Endophytic *Trichoderma* strains increase pre-harvest quantity and quality of grapes

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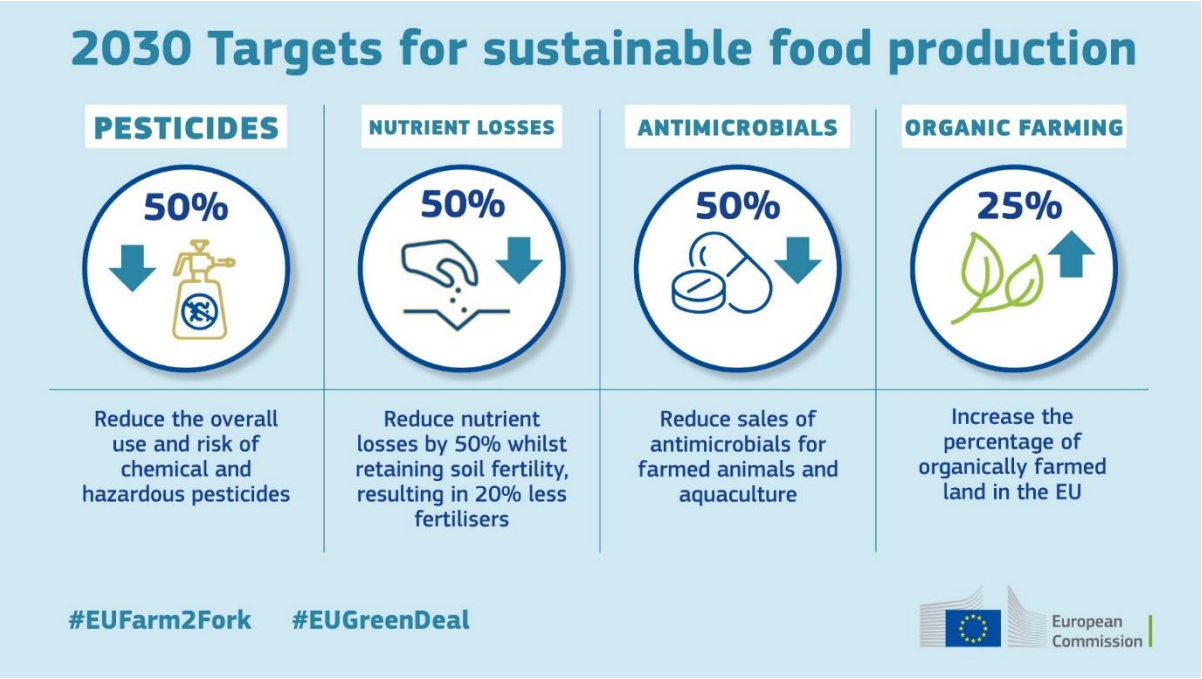
Importance of pre-harvest strategies

Improve product qualities ⇨ reduction of pre- and postharvest loss.

Source: FAO-SOFA 2019

Sustainable, alternative methods in agriculture

- European Green Deal - Farm to Fork strategy



Sustainable, alternative methods in agriculture

- European Green Deal - Farm to Fork strategy
- Consumers' requirements
  - Lower pesticide residues
  - Chemical pollutants
- Problems with chemical pesticides
  - Emergence of resistance among pests (Poster P22)

Ecotoxicology (2023) 32:102–113
https://doi.org/10.1007/s10646-023-02619-w

Recommended rates of azoxystrobin and tebuconazole seem to be environmentally safe but ineffective against target fungi

Libânia Queirós[1,2] · Nuno Aguiar[1] · Patrícia Pereira[1,2] · Fernando J. M. Gonçalves[1,2] · Artur Alves[1,2] · Joana Luísa Pereira[1,2]

FoodWaStop

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Sustainable, alternative methods in agriculture

- European Green Deal - Farm to Fork strategy
- Consumers' requirements
  - Lower pesticide residues
  - Chemical pollutants
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  - Toxicity

Planta (2009) 229:497–506
DOI 10.1007/s00425-008-0849-3

ORIGINAL ARTICLE

Botryticides affect grapevine leaf photosynthesis without inducing defense mechanisms

Anne-Noëlle Petit · Geneviève Wojnarowiez · Marie-Laure Panon · Fabienne Baillieul · Christophe Clément · Florence Fontaine · Nathalie Vaillant-Gaveau

Sustainable, alternative methods in agriculture

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- Consumers' requirements
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→ Increasing demands for alternative treatments

