Scuola di Agraria
e Medicina Veterinaria
dell'Università degli Studi di Napoli Federico II

IL caffè SCIENTIFICO

Metaboliti fungini: non solo antibiotici…

Francesco Vinale

8 Novembre 2023

UNIVERSITÀ
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UNIVERSITÀ
DEGLI STUDI DI NAPOLI
FEDERICO II

IL caffè
SCIENTIFICO

Indice

- Breve biografia
- Il metabolismo secondario dei funghi
- Il ruolo dei metaboliti secondari fungini nelle interazioni pianta/patogeno/microrganismi benefici
- Formulazioni per l'agricoltura a base di metaboliti secondari fungini
- Prospettive future

BREVE BIOGRAFIA

AA 1995-96	Laurea
AA 1997-98	Specializzazione in Biotecnologie Agroalimentari
2003	PhD in Patologia Vegetale
2003–2005 2006-2008	Assegno di ricerca (UNINA)
2009	*Endeavour Research Fellowship Award* (*UWA*)
2010	Ricercatore CNR - IPSP
2019	RTD B in Patologia Vegetale AGR/12 DMVPA UNINA
2022	PA in Patologia Vegetale AGR/12 DMVPA UNINA

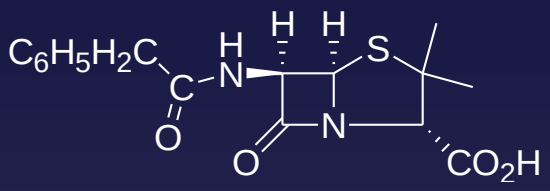
1998–1999	*Visiting scientist* presso l'Università di Monaco di Baviera
2005	*Visiting scientist* presso la *UWA*
2008	*Short Mobility* UNINA presso la *UWA*
2013	*Visiting scientist* presso *University of Stellenbosch* (SA)

Il metabolismo secondario dei funghi

non solo antibiotici…

Penicillina G

Antibiotico prodotto da *Penicillium notatum*



La sua scoperta nel 1932, che segna l'inizio dell'era degli antibiotici, spetta a ??????????

Da: Ricomincio da tre 1981

Il metabolismo secondario dei funghi

I funghi sono una miniera ancora in parte inesplorata di sostanze naturali attive (compresi gli antibiotici)

Pergamon

Tetrahedron, Vol. 53, No. 9, pp. 3135-3144, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00024-0

A New Fungal Growth Inhibitor from *Trichoderma viride*

Luisa Mannina*, Anna Laura Segre

Istituto di Strutturistica Chimica and NMR Service, C.P. 10,

00016 Monterotondo Scalo, Roma, Italy.

Alberto Ritieni, Vincenzo Fogliano, Francesco Vinale, Giacomino Randazzo

Dipartimento di Scienza degli Alimenti, Università di Napoli "Federico II".

Lucia Maddau, Antonio Bottalico

Istituto di Patologia Vegetale, Università di Sassari.

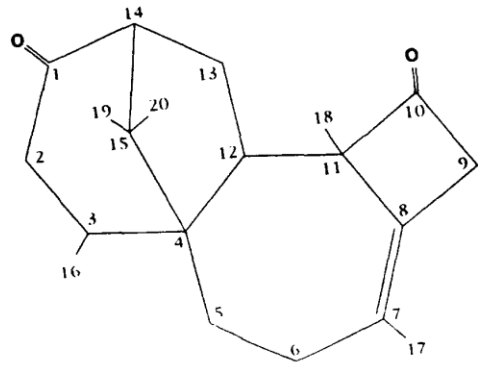


Fig. 7. Sketch of structure of isoharzandione. Notation was kept according to the biosynthetic rule.

Secondary metabolites

- Substances produced mainly by microorganisms and plants.

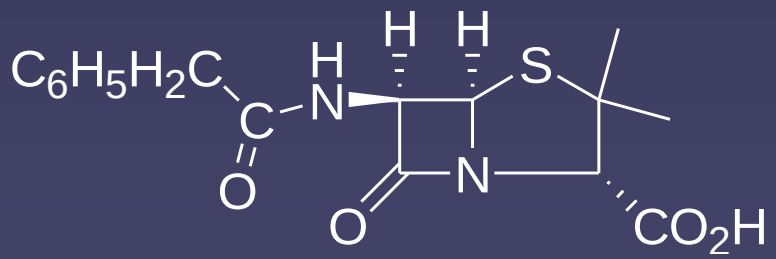
- Characteristic of a limited range of species.

- Heterogeneous group of chemically different natural products,

- Exhibit a wide range of biological activities,

i.e.: i) competitive weapons used against bacteria, fungi, plants, insects and animals; ii) metal transporting agents; iii) agents of symbiosis between microbes and plants, nematodes, insects; iv) sexual hormones.

Include antibiotics = natural products capable of inhibiting or killing microbial competitors



β - Lactame structure of Penicillin G

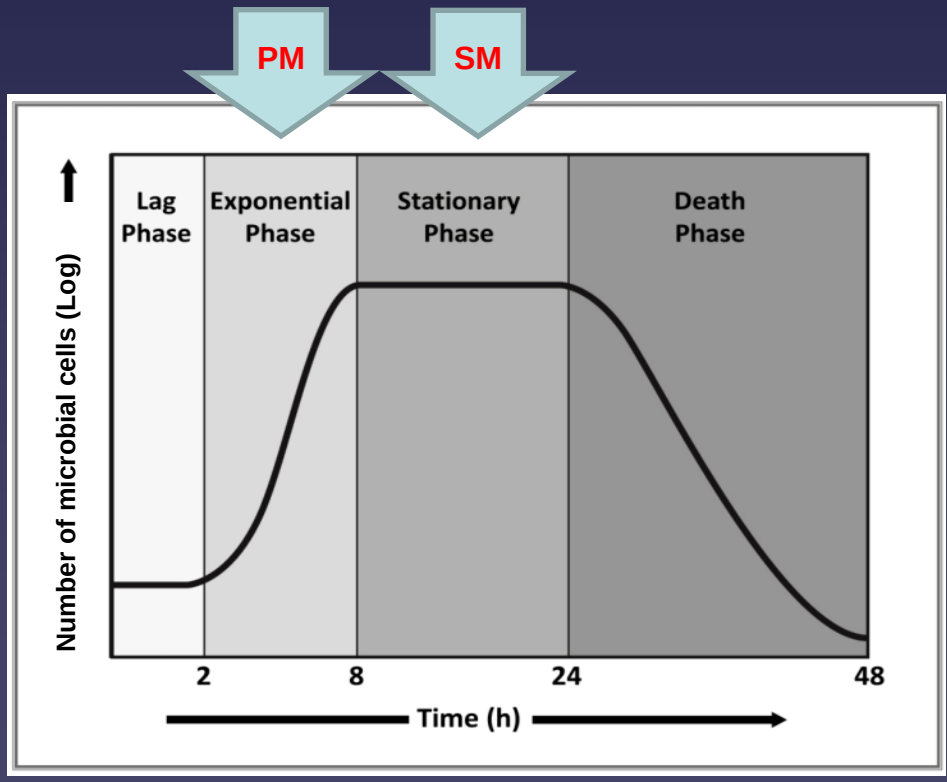
Primary *vs* Secondary metabolism

Primary metabolism = biochemical reactions that lead to metabolites which are required for the growth and maintenance of cellular functions.

Nucleic acids, proteins, carbohydrates and lipids.

Vital primary metabolism is related with the phase of rapid microbial growth (logarithmic, log or **exponential phase**).

