Book of Abstracts

Trichoderma

Molecular mechanisms and applications of biocontrol in agriculture

Technion, Haifa, Israel
October 12-15, 2010

Cover photo credits: *Trichoderma* SEM image, Benjamin A. Horwitz and Naomi Bahat 1983;
Haifa, Karen Horwitz 2009

SCHEDULE

Tuesday October 12 – arrival at hotels

Wednesday October 13

8:00 **Registration desk open – lobby of Butler Auditorium, Neaman Institute, Technion**

8:15 **Shuttle leaves hotels after breakfast**

9:00-9:15 Welcome address: Benjamin A. Horwitz
9:15-9:30 Edo Chalutz "BARD Operations, Achievements and Funding Opportunities"
9:30-10:10 Gary Harman (Cornell University) Trichoderma--not just for biocontrol anymore

10:15-11:15 **Coffee, registration desk open, put up posters**

Session 1 – Genomes
Chair: Benjamin A. Horwitz

11:15-11:45 Diego Martinez (Broad Institute, MIT) Global Organization of Trichoderma Genomes
11:45-12:15 Scott Baker (Pacific Northwest National Laboratory) Trichoderma genomics and bioenergy

12:15 – 13:45 **Lunch and Posters (Butler Auditorium)**

Session 2 – Genomics, Gene expression, Enzymes and Secondary Metabolites
Chair: Ada Viterbo and Prasun K. Mukherjee

13:45-14:15 Alfredo Herrera-Estrella (CINVESTAV, Irapuato, Mexico) A genomics view of conidiation
14:15-14:35 Barbara Reithner (T.U. Vienna, Austria) The complexity of *Trichoderma atroviride* – host interaction: observations on the transcriptome level
14:35-14:50 Matthias Steiger (T.U. Vienna, Austria) A transformation system for Hypocrea (Trichoderma) that favours homologous integration and that uses reusable bi-directionally selectable markers
14:50-15:20 Magnus Karlsson (SLU-Uppsala, Sweden) Comparative molecular evolution of Trichoderma chitinases in response to mycoparasitic interactions
15:20-15:50 Verena Seidl-Seiboth (T.U. Vienna, Austria) Trichoderma CAZomes: the carbohydrate degradation potential of mycoparasites
15:50-16:05 Ludovit Varecka (Slovak University of Technology) A study of the protease activity secretion from *Trichoderma viride*

16:15-17:15 Coffee and Posters

17:15-17:45 Prasun K. Mukherjee (Texas A&M University/BARC, Mumbai, India) Secondary metabolism and its regulation in *Trichoderma virens*

17:45-18:00 Enrique Monte (U. Salamanca, Spain): Overexpression of the trichodiene synthase gene *Tbtri5* increases trichodermin production and antimicrobial activity in *Trichoderma brevicompactum*

18:00-18:15 Richard Hung (Rutgers University, NJ, USA) The effect of 1-octen-3-ol, a volatile organic compound produced by *Trichoderma* species, on *Arabidopsis thaliana*

18:15-18:30 V. Shanmugam (CSIR, Palampur, India) Fusaric acid detoxification and chitinase expression in an antagonistic Trichoderma harzianum S17TH

Short walk on campus to Coler Visitors' Center

19:00 Dinner at Coler Visitors' Center, including short movie about Technion

20:40 Shuttle to central Carmel/hotels

Thursday October 14

8:45-9:45 Visit to Baha'i Gardens: meet at upper entrance, 61 Yefe Nof Street near hotels. The tour ends at the main entrance to the gardens on Hatzionut Avenue; bus to Technion.

Coffee & tea at Butler Auditorium

Session 3 – Fungal-fungal and fungal-plant interactions relevant to biocontrol

Chair: Tong Xu and Yigal Elad

10:30-11:00 Ada Viterbo (Hebrew University, Rehovot, Israel) *Trichoderma*-Plant Interactions: insights on induced resistance to biotic and abiotic stresses

11:00-11:30 Matteo Lorito (U. Napoli, Italy) An example of translational research on Trichoderma: when 'omics data improve field applications

11:30-12:00 Markus Omann (Technical University, Vienna, Austria) The role of G-Protein signaling in the biocontrol agent *Trichoderma atroviride*

12:00-12:30 Enrique Monte (University of Salamanca, Spain) The *Trichoderma*-plant cross talk model

12:30-12:45 Johanna Steyaert (Lincoln University, New Zealand) The influence of soil biotic factors on the ecology of two *Trichoderma* biological control agents

12:45-13:00 Yariv Brotman (MPI Potsdam, Germany) Analyzing the transcriptome and metabolome of Arabidopsis inoculated with *Trichoderma* and the pathogen *Pseudomonas syringae*

13:00-13:15 Michal Shoresh (ARO Volcani, Israel) Differential expression of maize chitinases in the presence or absence of *Trichoderma harzianum* (T22) and indications of a novel exo-endo-heterodimeric chitinase activity

13:15-14:15 **Lunch: sandwiches and posters, Butler Auditorium**

Session 4 – Applications

Chair: Yitzhak Hadar and Johanna Steyaert

14:15-14:45 U.S. Singh (South Asia Regional Project Coordinator, IRRI, India) Trichoderma: A potential agent for the management of abiotic stresses in plants

14:45-15:15 Willem Ravensberg (Koppert Biological Systems, The Netherlands) A roadmap for the successful development of a microbial plant protection product

15:15-15:45 Miguel Obregon (Costa Rica) Uses of *Trichoderma* in the agricultural field in Costa Rica

15:45-16:00 **Coffee & tea, Butler Auditorium**

16:00-16:30 Yitzhak Spiegel (ARO, Israel): Trichoderma as a biocontrol agent against plant-parasitic nematodes: Veni, vidi...vici?

16:30-16:45 Jie Chen (Shanghai Jiaotong University, China) Construction and function analysis of Trichoderma transformant with Metarhizium genes against insects

16:45-17:00 Robert Hill (Bio-Protection Research Centre, Lincoln University, New Zealand) Enhancing growth and health of *Pinus radiata* in New Zealand and *Acacia mangium* in Malaysia with selected *Trichoderma* isolates

17:00-17:15 Robert Hill (Bio-Protection Research Centre, Lincoln University, New Zealand) Non-target impacts of the biocontrol agent *Trichoderma atroviride* on two native ecosystems in New Zealand

17:15-17:30 Ofer Kleifeld (Mycontrol Ltd., Israel) The conflict between commercial and biological requirements in the development of commercial formulation of bio-fungicides

17:30-18:15 **Round Table Discussion - Leader:** Ilan Chet (Hebrew University); participants from molecular genetics, physiology, and industry; open discussion

18:15 Presentation of the Trichoderma and Gliocladium Research Excellence Award

18:30 Shuttle to hotels/Central Carmel

19:30 **Dinner (restaurant in central Carmel, walking distance from hotels)**

Friday October 15

Departures

ABSTRACTS OF TALKS

Global Organization of Genes in Trichoderma Genomes

Diego A. Martinez[1,2], Christine Chee[1], Joseph Kunkel[3], Charles Sanchez[1], Mary Anne Nelson[1] .
[1]University of New Mexico, Albuquerque, NM USA. [2]Broad Institute, Cambridge, MA USA, [3]University of Arizona, Tucson, AZ USA

Our previous work on the genome of the filamentous fungus *Trichoderma reesei* suggested that the location of genes in genomes is not random; instead they may be organized in a way that is beneficial to cellular processes and the organism. While a few studies have investigated the organization of genes on a whole genome scale, they were limited in the functions of genes used in the search and in the number and type of genomes searched. With the recent release of other Trichoderma genomes it is now possible to obtain a global view of the level of clustering in the genomes for this clade. To find gene clusters in many genomes, we have constructed a robust and flexible algorithm that runs in trivial time. In parallel, for comparison, we have annotated 72 fungal genomes using four automated annotation tools that provide information about protein function, protein targeting, involvement in biochemical pathways and paralogous gene families. We discovered that all the genomes contained clusters of related genes, and that in several cases the clusters included genes involved in processes that were specific to the species in which they are found. This has dramatically expanded our knowledge of both the types of clusters and the number of genomes known to contain clusters. This study has generated information that will assist researchers in addressing many questions central to molecular and cell biology as well as evolutionary studies. All data will be made available to interested researchers.
Email: admar505@gmail.com

A genomics view of conidiation

Hernández-Oñate, M., Ibarra, E., Vega, J., Esquivel-Naranjo, U., and Herrera-Estrella, A[1]. [1]National Laboratory of Genomics for Biodiversity, Cinvestav Campus-Guanajuato, 36821 Irapuato, Gto. Mexico.
Email: aherrera@langebio.cinvestav.mx

The main mechanism of survival and dispersal of *Trichoderma* species is through the production of asexual spores (conidia). Several decades ago it was shown that conidiation of *Trichoderma* can be triggered by several environmental factors such as light and nutrient availability, and by different types of stress, including desiccation and space limitation. Our group has focussed its attention in the conidiation process from a molecular point of view. In total darkness, *T. atroviride* grows indefinitely as a mycelium provided that nutrients are not limiting. However, a brief pulse of blue light given to a radially growing colony induces synchronous sporulation. The BLR1/BLR2 proteins, homologues of the photoreceptor complex of *Neurospora crassa* WC1/WC2, regulate this response to blue light. A few years ago we demonstrated that conidiation could also be induced by mycelial injury. The production of conidia in response to mechanical injury is, however, independent of *blr1* and *blr2*.
Recently, we applied high-throughput sequencing technology to the study of the *Trichoderma atroviride* transcriptome. RNA samples from the wild type strain grown in the dark or after exposure to a pulse of white light, as well as from a photoreceptor mutant (Dblr-1) exposed to light. Based on statistical tests and the relative abundance of a given transcript among all three samples we have been able to identify hundreds of light responsive genes and if their abundance depends on a functional *blr-1* gene. Among them we identified 12 transcription factors (TFs), DNA-repair enzymes, and a set chaperons, including heat shock proteins, suggesting that light is perceived as an stress signal by *Trichoderma*. We have initiated the study of light responsive genes through gene disruption of several TFs, and other key genes. Our results indicate that there positive and negative regulation of conidiation by some of these transcription factors, while other might regulate other responses to light. Using a similar strategy we analyzed injury induced conidiation. In this case, our data suggest that mechanical injury induces conidiation by triggering a burst of reactive oxygen species and we have identified key genes involved in this process by targeted gene disruption.

