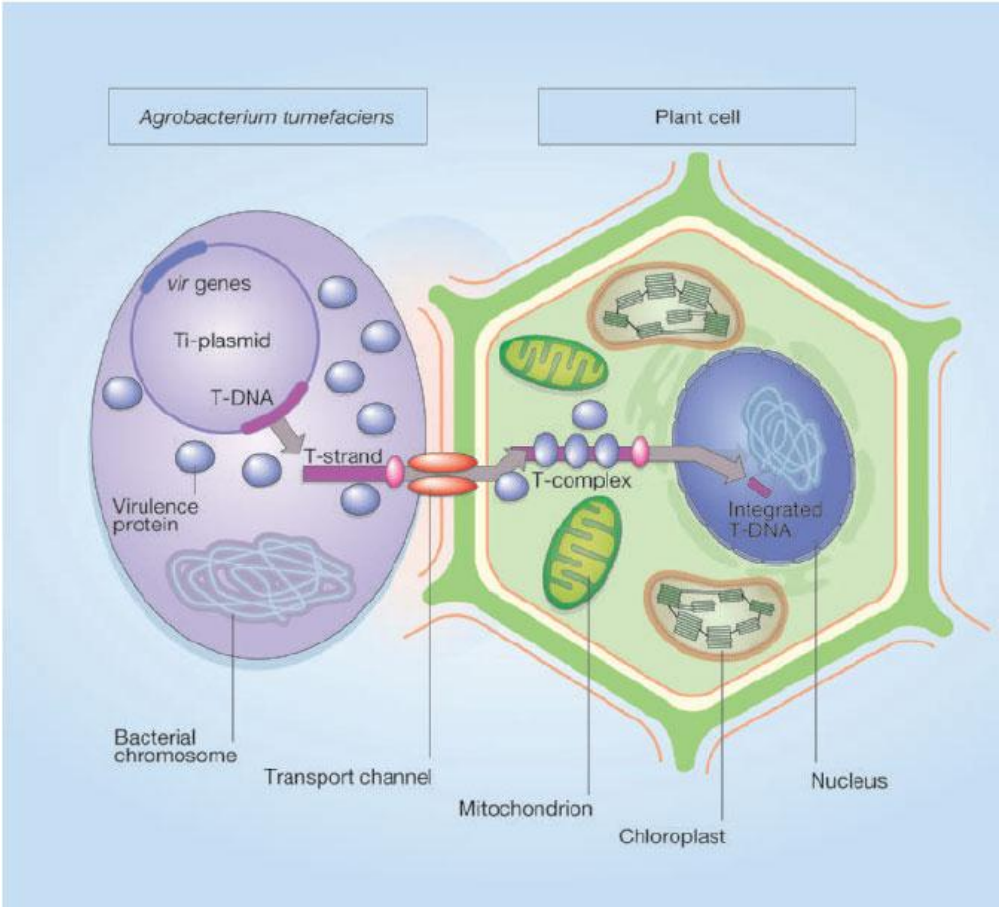
2) Bacterial parasites

Pasteuria penetrans
Rhizobacteria
Bacillus spp.

Twenty years have elapsed since the [Stirling's] *book was published dedicated to biological control of nematodes and to this day a robust commercially successful biological control agent for plant-parasitic nematodes is not routinely used*.

Keith Davies and Yitzak Spiegel (Eds) 2011:
Biological Control of Plant –Parasitic Nematodes

Transformation technology: heuristic approach



Heuristic approach by which we can learn about host parasite interactions..

Synthetic designer plants may be a little way off… but synthetic designer bacteria???

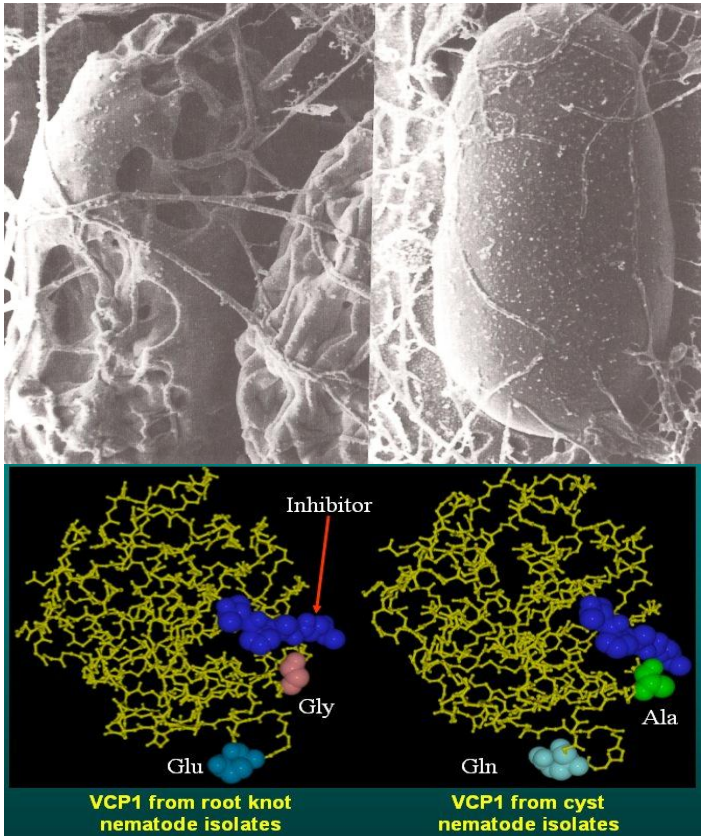
Mechanisms are key to understanding host parasite interactions?

