Advancement in Biocontrol Research: A Cellular Level Approach to Study Trophic Interactions

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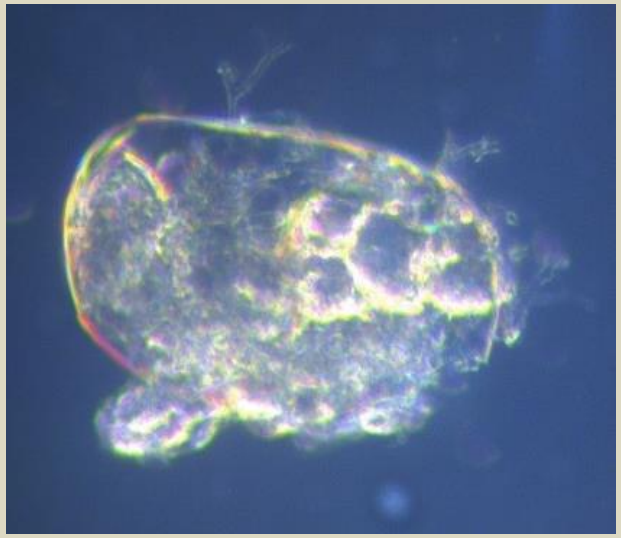
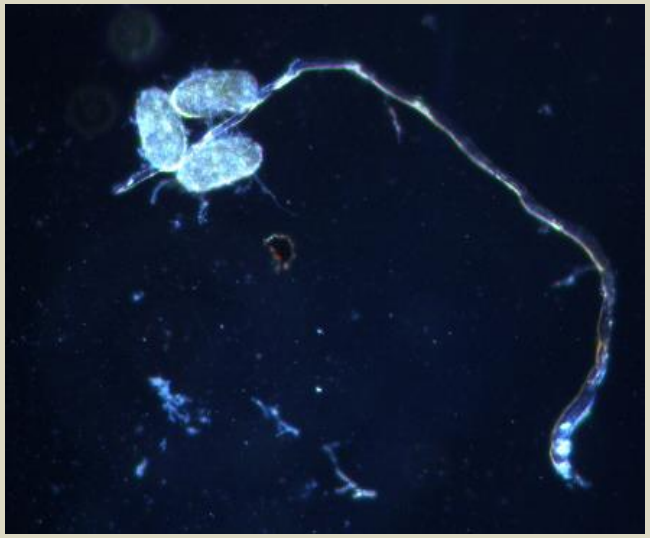
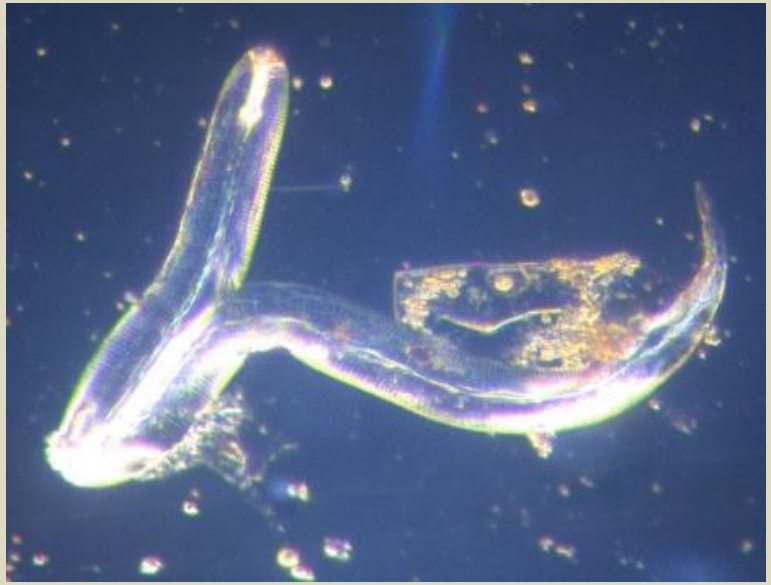
Outline

- Rapid screening of biocontrol agents
- Microaspiration technology to study interactions at the cellular level
- RNA extraction method for cell specific transcriptome
- Suppression of potential nematode parasitism genes due to biocontrol agents