The complexity of *Trichoderma atroviride* – host interaction: observations on the transcriptome level

Reithner Barbara [(1)] and Herrera-Estrella Alfredo [(2)].
(1) Institute of Chemical Engineering, Vienna University of Technology, Gumpendorferstraße 1a/1665, A-1060 Wien, Austria
(2) Laboratorio Nacional de Genómica para la Biodiversidad, CINVESTAV Campus Guanajuato, Km 9.6 Libramiento Norte Carretera Irapuato-León, A.P. 629, Irapuato 36500, Guanajuato, México
Email: barbara.reithner@tuwien.ac.at

Trichoderma spp. are capable to recognize and attack plant-pathogenic fungi of distinct phyla like *Rhizoctonia solani* (Basidiomycete), *Botrytis cinerea*, *Fusarium graminearum* (both Ascomycetes), *Phytophthora* spp, and *Pythium* spp (both Oomycetes). The scope of action involving recognition, secretion on cell wall lysing enzymes, secretion of secondary metabolites, formation of penetration structures, and lysis of the host fungus is commonly summarized as mycoparasitism.
In the present work the transcriptomes of different stages of interaction of *T. atroviride* IMI206040 with *R. solani* were sequenced using 454 Life Science Technologies high-throughput sequencing. A total number of 277,769 high quality reads with an average length of 84.44-bp could be attributed to approximately 5000 different gene models, depending on the stage of interaction between *T. atroviride* and the host fungus. Statistical analysis revealed 244 distinct differentially expressed genes. Whereof 13, representing different KOG classification groups like Defense mechanisms, Signal transduction mechanisms and Secondary metabolites biosynthesis, transport and catabolism, amongst others, were chosen for quantitative RT-PCR to obtain relative gene expression levels during the mycoparasitic interaction of *T. atroviride* with *P. capsici*, *B. cinerea* and *R. solani*. The majority of the genes tested showed a related, host-independent expression pattern with variations in their induction or repression intensity. But 3 genes were found to be transcribed in a host-dependent way. These were identified as a Trypsin (KOG3627), a Ferric reductase, NADH/NADPH oxidase and related proteins (KOG0039), plus an unclassified protein having a fungal specific transcription factor domain.
Based on these results we try to find another puzzle piece of *Trichoderma* mycoparasitic response.

A transformation system for *Hypocrea* (*Trichoderma*) that favours homologous integration and that uses reusable bi-directionally selectable markers

Matthias G. Steiger[1], Marika Vitikainen[2], Kurt Brunner[1], Gerhard Adam[3], Markku Saloheimo[2], Robert L. Mach[1] and Astrid R. Mach-Aigner[1]

[1] Gene Technology, Gene Technology and Applied Biochemistry, Institute of Chemical Engineering, TU Wien, Getreidemarkt 9/166/5/2, A-1060 Wien, Austria
[2] VTT Technical Research Centre, Biotechnology, FIN-02044 VTT, Espoo, Finland
[3] Department für Angewandte Genetik und Zellbiologie, BOKU Wien, Muthgasse 18, A-1190 Wien, Austria

Email: matthiassteiger@gmx.net

Genetic engineering of filamentous fungi is a very important tool to answer a plethora of scientific questions and to design strains for tailor-made applications. Although a lot of different transformation systems for *Hypocrea* were reported in the last decades, it still remains a challenging task to obtain the desired recombinant strains because of non-homologous end joining events, a limited set of available markers and the absence of an efficient recycling system for those markers. Furthermore, the construction of gene replacement cassettes is laborious because long homologous flanking regions are required in the transformation procedure.
Therefore, we report the development of a transformation system for *Hypocrea jecorina* that combines highly efficient gene targeting (by using a *tmus53* (human LIG4-homolog) deletion strain), marker recycling (with a Cre/loxP-based excision system), and bi-directional positive selection (by combining resistance to hygromycin B and loss of sensitivity to fluoroacetamide). Furthermore, the bi-directional marker *pyr4* is exploited to remove the *cre* gene again. The construction of deletion cassettes is facilitated applying the Cre/loxP system *in vitro*. Implementation of components of this system in *H. atroviridis* and *Fusarium* has highlighted it as a universal transformation tool for filamentous fungi.

Comparative molecular evolution of *Trichoderma* chitinases

Ihrmark K[1], Asmail N[1], Ubhayasekera W[2], Melin P[3], Stenlid J[1], Funck Jensen D[1] and Karlsson M[1], [1]Mycology and Plant Pathology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, [2]MAX-lab, Lund University, Lund, Sweden / Medicinal Chemistry, University of Copenhagen, Copenhagen, Denmark, [3]Microbiology, SLU, Uppsala, Sweden.
Email: magnus.karlsson@mykopat.slu.se

The emerging concept of effector biology of plant-associated microbes suggests that microbes secrete proteins and other molecules that modulate plant defence and to enable successful colonization of plant tissues. These secreted molecules are collectively known as effectors and understanding their function during plant interaction is critical for a mechanistic understanding of plant disease development. Here we expand the effector concept from plant/pathogen interactions to fungal/fungal interactions and use it as a framework for evolutionary studies of the *Trichoderma* chitinolytic system. In *Trichoderma*, whole-genome sequencing reveal between 20 and 36 different genes encoding chitinases, and several of these genes have been shown to be induced during the mycoparasitic attack. We employ maximum likelihood based methods to show that three different chitinase subgroups (B1, C1 and C2) have expanded in copy number in *T. atroviride* and *T. virens*, suggesting an important role during fungal/fungal interactions. Sequences of *Trichoderma* chitinase genes *chi18-5*, *chi18-13*, *chi18-15* and *chi18-17*, that all exhibit specific expression during mycoparasitism-related conditions, were determined from up to 13 different taxa and studied with regard to their evolutionary patterns. *Chi18-13* contained two codons that evolve under positive selection and seven co-evolutionary site networks. Regions of high amino acid variability were preferentially localized to substrate- or product side of the catalytic cleft. *Chi18-15* displayed a unique codon-usage and contained five codons that evolve under positive selection and three co-evolutionary site networks. Regions of high amino acid variability were preferentially localized to coil-regions adjacent to certain alpha-helixes, suggesting structural adaptations of enzyme architecture. In addition, differences in amino acid variability / conservation patterns of *chi18-15* were observed between different *Trichoderma* clades and an ortholog, *Streptomyces chiJ*. These observations show that *Trichoderma* chitinases *chi18-13* and *chi18-15* evolve in a manner consistent with rapid co-evolutionary interactions and identifies putative target regions involved in determining substrate-specificity and structural modifications of the family 18 chitinase TIM-barrel structure. Our results suggest that fungal/fungal interactions can drive adaptive changes in enzymatic properties as a response to specific ecological contexts of different *Trichoderma* species.

Trichoderma CAZomes: the carbohydrate degradation potential of mycoparasites

Verena Seidl-Seiboth[1], Pedro M. Coutinho[2], Bernard Henrissat[2] and Christian P. Kubicek[1].

[1]Gene Technology and Applied Biochemistry, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria. [2] Architecture et Fonction des Macromolécules Biologiques, CNRS, Universités Aix-Marseille I & II, Marseille, France. Email: vseidl@mail.tuwien.ac.at

The genome databases of the first sequenced mycoparasites *Trichoderma atroviride* and *T. virens* were used to assess their CAZome (Carbohydrate Active EnZmes genome content), which was subsequently compared with the corresponding gene subsets from 15 other fungi (11 ascomycetes and 3 basidiomycetes). In comparison to *T. reesei*, which is surprisingly poor in genes encoding glycoside hydrolases (GHs), the total number of 259 and 258 GH-encoding genes for *T. virens* and *T. atroviride* ranks them on the forefront of ascomycetes.

The potential of the mycoparasites to degrade the carbohydrate armour of fungal cells to invade the preys' hyphae is perfectly reflected in the increased abundance of chitinolytic enzymes and β-1,3-glucanases in comparison to other fungi. *T. virens* contains the highest number of chitinolytic enzymes of all so far described fungi. It was conspicuous that not only the number of chitinolytic enzymes is elevated, but that many of these additional proteins contain CBMs, suggesting an increased ability of the enzymes to adhere to insoluble substrates. Also the number of GH75 putative chitosanases is higher than in other fungi.

T. reesei is an efficient degrader of cellulosic plant matter, but its pool of cellulases and xylanases is relatively small in comparison to other ascomycetes. The number of identified cellulases and xylanases has not significantly expanded in *T. virens* and *T. atroviride*, suggesting that the observed low variety of cellulases and xylanases is a common feature of the genus *Trichoderma*. Furthermore, a reduced capacity to interact with the pectin backbone and sidechains in comparison to plant cell-degrading ascomycetes was observed in all three investigated *Trichoderma* species. A reduced capacity to degrade "soft" plant cell wall components, although still compatible with a saprophytic life-style, could be particularly relevant for a symbiotic life style with plants for *Trichoderma* species.

A study of the protease activity secretion from *Trichoderma viride*

Martin Šimkovič, Anita Kurucová, Zuzana Zemková, Ľudovít Varečka, [1]Department of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 – Bratislava, Slovak Republic
Email: ludovit.varecka@stuba.sk

The submerged cultivation of *Trichoderma viride* with purified protein substrate (bovine serum albumin, ovalbumin) led to the secretion of protease activity into the medium (measured with the chromolytic substrate). The secretion was induced under conditions avoiding cellular stress caused by the limitation of nutrients. Under these conditions, a biphasic secretory response was observed. In the first phase (after ~ 30 h) a high protease activity was secreted with maximum after ~3 d, which faded away after prolonged cultivation (up to 8 d). Zymography of secreted protease(s) (SDS-PAGE with 0.2 % gelatine) revealed high m.w. protease(s) (~200 kDa) with high autoproteolytic activity as the only secretory product. Its activity has been partially blocked by PMSF, pCMB, EDTA, or pepstatin depending on the inducer protein. Low-m.w. protease known from the work of other authors was seen only after prolonged cultivation as a band with m.w. about 27 kDa. Expression of known *Trichoderma sp.* genes encoding secreted proteases Prb1, ProA showed that only Prb1 was expressed after 3-4 days of cultivation, i.e., after fading out the early secretion phase suggesting that it was induced by the exhaustion of nutrients. The secretory activity of the earlier phase was impaired by tunicamycin and brefeldin A and was significantly stimulated by uncoupler. Attempts to purify the high-m.w. protease(s) were not successful. When complex substances, such as fungal cell walls, or orange rinds were used as protease inducers, the secreted protease activities were also resident in high-m.w. proteases similar to those observed in experiments with purified proteins. Thus, these proteases may participate in saprophytism and in mycoparasitism.
This work was supported by the VEGA Grant Agency (projects Nr. 1/0434/08 and 1/3251/06) and by the Science and Technology Assistance Agency under the contract No. 00642-07 and VVCE 0064-07.

SECONDARY METABOLISM AND ITS REGULATION IN *TRICHODERMA VIRENS*

Prasun K. Mukherjee[1] and [2]Charles M. Kenerley
[1]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India and [2]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, USA
Email: prasunmukherjee1@gmail.com

Trichoderma spp. are rich sources of secondary metabolites of agricultural and pharmaceutical significance. Recent genome sequencing revealed that the *T. virens* genome harbors more than 500 genes related to secondary metabolism, many of them are in clusters. However, to date only a few metabolites, notably gliotoxin, dimethoxy gliotoxin, gliovirin, heptelidic acid, viridin and viridiol and an 18-mer peptaibol have been characterized. There is thus a vast reservoir of unexplored secondary metabolites waiting to be discovered; the greatest challenge will be to obtain expression of these gene clusters, which might lead to discovery of novel metabolites. Using a high throughput gene knockout, we have identified some secondary-metabolism related genes responsible for root-colonization and induced resistance. We have studied the regulation of secondary metabolism in *T. virens* by using induced mutation as well as gene knockout approach. A radiation-induced mutant defective in morphogenesis and secondary metabolism has been isolated and used for identification of a gene cluster putatively involved in a terpene biosynthesis. Another mutant of *T. virens* has been isolated that exhibits a brown conidial pigment and produces three times more viridin and viridiol, by radiation-induced mutagenesis. Using gene knockout we have identified the *vel1* gene to be responsible for conidiation, chlamydospore formation, hydrophobicity as well as regulation of several genes involved in secondary metabolism and biocontrol in *T. virens*.