Stationary phase: the metabolites derived from primary metabolism may be further transformed to other products including **secondary metabolites (SMs)**

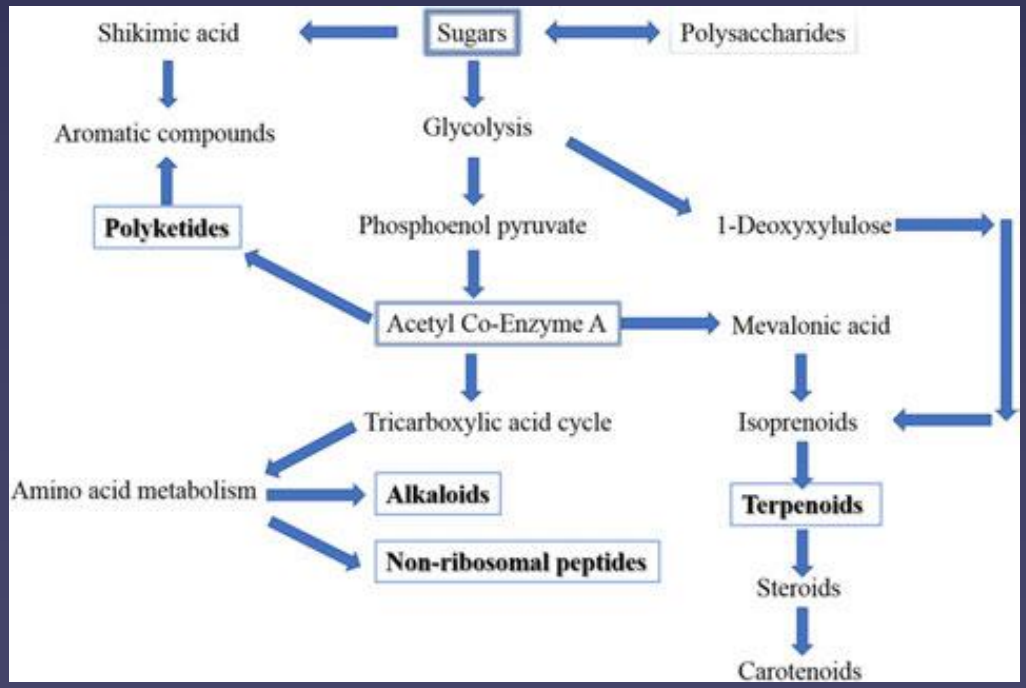


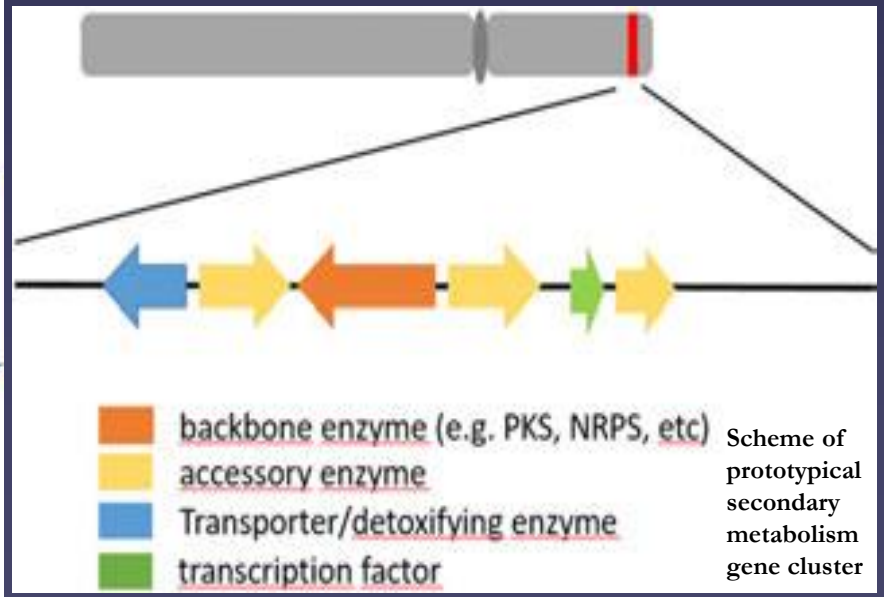
Primay *vs* Secondary metabolism

Fungal Secondary Metabolism = is not essential for vegetative growth but is <u>often related to differentiation and sporulation</u>.

Batch rather than continuous culture usually favors SM production.

These natural compounds show an enormous variety of biosynthetic origins (genes are often clustered), and there have been many examples of novel SMs (previously unreported).





Scheme of prototypical secondary metabolism gene cluster

Primay *vs* Secondary metabolism

-- Secondary metabolism is a rich and important source of chemical compounds with potential applications in several fields

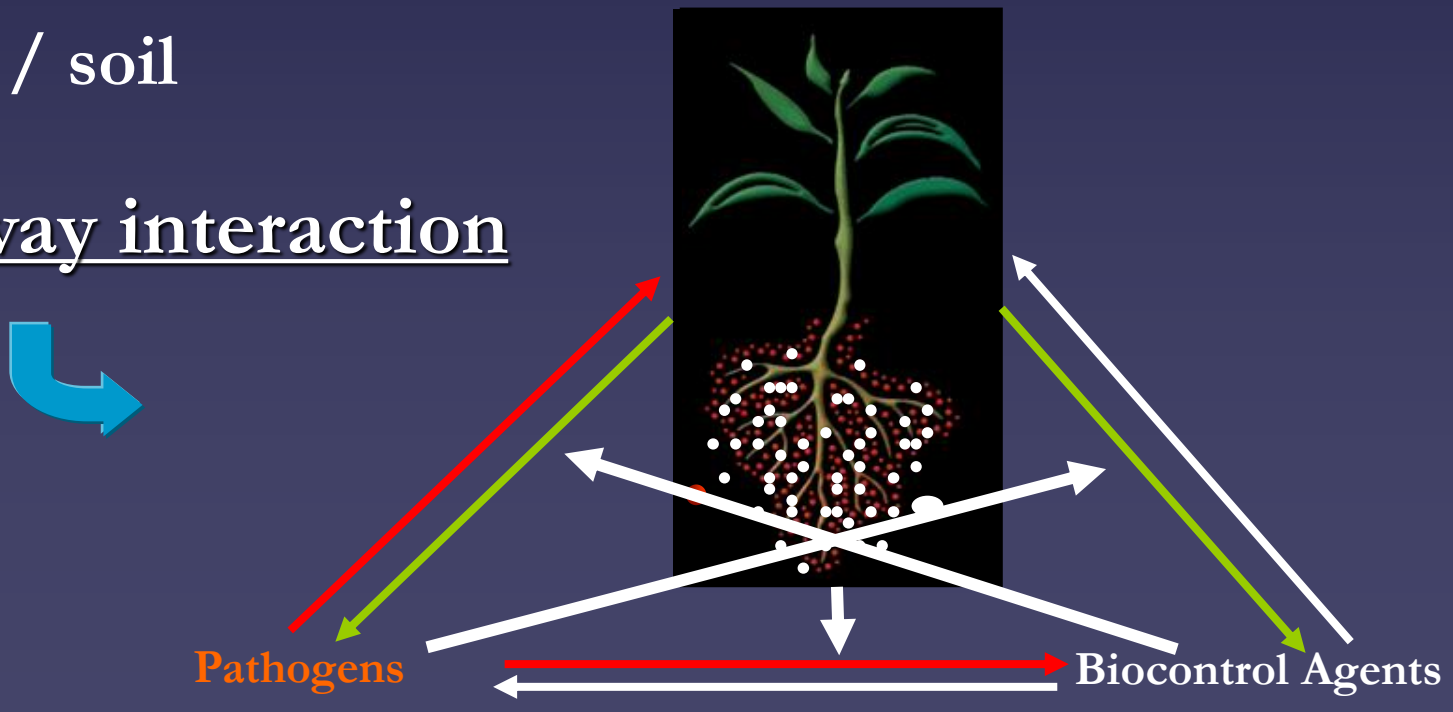
-- With beneficial or not-beneficial effects = from antibiotics to mycotoxins or phyto toxins(virulence factors).

