

# Classification of Endophytes



Micro-organisms as agents between  
fertilization and plant protection

6th International Symposium  
jointly organised by DPG, JKI and HU-Berlin  
13 – 14 May 2014 Braunschweig, Germany

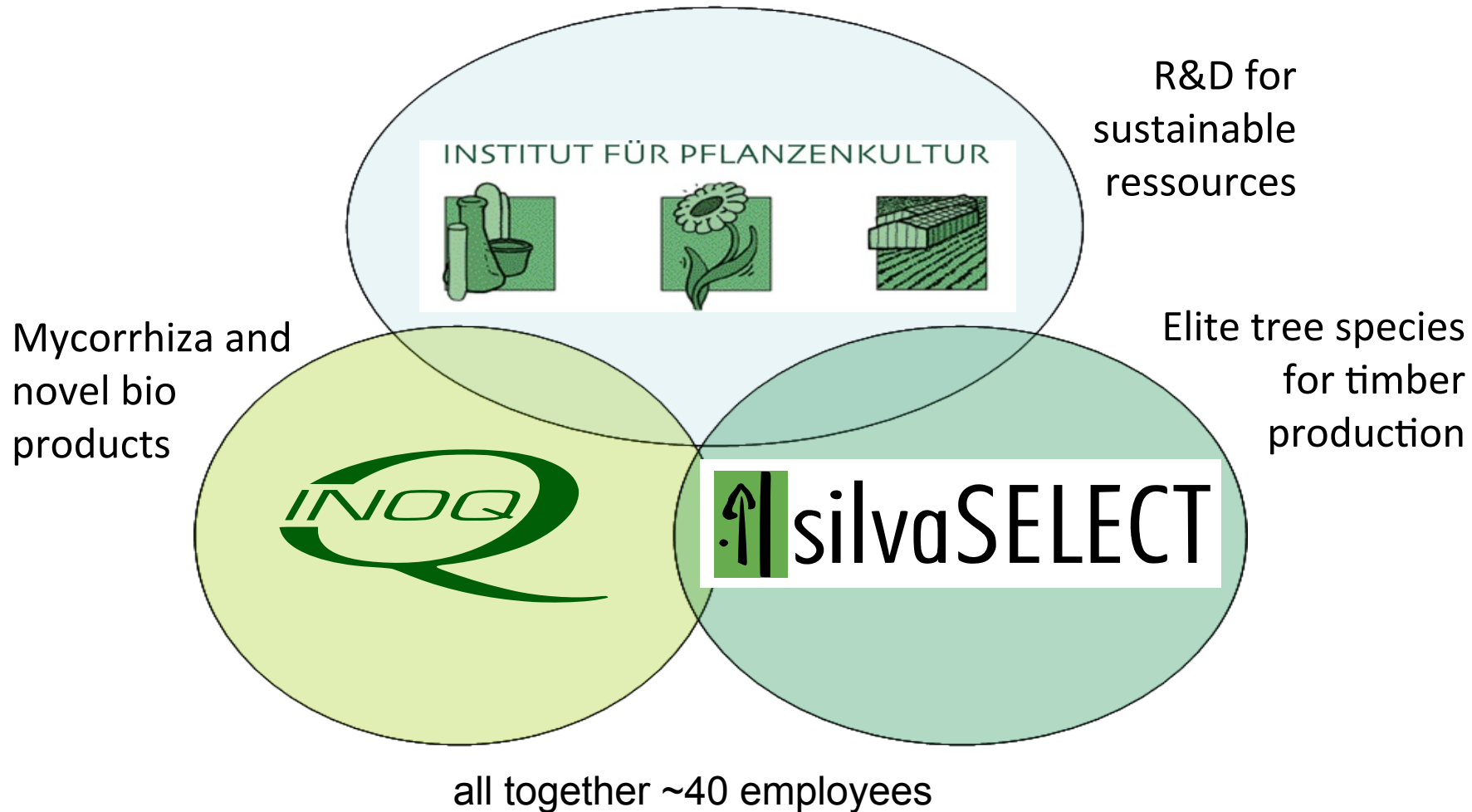
Philipp Rödel, INOQ GmbH, Solkau, Germany

# Topics



- INOQ - an SME dealing with microorganisms
- classification of endophytes
  - an endophyte project of INOQ
  - an example from endophyte research
  - grasslanz grass endophytes in NZ
- Are endophytes exotic in the context of biofertilizers and plant protection?