Trichoderma spp. (*Hypocreaceae*)

- Mycoparasitic and antagonistic on
  - Basidiomycetes - general property of the *Hypocreaceae*
  - Ascomycetes – special of *Trichoderma* spp.
- Their mycoparasitism known for a long time
  - Against *Fusarium oxysporum* (Tjamos et al., 1922)
  - Role in woody disease control (Weindling, 1928)

Trichoderma spp. - Plant protection mechanisms

On pathogens

- Producing secondary metabolites ⇨ **Prevent spore germination** and **growth**
- Cell wall-degrading enzymes ⇨ **Destroy**
- Rapid growth ⇨ **Space competitor**s

Hyphal coiling of *T. afroharzianum* (TR04 strain) on *Diplodia seriata* hypha
https://doi.org/10.3390/pathogens10121612

Trichoderma spp. - Plant protection mechanisms

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On plants

- Inducing systemic and local defense of the plant ⇨ **Increase resistance** towards pathogens
- **Promotion** of plant growth, development, and nutrient uptake ⇨ **Increase** tolerance to abiotic (e.g.: drought) stress

Trichoderma spp. - Biostimulants

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On plants

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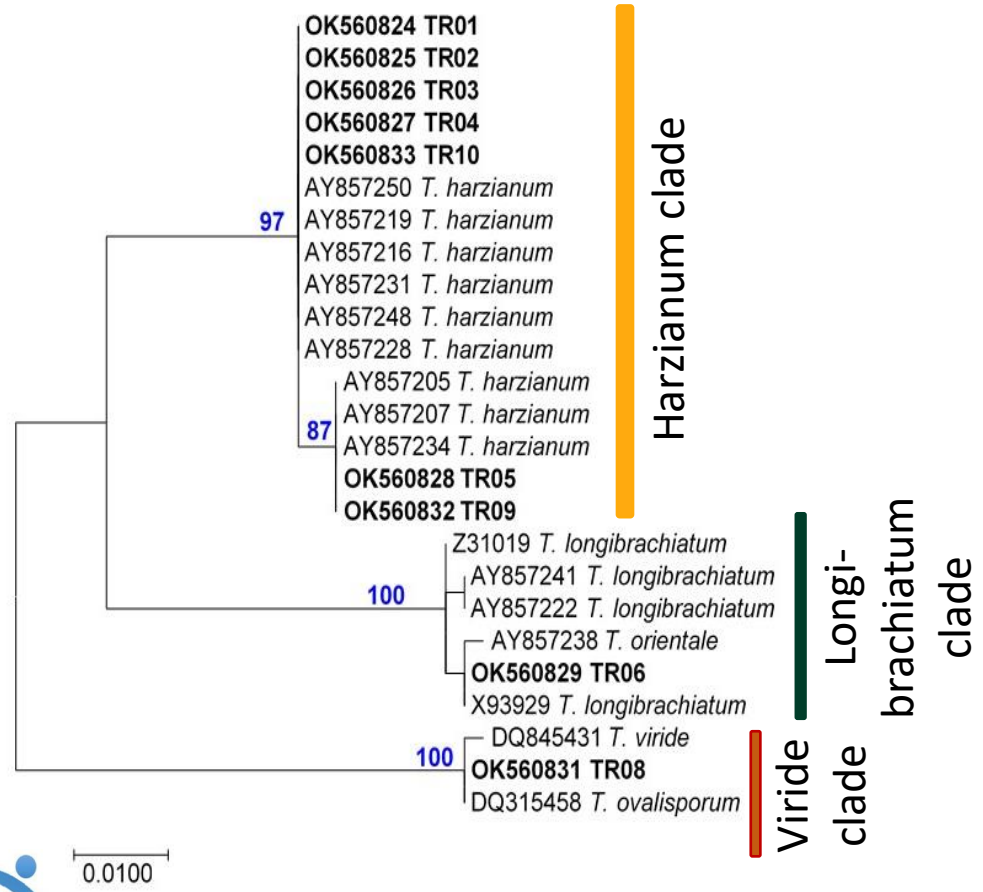
Endophytic *Trichoderma* strains