Overexpression of the trichodiene synthase gene *Tbtri5* increases trichodermin production and antimicrobial activity in *Trichoderma brevicompactum*

Anamariela Tijerino[1], R. Elena Cardoza[2], Javier Moraga[3], Mónica G. Malmierca[2], Francisca Vicente[4], Josefina Aleu[3], Isidro G. Collado[3], Santiago Gutiérrez[2], Enrique Monte[1] and Rosa Hermosa[1],
[1]Spanish-Portuguese Centre for Agricultural Research (CIALE), University of Salamanca, Salamanca (Spain), [2]University of León, Ponferrada (Spain), [3]University of Cádiz, Puerto Real (Spain), [4]Medina Foundation, Granada (Spain).
Email: emv@usal.es

Trichoderma spp. are used as biocontrol agents of plant diseases in many crops and their antimicrobial activities, other than cell-wall-degrading enzymes, against a wide variety of microorganisms, including bacteria, yeasts and filamentous fungi, have been screened in different species of the genus. Trichothecenes are sesquiterpene epoxides that are being deeply studied in *Fusarium* spp. because of their high agrifood relevance. Most genes involved in *Fusarium* trichothecene biosynthesis are clustered in a 26-kb region, being the *tri5* gene, coding the enzyme trichodiene synthase that catalyzes the conversion of farnesyl diphosphate (FPP) to trichodiene, the first step of the trichothecene pathway.

Although some authors have claimed production of trichothecene metabolites by different species of *Trichoderma*, in most of the studies nonspecific analytical methods were used and no details of taxonomic identification were given. Two trichothecene micotoxins have been detected in members of the Brevicompactum clade of *Trichoderma* with a common pathway from FPP to trichodiene, to trichodiol and to trichodermol, the divergence point for harzianum A in *T. arundinaceum*, *T. turrialbense* and *T. protrudens*, and for trichodermin in *T. brevicompactum*. We carried out the isolation and characterization of the *Tbtri5* gene in *T. brevicompactum* IBT40841, and its functional analysis by a gene overexpression strategy. Transformants with higher *Tbtri5* expression levels showed more antifungal activity than wild-type strain against the yeasts *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, and three human pathogenic *Candida* spp., and a nosocomial strain of the filamentous fungus *Aspergillus fumigatus*. Trichodermin and the phenolic antioxidant tyrosol [2-(4-hydroxyphenyl) ethanol] were identified by HPLC as the main differentially overproduced metabolites in the *Tbtri5* overexpressing transformants. Bioassays carried out with acetone extracts and the purified metabolites relate *Tbtri5* gene to the production of trichodermin and the antifungal activity of *T. brevicompactum* IBT40841.

The effect of 1-octen-3-ol, a volatile organic compound produced by *Trichoderma* species, on *Arabidopsis thaliana*

Richard Hung, Samantha Lee, Prakash Masurekar and Joan W. Bennett

Abstract

Sterile *Arabidopsis thaliana* plants exposed to *Trichoderma harzianum* and *Trichoderma longibratum* grown on potato dextrose agar (PDA) showed increased anthocyanin production, leaf senescence, mottling, color change, increased root mass, stunting, and delayed flower production. The observed effects suggest that the volatile organic compounds (VOCs) produced by *Trichoderma* have an adverse effect on seedling development. We then tested 1-octen-3-ol, one of the principle VOCs produced by *Trichoderma* and many other fungi. It is a biologically active compound commonly found in nature that attracts many arthropods. In our experiments, we exposed seeds as well as two-week-old plants to 1ppm, 2ppm, and 3ppm of racemic 1-octen-3-ol (Aldrich O5284-25G) for 3 days. Control seeds gave 95% germination to seedling while all three concentrations of 1-octen-3-ol inhibited germination in a dose dependent manner with 98% inhibition at 3ppm. Two-week-old seedlings were exposed for three days with destructive data collection at 24 hour intervals. Physical symptoms of exposure of the two week old plants were stunting, bleaching, mottling, leaf morphological changes, and senescence when compared to the controls. The test plants also showed lower concentrations of chlorophyll in a dose and time dependent manner.

Fusaric acid detoxification and chitinase expression in an antagonistic *Trichoderma harzianum* S17TH

Vivek Sharma, Pamita Bhandari, Bikram Singh, Amita Bhatacharya, V. Shanmugam

Institute of Himalayan Bioresource Technology (Council of Scientific and Industrial Research), Palampur 176 061, Himachal Pradesh, India

Email: shanpatho@yahoo.com

Detoxification of phytotoxin apart from lytic enzyme production is one of the strategies employed by *Trichoderma* spp. for their biocontrol efficacy. Antagonistic *Trichoderma* spp. were screened for their ability to detoxify the fusaric acid (FA), the phytotoxin produced by *Fusarium* spp. One isolate S17TH was able to tolerate high level of FA (upto 500 ppm) with similar level of antagonism over control but was unable to detoxify the toxin to significant level amended in minimal synthetic broth. However, the isolate was able to detoxify 400 ppm FA amended in the liquid medium after 7 days of incubation. In studies on the effect of FA (400 ppm) detoxification in relation to chitinase production by S17TH, the enzyme activity was significantly reduced in comparison to untreated control. SDS-PAGE of extra cellular proteins from the detoxified filtrate detected specific proteins of 20, 16 and 14 kDa, which were absent in the control. No chitinases were detected in zymogram analysis of the extracellular proteins. In semi-quantitative PCR assays for chitinase gene expression, while *ech42* expression was slightly repressed at 400 ppm, *nag1* was completely repressed at 300 ppm of FA. Selection of S17TH as a toxin insensitive or toxin degrading isolate makes it a potential antagonist against *Fusarium* incited plant diseases to commensurate the effect of negative signaling.

Trichoderma-Plant Interactions: insights on induced resistance to biotic and abiotic stresses

Ada Viterbo[1], Sergey Malitsky[2], Udi Landau[1], Asaph Aharoni[2] and Ilan Chet[1]

[1]Department of Plant Pathology and Microbiology, The Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100 Israel. [2]Department of Plant Sciences, Weizmann Institute of Science, PO Box 26, Rehovot 76100, Israel. Email: viterbo@agri.huji.ac.il

Trichoderma are versatile beneficial fungi which can stimulate growth and plant resistance to biotic and abiotic stresses. Understanding the molecular basis of their diverse modes of action is a central goal of our research and an important prerequisite to better implementation of these organisms in agriculture.

In our ongoing studies most recently we have tested the ability of *T. asperellum* T203 to allow protection against salinity which negatively affects germination and growth of cucumber seedlings. *Trichoderma* seed treatment can improve plant tolerance to such stressful conditions. The pool of reduced ascorbic acid is significantly increased in *Trichoderma* treated plants as is the expression of catalase *(cat)* and Mn/Cu-dependent superoxide dismutase *(SOD*) genes. ACC-deaminase impaired mutants cannot provide tolerance to salt stress, suggesting that *Trichoderma*, similarly to ACC deaminase producing bacteria, can ameliorate plant growth under abiotic stressful conditions by lowering deleterious elevated ethylene levels accompanied by an elevated antioxidative capacity.

A model system for *Trichoderma* induced resistance to biotic stresses is provided by study of the induction of a systemic response against angular leaf spot of cucumber (*Pseudomonas syringae* pv. *lachrymans*) following application of *T. asperellum* to the root system. As revealed by electron microscopy, bacterial cell proliferation in these plants is halted by the accumulation of secondary metabolites. The bulk of the antimicrobial activity is found in the acid-hydrolyzed extract containing phenolic compounds in their aglycone form. Metabolic alteration is a common response to both compatible and incompatible plant–microorganism interactions. A major future challenge will be to understand how induced resistance emerges from all of the chemical changes affected by elicitation. Non-targeted metabolic profiling performed using a UPLC-qTOF-MS instrument, revealed more then 40 different compounds strongly up-regulated by *Trichoderma* pre-inoculation and pathogen challenging. We are currently in the process of identifying the differential metabolites in order to characterize the metabolic pathways implicated in the systemic and defense response.

An example of translational research on Trichoderma: when 'omics data improve field applications.

M. Lorito[1, 2], M. Ruocco[2], F. Vinale[1], S. Lanzuise[1], R. Marra[1], M. Faraji[1], A.M. El-Tabey[1], M. Nigro [1]and S.L. Woo[1]
[1] Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università di Napoli Federico II and [2] Istituto per la Protezione delle Piante, Consiglio Nazionale delle Ricerche, via Università 133, 80055 Portici (ITALY).
E.mail: lorito@unina.it

Trichoderma spp. are among the most important microbes beneficial for agriculture. Many strains are produced and commercialized as a few hundred bio-products in about 50 countries of 5 continents. Different models of technology transfer and implementation have been followed, depending on the type of application (biopesticides and/or biofertilizers) and the related legal constrains (registration), but also on the local economical and political conditions. *Trichoderma*-based products have been taken to the market by following either the chemical pesticide, a “cottage industry” or government monopoly models. However, applications are directly related to the level of understanding of the multiple mechanisms of action, activities, effects and interactions of these biocontrol agents. Not only they directly kill or inhibit pathogens, but also activate extensive metabolic changes in treated plants and indirectly alter plant-pathogen interactions. They establish a symbiotic-like relationship with the roots, resulting in growth promotion and increased resistance to both biotic and abiotic stress. For instance, we analyzed the “interactome” of the multiple players (*Trichoderma*, plant and pathogen) involved in the complex, three-way molecular cross-talk occurring in the rhizosphere, and identified several key MAMPs (Microbes-Associated Molecular Patterns) and “effectors” that modulate the interaction. The results obtained by genomics studies were used directly for developing a more effective in-the-field application technology and a new model of implementation, as already tested in several countries worldwide.

The role of G-Protein signaling in the biocontrol agent *Trichoderma atroviride*

Markus Omann[1], Carolina Escobar[1], Susanne Zeilinger[1]

[1]Vienna University of Technology, Institute for Chemical Engineering, Research Area of Gene Technology and Applied Biochemistry, Getreidemarkt 9/166, 1060 Vienna, Austria
omann@mail.tuwien.ac.at

The mycoparasite *T. atroviride* acts as a potent biocontrol agent against a number of plant pathogenic fungi, whereupon the mycoparasitic attack includes host recognition followed by direct interaction of the mycoparasite with phytopathogenic fungi. Investigations on the underlying signal transduction pathways revealed an essential role of heterotrimeric G proteins and cAMP signaling during the antagonist-host interaction.
Tga1, a Gα subunit of subgroup I of the heterotrimeric G protein was found to be involved in regulating conidiation. It further affects multiple processes including production of antifungal metabolites and chitinase formation, which are at least partially involved in *Trichoderma* biocontrol. Tga3, a Gα subunit belonging to subgroup III, is also involved in mycoparasitism as *Δtga3* mutants are avirulent and are defect in chitinase secretion and infection structure formation. Furthermore Tga3 directly influences the regulation of mycoparasitism-related chitinase gene transcription. Summarising, the two G-protein α subunits of *T. atroviride* have overlapping roles in conidiation, mycoparasitism and both influence the internal cAMP level.
Investigations on G protein-coupled receptors of *T. atroviride,* resulted in the isolation of the *gpr1* gene encoding a G protein-coupled receptor of the cAMP receptor-like class. Silencing of *gpr1* led to avirulent mutants unable to recognize and attach to host hyphae and undergo mycoparasitism-related infection structure formation. The mutants were deficient in producing antifungal metabolites and in responding to the presence of living host fungi with the production of chitinases and proteases. Exogenous cAMP restored infection structure formation in *gpr1*-silenced mutants but not mycoparasitic overgrowth. A search for downstream-targets of the signaling pathway(s) involving Gpr1 resulted e.g. in the isolation of a member of the cyclin-like superfamily and Gpr1 was shown to influence hydrophobin gene expression. Although *gpr1* silencing caused defects similar to those of mutants missing the Tga3 G-alpha protein, no direct interaction between Gpr1 and Tga3 was observed in yeast two-hybrid assays; instead we found new putative Gpr1 interactors.

