Quality Control of BioGro:

Development, practice and recommendations

Development Marketplace

THE UNIVERSITY OF SYDNEY

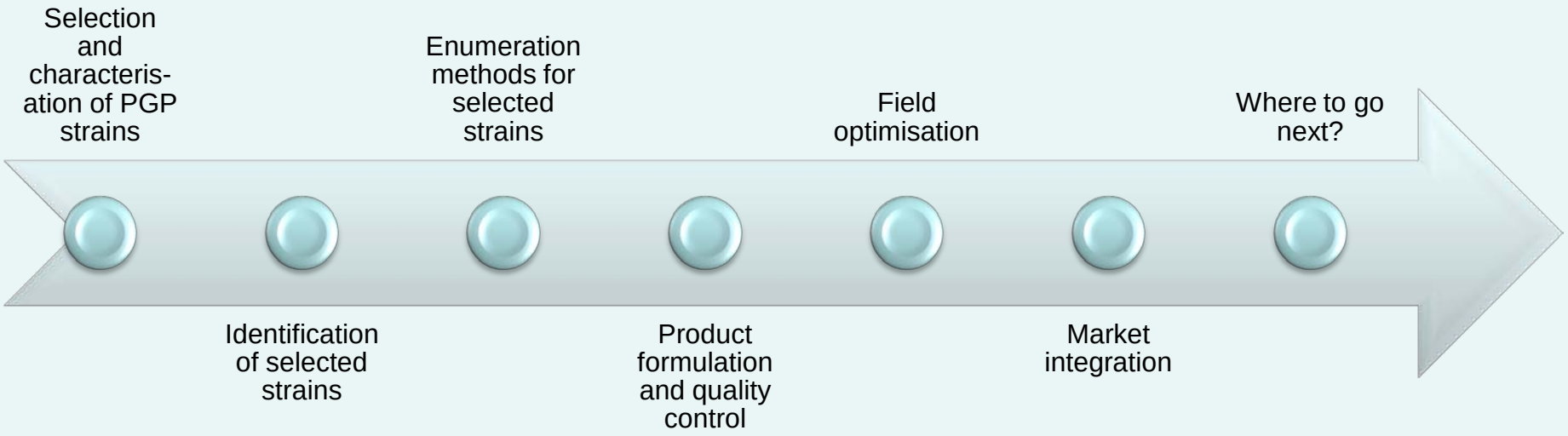
ĐẠI HỌC CẦN THƠ

IAS

Australian Government

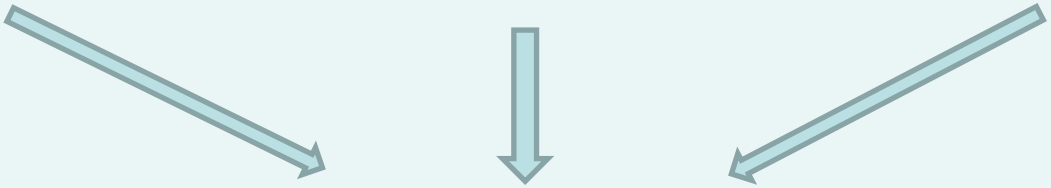
Australian Centre for International Agricultural Research

A framework for quality inoculant biofertiliser development



Selection and characterisation

1. Associative
2. Endophytic
3. Known



Culturable organisms

Positive effect on plant growth

Characterisation

Selection and characterisation

Fitness and compatibility in rhizosphere

- Fast growing on carbohydrates
- No plant inhibition
- Compatible with known beneficial microorganisms