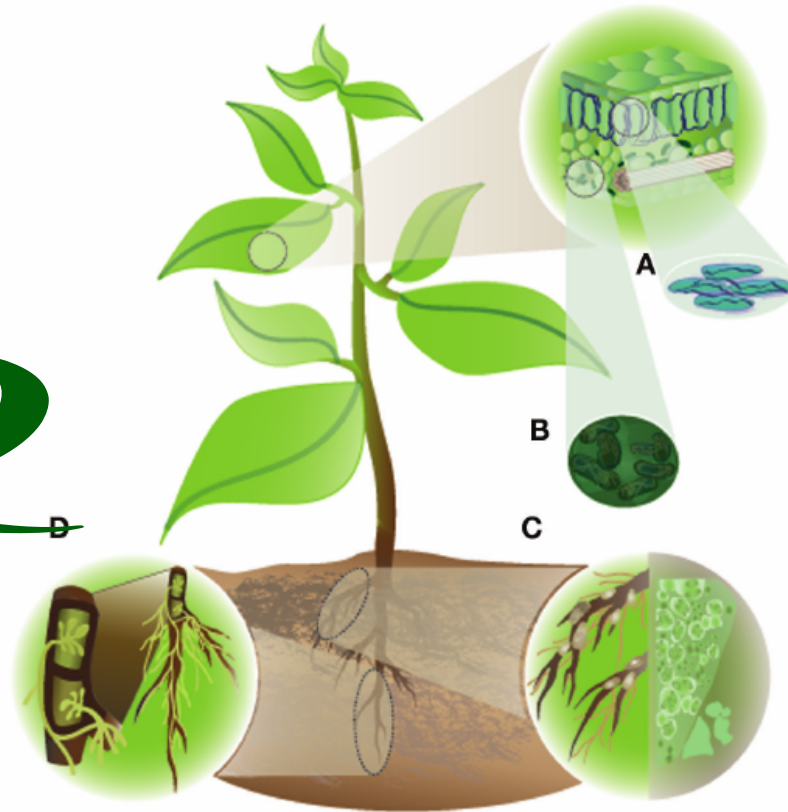
# Schneider Group – 19 years of business with science



# Why endophytes?



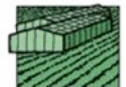
arbuscular  
mycorrhizal  
fungi



 silvaSELECT

fungal & bacterial  
endophytes

INSTITUT FÜR PFLANZENKULTUR



rhizobia

this image is a derivative of Figure 1 from: Partida-Martinez LPP, Heil M (2011) The microbe-free plant: fact or artefact? Frontiers in Plant Science DOI=10.3389/fpls.2011.00100. Cited under the Creative Commons Attribution License CC BY 3.0

# Classification of micro-organisms



Symposium flyer:

It is the **mode of action** and the **intention of use** which decides about the classification of the micro-organisms, the related registration requirements and processes in European member states.