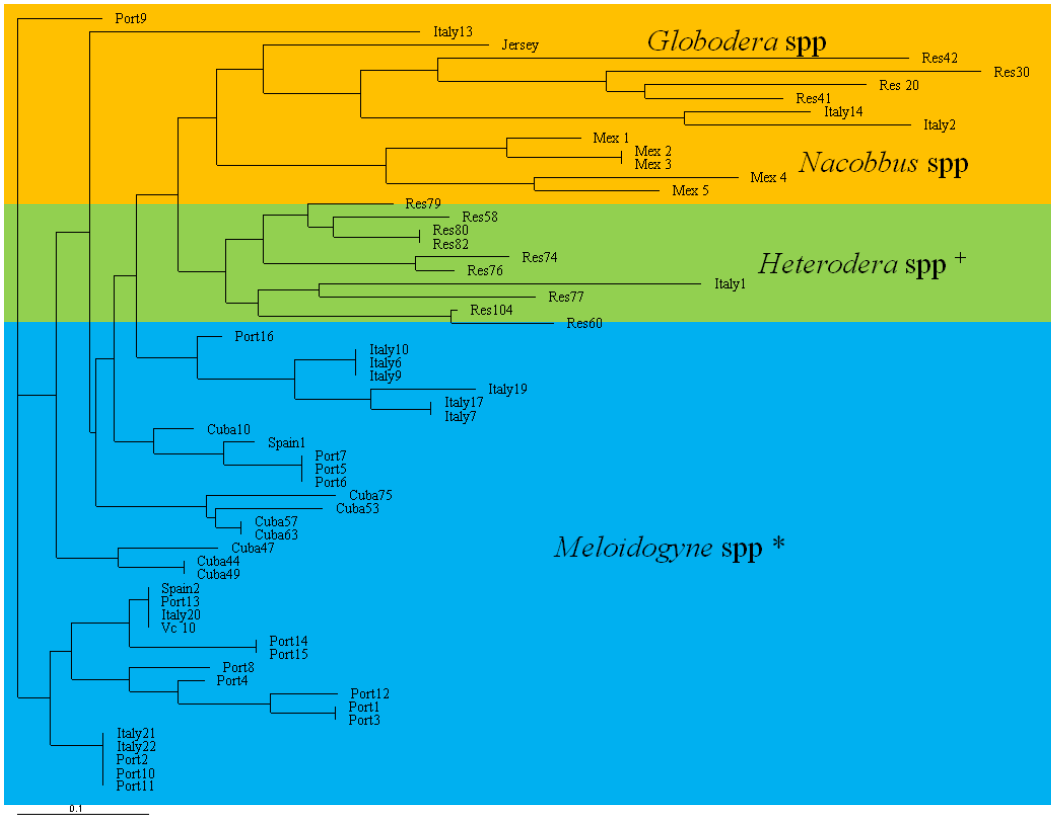
I am coming from the perspective of biological control

Agricultural biotechnology: Gene exchange by design Stanton B. Gelvin Nature 433, 583-584 (10 February 2005)

Example 1 *Pochonia*

Genetic variation of *Pochonia* biological control isolates ERIC fingerprinting

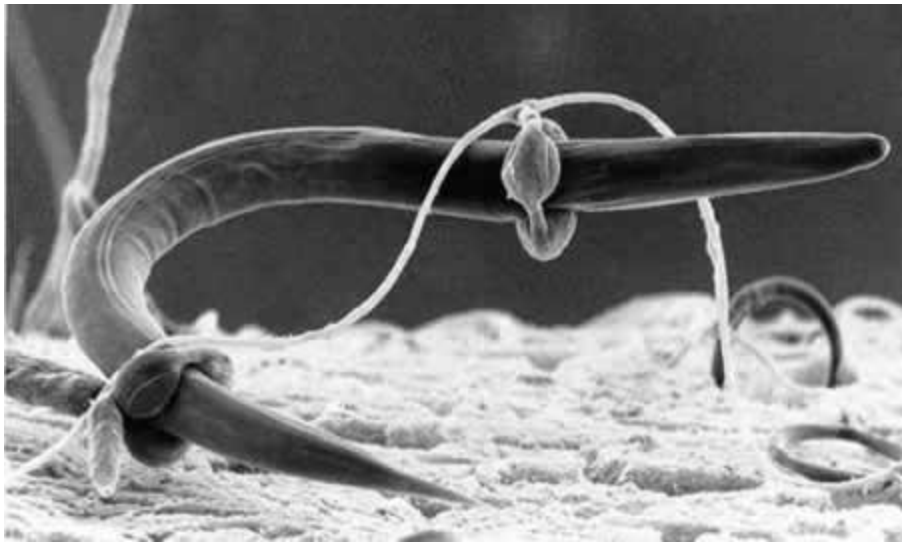




Host specific changes in the VCP1 protease

Kerry & Hirsch 2011: In Keith Davies and Yitzak Spiegel (Eds) *Biological Control of Plant –Parasitic Nematodes*