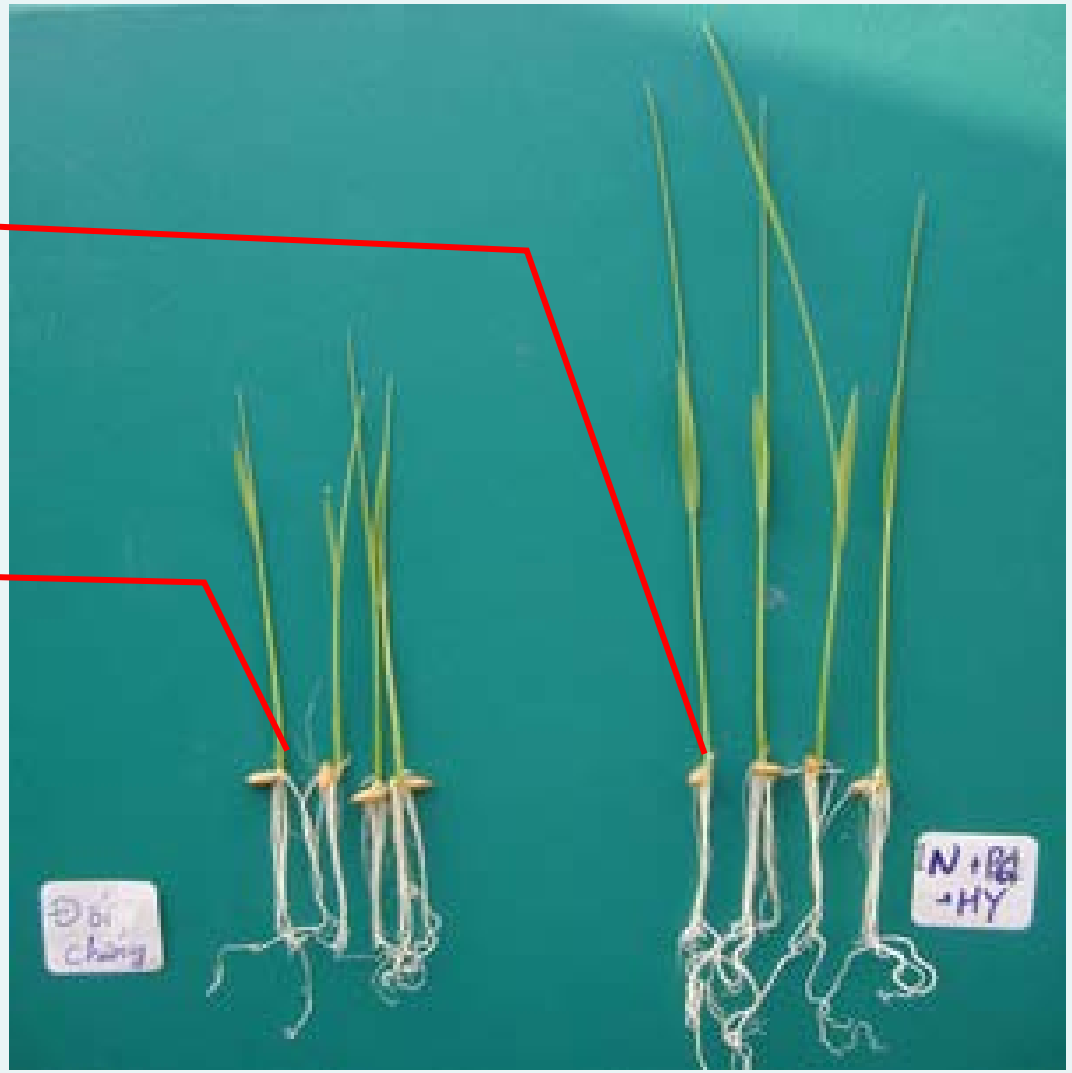
PGP characteristics

- Phytohormone production/ balancing – increased root development, increased stress tolerance
- Nitrogen fixation – increase access to reduced nitrogen
- Mobilisation of P and micronutrients
- Pathogen inhibition