Identification, growth characteristics

Endophytic *Trichoderma* strains

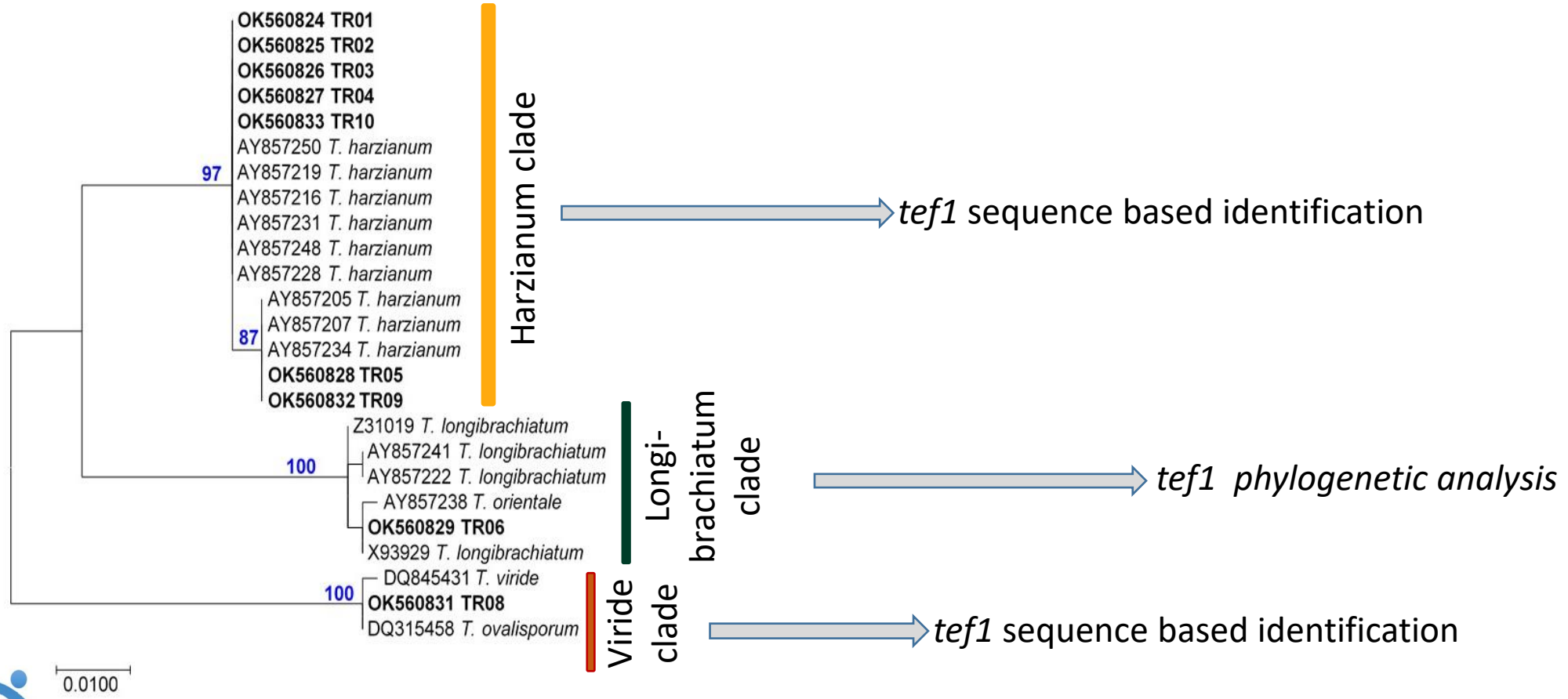
- Ten strains
- From the Tokaj Wine Region
- 22 years old vineyard showed > 34 % GTDs
- From healthy ‘Furmint’ cultivar of grapevine (*Vitis vinifera*, L.)
- Woody tissues (endophytic)

Endophytic *Trichoderma* strains



Greatest log likelihood **ITS** Maximum Parsimony phylogenetic tree generated from *Trichoderma* isolates (TR01 - TR10) and deponated sequences with Accession Number before species name. The length of branches is proportional to the number of nucleotide differences in the sequences, the scale is under the dendrogram. The numbers above branches show the results of the bootstrap analysis values from 1000 replicates.