fertilizer

plant  
protection  
product

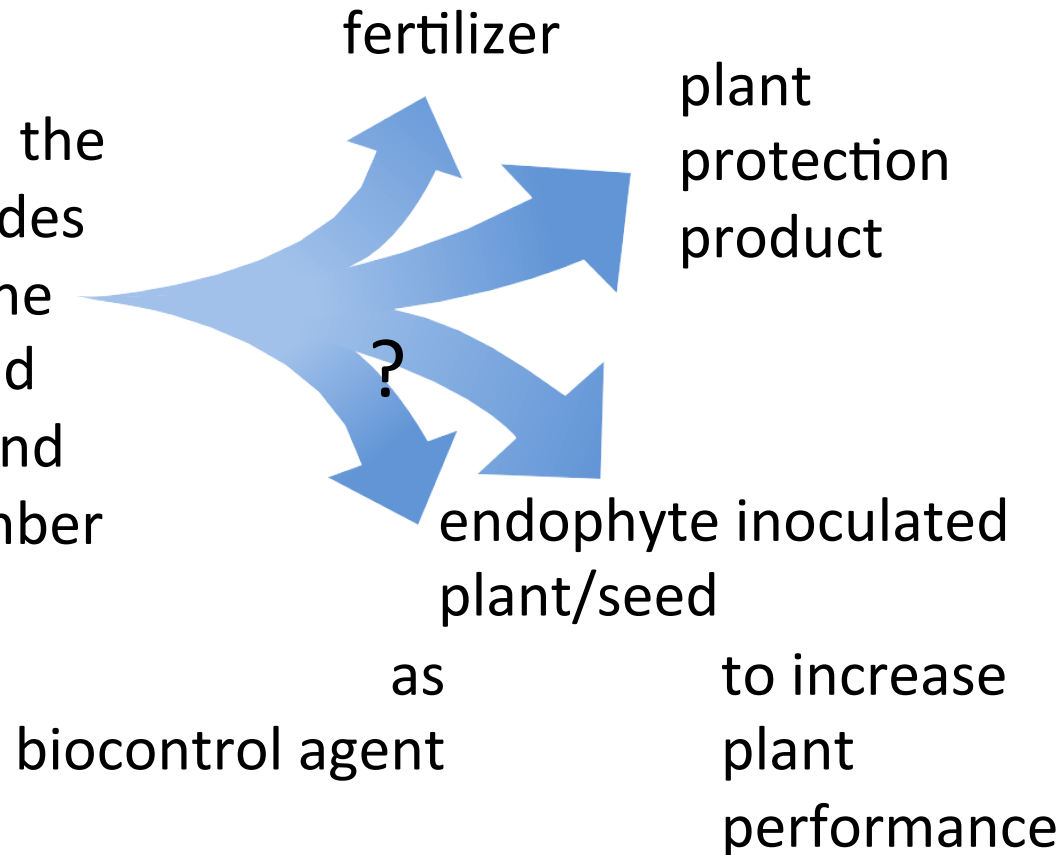
# What are endophytes?

- Endophytes live at least part of their life cycle inside of plants
- Endophytes can be
  - Bacteria
  - Fungi
  - Yeasts
- Several are beneficial and improve the performance of the plants
- Endophytes can be inherited vegetatively and some through seeds (vertical infection transmission)

# Classification of endophytes

Symposium flyer:

It is the **mode of action** and the **intention of use** which decides about the classification of the micro-organisms, the related registration requirements and processes in European member states.



# Example 1 – tree endophytes

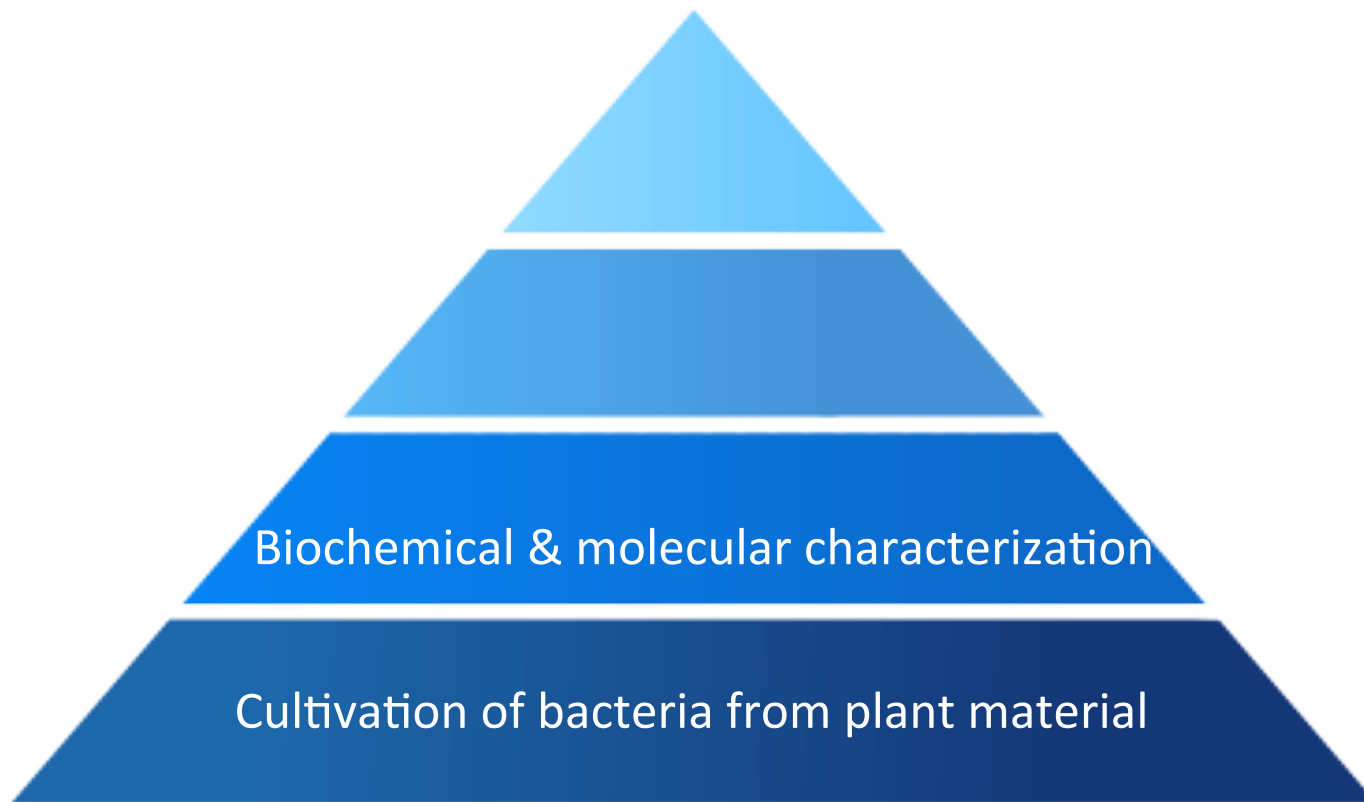
- vegetatively produced woody plants (*Prunus avium*)
- mixture of in vitro grown clones
- Some clones show better rooting and acclimatization than others
- could that be due to endophytes?