Secondary metabolites (SMs)

SM are involved in several biological interaction important in agriculture:

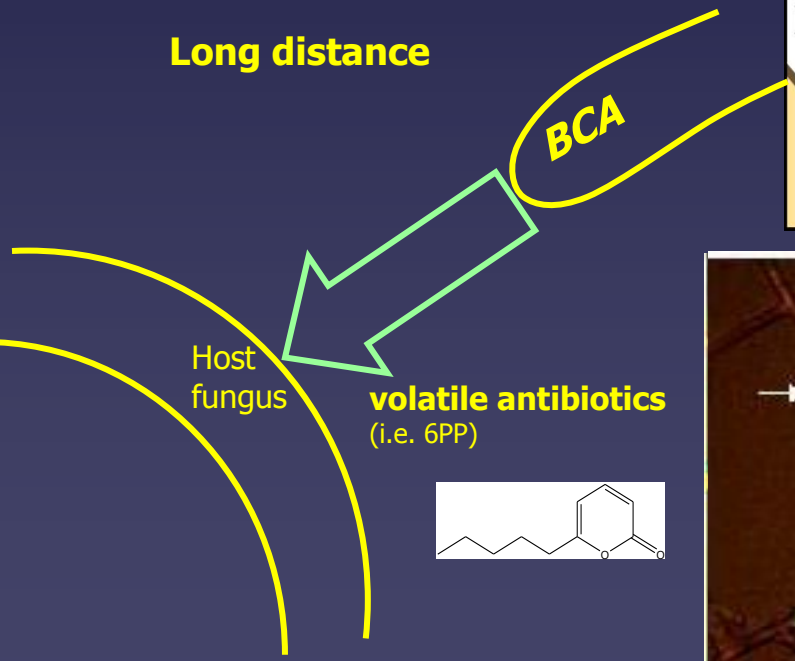
- Plant/pathogen
- Biocontrol agent (BCA)/pathogen
- Beneficial microbe /plant
- Microbe / soil

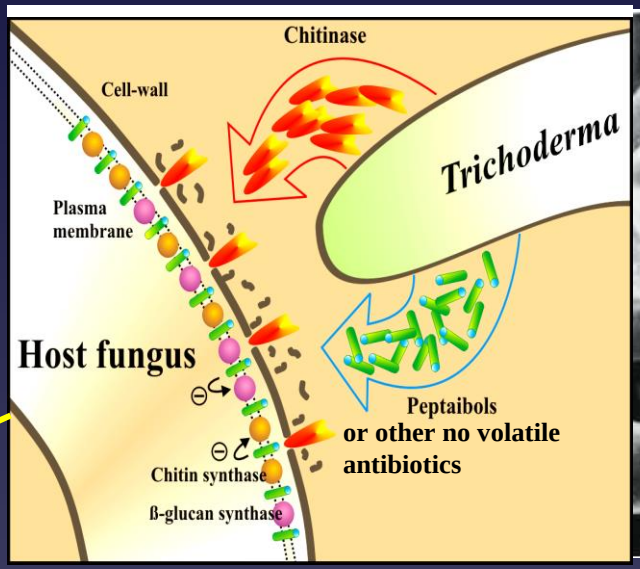
A three-way interaction

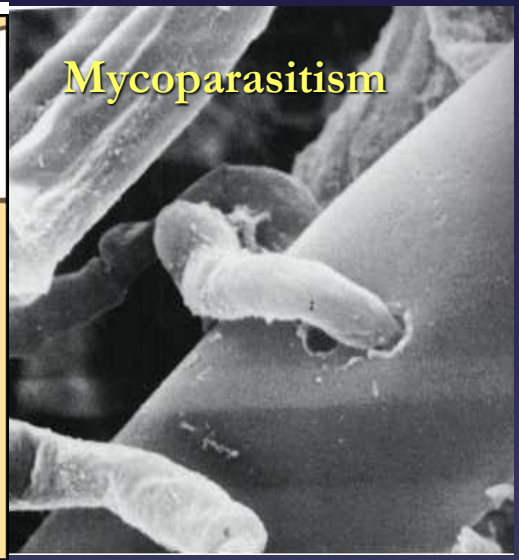


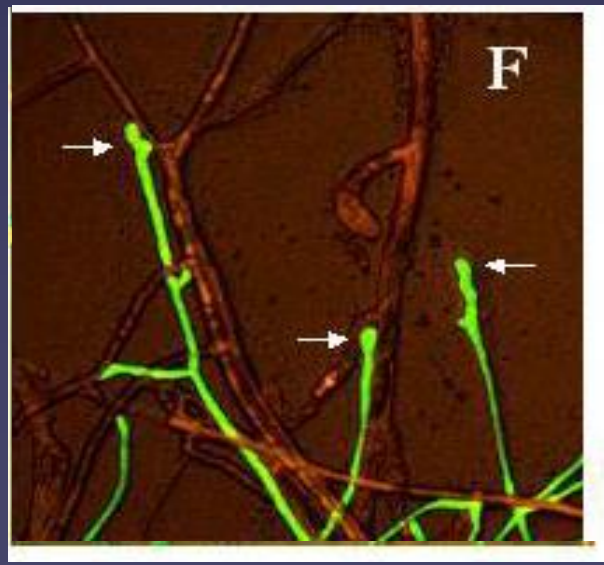
Beneficial microbes: interaction with the pathogen mediated by secondary metabolites

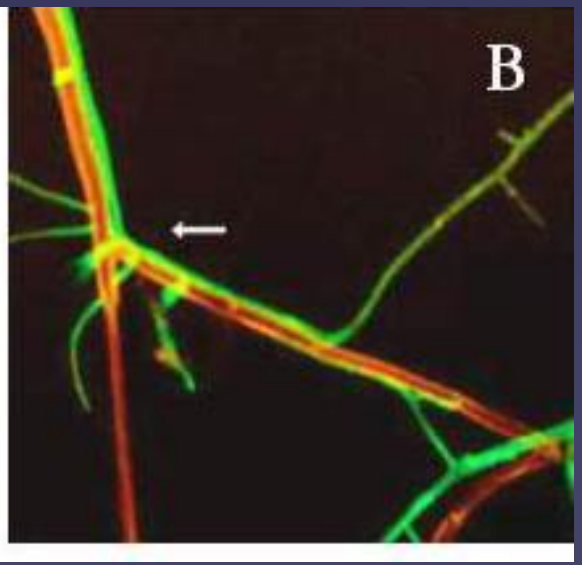
Antibiotic production is often correlated with disease control ability



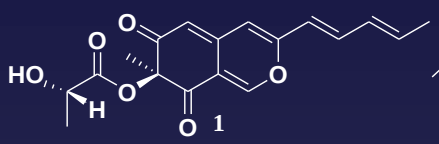






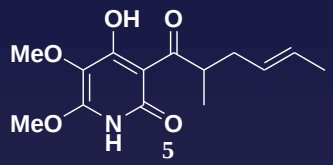


Antibiosis



1

2R = -CH_2COCH_3
3R = -$CH_2CHOHCH_3$
4R = -$CHCHCH_3$



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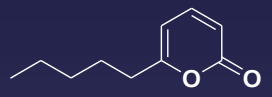

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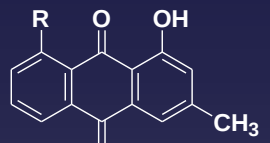
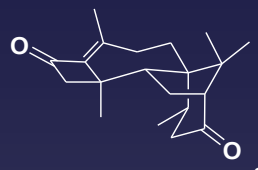


7R = H
8R = OH



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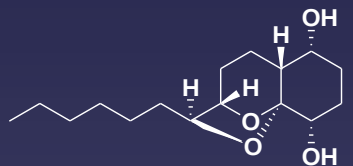

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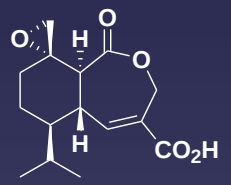

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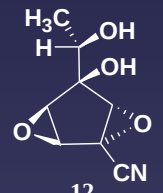

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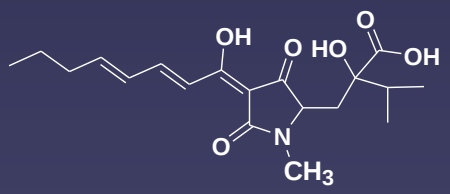

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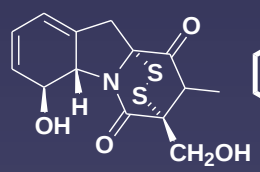