Example 2 Arthrobotrys



Peptides
"nemin"
elicitors

adhesins

Lectins

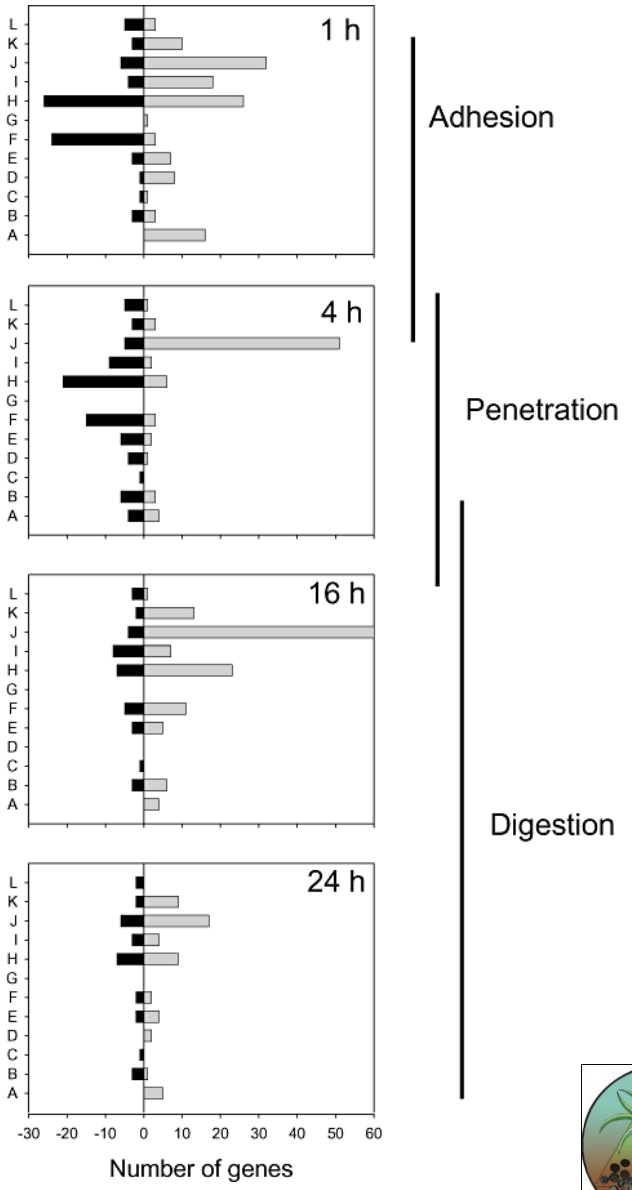
Serine
proteases

Subtilisins

Toxic metabolites
(linoleic acid)

Tunlid and Ahrén2011: In Keith Davies and Yitzak Spiegel (Eds) *Biological Control of Plant –Parasitic Nematodes*

Changes to fungal transcriptome



Example 3 *Trichoderma*

Molecular pattern recognition receptors

Adhesion factors

Lectin - fucose interactions

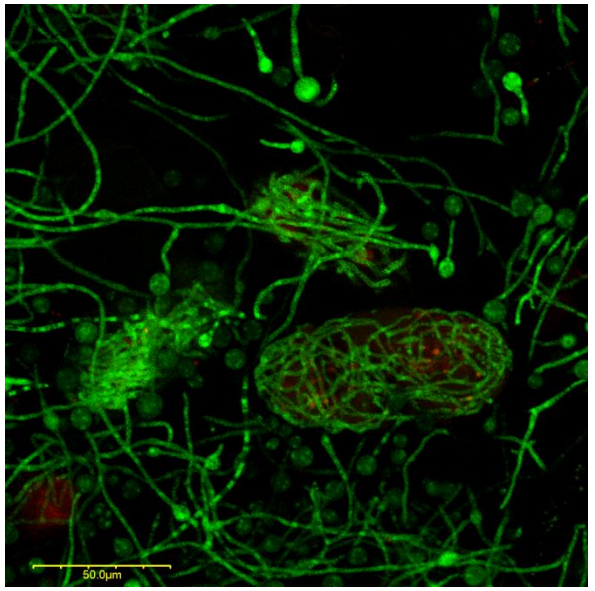
antibiotics

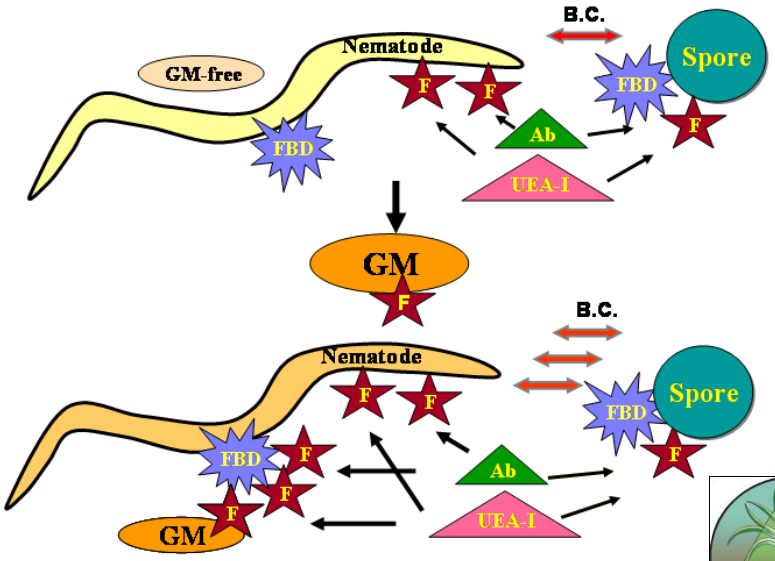
proteases

Signalling pathways

chitinases

Induced resistance





Sharon, Chet & Spiegel 2011: In Keith Davies & Yitzak Spiegel (Eds) *Biological Control of Plant –Parasitic Nematodes*