Endophytic *Trichoderma* strains



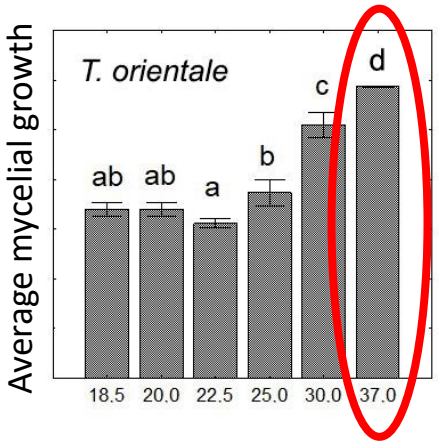
Endophytic *Trichoderma* strains

- ✓First properly identified endophytic *Trichoderma* spp. from grapevines from Europe

Trichoderma species	Strain No.
Harzianum clade	
T. afroharzianum	TR04
T. atrobrunneum	TR09
T. harzianum	TR07
	TR10
T. simmonsii	TR01
	TR02
	TR03
	TR05
Longibrachiatum Clade	
T. orientale	TR06
Viride Clade	
T. gamsii	TR08

Potential human pathogen →

Endophytic *Trichoderma* strains



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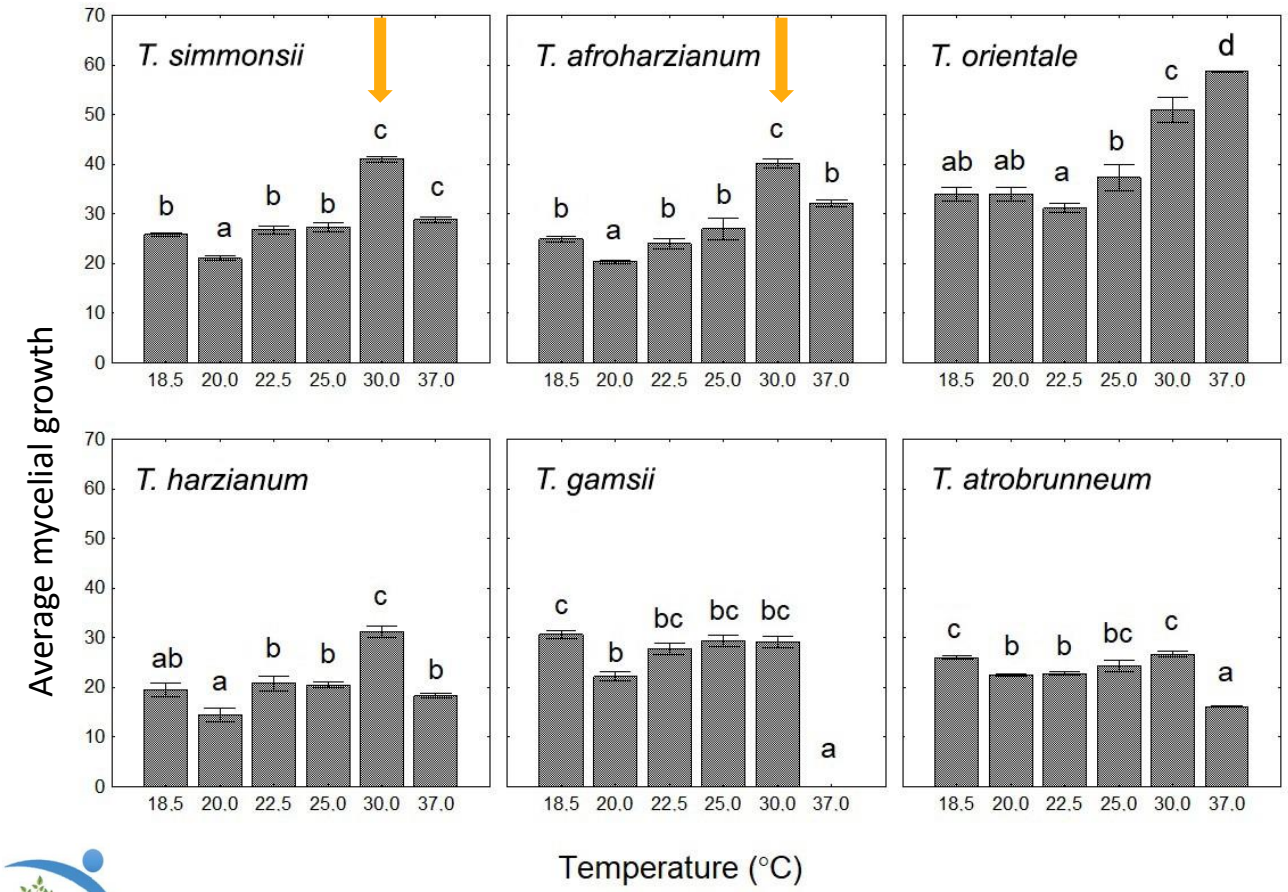
Endophytic *Trichoderma* strains