Interactions



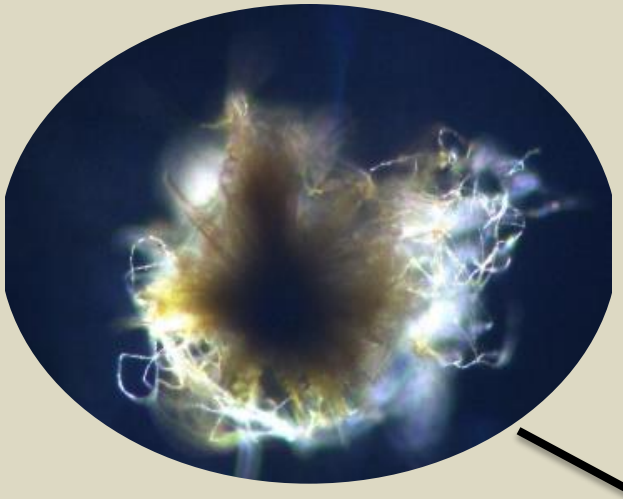





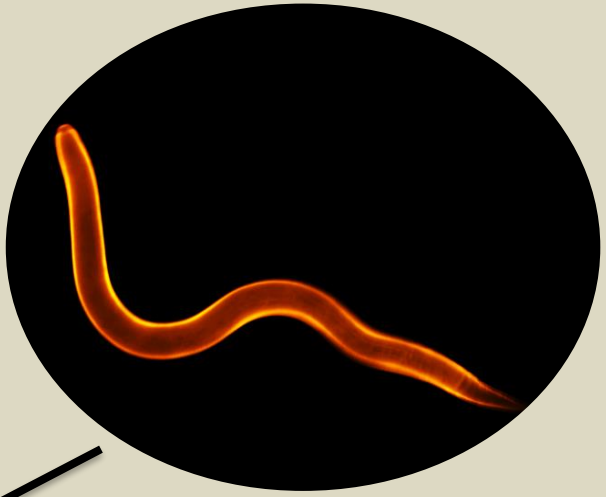
- **Nematophagous activity**
- **Targeting specific developmental stages of nematode life cycles**