Example 4 PGPR

Adhesion factors

Lipopolysaccharide

phytostimulators

Plant growth hormones

siderophores

antibiotics

Induced systemic resistance

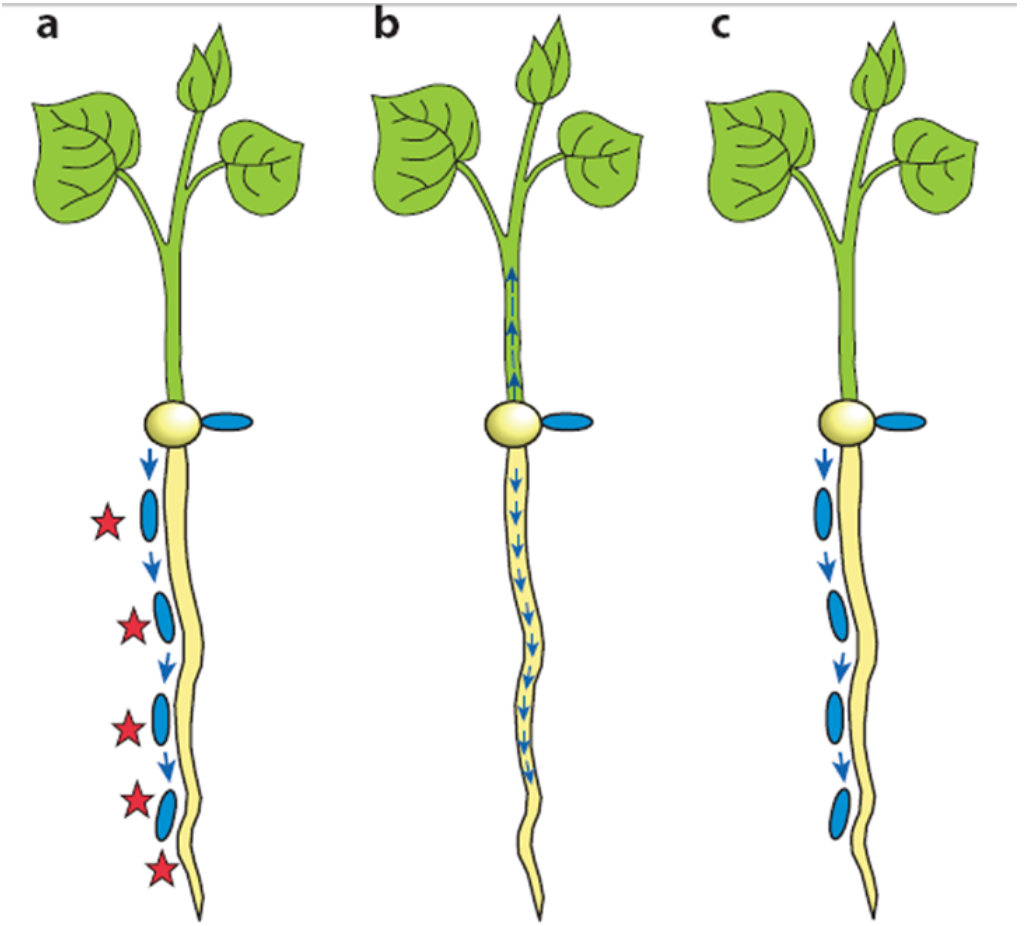


Figure 2

Illustration of the most important mechanisms of biological control of plant diseases by bacteria. In all cases illustrated here, biocontrol begins by coating seeds with the biocontrol bacterium. (*a*) Antibiosis. The bacterium colonizes the growing root system and delivers antibiotic molecules around the root, thereby harming pathogens that approach the root (*indicated by stars*).