Selection and characterisation

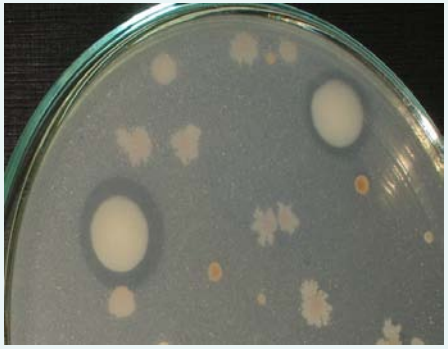
Inoculation with PGP strains

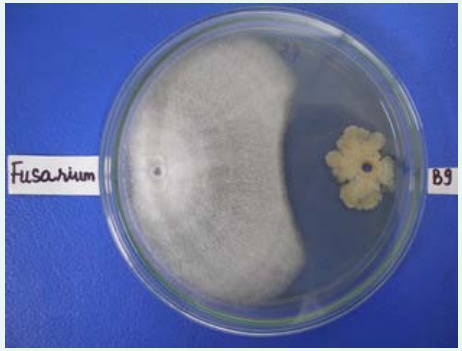
No inoculation



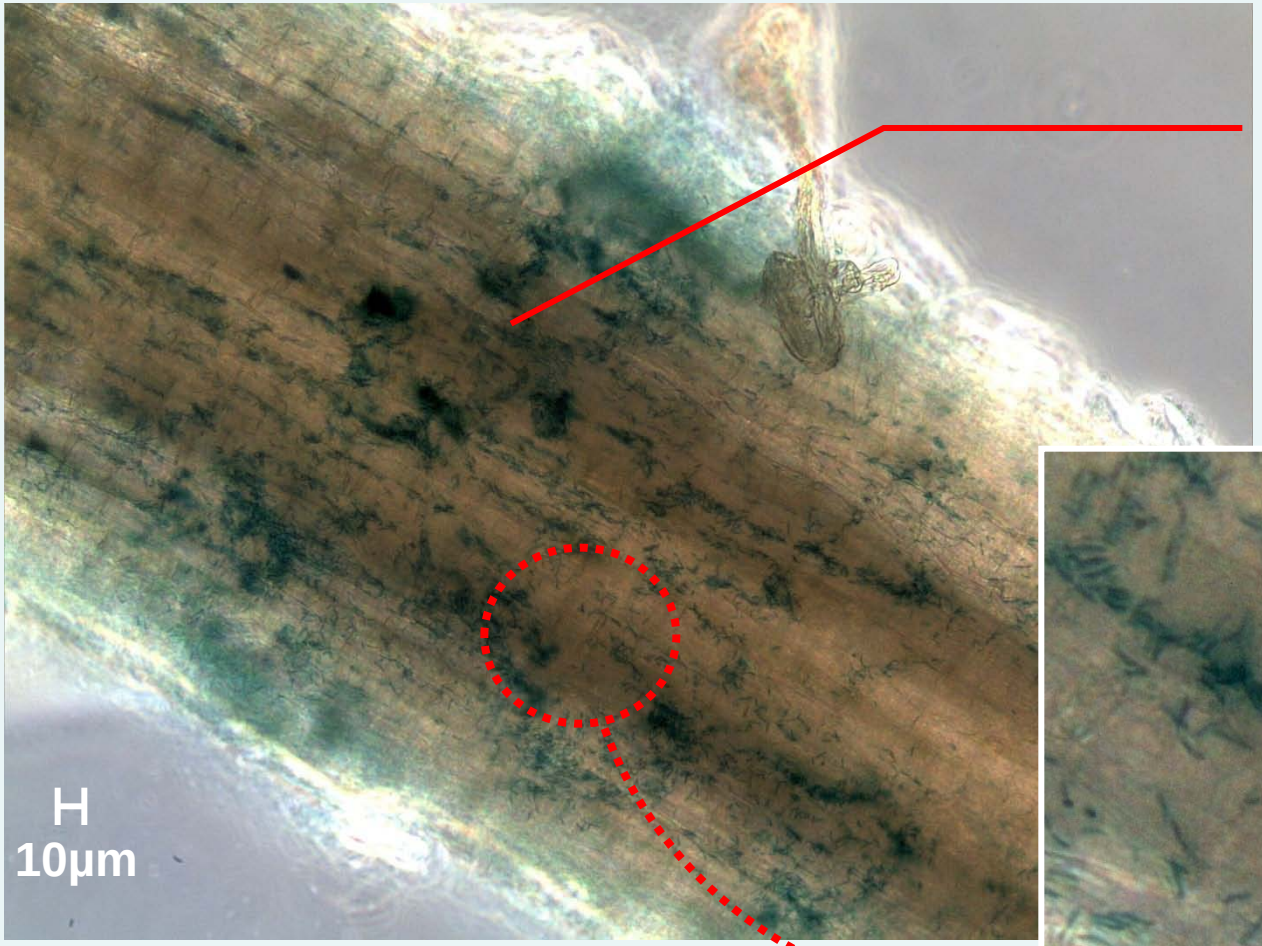
Selection and characterisation

Strain	Apparent contribution
1N	Auxin production (IAA), siderophores, biocontrol (DAPG)
HY	Phosphorus mobilisation, auxin production, ACC deaminase
E19	OM degradation, biocontrol
B9	OM degradation, biocontrol