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Endophytic *Trichoderma* strains

Mycelial growth at 5 °C

Species	8 days Mean	SE		14 days Mean	SE	
Harzianum Clade						
T. afroharzianum	16.17	2.20	ab	34.00	6.00	ab
T. atrobrunneum	17.00	1.44	a	27.50	1.61	a
T. harzianum	7.83	0.88	c	17.58	1.35	d
T. simmonsii	14.92	0.64	ab	28.79	0.85	a
Longibrachiatum Clade						
T. orientale	12.67	0.17	b	22.67	0.44	b
Viride Clade						
T. gamsii	8.00	1.15	c	22.17	0.60	bc

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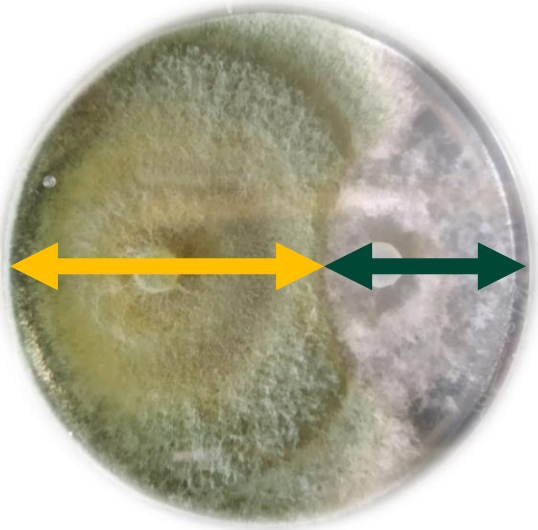
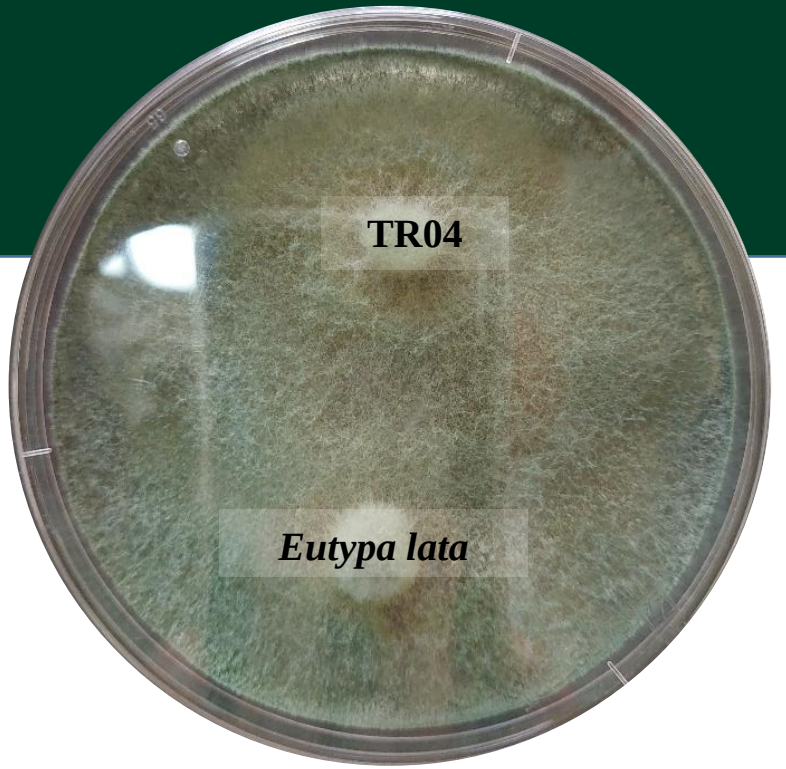
Endophytic *Trichoderma* strains