Lugtenberg and Kamilova 2009 *Ann Rev. Microbiol* 63 541-556

Example 5 *Pasteuria*

Velcro – like attachment

Innate immunity

collagen molecules

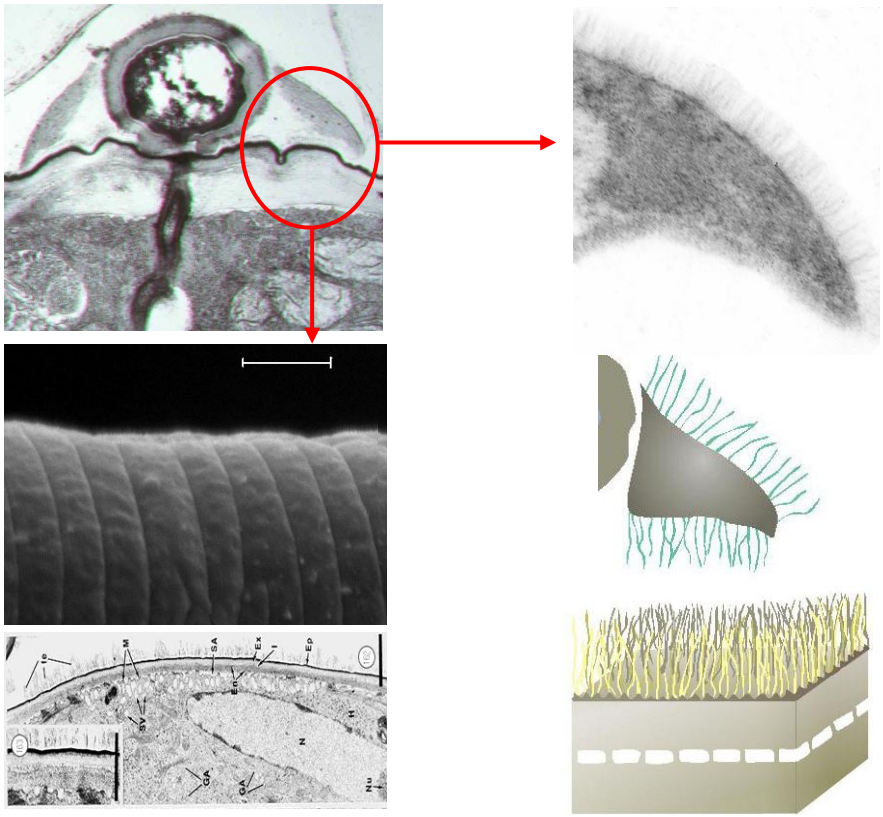
lectins

carbohydrates

Signalling pathways

Glycosyl-transferases

mucins



Davies K G 2009, *Adv. In Parasitol.* 68, 211 - 245

Nematode SC – plant interactions

signalling

Daf-2

Hormonal - peptides

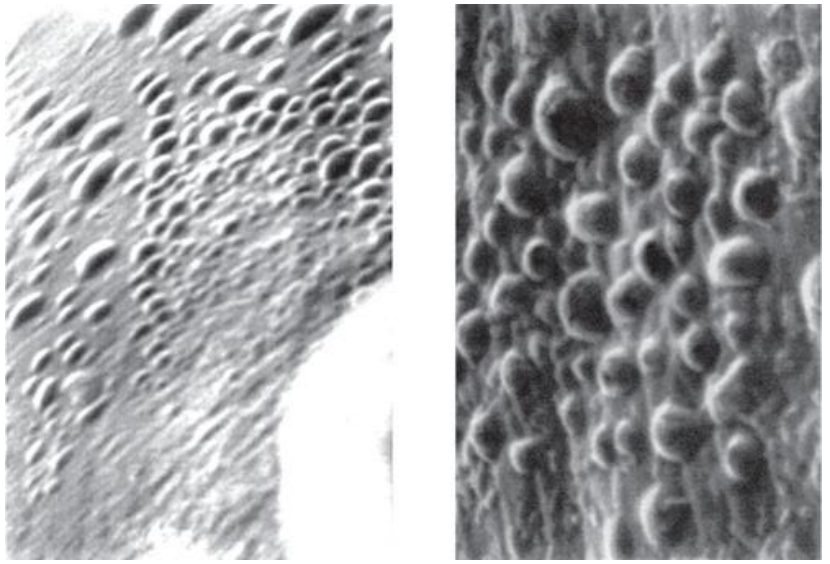
TGF-β

mucins

Innate immunity

proteases

Protease inhibitors



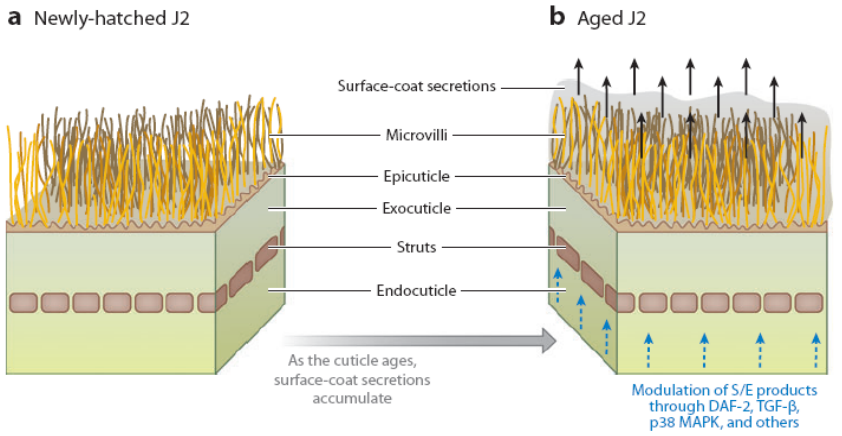
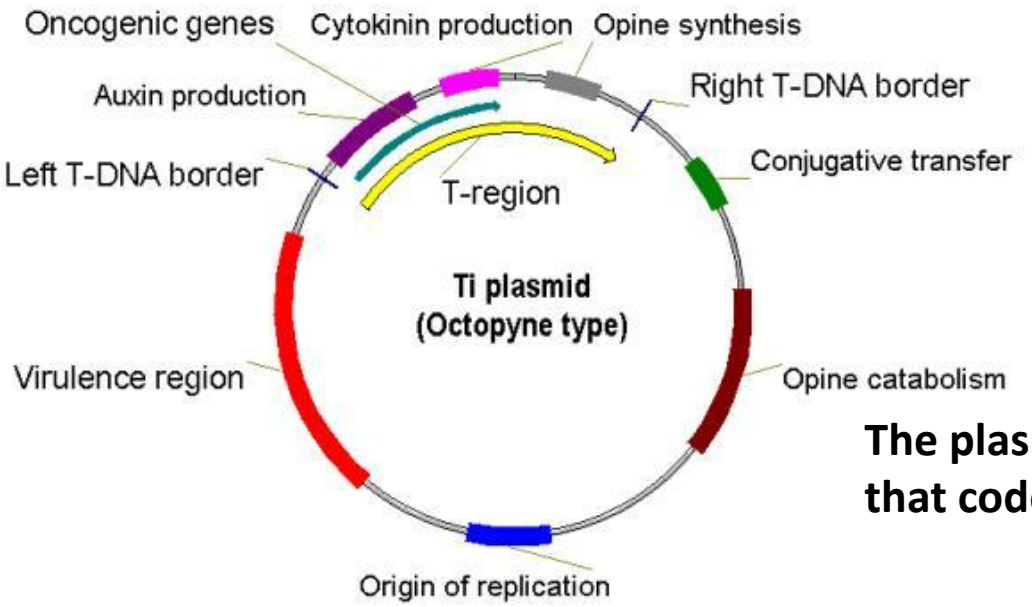


Figure 4

Generalized model of the infective juvenile nematode cuticle consisting of three easily definable layers (as described in **Figure 1**). The surface coat overlies the epicuticle and is an amorphous fuzzy structure that is carbohydrate rich and contains mucins and microvilli. As the infective juvenile ages, this layer changes and become less susceptible to *Pasteuria* endospore attachment (34). Preliminary experiments suggest that the surface coat secretions are modulated through signaling pathways, as exposure to peptides that inhibit IGF-1 stimulated cells involved in innate immunity (31, 54) also reduces endospore attachment.

Davies and Curtis, 2011, *Ann. Rev.Phytopath* 49

Do we need to reinvent the wheel?