The *Trichoderma*-plant crosstalk model

Enrique Monte, Belén Rubio, Carlos Nicolás and Rosa Hermosa
Spanish-Portuguese Centre for Agricultural Research (CIALE), University of Salamanca, Salamanca (Spain).
Email: emv@usal.es

Despite of their biocontrol capacity, *Trichoderma* spp. have been found to stimulate plant defense mechanisms against pathogens and enviromental stresses, and promote plant growth and seed germination, increasing root development, leaf greeness, thermal, drought or salinity tolerance, and facilitating the captation of nutrients. Several approaches have served to identify fungal effectors and plant responses involved in such *Trichoderma* abilities following a Jones and Dangl's zigzag model. Plant roots initiate crosstalk with *Trichoderma* by producing signals that are recognized by the fungus, which in turn produces signals that initiate colonization leading to changes in plant metabolism and local and systemic responses also effective against a broad spectrum of pathogens. Changes in hormone concentration or sensitivity triggered by *Trichoderma* mediate a whole range of adaptive plant responses. Extensive changes in the plant transcriptome and proteome caused by colonization with active *Trichoderma* strains have been studied in different plants and the involvement of many plant growth regulators in plant immunity suggests that the control of plant growth, development and defense is interconnected in a complex network of cross-communicating hormone signaling pathways. The intensity, duration, and outcome of the plant responses are significantly influenced by the inoculum concentration of *Trichoderma*, the duration of its interaction with the plant and the environmental conditions. Changes observed in *Trichoderma* transcriptome, proteome and metabolome have served to identify plant effectors (i.e. small secreted cysteine-rich proteins, hydrophobins, secondary metabolites, etc.) and genes/proteins involved in metabolic pathways, mainly enzymes for carbohydrate, lipid and amino acid metabolism, but also enzymes for vitamin and cofactor biosynthesis, and energy- and detoxification- related processes.

THE INFLUENCE OF SOIL BIOTIC FACTORS ON THE ECOLOGY OF TWO *TRICHODERMA* BIOLOGICAL CONTROL AGENTS

Alison Stewart[1], Yonas Workneh[1], Kirstin McLean[1] & Johanna Steyaert[1]
[1]Bio-Protection Research Centre, PO Box 84, Lincoln University, Lincoln 7647, New Zealand.
Email: Alison.Stewart@lincoln.ac.nz

Trichoderma atroviride and *T. hamatum* have been commercialised to control onion white rot and Sclerotinia lettuce rot, respectively in New Zealand. The influence of forty-eight soil microorganisms representing 12 fungal (*Acremonium*, *Alternaria*, *Aspergillus*, *Beauveria*, *Chaetomium*, *Cladosporum*, *Daldinia*, *Fusarium*, *Metarhizium*, *Paecilomyces*, *Penicillium* and *Verticillium*), 7 bacterial (*Agrobacterium*, *Azotobacter*, *Bacillus*, *Bukholderia*, *Flavobacterium* and *Paenibacillus*) and four Actinomycete genera (*Actinomyces*, *Arthrobacter*, *Rodococcus* and *Streptomyces*) on the ecology of the biocontrol agents was determined in preliminary lab and pot trial assays. In dual culture, six fungi (*Alternaria alternata*, *Paecilomyces lilacinus*, *Aspergillus niger*, *Chaetomium globosum*, *Metarhizium anisopliae* and *Daldinia eschscholzii*) and one bacterium (*Agrobacterium* sp.) significantly reduced ($P<0.05$) the colony growth of both *Trichoderma* biocontrol agents. The greatest inhibition (>85%) for *T. atroviride* and *T. hamatum* occurred with *Aspergillus niger* and *Alternaria alternata*, respectively. In general, *T. hamatum* was less sensitive to the test microorganisms than *T. atroviride*. In soil pot assays, the test microorganisms and the *Trichoderma* biocontrol agents were added to the soil at a concentration of 1 $x10^6$ cfu/g soil. *T. atroviride* populations were significantly ($P<0.05$) lower in soil treated with *Alternaria alternata* (4.0 x 10^4 cfu/g soil), *Aspergillus niger* (6.0 x 10^4 cfu/g soil), *M. anisopliae* (5.0 x 10^4 cfu/g soil), *P. lilacinus* (5.5 x 10^4 cfu/g soil) and *D. eschscholzii* (5.7 x 10^4 cfu/g soil) compared with the control treatment (1.1 x 10^5 cfu/g soil). *T. hamatum* was less sensitive to the test microorganisms than *T. atroviride* but the trend was similar. Preliminary studies indicated that the production of antifungal metabolites by the test microorganisms could be responsible for the inhibition of *Trichoderma* growth. The soil pot trial results most likely overestimated the potential negative effect on *Trichoderma* populations, since the concentrations of the test microorganisms used in the pot trial were higher than would be expected in the field. Further studies on the impact of soil microorganisms on the ecology of *Trichoderma* biocontrol agents are warranted.

Analyzing the Transcriptome and metabolome of Arabidopsis inoculated with *Trichoderma* and the pathogen *Pseudomonas syringae*

[1]Yariv Brotman, [1]Jan Lisec, [1]Lothar Willmitzer, [2]Ilan Chet, [2]Ada Viterbo
[1]Max-Planck-Institut fur Molekulare Pflanzenphysiologie, Potsdam, Germany. [2]Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot 76100 Israel.
Email: brotman@mpimp-golm.mpg.de

In the recent years there is an increasing number of publications on the molecular mechanism underline the interaction between *Trichoderma* – plant and plant pathogens. Few studies explore the global Transcriptome and proteome changes in plants challenge with *Trichoderma* and plant pathogens (Reviewed by Shoresh et al., 2010). In Arabidopsis, *Trichoderma* induces systemic resistance (ISR) against the foliar bacterial pathogen *Pseudomonas syringae* pv. Tomato (*Pst).* At present, the interaction between Arabidopsis and *Pst* is the best model for plant-pathogen interaction. This model may be exploited for a system approached study on the 3 way interaction between: plants, *Trichoderma* and plant pathogens. In this study Arabidopsis plants were growing in hydroponic system, roots were inoculated for 48 hours with *Trichoderma* subsequently infecting the leaves with *Pst*. The transcriptom of the leaves and roots samples was monitored using Agilent microarray chips. Results show that locally in the roots, *Trichoderma* elicited a substantial change in the expression of ~300 genes. However, systemically in the leaves, none of the genes tested showed a consistent changes in expression in response to colonization of the roots by *Trichoderma*, indicating that the onset of ISR in the leaves is not associated with detectable changes in genes expression. Lacks of detectable changes in gene expression is in agreement with the results of previous study in different plants species inoculated with *Trichoderma*. Same results were obtained with plant growth-promoting rhizobacteria (Verhagen, at al. 2004). *Trichoderma* can induce priming for enhanced defense in plants. Comparison of the genes expression pattern between leaves inoculated with *Pst* to plants primed by *Trichoderma* subsequently infected with *Pst* reveal quantitative rather than quality differences.

For the same samples, taken for transcriptome study, we monitor primary and secondary metabolites, using gas chromatography (GC), and liquid chromatography (LC) mass spectrometry (MS). With GC-MS we detected 157 primary metabolites (including amino acids, sugars and intermediates of the TCA cycle). Those compounds are essential for central plant metabolism and changes, they may reflect plant growth-promoting mediated by *Trichoderma* spp. Secondary metabolites are essential players in defense responses. Using a target approach we detect the change of 27 compounds involved in defense response of Arabidopsis against pseudomonas. Using non-target approach we search for a wide range of secondary metabolites that give a new insight into the spatial and temporal response of Arabidopsis to *ISR*.

Shoresh M, Harman G.E. and F. Mastouri, Induced systemic resistance and plant responses to fungal biocontrol agents, Annu. Rev. Phytopathol. 48 (2010) Reviews in Advance.
Verhagen BW, Glazebrook J, Zhu T, Chang HS, van Loon LC. and CM Pieterse, The transcriptome of rhizobacteria-induced systemic resistance in Arabidopsis, Mol Plant Microbe Interact. 2004 Aug;17(8):895-908.

This work was supported by Sino-Italy Joint Research Project (2006DFA32900)

Differential expression of maize chitinases in the presence or absence of *Trichoderma harzianum* (T22) and indications of a novel exo-endo-heterodimeric chitinase activity

Michal Shoresh

Institute of Soil Water and Environmental Sci. Volcani Center, POB 6, Bet-Dagan, 50-250, Israel
Tel: 03-9683698 e-mail: michalsho@agri.gov.il

Chitinolytic enzymes are important pathogenesis and stress related proteins. We identified 27 putative genes encoding endochitinases in the maize genome via *in silico* techniques and four exochitinases. The endochitinases included members of family 19 chitinases (classes I-IV of PR3, II of PR4) and members of family 18 chitinases (class III of PR8). Most of the genes expressed were identified from EST libraries from plants exposed to biotic or abiotic stresses but also from libraries from tissues not exposed to stresses. We isolated proteins from seedlings of maize in the presence or absence of the symbiotic root colonizing fungus *Trichoderma harzianum* strain T22, and analyzed the activity of chitinolytic enzymes using an in-gel activity assay. The activity bands were identified by LC/MS/MS using the database from our *in silico* study. The identities of the enzymes changed depending on whether or not T22 was present. The activity of specific chitinolytic enzymes was higher in plants grown from *Trichoderma* treated seeds than in control plants. One activity band of about 95 kDa was found to be a heretofore undiscovered heterodimer between an exo- and an endo-enzyme, and the endo portion differed between plants colonized with *T. harzianum* and those grown in its absence and between shoots and roots. The heterodimeric enzymes from shoots in the presence and absence of *T. harzianum* were purified and characterized. The dimeric enzyme from *Trichoderma*-inoculated plants had higher specific activity and greater ability to inhibit fungal growth than those from control plants.

Trichoderma as a biocontrol agent against plant-parasitic nematodes: Veni, vidi...vici?

Yitzhak Spiegel and Edna Sharon
ARO Volcani Center, Bet-Dagan Israel
e-mail: spiegely@volcani.agro.gov.il

Root-knot nematodes, *Meloidogyne* spp., are sedentary, obligatory root endoparasites cause devastating harm to crops worldwide. Means like chemical control are less 'popular' these days because of environmental reasons, where resistant plants are not always available or they are overcome by resistance-breaking nematode species. Biological control has been suggested as an alternative mean to control these species. *Trichoderma* species and isolates have been screened and evaluated as biocontrol agents against the root-knot nematodes with various crops and experimental conditions. Several isolates revealed significant nematicidal activity, exhibited by enhanced plant growth, reduction of galling indices and the number of eggs per root. Modes of action of the interaction between the fungus, nematode and plant have been investigated, and the involvement of enzymatic activities, direct parasitism, antibiosis and induced resistance were recorded.