Biocontrol, biostimulant properties

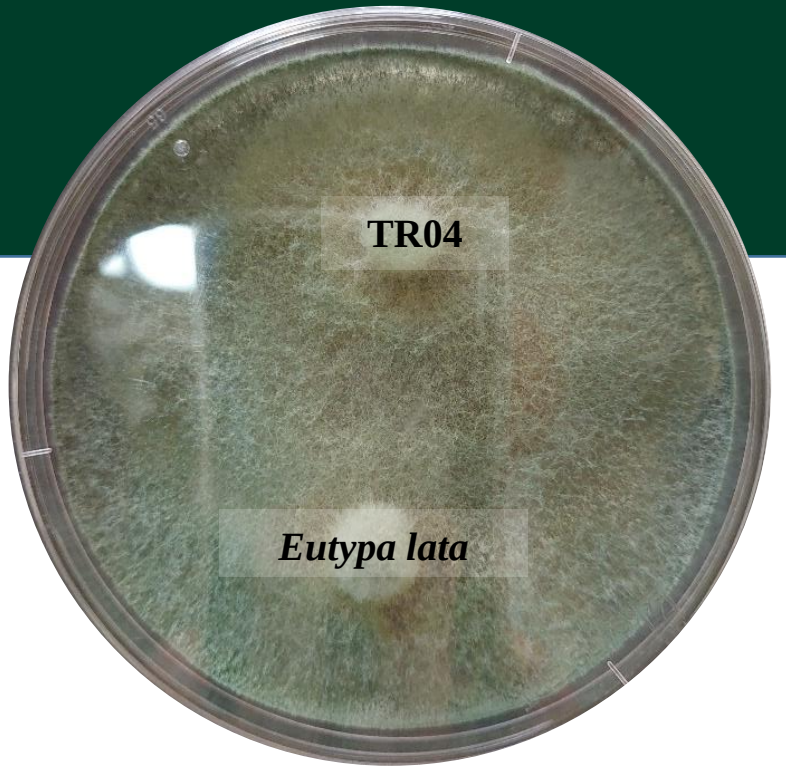
Biocontrol – confrontation test

BCI = 100 %



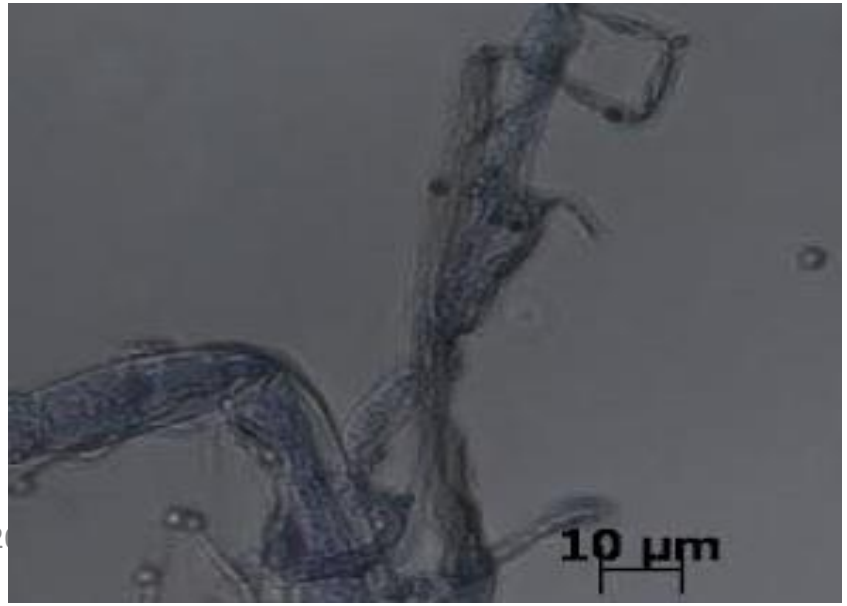


Biocontrol – confrontation test



BCI = 100 %

- ✓ Strain completely overgrew pathogens
- ✓Sporulated on their mycelia
- ✓Killing the pathogen colonies
- ✓Mycoparasitic activity was also detected by hyphal coiling and penetration



Biocontrol – confrontation test

Fungal pathogen	Host	Biocontrol Index (BCI %)	
		TR04	TR05
Oomycota			
Aphanomyces cochlioides	*Beta vulgaris*	90.37 (0.64)	84.81 (0.64)
Pythium acantophoron	*Ananas sativus*	**100.00** (0.00)	**100.00** (0.00)
Ascomycota			
Botryosphaeria dothidea	*Juglans regia*	**100.00** (0.00)	25.19 (0.64)
Diaporthe eres		**100.00** (0.00)	**100.00** (0.00)
Diplodia seriata	*Vitis vinifera*	**100.00** (0.00)	**100.00** (0.00)
Eutypa lata		**100.00** (0.00)	**100.00** (0.00)
Neofusicoccum parvum		95.19 (1.28)	90.00 (1.11)