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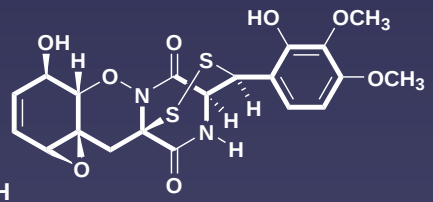

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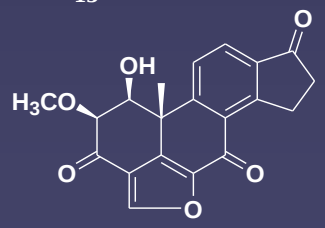

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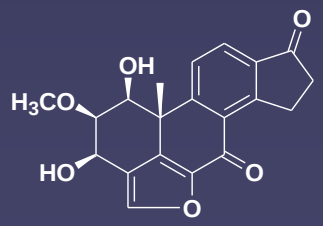


17

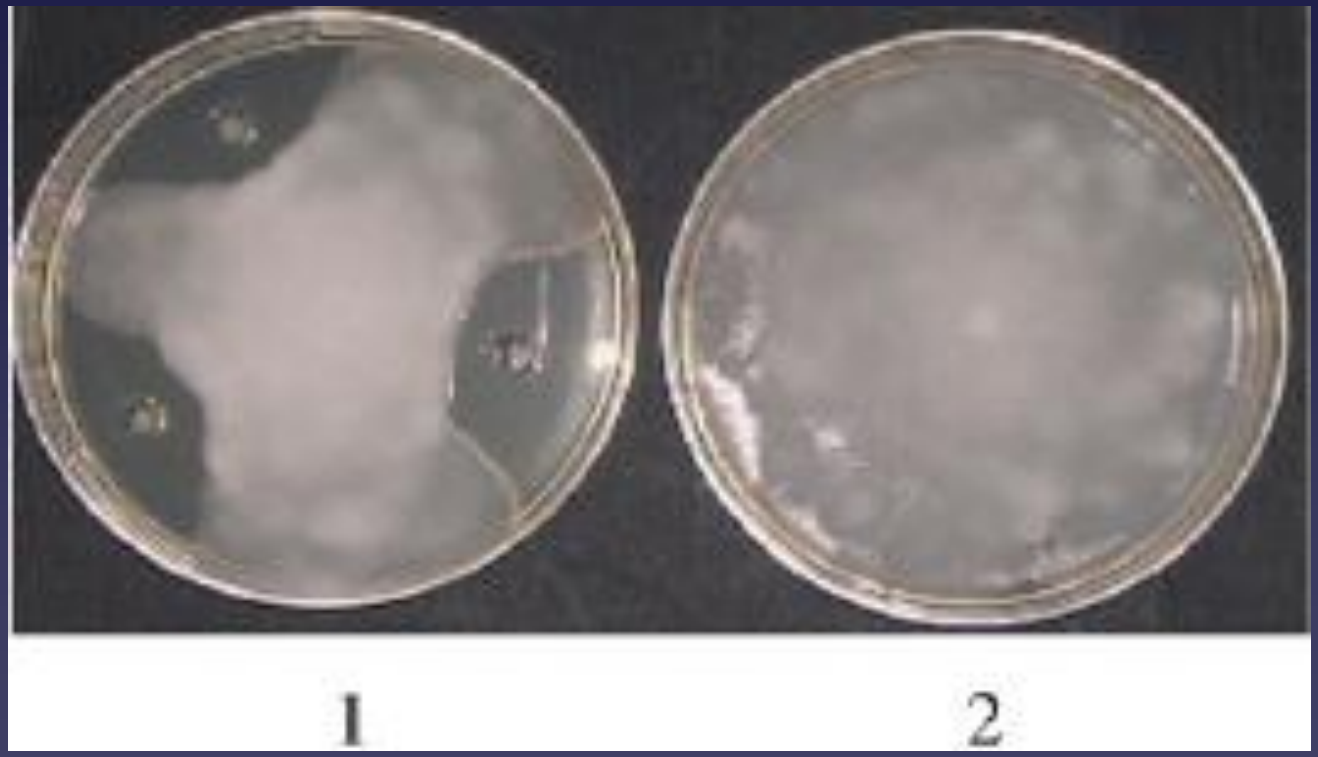
Ac-Aib-Ala-Ala-Aib-Aib-Gln-Aib-Aib-Aib-Ser-Leu-Aib-Pro-Leu-Aib-Ile-Gln-Gln-Leuol

18

Antibiotics of fungi used in agriculture for disease control: *Trichoderma* spp.

1: T22azaphilone;
2: T39butenolide;
3: harzianolide;
4: dehydro harzianolide;
5: harzianopyridone;
6: 6-pentyl-α-pyrone (6PP);
7: 1-hydroxy-3-methyl-anthraquinone;
8: 1,8-dihydroxy-3-methyl-anthraquinone;
9: harziandione;
10: koninginin A;
11: heptelidic acid;
12: trichoviridin;
13: harzianic acid;
14: gliotoxin;
15: gliovirin;
16: viridin;
17: viridiol;
18: trichorzianines.

Antibiosis



P. ultimum **(1): with 6PP; (2): without 6PP**

Antibiosis

JOURNAL OF NATURAL PRODUCTS

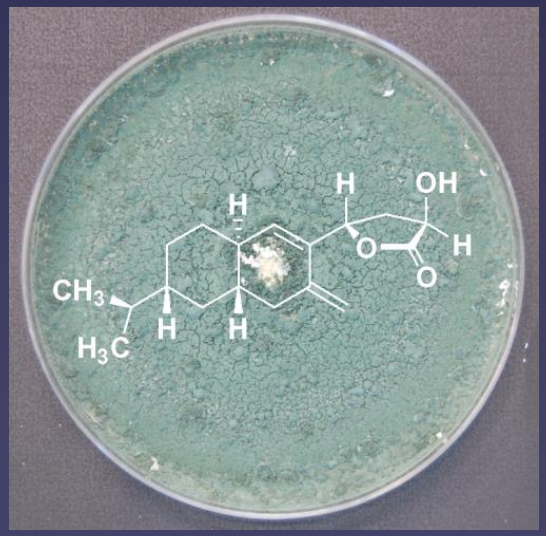
Note

pubs.acs.org/jnp

Cerinolactone, a Hydroxy-Lactone Derivative from *Trichoderma cerinum*

Francesco Vinale,*[†,‡] Isabel Arjona Girona,[§] Marco Nigro,[†,‡] Pierluigi Mazzei,[⊥] Alessandro Piccolo,[⊥] Michelina Ruocco,[†] Sheridan Woo,[†,‡] David Ruano Rosa,[§] Carlos López Herrera,[§] and Matteo Lorito[†,‡]

A novel metabolite named cerinolactone, has been isolated from culture filtrates *of T. cerinum*. Active against *Pythium ultimum*, *Rhizoctonia solani*, *and Botrytis cinerea.*



Beneficial microbes: interaction with the plant mediated by secondary metabolites??