Imaging techniques

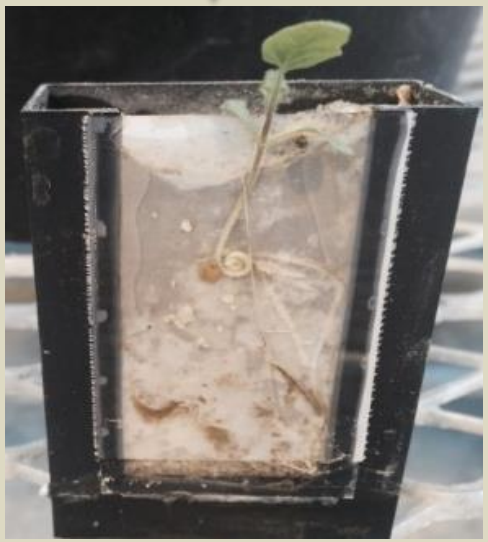
Biocontrol fungi



Potato plantlets



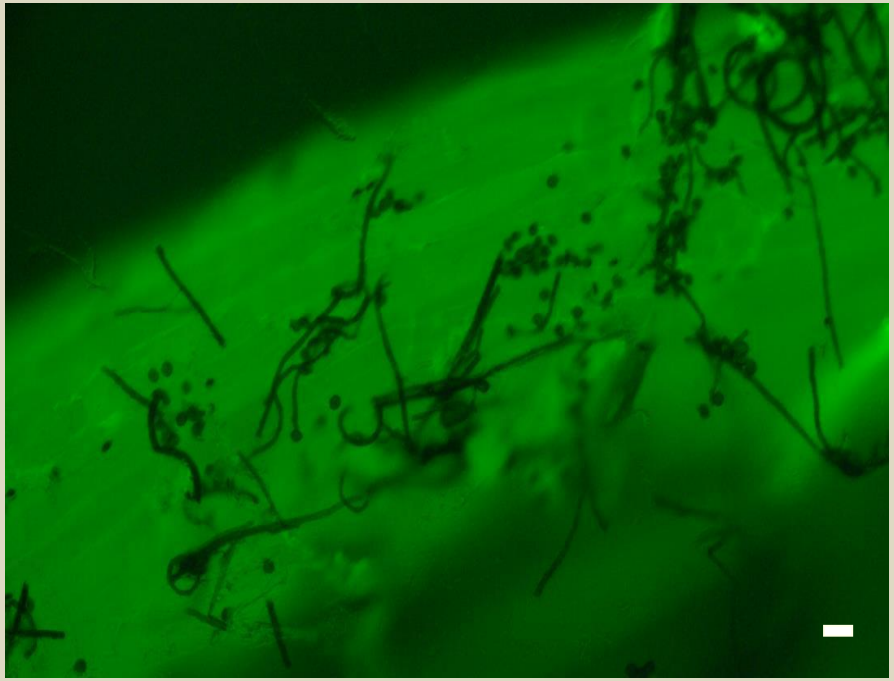
Microscopy Rhizosphere Chambers (Micro-ROCs)