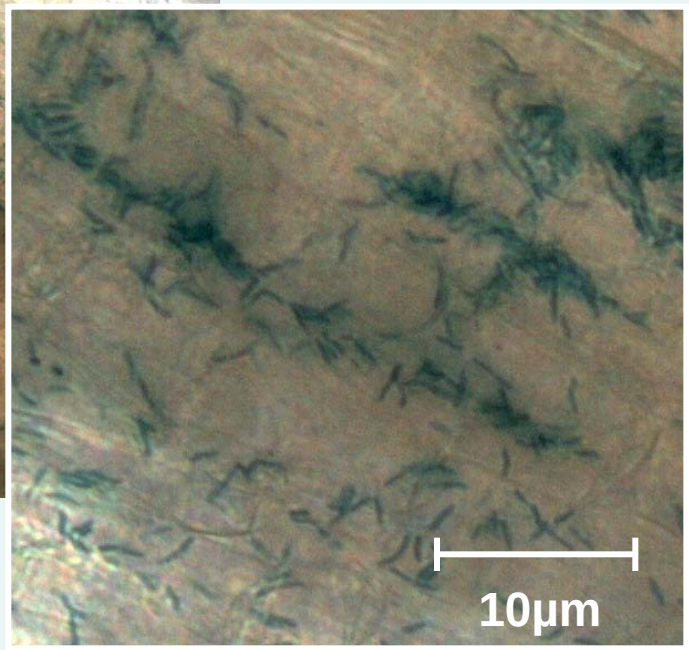


Colonisation of the rhizosphere



zone of elongation

Photographs: Khanok-on Amprayn



Rice (Amaroo) root

Identification

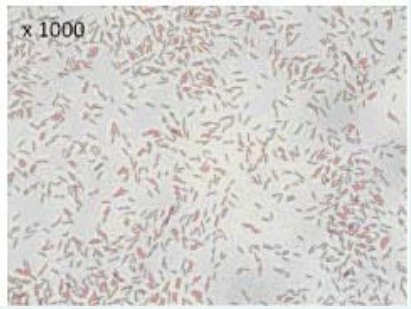
Physiological: nutritional, biochemical

- Appearance (rod, coccus, spores)
- Gram stain
- Enzyme activities (catalase, oxidase
- Aerobic/anaerobic
- Carbohydrate nutrition (e.g. API strips)

Molecular

- Fatty acid profiling
- 16SrDNA (bacteria)
- ITS (yeast/fungi)

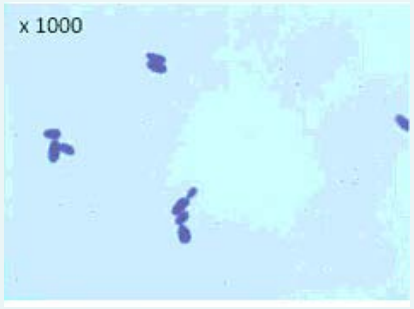
Identification



1N

- Gram negative bacteria

16SrDNA
Pseudomonas fluorescens
99%



HY

- Yeast

ITS region
Candida tropicalis
99%

E19

- Gram positive bacteria
- Spore-forming

16SrDNA
Bacillus amyloliquefaciens
96%

B9

- Gram positive bacteria
- Spore-forming

16SrDNA
Bacillus subtilis
98%

Enumeration

Enumeration Method	Capital Cost	Variable Cost	Time to develop	Assay Time	Specificity	Factors affecting accuracy in counting	Measure of viability
Plate count	Low	Low	Fast	Slow	Low	Colony growth*	Yes
Selective plate count	Low	Low	Fast	Slow	Medium	Colony growth*	Yes
Multiple-tube fermentation	Low	Low	Fast	Slow	Medium	Dilution factor and no. tubes	Yes
Indirect ELISA	Medium	Low	Slow	Fast	High	Sensitivity and calibration	Includes dead cells
Immunoblotting	Medium	Medium	Slow	Slow	High	Colony growth*	Yes
Sandwich ELISA	Medium	Low	Slow	Fast	High	Sensitivity and calibration	Includes dead cells
Quantitative Real Time PCR	High	High	Medium	Fast	Very High	Sensitivity and calibration	Includes dead cells (DNA)

