

Transcriptomic analysis of soil microorganisms in presence of a plant pathogen and its biocontrol agent

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Introduction

RNA-Seq sequencing

Armillaria mellea

is a pathogen of woody plants and causes root rot
is a serious disease for grapevine
infects grapevine roots and reduces growth
can not be controlled by conventional fungicides



Disease symptoms

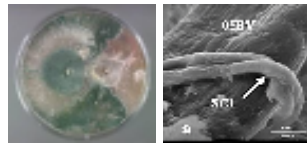
Infected roots

RNA-Seq Illumina sequencing

RNA extraction (PowerSoil Total isolation kit)
DNA removal (RNeasy Plus Micro kit)
rRNA depletion with adapted RiboMinus for fungi, yeast and bacteria
Library preparation by TruSeq SBS Kit v3
Paired ends sequencing (2 x 100 bp) by Illumina HiSeq

Trichoderma atroviride SC1

is a biocontrol agent isolated from soil
is an antagonist of several plant pathogens
is a mycoparasite of *A. mellea*
controls *A. mellea* spread in field conditions


 Control of *A. mellea* by *T. atroviride* SC1

 Parasitization of *A. mellea* by *T. atroviride* SC1

Quality filtering of RNA-Seq reads

Adaptor removal, quality trimming and quality filtering

Treatment	Replicate	Sequenced reads	Cleaned reads (%)
Microcosm Time 0h	1	54867635	48445515 (88)
	2	53137108	46727993 (88)
	3	47288179	42720816 (90)
Microcosm Time 24h	1	53923704	47273812 (88)
	2	57625098	50997549 (88)
	3	47957873	43625309 (91)
Microcosm <i>T. atroviride</i> SC1 Time 24h	1	53770221	48667484 (91)
	2	74788948	68814544 (92)
	3	60209616	52802432 (88)
Microcosm <i>A. mellea</i> SC1 Time 24h	1	45543588	41810447 (92)
	2	47244943	41394010 (88)
	3	54715166	48224300 (88)
Microcosm <i>T. atroviride</i> SC1 <i>A. mellea</i> SC1 Time 24h	1	51885907	45464087 (88)
	2	80245613	73309986 (91)
	3	47733908	42692300 (89)

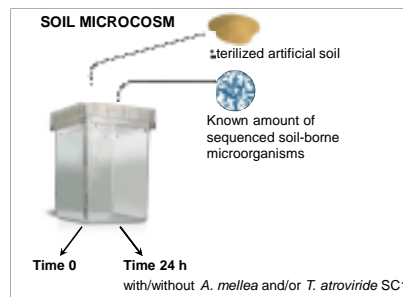
Aim

Understanding the transcriptional regulation of *T. atroviride* SC1 interactions with target (*A. mellea*) and non-target microorganisms at the early stages of soil colonization

Experimental design

Sterilized artificial soil

Component	% (w/w)
Sand	68.5
Kaolinite	19.5
Bentonite	9.8
CaCO ₃	0.2
Humic acid	2



Microbial community

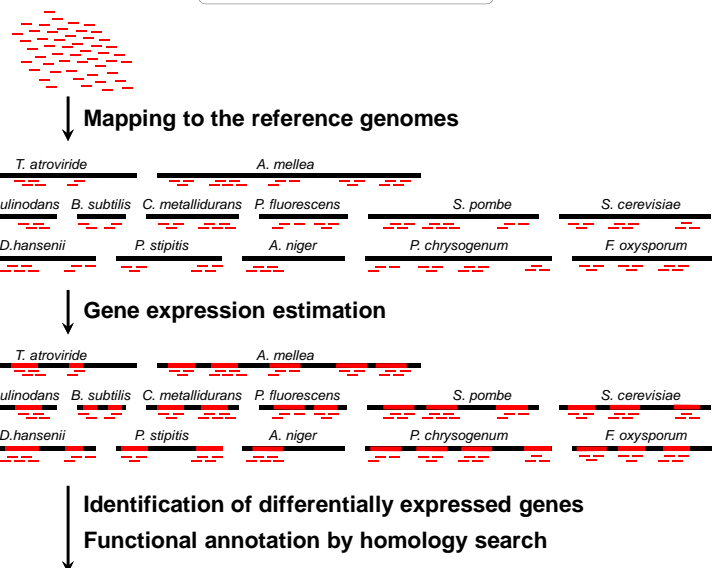
soil-borne microorganisms
culturable and countable
sequenced genome available

Microbial species	Concentration (CFU/g, <i>A. mellea</i> g/g)
<i>Azorhizobium caulinodans</i> DSM 5975	1.3 × 10 ⁷
<i>Bacillus subtilis</i> 168	2.0 × 10 ⁵
<i>Cupriavidus metallidurans</i> CH34	2.0 × 10 ⁷
<i>Pseudomonas fluorescens</i> Pf-5	2.0 × 10 ⁷
<i>Schizosaccharomyces pombe</i> 972h	3.0 × 10 ⁶
<i>Saccharomyces cerevisiae</i> S288C	6.2 × 10 ⁶
<i>Debaryomyces hansenii</i> CBS 767	6.0 × 10 ⁵
<i>Pichia stipitis</i> CBS 6054	2.4 × 10 ⁶
<i>Aspergillus niger</i> CBS 513.88	2.0 × 10 ⁶
<i>Penicillium chrysogenum</i> DSM 1075	2.4 × 10 ⁶
<i>Fusarium oxysporum</i> FGSC 9935	5.8 × 10 ⁶
<i>Trichoderma atroviride</i> SC1	8.8 × 10 ⁶
<i>Armillaria mellea</i> M6132	1.3 *

Sample collection

time 0h soil community
time 24h soil community
soil community + *A. mellea*
soil community + *T. atroviride* SC1
soil community + *A. mellea* + *T. atroviride* SC1
3 samples (replicates) for each microcosm

Gene expression analysis



Identification of genes involved in

- biocontrol mechanisms activated by *T. atroviride* SC1
- defense reaction of *A. mellea*
- response of the microbial community
- communication between the soil community and the biocontrol agent

References

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