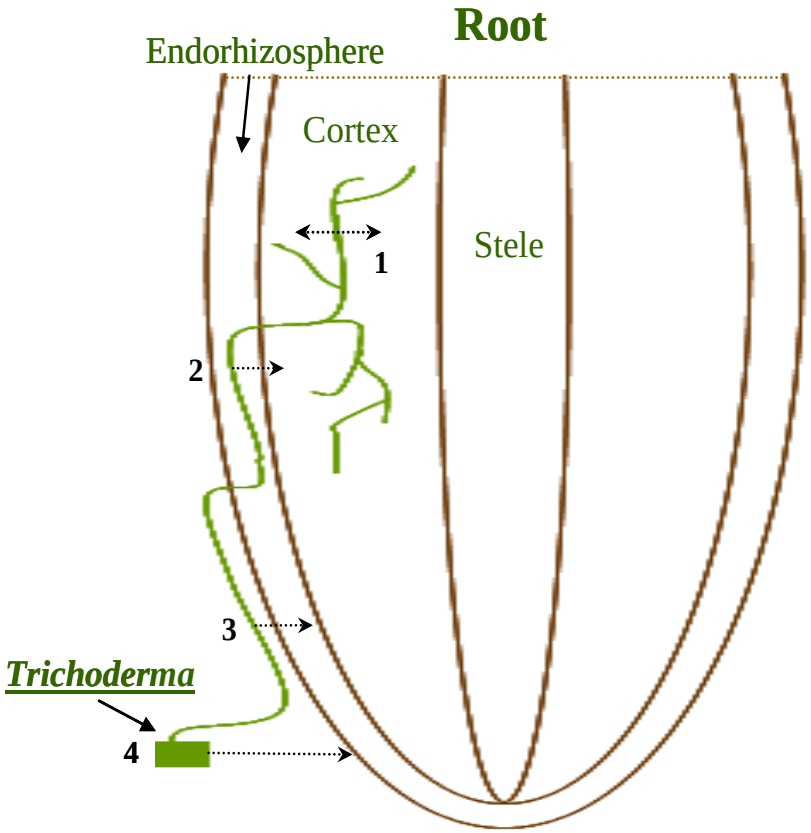
Effects of SM on plant??

-Promotion of plant growth

-Increased nutrient availability

-Improved crop production

-Enhanced disease resistance



Potential sites of production of *Trichoderma* metabolites that can affect plant host metabolism.

❶ Metabolites produced within live cortical cells.

❷ Metabolites produced in the root surface and within dead cortical cells.

❸ Metabolites produced in the rhizosphere.

❹ Metabolites produced in soil organic matter

Beneficial microbes: interaction with the plant mediated by secondary metabolites??

Physiological and Molecular Plant Pathology 72 (2008) 80–86

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

journal homepage: www.elsevier.com/locate/pmpp

A novel role for *Trichoderma* secondary metabolites in the interactions with plants

F. Vinale [a,*], K. Sivasithamparam [b], E.L. Ghisalberti [c], R. Marra [a], M.J. Barbetti [d], H. Li [b], S.L. Woo [a], M. Lorito [a]

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. 2019, 67, 1814−1822

pubs.acs.org/JAFC

Application of *Trichoderma* Strains and Metabolites Enhances Soybean Productivity and Nutrient Content

Roberta Marra,*[,†,‡] Nadia Lombardi,[†,§] Giada d'Errico,[†,‡] Jacopo Troisi,[⊥,▽] Giovanni Scala,[▽,○] Francesco Vinale,[§] Sheridan L. Woo,[‡,§,#] Giuliano Bonanomi,[†,‡] and Matteo Lorito[†,‡,§]

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Metabolomics by Proton High-Resolution Magic-Angle-Spinning Nuclear Magnetic Resonance of Tomato Plants Treated with Two Secondary Metabolites Isolated from *Trichoderma*

Pierluigi Mazzei,*[,†] Francesco Vinale,[‡] Sheridan Lois Woo,[‡,§] Alberto Pascale,[§] Matteo Lorito,[‡,§] and Alessandro Piccolo[†,§]

ELSEVIER

Crop Protection

Volume 92, February 2017, Pages 176-181

Trichoderma and its secondary metabolites improve yield and quality of grapes

A. Pascale [a], F. Vinale [b], G. Manganiello [a], M. Nigro [a], S. Lanzuise [a], M. Ruocco [b], R. Marra [a], N. Lombardi [a], S.L. Woo [a b], M. Lorito [a b]

NPC Natural Product Communications

2012
Vol. 7
No. 11
1545 - 1550

Trichoderma Secondary Metabolites that Affect Plant Metabolism

Francesco Vinale[a*], Krishnapillai Sivasithamparam[b], Emilio L. Ghisalberti[c], Michelina Ruocco[a], Sheridan Woo[d,a] and Matteo Lorito[d,a]

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ACS AUTHORCHOICE

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Article

Effect of *Trichoderma* Bioactive Metabolite Treatments on the Production, Quality, and Protein Profile of Strawberry Fruits

Nadia Lombardi,*[,¶] Anna Maria Salzano,[¶] Antonio Dario Troise, Andrea Scaloni, Paola Vitaglione, Francesco Vinale, Roberta Marra, Simonetta Caira,* Matteo Lorito, Giada d'Errico, Stefania Lanzuise, and Sheridan Lois Woo

Phytotherapy Research

REVIEW | Full Access

Beneficial effects of *Trichoderma* secondary metabolites on crops

Francesco Vinale, Krishnapillai Sivasithamparam

First published: 23 June 2020 | https://doi.org/10.1002/ptr.6728 | Citations: 55

Promotion of plant growth

Increased nutrient availability

Harzianic acid = a plant growth promoting metabolite fully characterized by x-ray studies

2032 *J. Nat. Prod.* **2009**, *72*, 2032–2035

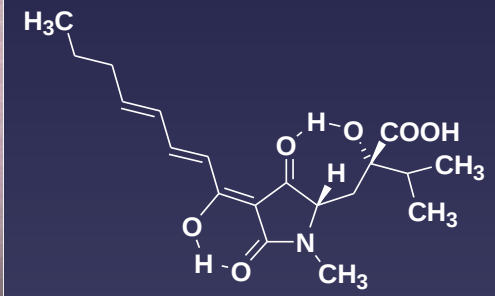
Harzianic Acid, an Antifungal and Plant Growth Promoting Metabolite from *Trichoderma harzianum*

Francesco Vinale,*[,†,⊥] Gavin Flematti,[‡] Krishnapillai Sivasithamparam,[†,∥] Matteo Lorito,[§] Roberta Marra,[§] Brian W. Skelton,[‡] and Emilio L. Ghisalberti[‡]

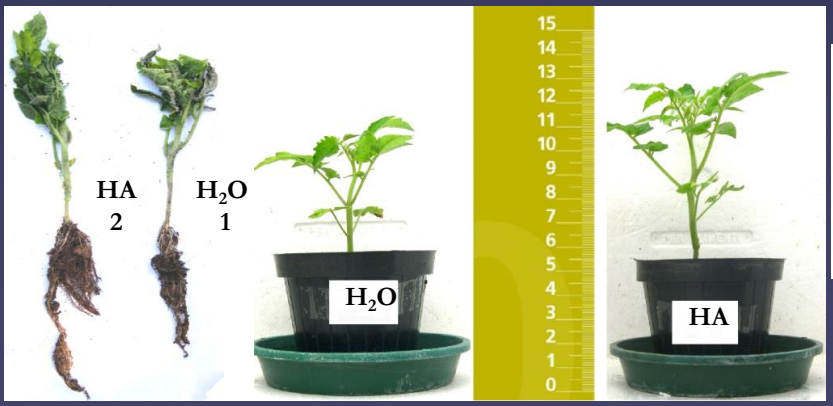
RESEARCH LETTER

Harzianic acid: a novel siderophore from *Trichoderma harzianum*

Francesco Vinale[1], Marco Nigro[1,2], Krishnapillai Sivasithamparam[3], Gavin Flematti[4], Emilio L. Ghisalberti[4], Michelina Ruocco[1], Rosaria Varlese[2], Roberta Marra[2], Stefania Lanzuise[2], Ahmed Eid[2], Sheridan L. Woo[1,2] & Matteo Lorito[1,2]



Harzianic acid = Fe^{3+} binding



Tomato plants treated with water (H_2O) (1) and *Trichoderma* metabolite Harzianic acid (HA) [10^{-6} M] (2)

molecules MDPI

Article

Bivalent Metal-Chelating Properties of Harzianic Acid Produced by *Trichoderma pleuroticola* Associated to the Gastropod *Melarhaphe neritoides*

Gaetano De Tommaso [1], Maria Michela Salvatore [1], Rosario Nicoletti [2,3], Marina DellaGreca [1], Francesco Vinale [4,5], Assunta Bottiglieri [3], Alessia Staropoli [3,5], Francesco Salvatore [1], Matteo Lorito [3,5], Mauro Iuliano [1,*] and Anna Andolfi [1,*]