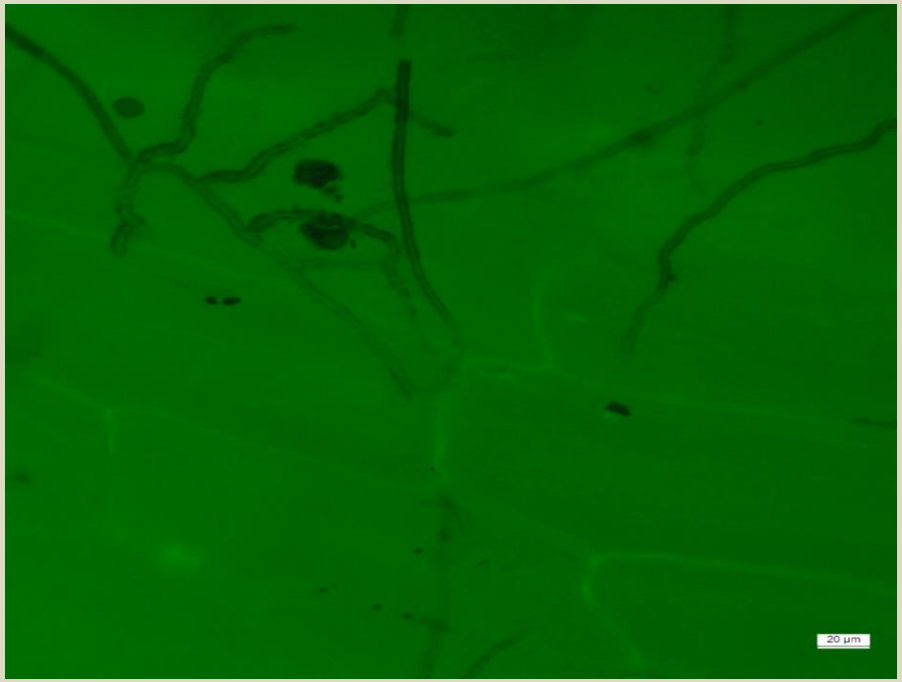


G. pallida juveniles stained with PKH26 (1 week after fungal inoculation)

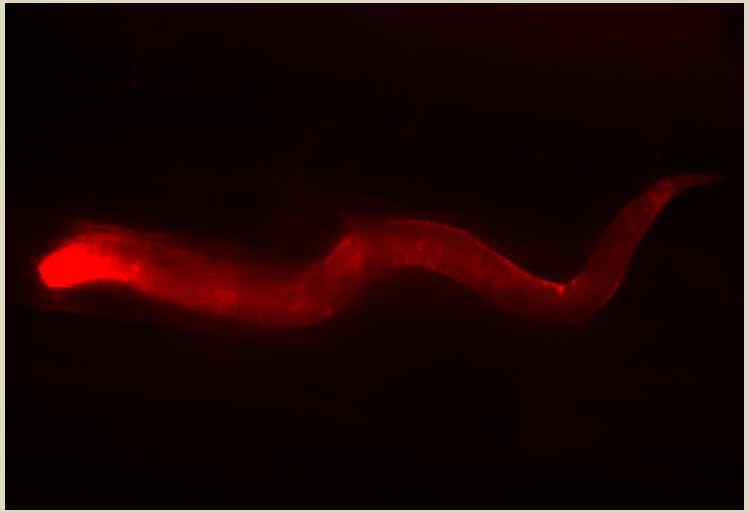
Microscopic observation after 4 days

Chaetomium globosum





Potato root colonization by *C. globosum*



***G. pallida* in potato root**

Effect of biocontrol fungi on infection by *G. pallida*

Treatments	*G. pallida* juveniles (J2s)/root system
Control	38.6 ± 9.6 a
F. oxysporum	24.0 ± 20.6 bd
M. bolleyi	23.2 ± 8.2 b
P. lilacinum	22.8 ± 12.1 b
F. solani	18.2 ± 12.8 ed
P. cucumerina	15.0 ± 7.8 ebd
F. tricinctum	13.2 ± 5.8 ced
C. globosum	7.4 ± 4.1 e

Values with the same letter are not significantly different at $P \leq 0.05$

Kooliyottil et al. (2017) Journal of Basic Microbiology 57:386–392.

Effect of PKH26 or acid fuchsin

Treatments	Number of infective juveniles (J2s) in potato roots	
	Acid fuchsin	PKH26
Non-inoculated control	30.6 ± 9.3 a	29.8 ± 6.8 a
C. globosum	8.4 ± 4.8 b	7.60 ± 3.3 b

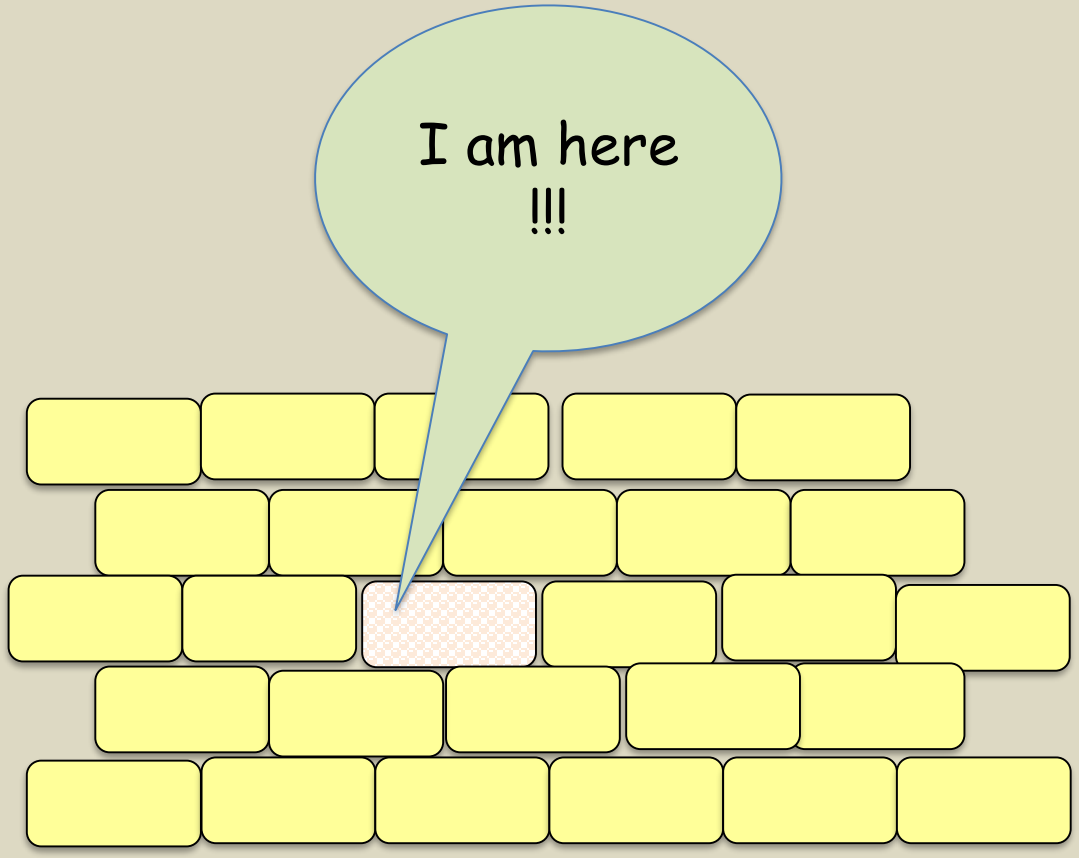
Values with the same letter are not significantly different at $P \leq 0.05$

Kooliyottil et al. (2017) Journal of Basic Microbiology 57:386–392.