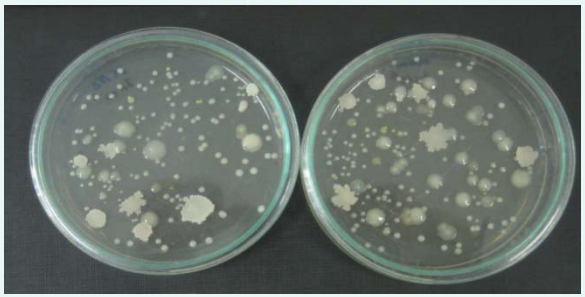
Enumeration

1. Selective Plate Counting

Strain	Apparent contribution
P. fluorescens 1N	Resistance to vancomycin, colony morphology
C. tropicalis HY	P-mobilisation, nutrition, colony morphology
B. amylo-liquefaciens E19	Spore forming (high temperature treated), colony morphology
B. subtilis B9	Spore forming (high temperature treated), colony morphology

1N on TSA + antibiotic (10^{-4} dilution)

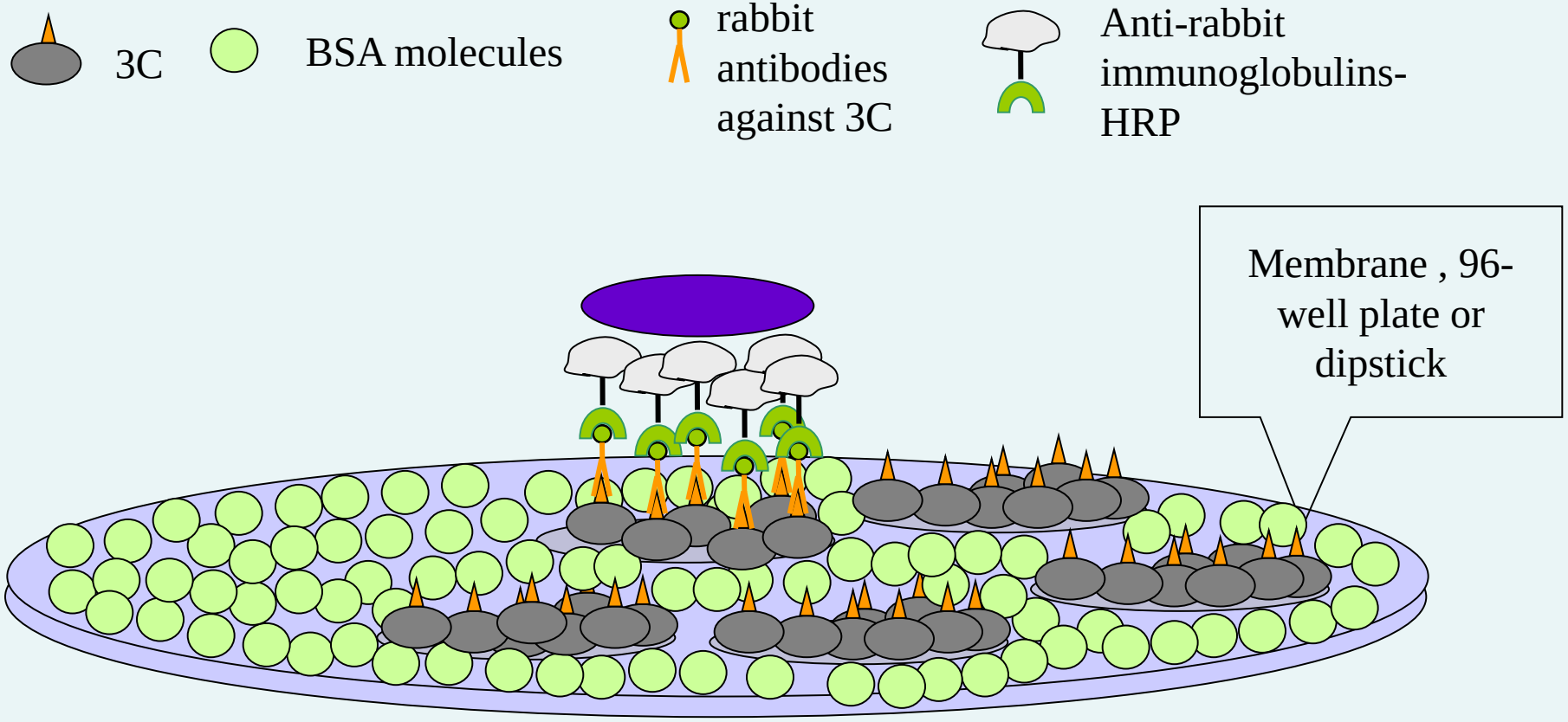
HY on Hansen (10^{-4} dilution)