The plasmid has 196 genes that code for 195 proteins

Mycoplasma mycoides
minimal genome project

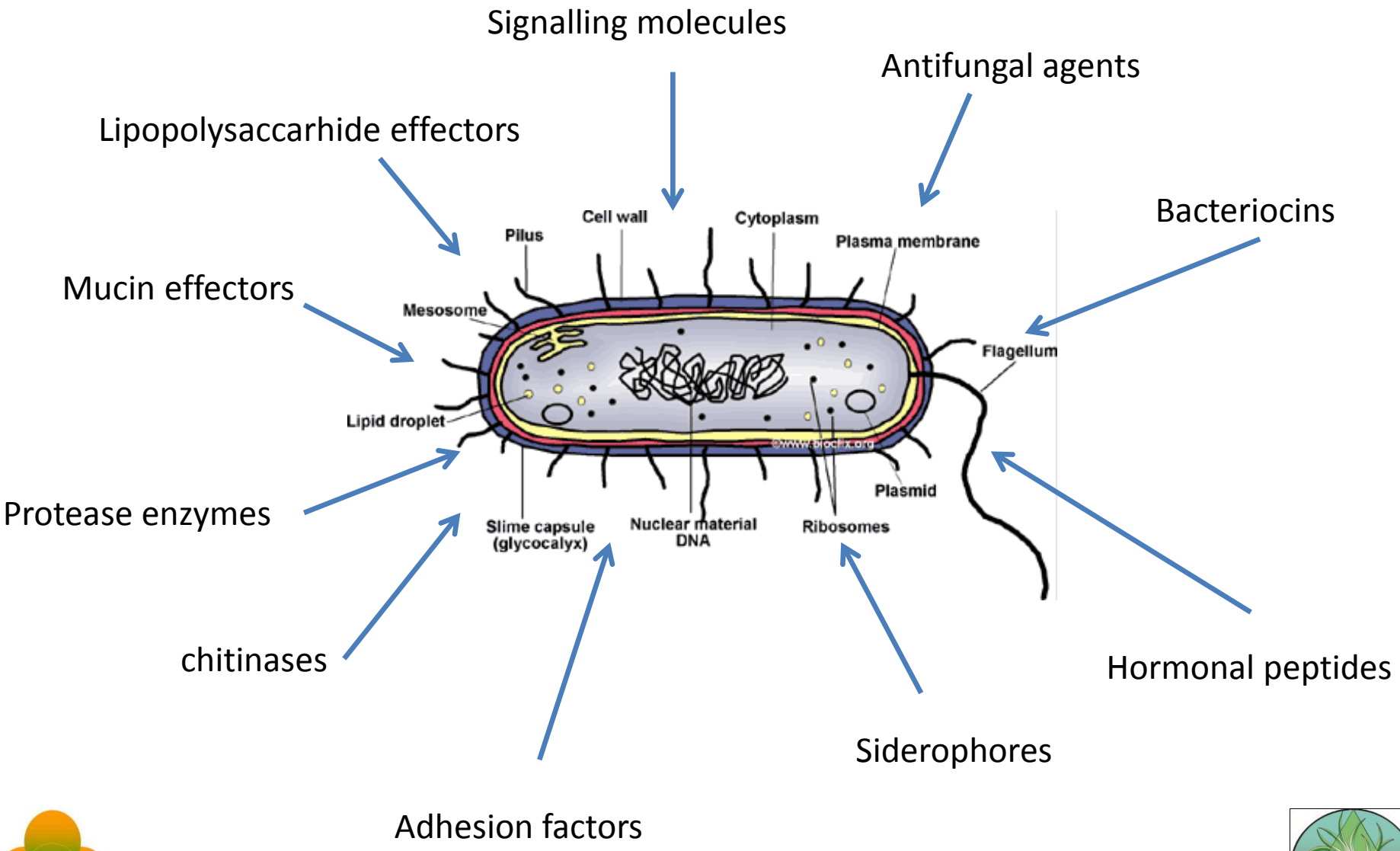
1.8 mega-base genome
582,970 base pairs = 482 genes
382 minimal gene set