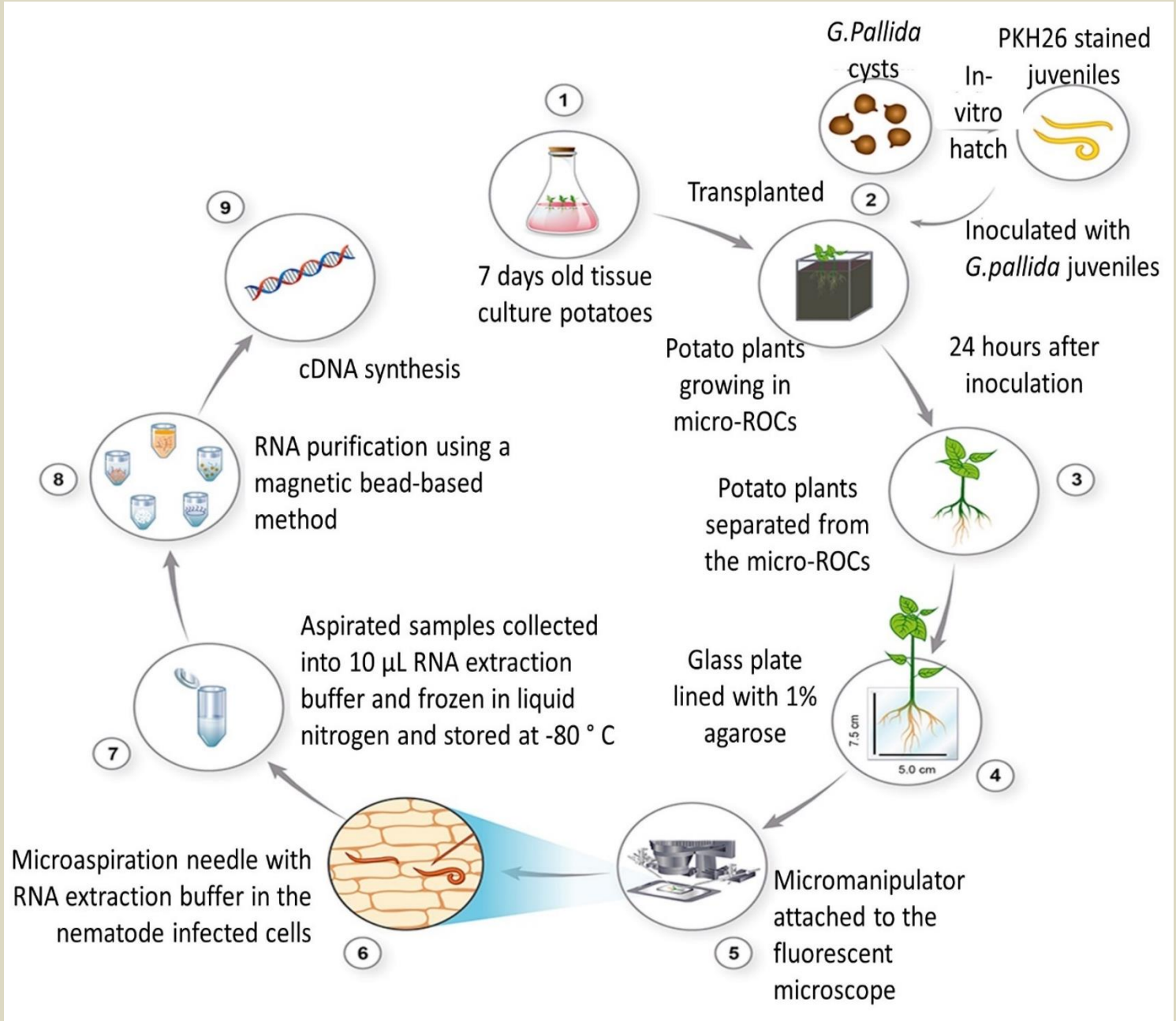
Molecular Methods

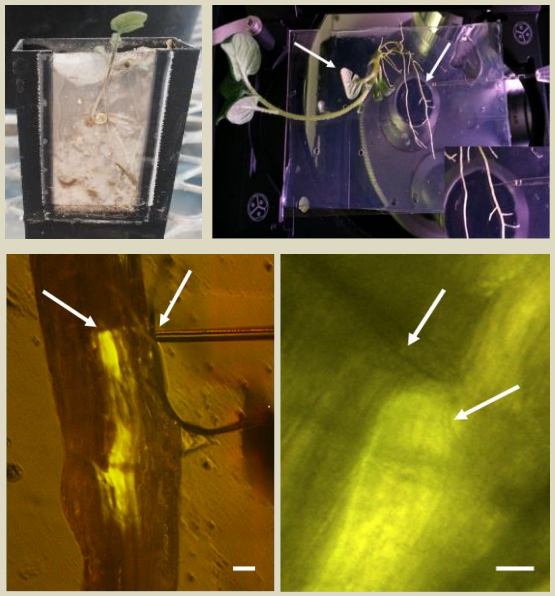
- Systemic and local responses
- Competition for nutrients and niches
- Biotic/abiotic factors