Organic LETTERS Letter pubs.acs.org/OrgLett

Total Synthesis and Biological Evaluation of the Tetramic Acid Based Natural Product Harzianic Acid and Its Stereoisomers

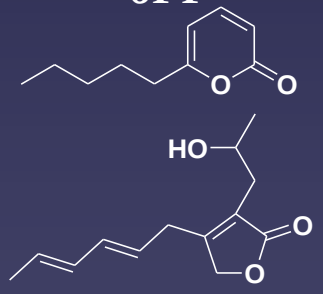
Alan R. Healy,[†] Francesco Vinale,[‡] Matteo Lorito,[‡,§] and Nicholas J. Westwood*[,†]

SMs and plant growth promotion

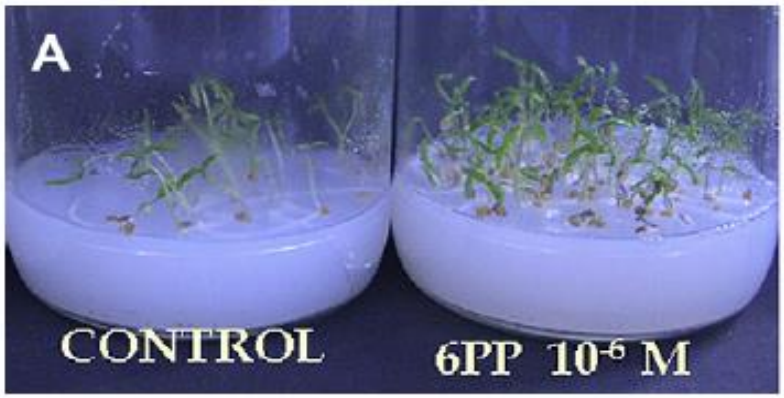
IAA

Some beneficial strains are able to produce indole-3-acetic acid (IAA), auxin analogues or other SM that act as plant growth regulators

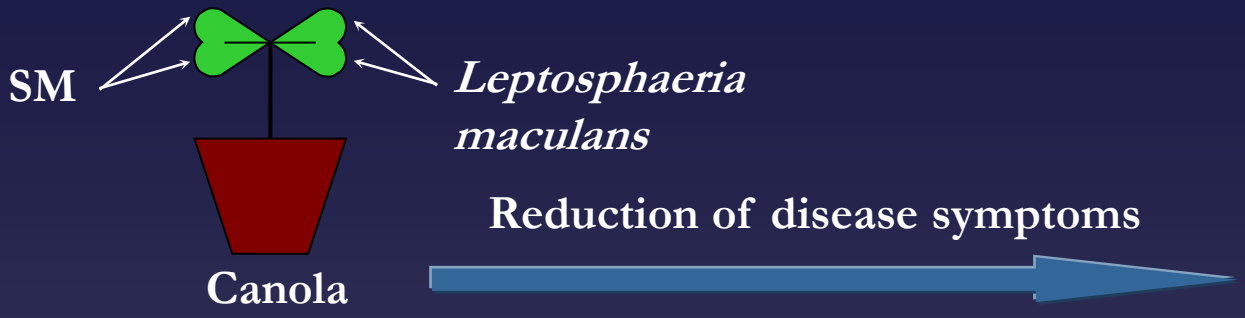
6PP

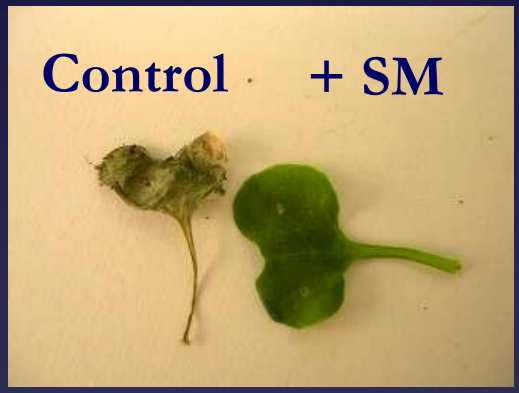


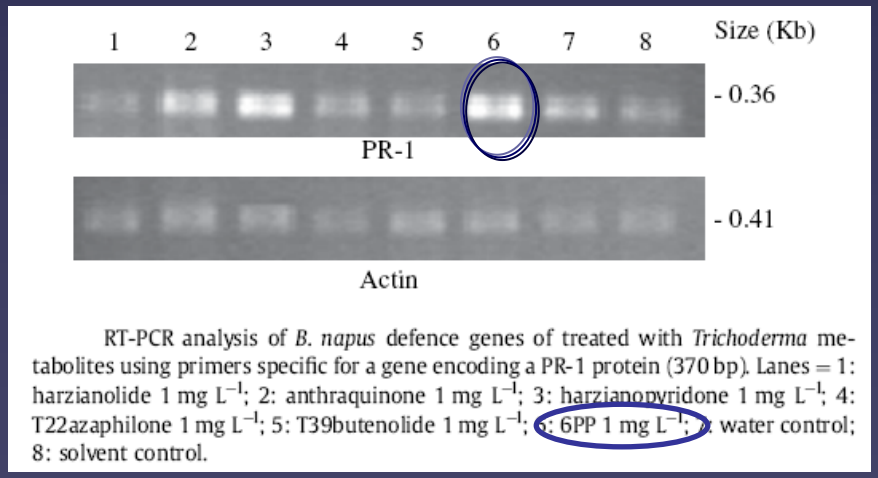
Harzianolide (HAR)



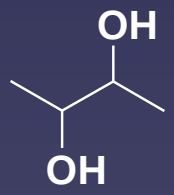
SMs and induction of plant defence responses







RT-PCR analysis of *B. napus* defence genes of treated with *Trichoderma* metabolites using primers specific for a gene encoding a PR-1 protein (370 bp). Lanes = 1: harzianolide 1 mg L^{-1}; 2: anthraquinone 1 mg L^{-1}; 3: harzianopyridone 1 mg L^{-1}; 4: T22azaphilone 1 mg L^{-1}; 5: T39butenolide 1 mg L^{-1}; 6: 6PP 1 mg L^{-1}; 7: water control; 8: solvent control.

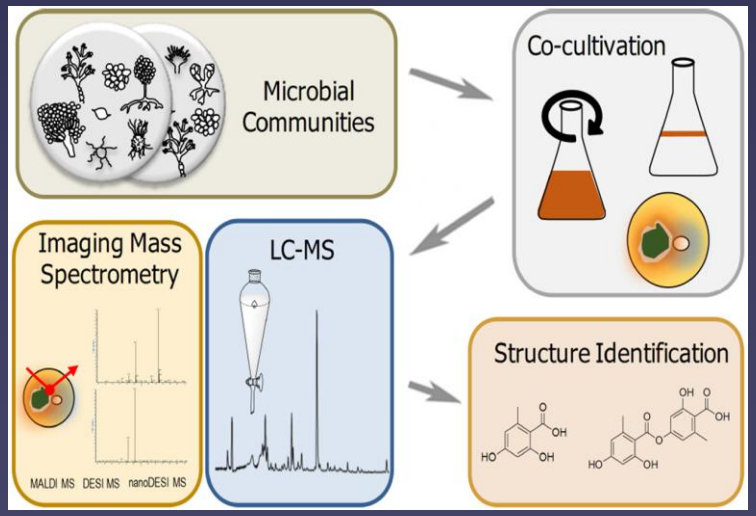


Peptaibols and other small peptides = plant defence elicitors.