Bacillus subtilis
minimal genome project

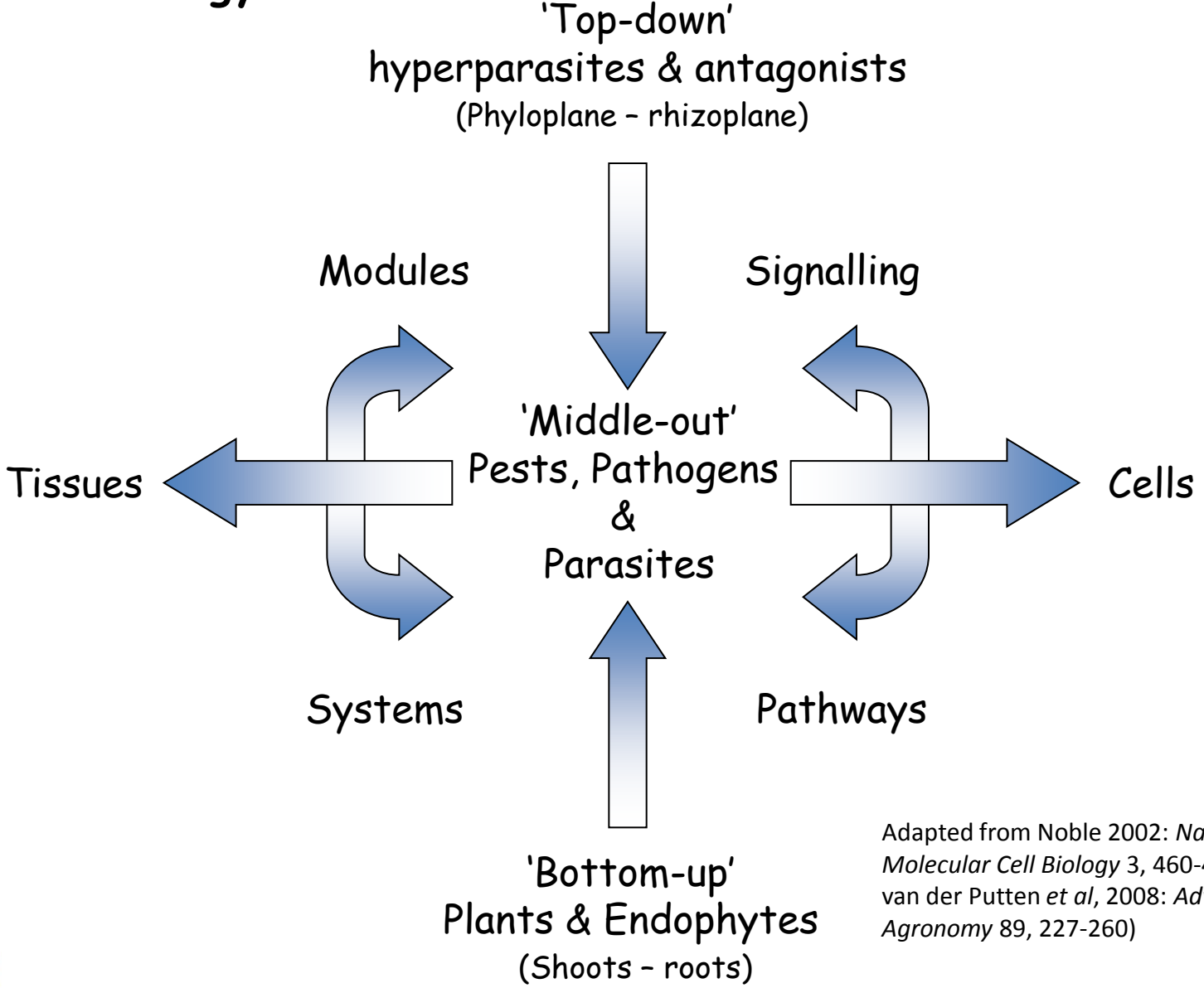
271 genes minimally

***Kobayashi et al*., 2003 *PNAS* 100, 4678-4683**

Designer biological control agents?



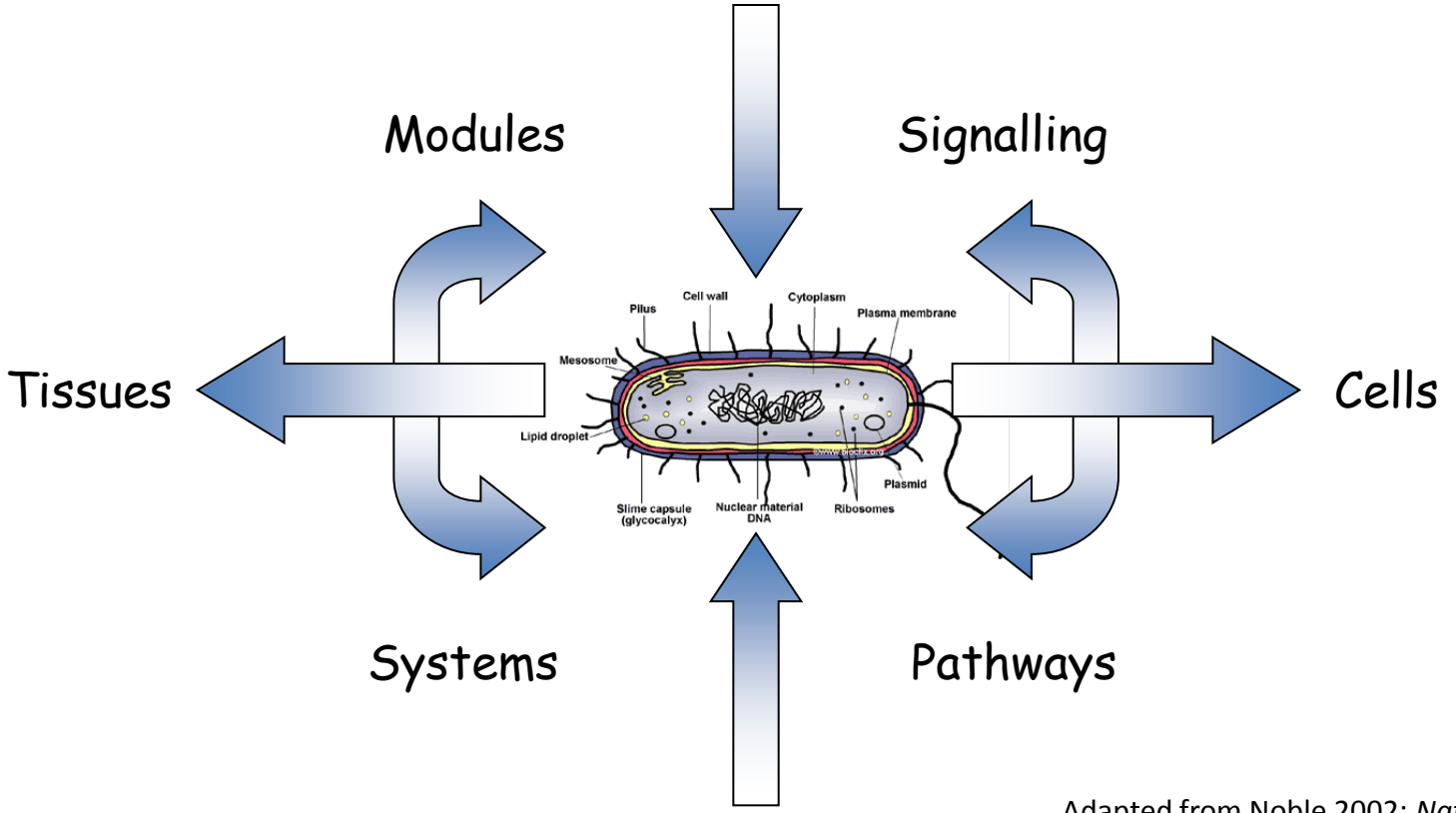
Multitrophic Plant-Patho-Systems Biology



Adapted from Noble 2002: *Nature Reviews Molecular Cell Biology* 3, 460-463 and van der Putten *et al*, 2008: *Advances in Agronomy* 89, 227-260)

Can a *designer* BCA be added into the system to develop robust control strategies?