- **Changes in gene expression at site of infection may be diluted by the relative abundance of uninfected cells.**



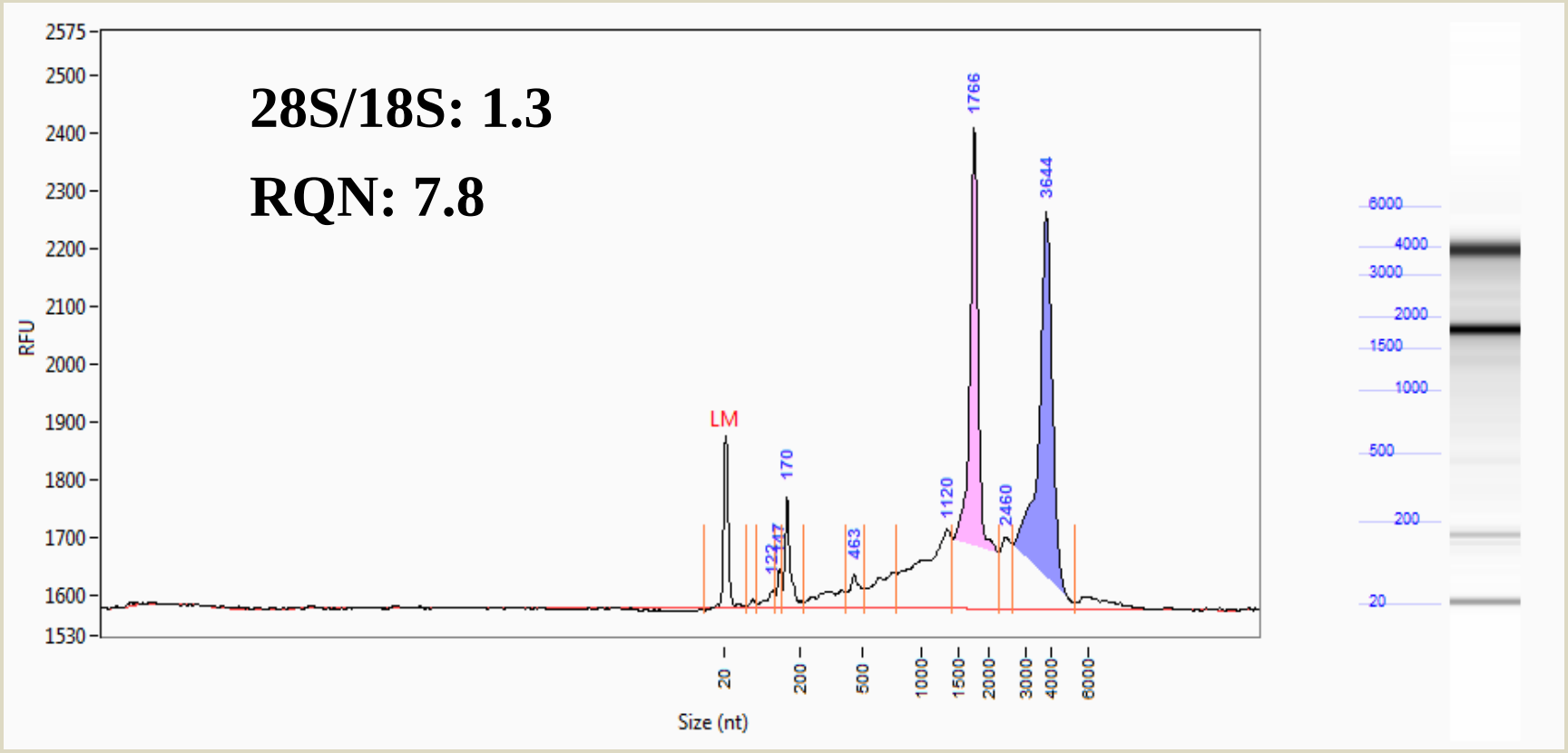
Process of microaspiration and RNA isolation