B9 on MNA (10^{-4} dilution)

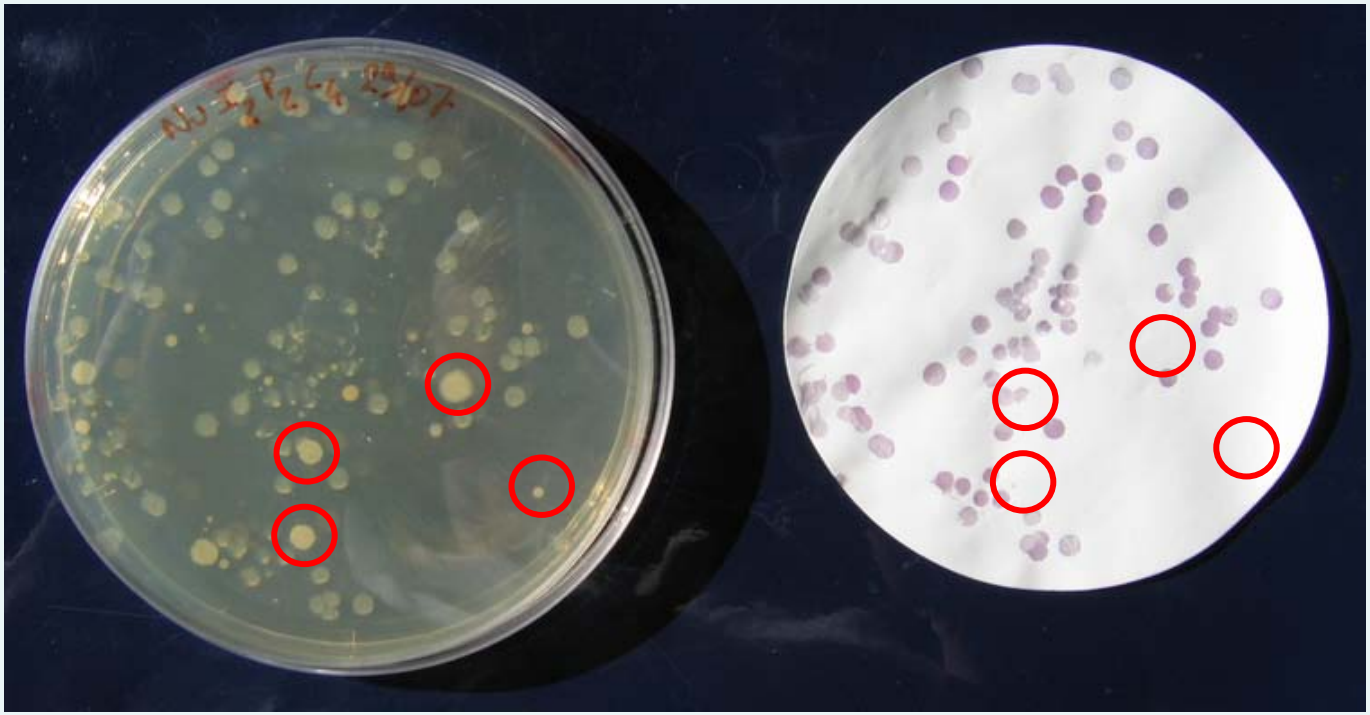
Enumeration

2. Immunological



Enumeration

2. Immunological



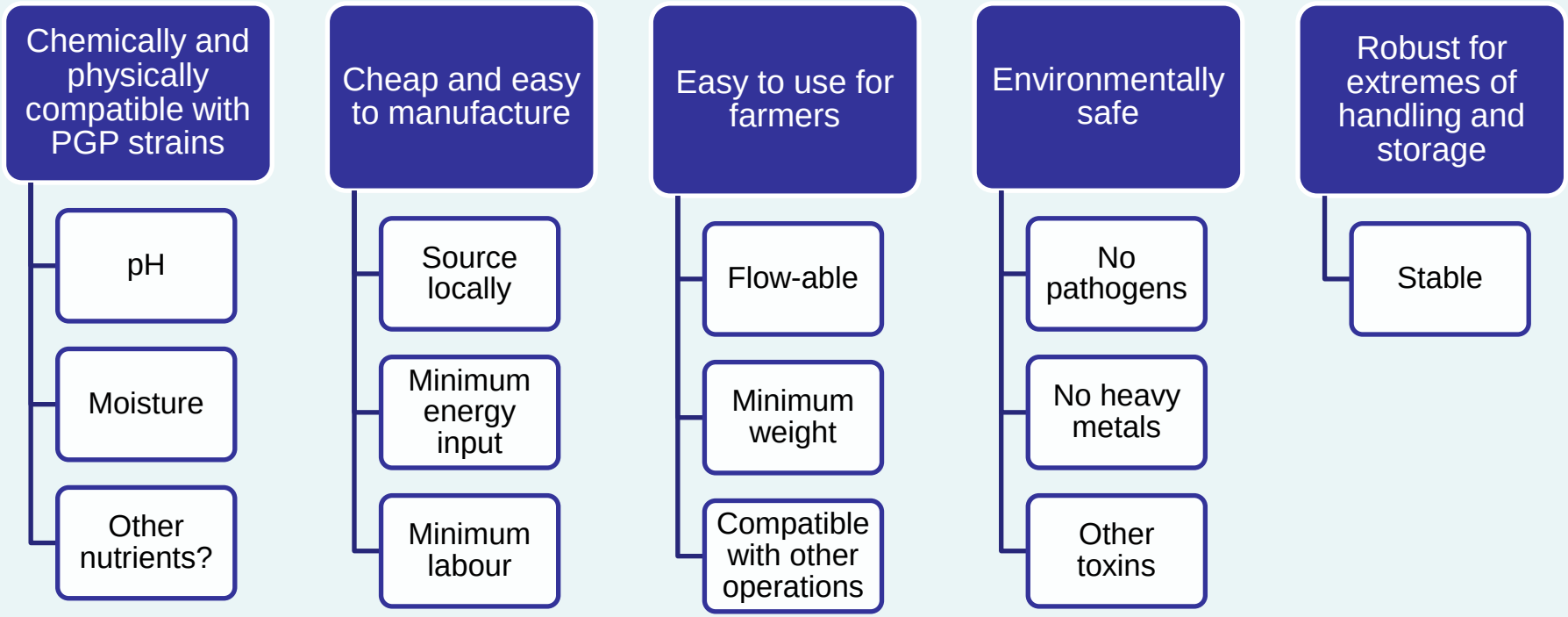
Pseudomonas fluorescens 1N isolated from rice rhizosphere 3 months after inoculation

Enumeration

2. Immunological

Product Formulation and QC

1. Optimal product characteristics



Product Formulation and QC

2. Carrier selection/development

Carrier	Advantages	Disadvantages
Peat	Well characterised, PGP bacteria are active	Not always available, prone to contamination
Agricultural/organic wastes	More easily available, can help solve waste problems, cheap	Highly variable, more difficult to characterise, very prone to contamination, shorter shelf-life
Inert polymers/minerals	Highly consistent, low probability of contamination, easy to formulate as granule/powder, good protection from stress	Expensive, materials and machinery may not be available
Freeze-dried cultures	No contamination, high-shelf life, very low handling/transport costs	Specialised equipment required, reduced numbers of viable cells
Stabilised liquid concentrates	No contamination, high-shelf life, low transport/handling costs	Still being developed, intellectual property

Product Formulation and QC

2. Carrier selection/development

Carrier	pH	%C	%N	%P	Water-holding capacity (%w/w)
Peat	2.5	12-15			46
Sugarcane mud	5.7	15-20	2.5		120
Coconut coir dust	4.9	30-55	0.4	0.85	656
Worm castings	6.9	10			63
Aquaculture mud	6.0	3-12	0.2-0.4	0.8-1.0	60

Future: integrated strategies

1. Climate change considerations
    - Life-cycle analysis
    - Biochar as a carrier - sterile, other benefits

2. Marketing considerations
    - Quality control logos

3. Optimisation for other cropping systems
    - E.g. Higher value horticulture