Construction and function analysis of *Trichoderma* transformant with *Metarhizium* genes against insects

Jie Chen , Yingying Li
Email: jiechen59@sjtu.edu.cn
Department of Resource and Environmental Science, School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 200240, China
Key Laboratory of Urban Agriculture (South), Ministry of Agriculture, People's Republic of China

Trichoderma has been extensively studied in the application to control various crop soil borne diseases. However, *Trichoderma* is not always effective in practical application particularly when insect pests and diseases occur simultaneously at the same space. Aiming to the problem we conducted the transfer of *chit42* and *pr1* cloned from *Metahizium anisopliae*, an entomogenous fungus, into *Trichoderma koningi* and examined both genes expression in *Trichoderma* transformants. *In vivo* assessment of transformants was performed to confirm affection of transformant on the growth of model insect (silkworm) and corn borer larvae. Totally we obtained more than 20 transformants ,in which eight transformants with *chit42* exhibited much higher activities of chintinase or protease than wild type *Trichoderma* strain , respectively. The average chitinase activity of transformants is approximately 3 times over wild type strain, of which TMC42-4 and TMC42-11 exhibited highest chitinase activity particularly at 6-day of transfromant culture in medium. *In vivo* assay showed that silkworm appeared more sensitive to transfromants than to wild type strains, for example, based on silkworm weight change after 12 days feeding transformant-dipped mulberry leaf, we demonstrated that transformant improve Trichoderma inhibtion against silkworm by 23.1% over wild type .Moreover, corn borer larvae were used to assess lethal effect of transformants to corn bore. Among all transformants, TMC42-4 and TMC42-11 exhibited most significant lethal effect to the larvae after two day -feeding.Besides, the transformants with *chit42* from *Metahizium anisopliae* still maintained a significant antagonistic activity to Fusarium verticillioides and F.graminearum ,two major maize stem rot pathogens.For further improving transformant inhibition efficiency to pest insects, we synthesized a range of fused genes with *chit42* and five chitin domains from different organisms, those fused genes have been transferred into *Trichoderma* wild type strain. The assessment of transformants performance against insect was under way.

Keywords: Transgenic; Trichoderma; chitinase ; protease

Reference

Song J Z, Yang Q, Liu B D and Chen D F. Expression of the chitinase gene from *Trichoderma aureoviride* in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol, 2005, 69: 39–43.

ENHANCING GROWTH AND HEALTH OF *PINUS RADIATA* IN NEW ZEALAND AND *ACACIA MANGIUM* IN MALAYSIA WITH SELECTED TRICHODERMA ISOLATES

Robert Hill[1], Alison Stewart[1], Pierre Hohmann[1], Mark Braithwaite[1], Annabel Clouston[1] & Rhys Minchin[2]
[1]Bio-Protection Research Centre, Lincoln University, Lincoln 7647, New Zealand
[2]Plant & Food Research, Private Bag 4704, Christchurch, New Zealand
Email: Robert.Hill@lincoln.ac.nz

The use of selected *Trichoderma* isolates with *Pinus radiata* in New Zealand forestry has improved health and vigour (by 10-20%) of seedlings and cuttings in containerised and soil bed nursery systems over the past 20 years. Nursery stock inoculated with a commercialised mixture of *Trichoderma atroviride* isolates (ABORGUARD™) reduced losses from *Armillaria* disease (by between 25 and 50%) in forestry plantations in New Zealand. Using the same concept in Malaysia, *Trichoderma* cultures were isolated from a variety of healthy plant roots sampled in the Planted Forest Zone in Sarawak. From these isolates, inoculum of pure cultures and selected mixtures was grown for trials using *Acacia* seedlings at the Samarakan Nursery. The best *Trichoderma* treatments were selected after ten replicated trials (Aug 2008 – Aug 2009). These increased seedling growth and health and the proportion of seedlings meeting specification for planting out into the forest by 66%, compared with the standard nursery practice. These results were validated on a large scale in the nursery, with exactly the same benefits observed as in the trials. A new mass production facility for *Trichoderma* inoculum was designed and built and is capable of producing sufficient *Trichoderma* to treat the total nursery throughout of around 30 million plants per year. *Trichoderma* inoculum was mixed into the nursery substrate for growing the seedlings prior to seed sowing. The nursery standard practice has now been changed from the use of multiple fungicide sprays to a single *Trichoderma* application before sowing the *Acacia mangium* seed.

NON-TARGET IMPACTS OF THE BIOCONTROL AGENT *TRICHODERMA ATROVIRIDE* ON TWO NATIVE ECOSYSTEMS IN NEW ZEALAND

Kirstin McLean[1], Sarah Dodd[2], Alison Stewart[1] & Robert Hill[1]
[1] Bio-Protection Research Centre, PO Box 84, Lincoln University, Lincoln 7647, New Zealand.
[2] Landcare Research, Private Bag 92170, Auckland Mail Centre, Auckland 1142, New Zealand.

Email: Kirstin.Mclean@lincoln.ac.nz

Trichoderma atroviride has been commercialised as Tenet® for control of onion white rot and as Sentinel® for control of Botrytis in grapes and glasshouse tomatoes in New Zealand. Non-target impacts of *T. atroviride* on native plant and soil microorganism communities were assessed in both native Podocarp forest and tussock grassland environments. A comprehensive plant health assessment determined that there was no significant difference in physical parameters between plants grown in *T. atroviride* treated and untreated soil from both ecosystems. Arbuscular mycorrhizal root colonisation and spore density in the soil was also unaffected by the presence of *T. atroviride* and there was no significant difference in mycorrhizal diversity (DGGE band) in the rhizosphere of plants grown in *T. atroviride* treated and untreated soil. *Pseudomonas* isolates were isolated from soil in both ecosystems and paired against *T. atroviride* in dual culture. Although *T. atroviride* overgrew all of the *Pseudomonas* isolates, all isolates remained viable beneath the *T. atroviride* colonies and could be recovered. The diversity of *Pseudomonas* species (DGGE band) was also unaffected by the addition of *T. atroviride* to the soil in all but two grasses. The presence of *T. atroviride* in the rhizosphere of *Festuca novae-zelandiae* and *Chionochloa rubra* resulted in a gain ($p=0.07$) and a loss ($p=0.04$) in species diversity, respectively when compared with the control treatment. Overall, the addition of *T. atroviride* to soil had a minimal impact on the plant health and beneficial soil microorganism communities in the native forest and grassland environments studied in New Zealand.

The conflict between the commercial and biological requirements, in the development of commercial formulation of Bio- fungicide

Ofer Kleifeld

Mycontrol Ltd. Alon Hagalil M.P. Hamovil 17920 Israel

Commercial development and production of Bio-fungicides dictates producing formulations which should fit to application by conventional methods.
But the efficacy of the biological products, which contain living microorganisms, is influenced by the formulation constituents and the production process.

The formulation affects not only the dispersal of the Bio-fungicide, the formulation used as - “Reservoir of nutrients" for the bio agent, and the formulation components, influence the “shelf life” and some other biological processes which are very significant to the activity of the microorganisms.

Combining the aspects mentioned above, some time are impossible, and very efficient microorganisms, loss their efficacy during the formulation process, aimed to produce a commercial product which will fit dispersion by commercial methods such as spraying.

In such cases scientists might produce an alternative formulation, which will fit to the microorganisms and will keep them effective.

Some good and efficient formulations of biological products include the microorganism with some organic additives, which function as a source of nutrient, and used as suitable environment t for the microorganism development. This kind of formulation requires application by alternative methods.

The aim of some producing companies to push biological products into the market is sometime leads to disappointment from the potential of the biological control, since the production and the use with biological products requires special attention to special and different factors.

Abstracts of Posters

[1]

Characterization of *Trichoderma* isolates of marine origin and assessment of their potential as biocontrol agents

Inbal Gal-Hemed[1], Lea Atanasova[2], Monika Komon-Zelazowska[2], Irina S. Druzhinina[2], Christian P. Kubicek[2], Ada Viterbo[1] and Oded Yarden[1] Email:bulix1@gmail.com
[1]Department of Plant Pathology and Microbiology, The Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100 Israel.
[2]Institute of Chemical Engineering, Research Area Gene Technology and Applied Biochemistry, Getreidemarkt 9/E1665, A-1060 Vienna, Austria

Since climate change may disrupt the effectiveness of biological control agents, search for new *Trichoderma* strains capable to overcome extreme environmental conditions is needed. Fungi from marine origin are an appealing source due to their potential in either ecological or pharmaceutical aspects. *Trichoderma* spp. were isolated from the Mediterranean sponge *Psammocinia* sp., collected near the shores of Israel. The identification of selected *Trichoderma* isolates was performed on the basis of the sequence analysis of genomic DNA loci [internal transcribed spacers 1 and 2 of rRNA, the large intron of translation elongation factor 1-alpha (*tef1*) gene, partial calmodulin (*cal1*) sequence and the RNA-polymerase subunit B II (*rpb2*)] using the on-line sequence similarity search tool *Tricho*BLAST and the identification program *Trich*OKEY (www.isth.info). *Trichoderma* isolates, representing different species and genotypes from three taxonomic sections of this genus (*T*. sect. *Trichoderma*, *T*. sect. *Pachybasium* and *T*. sect. *Longibrachiatum*) were chosen and screened for tolerance to increasing osmotic pressure and temperature while carbon assimilation was investigated using Phenotype Microarray technique with BioLog ^{TM} FF MicroPlates. The more promising isolates were screened for their antagonistic activity towards several plant pathogens and compared to the well-known biocontrol agent *T. asperellum T203*. Assays included antibiosis of secreted metabolites and volatiles, mycoparasitic activity in direct confrontation assays *in vitro*, chitinolytic activity and ability to colonize plant roots. Two marine isolates were as effective as the terrestrial biocontrol agent in greenhouse experiments against the phytopathogen *Rhizoctonia solani* and due to their capability to colonize plant roots can be good candidates for inducing defense responses in plants.

[2]

Distribution of rhizosphere competence within the genus *Trichoderma*

Natalia Guazzone[1], Robert Hill[1], Johanna Steyaert[1], Gary E. Harman[2], Alison Stewart[1]

[1]Bio-Protection Research Centre, PO Box 84, Lincoln University, New Zealand
[2]Department of Horticultural Sciences, Cornell University, Geneva, New York 14456, USA

Email: natalia.guazzone@lincolnuni.ac.nz

Rhizosphere competence, the ability of an organism to grow and function in the rhizosphere of developing roots, is a key component of successful biocontrol. Rhizosphere competence has been observed in *Trichoderma harzianum* UV-mutants and protoplast fusants and these strains exhibited enhanced biocontrol ability compared with the wild-type and/or parent strains. This study asks whether rhizosphere competence is common to all *Trichoderma* species, and, if not, whether it is a species- or isolate-specific trait. We have selected 22 *Trichoderma* isolates from the Lincoln University culture collection which represent seven different biocontrol species (*T. asperellum*, *T. atroviride*, *T. hamatum*, *T. harzianum*, *T. virens* and *T. viride*) from diverse locations within New Zealand. Using previously established methods [1] [2], we will assess the ability of these isolates to colonize the rhizosphere of developing roots of sweet corn (*Zea mays*) and develop endophytic relationships in non-sterile soil. Shoot and root lengths will be measured to assess growth promotion. Soil tests will be performed (total C, total N, biomass C and biomass N, dehydrogenase activity) to characterize the soil. *Trichoderma harzianum* strain T22 will be used as a positive control, as it has been proven to be rhizosphere competent and a very effective biological control agent. Unlike previous studies, all isolates have been identified on the basis of ITS1/2 and *tef1* sequence analysis. The outcomes of this study will contribute to a greater understanding of *Trichoderma* biocontrol systems, the improvement of commercial products and might lead to the identification of isolates with enhanced rhizosphere competence capabilities. Results of these studies will be presented and discussed in relation to the current knowledge on rhizosphere competence in *Trichoderma*.