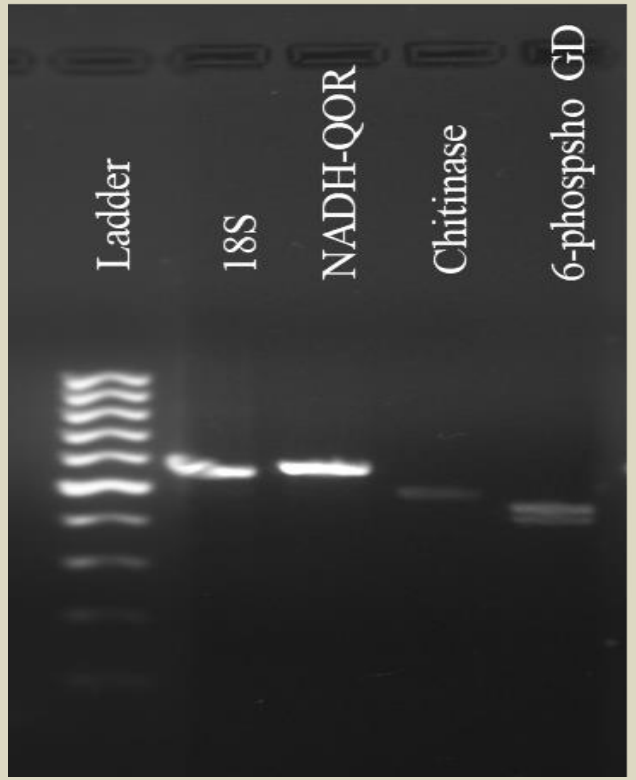




- **Allows isolation of RNA, DNA, proteins and metabolites from a heterogeneous cell population**
- **“Touch-free” approach: Contents from the cells of interest can be aspirated**

Quality assessment of RNA isolated from microaspirated samples





Genes amplified from microaspirated cell specific cDNA

Pathogen defense against biological control

- Understanding pathogen self-defense mechanisms against biocontrol agents
- Developing approaches to improving the durability of biocontrol agents
- Implications for the deployment of transgenes

Duffy *et al*. (2003) Annual Review of Phytopathology. 41:501–38

Nematode inoculation and RNA isolation

- Single roots of *S. tuberosum* and *S. sisymbriifolium* were inoculated with sterile *G. pallida*.
- Extraction of infected nematodes from the plant roots
- RNA isolation and transcriptome analysis

Single root inoculation prototype

Differential expression of nematode defence genes

Clp-1

CTSS

Exn-1

Bath-38

Concluding Remarks

- **Tools are required to study trophic interactions at the molecular level.**
- **Recent developments in microscopic and “Omics based” techniques are promising to study these interactions at the cellular level.**

Acknowledgements

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Thank you