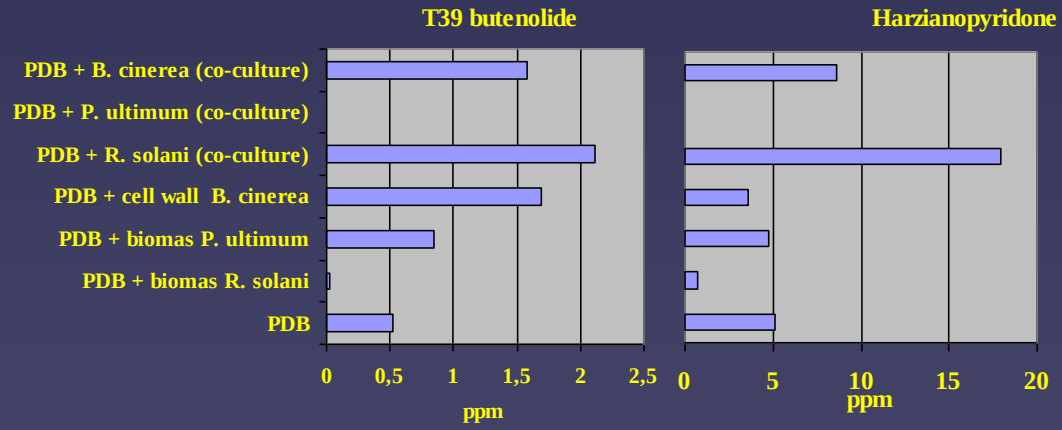
Co-culture of plant beneficial microbes as source of bioactive metabolites

- Re-isolation of known compounds is frequent
- Only some biosynthetic genes are transcribed in lab conditions
- Gene clusters coding for secondary metabolites can be activated

<u>To overcome these limitations is possible to cultivate microorganisms by simulating naturally occurring conditions, where microbes co-exist within complex communities, generally referred as the "microbiome"</u>



Netzker et al., 2015 (Front. Microbiol.)



Letters in Applied Microbiology ISSN 0266-8254

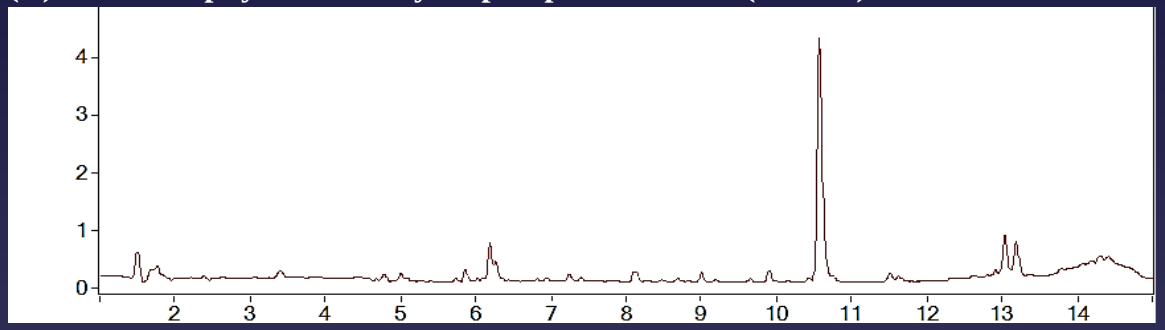
ORIGINAL ARTICLE

Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens

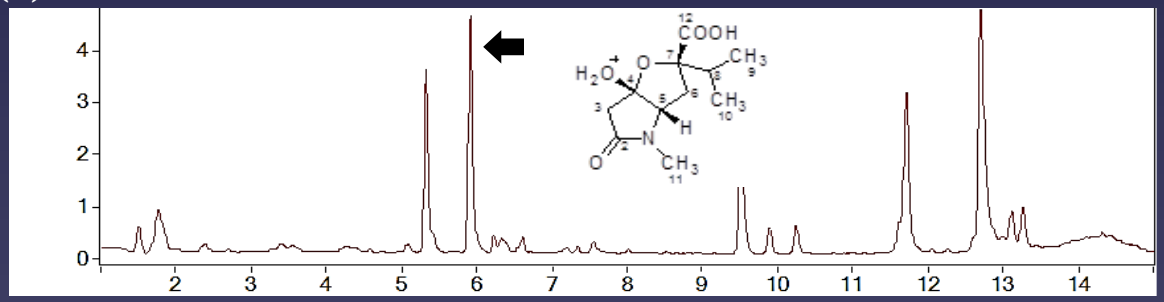
F. Vinale[1], E.L. Ghisalberti[2], K. Sivasithamparam[3], R. Marra[1], A. Ritieni[4], R. Ferracane[4], S. Woo[1] and M. Lorito[1]

Co-culture of plant beneficial microbes as source of bioactive metabolites

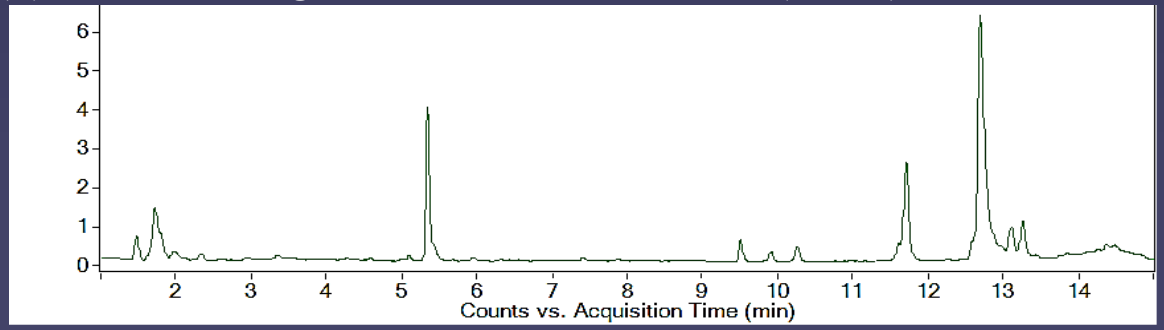
(A) **Endophyte Talaromyces pinophilus F36CF (control)**



(B) **Co-culture**



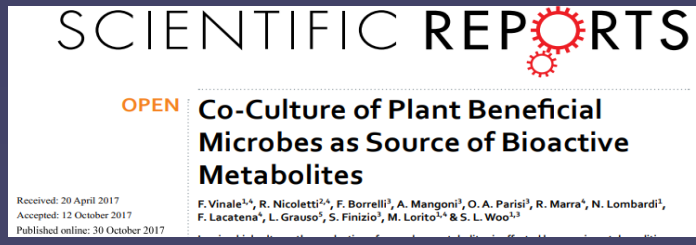
(C) **Biocontrol agent *Trichoderma harzianum* M10 (control)**



Co-culture = *T. harzianum* + endophyte *Talaromyces pinophilus*

Metabolomic analysis

New metabolite = harziaphilic acid



Co-culture of plant beneficial microbes as source of bioactive metabolites

OSMAC (One Strain MAny Compounds) strategy based on application of a variety of growing environments on a selected fungal (or other microbial) strain to induce biosynthesis of SMs

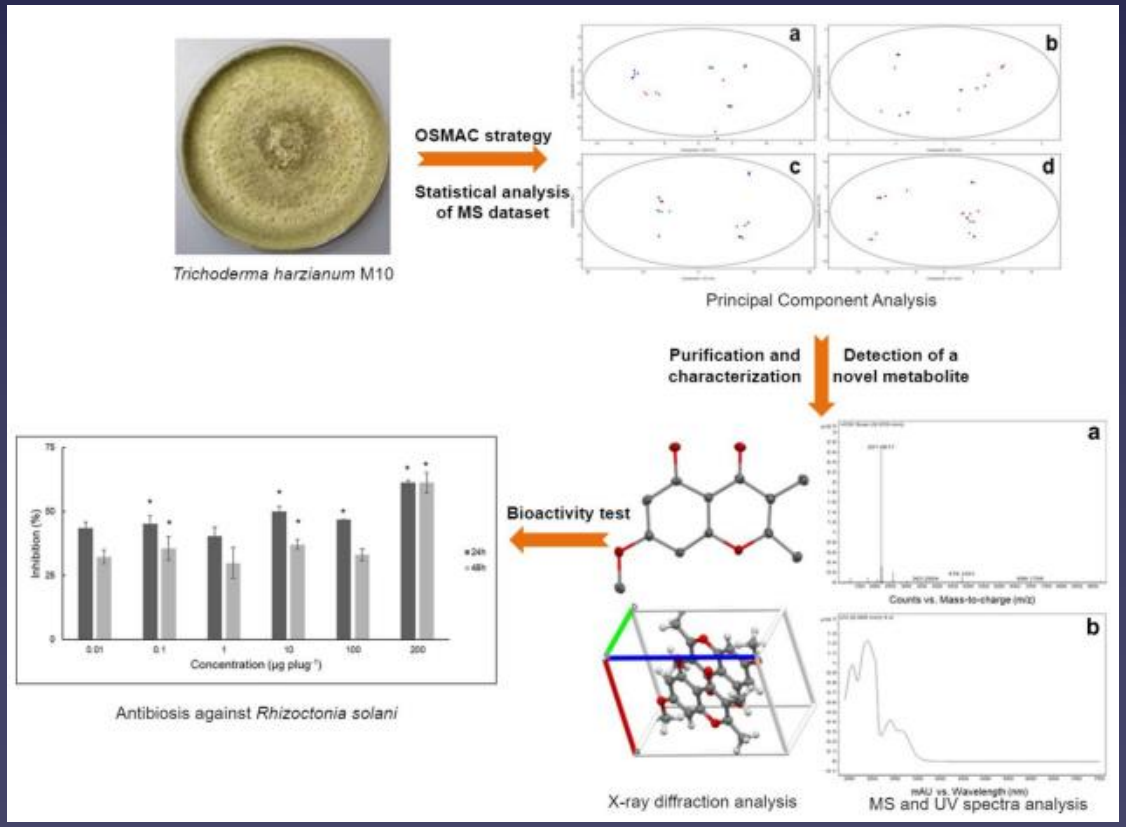
Staropoli *et al. Chem. Biol. Technol. Agric.* (2023) 10:28
https://doi.org/10.1186/s40538-023-00383-x