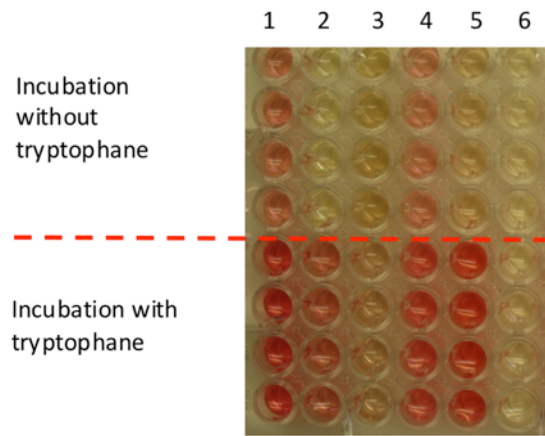
<http://www.silvaselect.de/>



# How we find our candidates

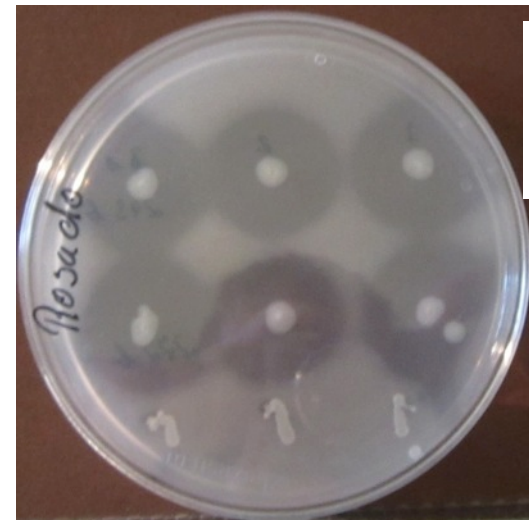


# Biochemical characterization



Test of bacteria for auxin synthesis from tryptophane

→ beneficial effect upon plant growth



phosphate solubilizing yeast

# Phylogeny of the 16S-rDNA sequences

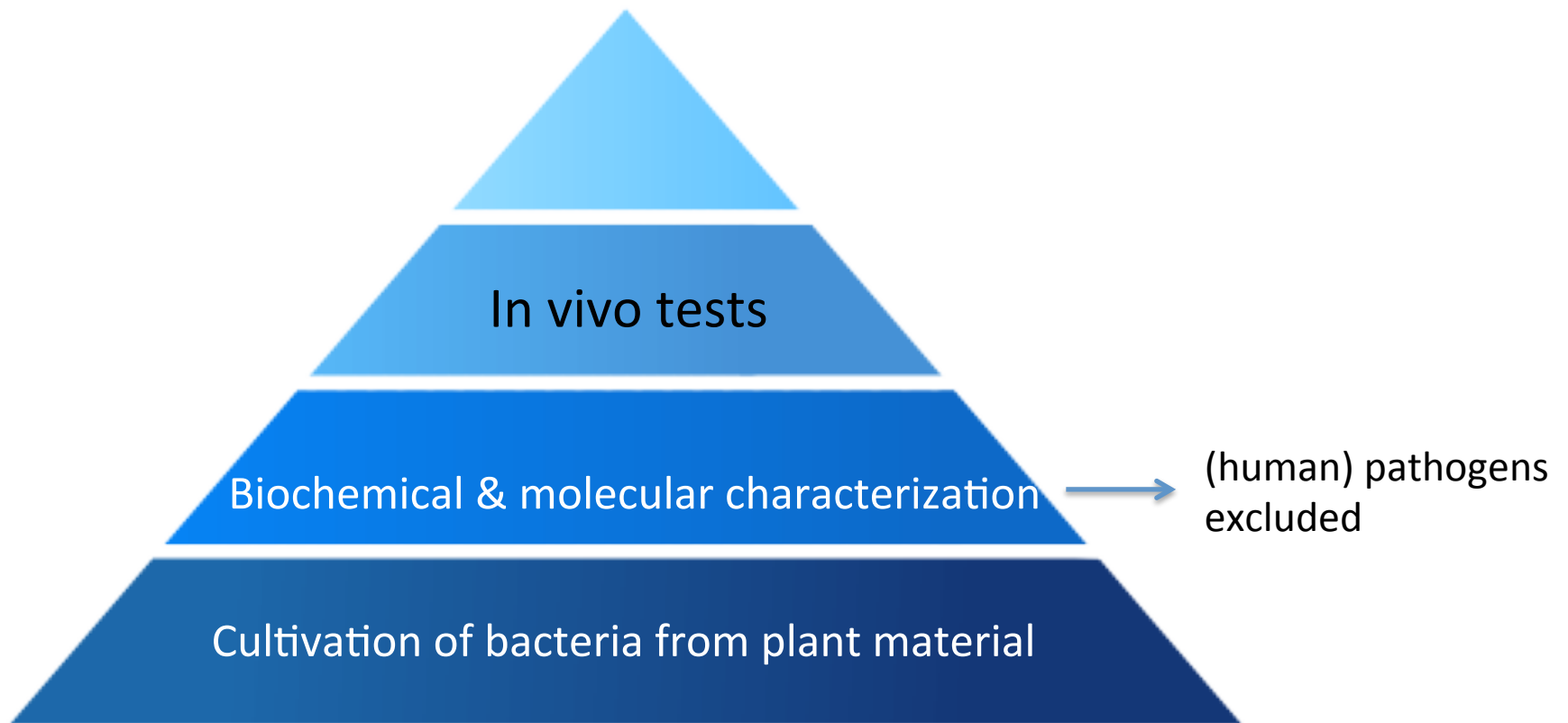


The 16S-rDNA phylogeny is used to identify potential pathogens and exclude them from further investigations.

figure removed due to copyright considerations

modified after Figure 5: Quambusch M, Pirttilä MA, Tejesvi MV, Winkelmann T and Bartsch M (2014) Endophytic bacteria in plant tissue culture: differences between easy- and difficult-to-propagate *Prunus avium* genotypes. *Tree Physiology* doi:10.1093/treephys/tpu027.