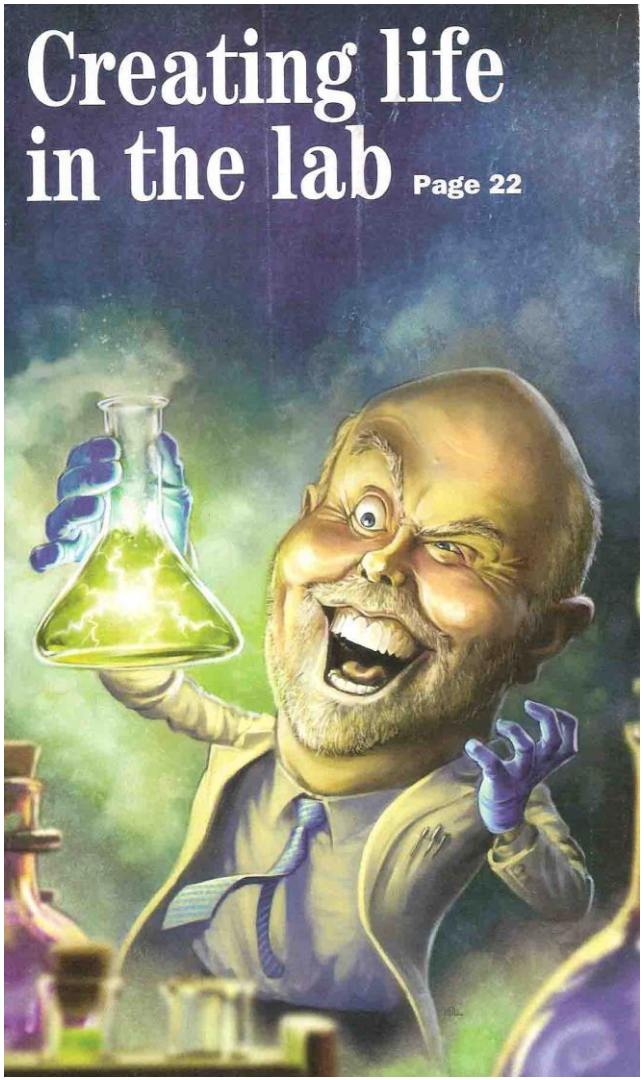
'Top-down'
hyperparasites & antagonists
(Phyloplane – rhizoplane)



'Bottom-up'
Plants & Endophytes
(Shoots – roots)

Adapted from Noble 2002: *Nature Reviews Molecular Cell Biology* 3, 460-463 and van der Putten *et al*, 2008: *Advances in Agronomy* 89, 227-260)

From Frankenstein to *Venterstein*?

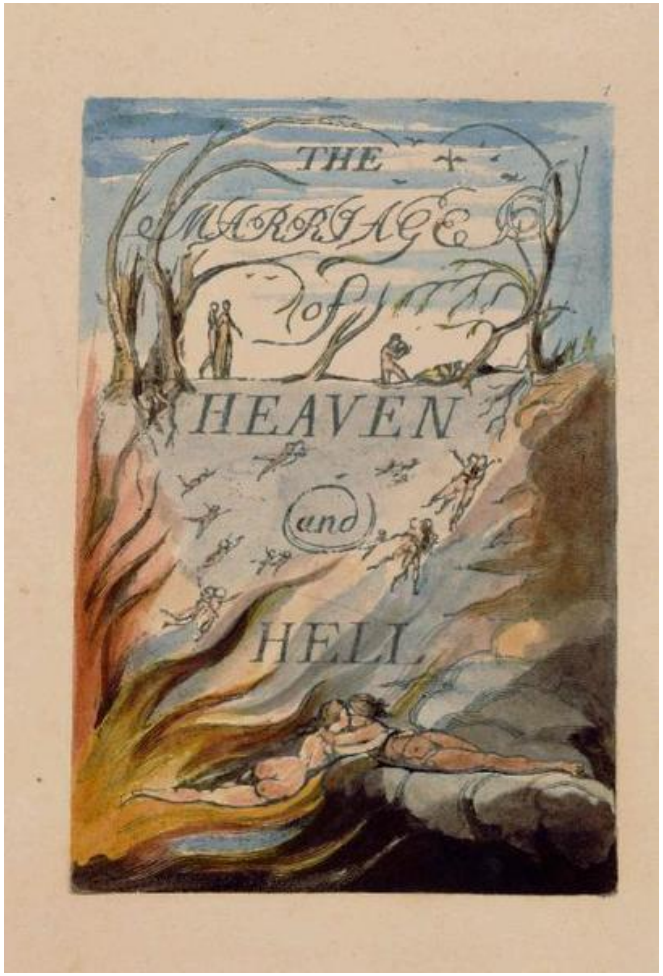


“WHAT I CANNOT BUILD, I CANNOT UNDERSTAND.”

– attributed to Richard Feynman

“TO LIVE, TO ERR, TO FALL, TO TRIUMPH, TO RECREATE LIFE OUT OF LIFE.”

– from James Joyce’s



Frontispiece
Marriage of Heaven and Hell

If you think this is all a little bit far fetch I will quote the Artist and Poet William Blake

What is now proved was once only imagin'd

William Blake (1757 – 1828)