[1] Ahmad, J. S., and Baker, R. 1987. Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77: 182-189.
[2] Sivan, A., and Harman, G. E. 1991. Improved rhizosphere competence in a protoplast fusion progeny of *Trichoderma harzianum*. Journal of General Microbiology 137: 23-29.

[3]

Overexpression in Arabidopsis plants of *Trichoderma* chitinases confers resistance to saline and heavy metal stresses through the LysM receptor-like kinase LysM RLK1

[1]Udi Landau, [2]Yariv Brotman, [3]Smadar Pnini, [3]Aviah Zilberstein, [2]Lothar Willmitzer, [1]Ilan Chet, [1]Ada Viterbo

[1] Department of Plant Pathology and Microbiology, The Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100 Israel, *[2]Max-Planck-Institut fur Molekulare Pflanzenphysiologie, Am Muhlenberg 1, 14476 Potsdam-Golm, Germany. [3]Department of Plant Science, Tel Aviv University, Tel-Aviv 69978 Israel.* Email:udiland@hotmail.com

Arabidopsis plants over-expressing the endochitinase *chit36* and the hexoaminidase *exy2* genes from the fungus *Trichoderma asperellum* T203 were found to be resistant to abiotic stresses like saline and heavy metals. Resistant lines, presenting different expression levels of chitinase activity, were crossed to *Arabidopsis* null mutants of the LysM Receptor-Like Kinase (LysM RLK1) which is known to play a critical role in chitin signaling in *Arabidopsis*. Independent homozygous lines resulting from reciprocal crossing were selected and subjected to abiotic stresses. Loss of resistance in spite of high chitinase activity, suggests that chitinase constitutive over-expression may release chitin-oligosaccharides from the plant cell which through binding to the LysM RLK1 receptor start a signaling cascade affecting resistance to abiotic stresses.

[4]

PHOTOREGULATORY FUNCTION OF PHR1, *TRICHODERMA ATROVIRIDE* PHOTOLYASE, AT PRESENT

Gloria Berrocal-Tito[1], Benjamin Horwitz [2], Edgardo Ulises Esquivel -Naranjo[1] & Alfredo Herrera-Estrella, [1]
[1] Laboratorio Nacional de Genómica para la Biodiversidad, CINVESTAV Irapuato, Irapuato, Gto., Mexico.
[2] Biology Department, TECHNION, Haifa 3000 Israel
Email: gberrocal@aliceadsl.fr

The photolyases, DNA repair enzymes that use visible and long-wavelength UV light to repair cyclobutane pyrimidine dimers (CPDs) created by short-wavelength UV, belong to the larger photolyase-cryptochrome gene family. Cryptochromes (UVA-blue light photoreceptors) lack repair activity, and sensory and regulatory roles have been defined for them in plants and animals. Evolutionary considerations indicate that cryptochromes diverged from CPD photolyases before the emergence of eukaryotes. In prokaryotes and lower eukaryotes, some photolyases might have photosensory functions. *phr1* codes for a class I CPD photolyase in *Trichoderma atroviride*. *phr1* is rapidly induced by blue and UVA light, and its photoinduction requires functional blue light regulator (BLR) proteins. Here we show that deletion of *phr1* abolished photoreactivation of UVC (200 to 280 nm)-inhibited spores and thus that PHR1 is the main component of the photorepair system. The 2-kb 5' upstream region of *phr1*, with putative light-regulated elements, confers blue light regulation on a reporter gene. To assess phr1 photosensory function, fluence response curves of this light-regulated promoter were tested in null mutant (∆phr1) strains. Photoinduction of the phr1 promoter in ∆phr1 strains was >5-fold more sensitive to light than that in the wild type, whereas in PHR1-overexpressing lines the sensitivity to light increased about 2-fold. Our data suggested that PHR1 may regulate its expression in a light-dependent manner, perhaps through negative modulation of the BLR proteins. This was the first evidence for a regulatory role of photolyase, a role usually attributed to cryptochromes.
To date, other fungal photolyases have been reported to have regulatory roles in addition to photolyase function. ENV1 (*N. crassa* VIVID homolog) another light regulator involved in photoadaptation and in carbon metabolism, was identified in *T. reseei*. The *velvet* complex in *A. nidulans* that synchronizes developmental and metabolic changes to the disappearance of light was described. Finally a putative DASH type cryptochrome, which modulates circadian entrainment in *N. crassa*, is present in *T. atroviride*. Thus, in this poster were going to discuss our model in the scenario of current data.

[5]

Comparative study of gene expression of three different *Trichoderma* species in the presence of tomato plants, using a high-density oligonucleotide microarray

Belén Rubio, Rosa Hermosa and Enrique Monte

Spanish-Portuguese Centre for Agricultural Research (CIALE), University of Salamanca, Salamanca (Spain).
Email: emv@usal.es

Members of the genus *Trichoderma* contain important examples of biocontrol agents, they antagonize plant-pathogenic fungi and are capable of interacting with plant roots directly, behaving as symbiotic microorganisms. Microarray analyses are becoming a powerful tool for large-scale gene expression studies. A high-density oligonucleotide (HDO) microarray encompassing 14,081 Expressed Sequence Tag (EST)-based transcripts from eight *Trichoderma* spp. and the on line available *T. reesei* and *T. virens* genomes was constructed and used to examine the gene expression of three strains, representing different *Trichoderma* species (*T. harzianum* CECT 2413, *T. virens* T87 *and T. hamatum* T7), either alone or in the presence of tomato plants.

Sterilized tomato seeds were germinated and grown in multi-cell growing trays containing MS medium at 25ºC for 2 weeks. Mycelium of *Trichoderma* strains was obtained from 2-days minimal medium, supplemented with 2% glucose, cultures, and used for tomato plant inoculations. All cultures were maintained at 25°C and 80 rpm for 20 h. After this time, *Trichoderma* mycelia were harvested by filtration (the mycelium on the plant roots was recovered with a direct water jet). Mycelia were washed twice with sterile distilled water, frozen in liquid nitrogen and lyophilized. RNA was extracted using Trizol Reagent® and purified with the RNeasy kit. cDNA synthesis, labeling, hybridization, microarray scanning and data acquisition were performed by Roche-NimbleGen. From a total of 34,138 probe sets, those with differential expression values (fold change >2 and P< 0.05) in comparison to alone *Trichoderma* MS cultures were selected. Differential regulated transcripts were annotated using Gene Ontology (GO) terms. A total of 200 genes (166-up and 34-down) were differentially expressed in 20 h *T. hamatum* T7-tomato plant interactions: 43.14% related with molecular function and 56.86% with biological processes. A total of 615 genes were differentially expressed in 20 h *T. harzianum* T34-tomato plant interactions: 33.5% related with molecular function, 34.5% with biological processes and 32.0% cellular components. And, a total of 370 genes were differentially expressed in 20 h *T. virens* T87-tomato plant interactions: 31.4% related with molecular function, 42.3% with biological processes and 26.3% cellular components. Just 7 differentially expressed genes were common to the three *Trichoderma* species.

[6]

Isolation, Characterization and Evaluation of *Trichoderma* species as bio-control agents against soil borne plant pathogens and plant parasitic nematodes in an Ornamental foliage nursery, Sri Lanka.

[1]Jegathambigai, Velmurugu., [2] Wilson Wijeratnam, R.S.,
[3] Wijesundera, R.L.C, [1]Arne E. Svinningen

1. Green Farms Ltd., Marawila, Sri Lanka.
2. Deputy Director, Industrial Technology Institute, 363, Bauddhaloka Mawatha, Colombo 07, Sri Lanka.
3. Professor, Department of Plant Science, University of Colombo, Sri Lanka.
Email: presenting_author@address jega@greenfarms.lk

Abstract

The fungi *Trichoderma* species were isolated from organic rich soil samples obtained from Green farms Limited (Ornamental Foliage Nursery), Marawila, Sri Lanka. The fungal species were identified on the basis of their morphological, reproductive and molecular characteristics. Five isolates were identified as *Trichoderma viride* strain NRRL 6418, *Trichoderma asperellum* strain D11 18S, *Trichoderma* sp. Hy6, *Trichoderma* sp. ZAUT013, *Hypocrea lixii* isolate TWC1 / *Trichoderma harzianum.* The efficacy of locally isolated *Trichoderma* species in control of three soil borne plant pathogen and one plant parasitic nematode were studied *in-vitro* and *in vivo* conditions. Tested isolates had antagonistic effects against soil borne plant pathogens such as *Sclerotium rolfsii*, *Fusarium oxysporum, Helminthosporium* and plant parasitic nematode *Meloidogyne incognita. Sclerotium rolfsii*, *Fusarium oxysporum, Helminthosporium*, causative fungi of collar rot on *Zamioculcas zamiifolia,*vascularr wilt on *Crossandra infundibuliformis,* leaf spot on *Dypsis lutescens* respectively and *Meloidogyne incognita* causative agent of root knot on *Livistona rotundifolia .*The three most effective isolates were *Trichoderma viride* strain NRRL 6418, *Trichoderma asperellum* strain D11 18S and *Hypocrea lixii* isolate TWC1 / *Trichoderma harzianum. Trichoderma* treatment significantly enhanced growth parameters in terms of shoot length/weight, root length/weight on ornamental foliage plants as compared to untreated plants.
On farm mass production of these isolates of *Trichoderma* spp was developed to help facilitate the establishment of an integrated eco-friendly disease management system for growers. The media evaluated in this study included the solid substrates barley seeds, paddy, cow pea (2 varieties) maize and sorghum and semi solid, liquid substrates such as potato dextrose, rice extract, paddy extracts respectively. Mycelial growth was fastest in barley and paddy media, the highest yield of spores of the *Trichoderma* isolate was also observed 7 days after inoculation in Barley and Paddy media.
Key words: - *Trichoderma*, soil borne disease, parasitic nematodes, ornamental foliage plants.