Chemical and Biological Technologies in Agriculture

RESEARCH Open Access

Induced secondary metabolites of the beneficial fungus *Trichoderma harzianum* M10 through OSMAC approach

Alessia Staropoli[1,2], Giuseppina Iacomino[1], Paola De Cicco[3], Sheridan L. Woo[3,5], Luigi Di Costanzo[1] and Francesco Vinale[2,4,5*]

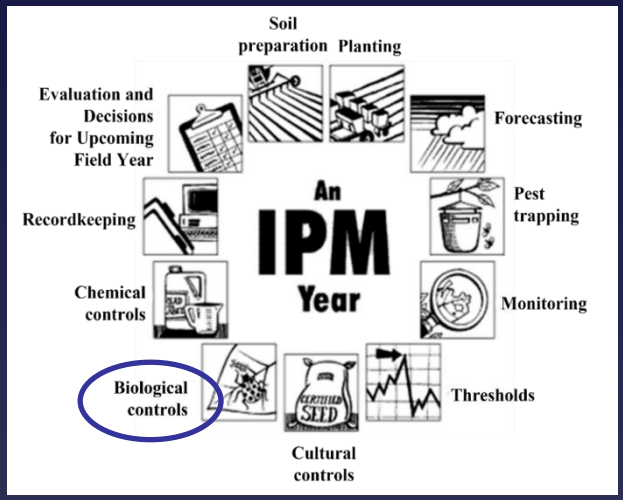


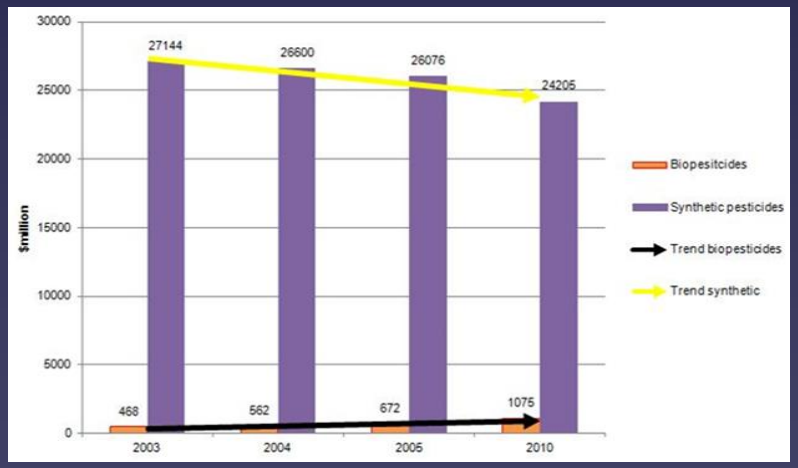
Future perspective

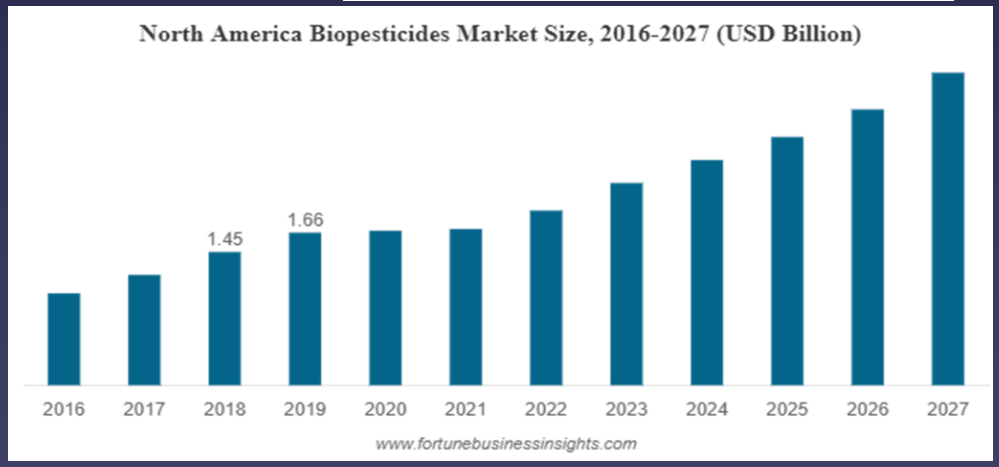
Integrated Pest Management

Use of alternatives to pesticides and all methods available for crop, including:

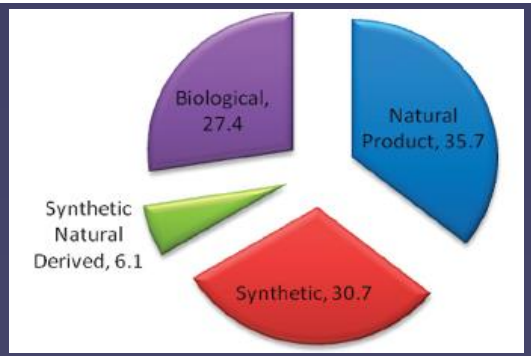
- **beneficial microbes and/or their products (SMs)**







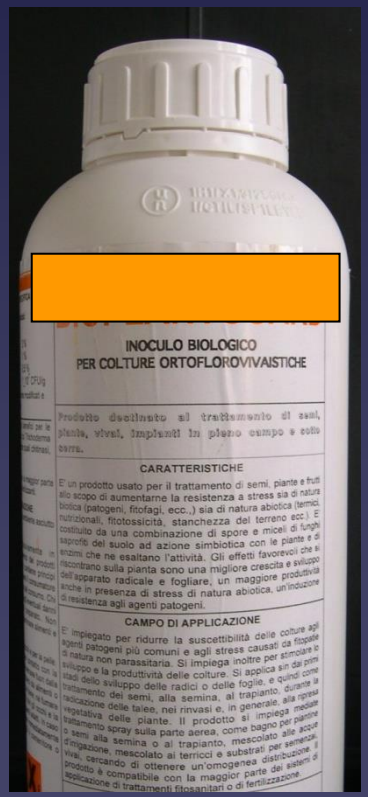
Certain beneficial properties are related with **"effector metabolite"** that beneficial microbes provide during the interaction with the plant and the other microbes.



Future perspective

New SMs - based products

- Produced cheaply in fermentor in selected inexpensive media
- Good shelf-life
- Antifungal, plant growth promotion and induced resistance
- Combined easily with chemicals, additives, other BCA, etc.



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IL caffè SCIENTIFICO

Prossimo incontro:

22 Novembre 2023

Francesco Serrapica

Qual è il futuro dei foraggi fuori suolo in alimentazione animale

Thank you for your attention