Bostimulant – plant loss

Cultivar	n	U-test	Plant loss (%)	
			Control	Trichoderma
All	68	P=0.4945	**5.33±1.12**	**3.71±0.54**
Cabernet Franc	16	P=0.1770	1.88±0.60	0.78±0.36
Blaufraenkisch	30	P=0.1187	3.17±0.68	4.83±0.74
Cabernet Sauvignon	22	P=0.0943	9.46±2.46	4.38±1.03

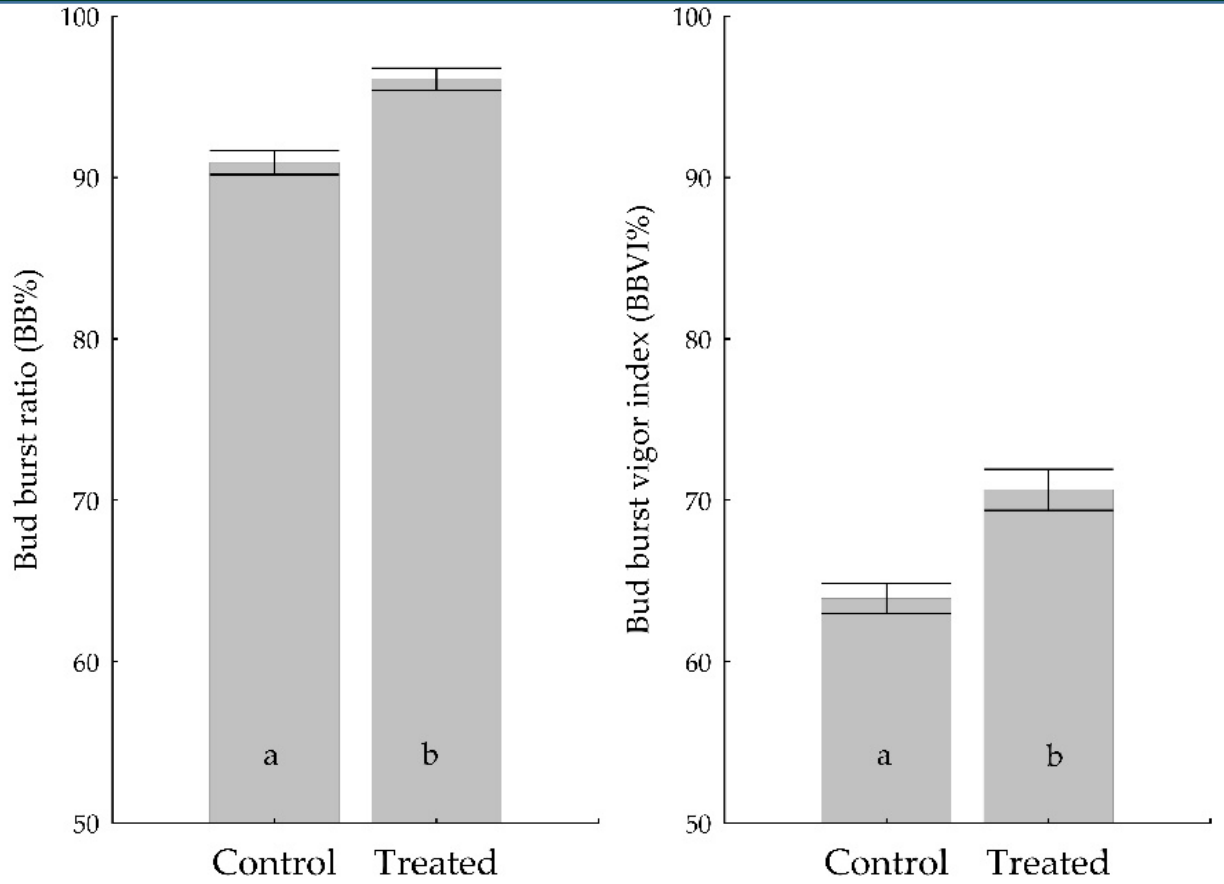
n: number of statistical samples (40 plants)

Bostimulant – plant development

Pre-planting soak in ***Trichoderma*** spore solution

Pre-planting soak in **water**

Bostimulant – bud burst



4 years following *Trichoderma* treatment with combination of *Trichoderma* strains. Mann-Whitney U-test (P<0.05).