[7]

Construction of Trichoderma transformants with high copper bioaccumulation capability and analysis of related gene function

Kehe Fu, Lili Fan, Jie Chen
Email: fkh_0112@163.com

School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 200240, China
Key Laboratory of Urban Agriculture (South), Ministry of Agriculture, People's Republic of China

Over accumulation of copper in agricultural envirenment would lead to potential risk of food safety concerned,therefore our investigation on Trichoderma mechanism absorbing copper are imperative for Trichoderma to used for bioremediation of polluted soil.We established a modified ATMT (*Agrobacterium tumefaciens*-mediated transformation) method for construction of mutants with improved copper bioaccumulation ability in *Trichoderma reesei*. One mutant, AT01, exhibited highest copper accumulation capability. When the copper concentration in growth media was 0.7 mM, AT01 reached the maximum copper removal rate at 96.1 %, while the maximum removal rate of wild-type strain was merely 49.6 %. AT01 grown in copper-contained medium appeared verdigris in color as compared with wild type strain, which may be an indicator of highly absorbing copper. Electron microscope observation revealed that copper was mainly accumulated in cell vacuoles. Furthermore, using bioinformatics method to search copper transporter in *Trichoderma reesei* genome (v2.0) according to the conservation domain of CTR gene family, we identify two genes *TCtr2* and *TCtr3*. *Tctr2* contained a conserved transmembrane domain MLX_2M at the C-terminal ends, which present in other copper transporters family members. The expression of *Tctr2* is activated by copper and repressed while the copper chelator BCS was added to medium. To examine the *in vivo* function of *Tctr2* gene, we disrupted the target gene by the homologous recombination with *Agrobacterium tumefaciens*-mediated transformation. The result showed that mutant of *Tctr2* gene by homologous replacement was decreased in copper uptake capability, contrary to the function of *Ctr2* in *Saccharomyces cerevisiae*. *Tctr3* gene was found with close involvement in high affinity copper transport. Real-Time PCR approach demonstrated that the expression of *Tctr3 gene* was strongly regulated at the transcriptional level by copper availability, being activated by copper starvation and repressed under condition of copper excess. Deletion of *Tctr3* results in the cellular responses to extracellular copper and loss of high affinity copper transport ability. Gene complementation experiments by co-expression of the fusion protein *Tctr3*+EGFP verified the gene function of copper transport. In addition, we found that the gene *Tctr3* was expressed on the plasma membrane by fluorescence microscopy.

Key words: Trichoderma; bioaccumulation; copper ; ATMT

Reference1. Dancis A, Haile D, Yuan DS, Klausner RD. The Saccharomyces cerevisiae copper transport protein (Ctr1p). Biochemical characterization, regulation by copper, and physiologic role in copper uptake. J Biol Chem 1994;269:25660 –7.

2. Pena MM, Puig S, Thiele DJ. Characterization of the Saccharomyces cerevisiae high affinity copper transporter Ctr3. J Biol Chem 2000;275:33244–51.

[8]

SECONDARY METABOLISM AND ITS REGULATION IN *TRICHODERMA VIRENS*

Prasun K. Mukherjee[1] and [2]Charles M. Kenerley

[1]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India and [2]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, USA
Email: prasunmukherjee1@gmail.com

Trichoderma spp. are rich sources of secondary metabolites of agricultural and pharmaceutical significance. Recent genome sequencing revealed that the *T. virens* genome harbors more than 500 genes related to secondary metabolism, many of them are in clusters. However, to date only a few metabolites, notably gliotoxin, dimethoxy gliotoxin, gliovirin, heptelidic acid, viridin and viridiol and an 18-mer peptaibol have been characterized. There is thus a vast reservoir of unexplored secondary metabolites waiting to be discovered; the greatest challenge will be to obtain expression of these gene clusters, which might lead to discovery of novel metabolites. Using a high throughput gene knockout, we have identified some secondary-metabolism related genes responsible for root-colonization and induced resistance. We have studied the regulation of secondary metabolism in *T. virens* by using induced mutation as well as gene knockout approach. A radiation-induced mutant defective in morphogenesis and secondary metabolism has been isolated and used for identification of a gene cluster putatively involved in a terpene biosynthesis. Another mutant of *T. virens* has been isolated that exhibits a brown conidial pigment and produces three times more viridin and viridiol, by radiation-induced mutagenesis. Using gene knockout we have identified the *vel1* gene to be responsible for conidiation, chlamydospore formation, hydrophobicity as well as regulation of several genes involved in secondary metabolism and biocontrol in *T. virens*.

[9]

The cloning of *hex1* from *Trichoderma atroviride* and gene expression analysis under dichlorvos stress

Jun Tang ,Jie Chen
Email: tj751@sjtu.edu.cn
School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 200240, P. R. China
Key Laboratory of Urban Agriculture (South), Ministry of Agriculture, P. R. China

Trichoderma spp., as important free-living fungi, have been widely used around the world in the biocontrol of soilborne crop diseases. In recent years, *Trichoderma* spp. has been extensively studied for bioremediation for environmental pollutants. The proteomic technique has been considered as an effective method to understand the complex bioremediation process. This also will increase our understanding on resistance or degradation mechanisms of *Trichoderma* against organophosphate pesticides in agricultural environment. Based on the proteomic approach, some proteins including HEX1 were differentially expressed at presence of organophosphate pesticide dichlorvos. A 741-bp open reading frame encoding a 162-amino acid hex1 mature protein in *Trichoderma atroviride* has been successfully cloned. The recombinant plasmid pET28a-hex1 gene was expressed in *E. coli* BL21 (DE3). The crude recombinant protein was extracted from *E. coli,* and then purified by Ni^{2+}-affinity chromatography.

Southern blotting analysis showed the hex1 gene is present as single copy in *Trichoderma atroviride*. Furthermore, the targeted gene disruption vector conferring resistance to hygromycin B was designed and transformed into the protoplasts prepared from the wild *T. atroviride* T23. And the successful disruption of *hex1* gene in the wild type was identified by PCR and Southern blotting. The Δ*hex1* mutant showed poor growth on PDA plates and decreased tolerance to organophosphate dichlorvos. To determine the subcellular localization of HEX1 protein, we construct the fusion vector with the *egfp* gene (enhanced green fluorescent protein). *Hex1*-egfp fusions accumulate at septal plugs.

Key words:Trichoderma ; *hex1 ;* organophosphate pesticides; bioremediation

References

1.Zhou, X.Y., Xu, S.F., Liu, L.X., Chen, J., 2007. Degradation of cyanide by Trichoderma mutants constructed by restriction enzyme mediated integration (REMI). Bioresour.Technol. 98, 2958-2962.

2.Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M. 2004. Trichoderma species-opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2, 43–56.

[10]

Trichoderma virens genes encoding small secreted proteins

Mala Mukherjee[1], Idit Kosti[1], Fabian Glaser[1,4], Natthiya Buensanteai[2], Netta Dahan-Lamdan[1], Naomi Trushina[1], Prasun K. Mukherjee[3], Charles M. Kenerley[2] and Benjamin A. Horwitz[1]

[1]*Department of Biology, Technion, Haifa 32000, Israel;* [2]*Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA,* [3]*Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400 085, India,* [4]*Bioinformatics Knowledge Unit, Technion, Haifa 32000, Israel.*
Email: malamukherjee1@gmail.com

Small secreted proteins (SSPs) released by fungi can elicit defense or other responses during interaction with other organisms. The roles of some SSPs are known from plant pathogens and mycorrhizae. In *Trichoderma*, the SSP Sm1 is a major elicitor of induced systemic resistance (ISR) [1]. We therefore searched the *T. virens* genome [2] systematically for genes predicted to encode proteins of 300 amino acids or smaller, with at least 4 cysteine residues, and a signal peptide predicted at high confidence by SignalP. This initial screen identified over 200 SSP genes in *T. virens* and *T. atroviride*, while *T. reesei* has fewer. The SSPs that we identified include, for example, additional genes with similarity to *Sm1*, MISSPs (similarity to mycorrhiza-induced SSPs of *Laccaria bicolor*), a new family of SSPs of unknown function, and previously described hydrophobin genes. To obtain an initial classification, we analyzed the set of sequences including all the SSPs from the three species for similarity using BLASTclust (http://toolkit.tuebingen.mpg.de/blastclust). Several clusters were identified by this method, and these clusters were used to generate phylogenies across the three sequenced *Trichoderma* species ([2], http://genome.jgi-psf.org/). Each predicted SSP protein sequence was also used to search the protein models of all three species using BLASTP. The result of this second search showed that a large fraction of the SSPs in each species have no obvious homologs in the same or in the other two species, at the cutoff level chosen [2]. This suggests a high level of diversity among the three species. SSPs may be evolving rapidly, as might be expected if they are involved in communication between *Trichoderma* and its plant root or fungal partners in the soil and rhizosphere. In order to determine whether any of these novel SSPs, in addition to the well-studied Sm1, have elicitor activity, we are studying the root-inducibility of these genes and also expressing representatives of several groups of SSPs in *Pichia pastoris*. Recombinant *Sm1* produced in *Pichia* is indeed active in an assay for ISR [3].

[1] Djonovic *et al.* (2007). Plant Physiol. 145:875-89.
[2] Kubicek *et al.* (2010) Understanding mycoparasitism and biocontrol by integrated genomics and transcriptomics analysis of three *Trichoderma* species. Submitted.
[3] Buensanteai *et al.* (2010) Protein Expr Purif. 72:131-8.
Supported in part by BARD, TB-8031-08

[11]

Expression of small secreted proteins in response to co-culture of *Trichoderma virens* with maize roots

Naomi Trushina[1], Prasun K. Mukherjee[2,3], Charles M. Kenerley[3] and Benjamin A. Horwitz[1]

[1]Department of Biology, Technion, Haifa, Israel, [2]BARC Mumbai India, [3]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA

Small secreted proteins (SSPs) have roles in the interaction of *Trichoderma* with plant roots, and with fungal hosts in mycoparasitic interactions occurring in the rhizosphere and in the soil. To test the hypothesis that expression of SSP genes depends on signals generated by co-culture with maize roots, we measured the relative transcript levels of a large set of SSPs in RNA isolated from *T. virens* grown in interaction with maize roots in hydroponic culture. For comparison, transcript levels were measured for RNA isolated from mycelia grown axenically. Some of SSP genes were examined and found to be upregulated (the SSPs with JGI protein ID numbers 47273, 82045, 110852, 111830, 72996, 63571, 60325, 82827, 56831, 66108, 66126, 64656, 63669, 48810, 53060, 65112); and down-regulated (the SSPs with JGI protein ID numbers 61099 and 29260) in a series of semi-quantitative RT PCR experiments. Most of these proteins have no known function, except for 110852 which is the elicitor gene *Sm1* [1]. Automated annotation at JGI [2] found significant homology of 60325 to casein alpha/beta, and of 82827 to C-type lectin. We are currently exploring the expression of SSP genes on a larger scale using oligonucleotide microarrays. The results will help guide our choice of SSP genes for study for their possible roles in induced systemic resistance.

[1] Djonovic *et al.* (2007). Plant Physiol. 145:875-89.
[2] http://genome.jgi-psf.org/TriviGv29_8_2/TriviGv29_8_2.home.html

We are grateful to Nadin Ben-Zeev for technical assistance in BAH lab. This work was supported in part by BARD, TB-8031-08.