# How we test our candidates



# Improved rooting success for in vitro plants

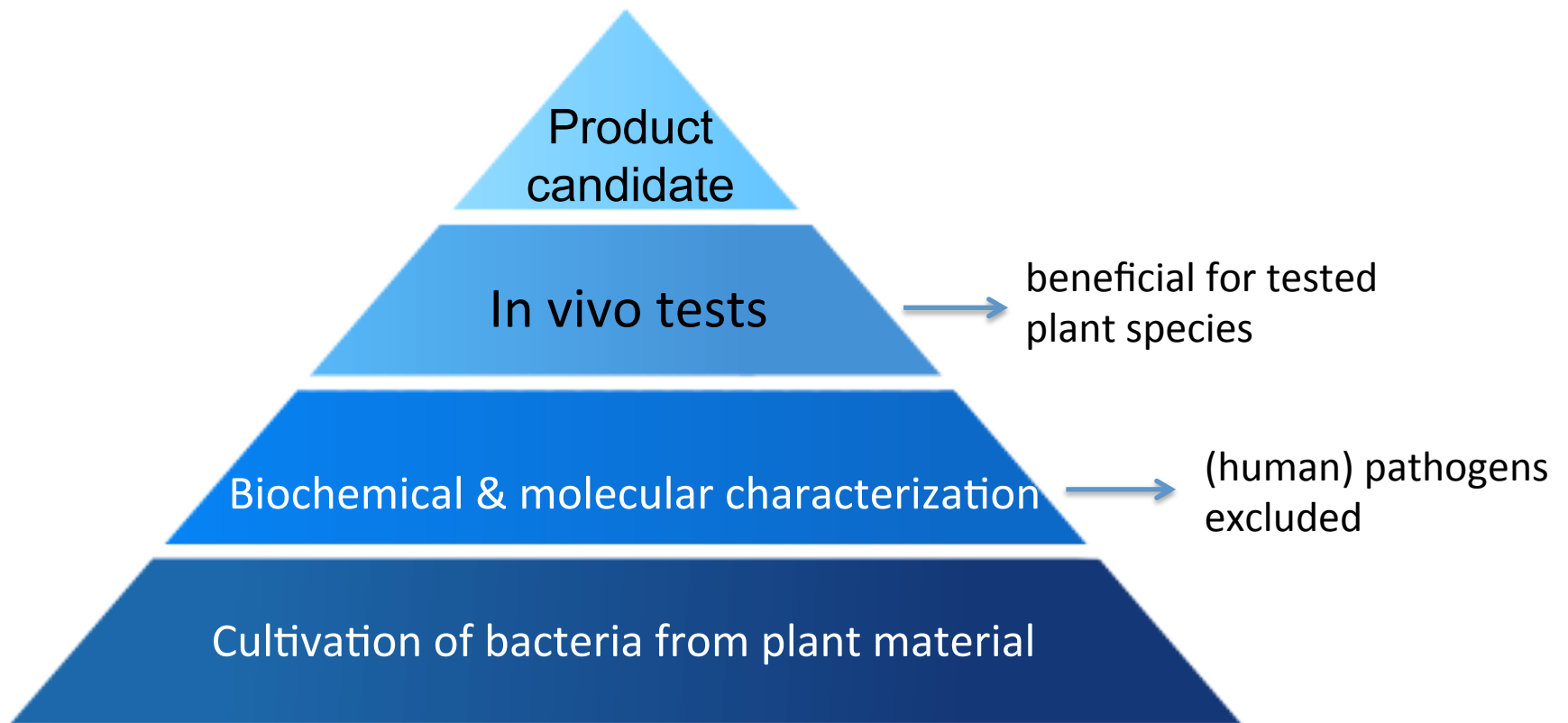


2 tested bacteria improve the rooting of *Prunus avium* significantly.