Bostimulant – quantity parameters

Four years after *Trichoderma* treatment

Blaufraenkisch clone	Treatment	No. of planted grapevines	Plant loss (%) [1]	Yield (kg/plant) [1]	
				realized	potential
A4/1	Control	808	2.24(±0.57)	2.48(±0.27)	2.54(±0.28)
	Trichoderma	990	2.12(±0.51)	2.74(±0.11)	2.81(±0.13)
Kt1	Control	748	5.35(±1.77)	1.88(±0.29)	2.00(±0.33)
	Trichoderma	1309	7.10(±0.99)	2.26(±0.14)	2.43(±0.13)
All	Control	1556	3.62(±0.96)	2.21(±0.21)	2.30(±0.22)
	Trichoderma	2299	4.80(±0.91)	2.48(±0.11)	2.60(±0.10)

[1]mean(±SE)

Bostimulant – quantity parameters

Two years after *Trichoderma* treatment

Blaufraenkisch clone	Treatment	No. of planted grapevines	Plant loss (%) [1]	Yield (kg/plant) [1]	
				realized	potential
A4/1	Control	808	2.24(±0.57)	2.48(±0.27)	2.54(±0.28)
	Trichoderma	990	2.12(±0.51)	2.74(±0.11) **+10.5%**	2.81(±0.13)
Kt1	Control	748	5.35(±1.77)	1.88(±0.29)	2.00(±0.33)
	Trichoderma	1309	7.10(±0.99)	2.26(±0.14) **+20.2%**	2.43(±0.13)
All	Control	1556	3.62(±0.96)	2.21(±0.21)	2.30(±0.22)
	Trichoderma	2299	4.80(±0.91)	2.48(±0.11) **+12,2%**	2.60(±0.10)

[1]mean(±SE)

Bostimulant – quality parameters

Two years after *Trichoderma* treatment

Must parameter	Control	*Trichoderma*
Brix (°Bx)	22.80	**23.70**
Extract (g L^{-1})	256.95	268.10
Reducing sugar (g L^{-1})	230.50	**241.20**
Glucose (g L^{-1})	109.65	**114.90**
Fructose (g L^{-1})	117.50	**121.85**
Glycerol (g L^{-1})	0.60	**0.80**
pH	3.39	3.42
Total Acidity (g L^{-1})	6.30	6.40
Volatile Acidity (g L^{-1})	0.09	0.11
Potassium (mg L^{-})	1114.50	**1153.00**

Biostimulation

- 'Hárslevelű' **grapevine**
  - 1 kg/ha Tricho Immun (GEP test)
    - ➢ 4% higher sugar content
    - ➢ 14% higher yield
- 'Cabernet Sauvignon' **grapevine**
  - 1 kg/ha Tricho Immun (GEP test)
    - ➢ 6% higher sugar content
    - ➢ 7% higher yield

Plant colonization

- ✓Colonized permanently the woody tissue of the different **grapevine** cultivars ‘Furmint’, ‘Hárslevelű’, ‘Cabernet Franc’, ‘Blaufraenkisch’, ‘Cabernet Sauvignon’ - in contrary of intensive pesticide usage

Conclusions

1. Potential human pathogens may colohyze plant tissues
   - ➢ Necessary of proper identification and characterization
2. Advantages of endophytic biocontrol strains with biostimulant activity isolated from commercial fields
   - ✓ Adapted to agricultural practices (pesticides)
   - ✓ Adapted to climatic conditions (cold tolerance)
   - ✓ Adapted to host and its microbiome
   - ✓ Lower risk for soil microbiome
   - ✓ Pesticide applications or antagonists will not decrease their activity following plant colonization

→ Stabile colonization
Longtime effect

Plant colonization

pathogens

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Article

The Biocontrol Potential of Endophytic *Trichoderma* Fungi Isolated from Hungarian Grapevines. Part I. Isolation, Identification and In Vitro Studies

Csilla Kovács [1], András Csótó [2,3], Károly Pál [4], Antal Nagy [2], Erzsébet Fekete [5], Levente Karaffa [5,6], Christian P. Kubicek [7] and Erzsébet Sándor [4,*]

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The Biocontrol Potential of Endophytic *Trichoderma* Fungi Isolated from Hungarian Grapevines, Part II, Grapevine Stimulation

András Csótó; Csilla Kovács; Károly Pál; Antal Nagy; Ferenc Peles; Erzsébet Fekete; Levente Karaffa; Christian P. Kubicek; Erzsébet Sándor

Pathogens 2023, Volume 12, Issue 1, 2

Thank you for your attention!

Trichoderma treated

Control