figure removed due to copyright considerations

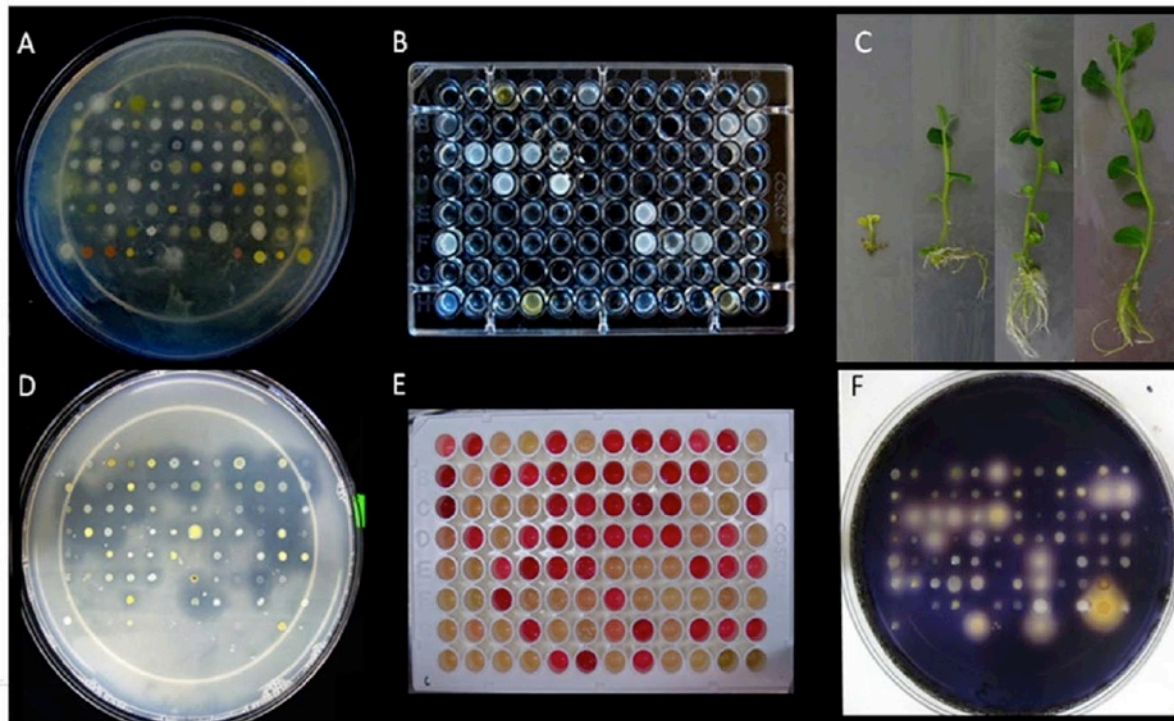
drawn after data from Table 2: Quambusch M, Pirttilä MA, Tejesvi MV, Winkelmann T and Bartsch M (2014) Endophytic bacteria in plant tissue culture: differences between easy- and difficult-to-propagate *Prunus avium* genotypes. *Tree Physiology* doi:10.1093/treephys/tpu027.

# How we test our candidates



# Example 2

## PGP-endophytes in maize



- (A) antagonism to *E. coli*
- (B) growth in nitrogen free media
- (C) growth promotion of tissue cultured potato
- (D) ability to solubilise tricalcium phosphate
- (E) acetoin and butanediol production (systemic resistance elicitor)
- (F) extracellular digestion of cellulose

this image is a derivative of Figure 8: Johnston-Monje D, Raizada MN (2011) Conservation and Diversity of Seed Associated Endophytes in Zea across Boundaries of Evolution, Ethnography and Ecology. PLoS ONE 6(6): e20396. Cited under the Creative Commons Attribution License CC BY 3.0

# Seed-borne maize endophytes



OPEN ACCESS Freely available online



## Conservation and Diversity of Seed Associated Endophytes in *Zea* across Boundaries of Evolution, Ethnography and Ecology

David Johnston-Monje, Manish N. Raizada\*

Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada

*Bacillus subtilis*, *Bacillus megaterium*, *Pseudomonas fluorescens*, *Pseudomonas putida*,  
*Escherichia coli*,  
*Klebsiella pneumoniae*, *Enterobacter cloacae*,  
*Micrococcus*, *Arthrobacter*, *Paenibacillus*, *Streptomyces*,  
*Bradyrhizobium japonicum*



# Endophytes in biofertilizer products



## Bacteria

- *Azospirillum brasilense*
- ***Enterobacter***
- ***Klebsiella***
- ***Pseudomonas fluorescens***
- *Bacillus cereus*
- *Bacillus amyloliquefaciens*
- ***Bacillus megaterium***
- ***Bacillus subtilis***
- ***Paenibacillus azotofixans***

## Fungi

- *Trichoderma virens*
- *Trichoderma harzianum*
- *Clonostachys rosea* f. *rosea* (*Gliocladium roseum*)

intersection with seed-borne maize endophyte in bold

# Example 3 – Grasslanz endophyte infected seeds



The endophyte shows vertical transmission, i.e. inheritance to the offspring via the seeds.

figure removed due to copyright considerations

[www.grasslanz.com](http://www.grasslanz.com)

pasture grasses tall fescue (*Festuca arundinacea*) and ryegrass (*Lolium*)

# Example 3 – Grasslanz endophyte infected seeds



A natural fungus (endophyte) that lives in grass and produces chemicals that give resistance to insect pests, which otherwise cost 10-20 % dry matter.

Alkaloids:  
**Peramine**  
**Ergovaline**  
**LolitremB**  
**Loline**

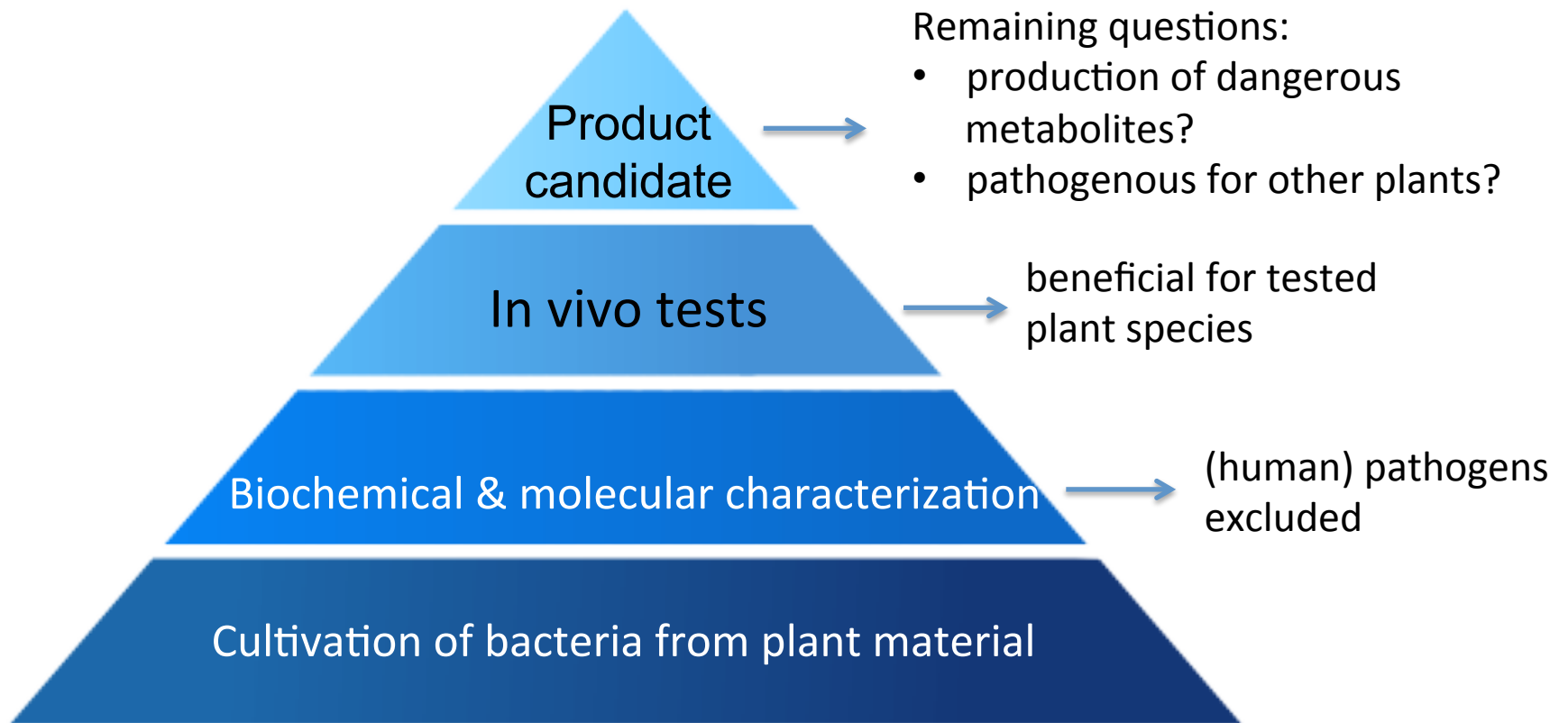


Insect pests of ryegrass are  
poisoned by the endophytes  
alkaloids.

figure removed due to copyright  
considerations

<http://www.grasslanz.com/UnderstandingtheScience/AR37Endophyte.aspx>

# Endophyte product candidates



# Discussion



Classification – Fertilizer (Plant protection product)

What must be registered?

- Nothing at all? Endophytes are already in the plant...
  - The endophyte?
  - The plant with the endophyte?
  - Every plant endophyte combination?
- 
- Standards Operating Procedure (SOP) for testing quality, safety and efficacy and for labeling and registration ?

Thank you for listening