[12]

Detection by proteomics of small secreted proteins in a *Trichoderma virens* – maize interaction

Netta Dahan-Lamdan and Benjamin A. Horwitz, Department of Biology, Technion,

Haifa, Israel

Small, cysteine-rich secreted proteins (SSPs) are known to play an important role in fungal-plant interactions [1]. Search of the *Trichoderma virens* genome [2] revealed over 200 SSP-encoding genes, including, for example, the well-studied inducer *Sm1* [3], additional genes with similarity to *Sm1*, hydrophobins, and other known and novel classes. To study the SSPs expression pattern at the protein level, we performed secretome analysis of *T.virens* growing by itself or in interaction with maize roots. We collected medium from maize plants growing in hydroponic culture with or in the absence of *T. virens*. Two liters of medium were loaded by flow overnight onto a Q-Sepharose column and eluted with 1 M NaCl, 20 mM Tris pH 8 and than further concentrated through ultrafiltration spin columns. We used the peptide dimethylation with isotopically coded formaldehydes labeling technique for labeling our trypsin digest peptides. The peptides were analyzed by LC-MSMS. Data analysis was done by the Sequest 3.31 software *vs* the Joint Genome Institute database (http://genome.jgi-psf.org/TriviGv29_8_2/TriviGv29_8_2.home.html). In the two independent repeats of the experiment there was a similar number of proteins detected (50 and 54) at high confidence, with 25 proteins in common for the two repeats. We were surprised to identify a relatively high fraction of SSPs among the secreted proteins (~16% of the total), including the well characterized Sm1 [3]. A total of 13 SSPs was detected. BLASTP searches indicate that all of these have additional homologues in *T. virens*, and/or in *T. atroviride* and *T. reesei* with the exception of one unique protein. Perhaps surprisingly, we have not detected proteins whose abundance is strongly increased by co-culture with maize roots. On the contrary, several proteins showed decreased abundance, suggesting that they might be sequestered by the roots.The secreted proteins are candidates for elicitors of systemic induced resistance; their roles can be studied by construction of loss-of-function and overexpressing lines of *T. virens*.

[1] Rep (2005) FEMS Microbiol Lett. 253:19-27; Martin *et al.* (2008) Nature 452: 88-92.
[2] Kubicek *et al.* (2010) Understanding mycoparasitism and biocontrol by integrated genomics and transcriptomics analysis of three *Trichoderma* species. Submitted.
[3] Djonovic *et al.* (2006) Mol Plant Microbe Interact.19: 838-53; Djonovic et al. (2007) Plant Physiol. 145:875-89.

We are grateful to Dr. Tamar Ziv, Smoler Protein Center, Technion, for the mass spectrometry analyses. Supported in part by BARD, TB-8031-08.

[13]

EXPLORING THE INVOLVEMENT OF TWO *Trichoderma harzianum* GENES IN PLANT INTERACTIONS

[1]Samolski, I., [2]Suárez, M.B., [1]Cardoza, R. E., [1]Gutiérrez, S., [3]Rincón, A., [4]Viterbo, A., [4]Chet, I. & [1]Monte, E.

[1]Centro Hispano-Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca, Spain. [2]Instituto de Microbiología-Bioquímica (IMB), CSIC/Universidad de Salamanca, Spain. [3]Departamento de Genética, Universidad de Sevilla, Spain. [4]Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot, Israel.
Email: ilanit@usal.es

It has been known for some years that *Trichoderma* species are able not only to antagonize soil-borne organisms that cause plant diseases, but also to interact directly with plant roots, behaving as opportunistic symbiotic microorganisms. Elucidating the molecular mechanisms occurring during this complex interaction process between *Trichoderma* spp. and plants is crucial for the understanding and subsequent exploiting of their bio-fertilizing and bio-protectant properties in agriculture.
In a previous work, we developed a high-density oligonucleotide microarray to examine the transcriptional response of *T. harzianum* CECT 2413 in contact with tomato plants. A number of genes were identified to be over-expressed in this biocontrol agent within the first hours of contact with the host plant. Among them, we selected two genes particularly interesting since they had been previously described in this strain but never before related to the *Trichoderma*-plant direct interaction.
One of these genes, *p6281*, encodes an extracellular aspartic protease previously associated with the mycoparasitic activity of *T. harzianum*. Unlike other mycoparasitism-related protease genes from this strain, *p6281* is specifically induced during contact with plant roots and inert surfaces such as nylon fibers. In order to characterize this gene and to study its implication during the *Trichoderma*-plant association, we have constructed *p6281* over-expressing and silenced mutants. Quantifications by real-time PCR showed no differences in their ability to colonize the roots. Assays to determine the capacity to activate defense-related genes in plants are in progress. The other gene, *qid74*, encodes a cell wall hydrophobin-like protein. This gene has been proposed to be implicated in cellular protection and adherence of *T. harzianum.* Contrary to our expectations, studies with *qid74* over-expressing and disrupted mutants revealed that all of them kept the same ability to colonize the plant roots as the wild type, suggesting that this protein is not essential in root attachment or in protection of the growing hyphae against plant defense compounds during the interaction. Interestingly, we found that the plant roots showed considerably less root hairs in contact with the *qid74* disrupted mutant. Since root hairs aid nutrient and water uptake increasing the area of soil exploitable by the plant, this finding could reveal a new mechanism of bio-fertilization by *T. harzianum* as a consequence of the improvement of the nutritional status of the plant.

[14]

Genetic stability and resistance to fungal diseases of transgenic rice plants with CWDE's genes from *Trichoderma atroviride*

Mei Liu[1,2], Zong-Xiu Sun[2], Jie Zhu[1], Tong Xu[1]

[1] College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310029, China; [2] China National Rice Research Institute, Hangzhou 310006, China.

Email: Tong Xu: xutong@zju.edu.cn

In our previous work, rice plants were transformed with a single or multiple of transgenes encoding CWDEs *ech42* (endochitinase), *nag70* (exochitinase) and *gluc78* (exo-1, 3-β-glucanase) from the biocontrol fungus *Trichoderma atroviride* via the binary vector pCAMBIA1305.2. The aim of the present work was to investigate the genetic stability of transgenic rice plants with CWDE's genes.

T_1 transgenic rice plants were detected with PCR and analyzed with hygromycin B resistance. Several lines showing resistance to hygromycin B were also analyzed by Southern blotting. About 70 percent of the T_1 lines with *ech* showed the segregation ratio of 3:1 (positive : negative), indicating that these lines had single integration locus, and about 20 percent of the T_1 lines displayed the segregation ratio of 15:1, indicating that these lines had two integration loci. But 80 percent of the T_1 lines with *nag* and 57 percent of the T_1 lines with *ech*＋*nag* showed the segregation ratio of 3:1 (positive : negative), no segregation ratio of 15:1 was observed. The results further confirmed that the transgenes were stably inherited to T_1 and generation.

In greenhouse experiment, the lesion height of sheath blight measured 30 d after heading of T_1 lines with *ech, nag and nag* was 16.3 cm, 12.7 cm and 14.8 cm, respectively, showing the significantly reduced symptoms in comparing with that of control (33.5 cm).

Comparing with the controls, most of the transgenic rice offspring lines showed enhanced resistance to *R. solani* Kühn in greenhouse or nursery at different level. Among them the transgenic line T64 (T_4) with endochitinase gene was tested for its resistance to 22 races of *Magnaporthe grisea* from Zhejiang Province in China, showing Immune to 21 races and moderate resistance to one (97-23-2D1).

This work was supported by Sino-Italy Joint Research Project (2006DFA32900)

[15]

Bioprospecting: Screening Large Collections for Cellulase Activity Using Metabolic Profiles

John Bissett[1*], Parivash Shoukouhi[1], Philippe Dufresne[2], Loreta Gudynaite-Savitch[2]
[1] ECORC, Agriculture and Agri-Food Canada, Ottawa, Canada; [2] Iogen Corp, Ottawa, Canada. [*]Email: John.Bissett@agr.gc.ca

Metabolic profiles based on growth and respiration on 95 carbon substrates, assessed on Biolog FF MicroPlates™, were developed for 236 *Trichoderma* isolates. Cellulase activity (resorufin cellobioside fluorometric assay) was assessed, and multiple regressions performed to calculate a predictive model for cellulase activity based on metabolic data. This model was applied to an additional 1628 *Trichoderma* isolates, and separately to 3750 isolates of 'food- and airborne' fungi (1189 *Penicillium* isolates, 769 *Aspergillus*, 428 *Fusarium*, 1364 other genera) for which metabolic data were available. Finally, cellulase activity was determined using the resorufin assay for 307 isolates with highest predicted activity (151 *Trichoderma* isolates, 156 other genera). *Trichoderma* isolates with highest cellulase activities were all from the Viride-Koningii and Lixii clades - 6 had higher cellulase activity (389-1033 pmol/min) than four industrial isolates of *Trichoderma reesei* used for commercial production of ethanol from cellulosic feedstocks. Average cellulase production increased more than 5-fold in the predicted group (172 pmol/min) compared to the survey group (31 pmol/min). For *Trichoderma*, cellulase activity in the resorufin assay correlated reasonably with qualitative assays on acid swollen cellulose (ASC). The overall highest cellulase activities were found in the second predicted group ('FABF'), represented especially by *Fusarium* isolates. 28 of these strains had higher cellulase activities than industrial strains of *T. reesei*, exceeding 2000 pmol/min in *Alternaria*. However, these results were not clearly correlated with ASC assays, or in preliminary MUC (4-methylumbelliferyl-β-D-cellobiose) or EGDA assays (esculin gel diffusion assay) indicating that predictions from the *Trichoderma* survey may be less transferable extrapolated beyond the Hypocreales. This study demonstrates the applicability of metabolic profiles from Biolog microplates to select isolates with enhanced cellulase activity, and equally adaptable to survey collections for other enzymes with industrial applications. We are studying strains selected from metabolic profiles for assimilation of glycosides, pentoses, amines, organic acids, polyols, etc. that may accumulate in the fermentation stream and degrade performance of the cellulosic ethanol bioreactor process.

[16]

MOLECULAR ASPECTS OF INTERACTIONS BETWEEN *TRICHODERMA* AND MYCOTOXIGENIC *FUSARIUM*

Matarese F.[1], Sarrocco S.[1], Gruber S.[2], Seidl-Seiboth V.[2] and Vannacci G.[1]

[1]Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi"- Sezione di Patologia Vegetale, Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italy. [2]Research Area Gene Technology and Applied Biochemistry, Institute of Chemical Engineering, Vienna University of Technology, Getreidemarkt 9/166-5, 1060 Vienna, Austria.
E-mail: sarrocco@agr.unipi.it

Fusarium Head Blight (FHB) is a re-emerging disease of wheat that causes extensive agricultural damage through direct losses in yield and quality due to the presence of *Fusarium* damaged kernels and their associated mycotoxins such as the trichothecene deoxynivalenol (DON). Biological control, including the treatment of crop residues with antagonists such as mycoparasitic *Trichoderma* species to reduce pathogen inoculum of FHB, holds considerable promise.
Ten *Trichoderma* strains, previously selected for their ability to grow in presence of DON, were investigated as potential antagonists against *Fusarium culmorum* and *F. graminearum* mycotoxigenic strains in plate confrontation assays. The results showed that three *Trichoderma* strains were successful antagonists and exhibited antibiosis and mycoparasitism. In order to evaluate the ability to prevent DON production by *F. graminearum*, competition tests on natural substrates of these 3 *Trichoderma* isolates with *F. graminearum* confirmed the positive results achieved on agar plates. In addition, we assessed the transcription of some chitinase-encoding genes, known for their involvement during mycoparasitic growth conditions, in various stages of plate confrontation assays with *F. culmorum* and *F. graminearum* by RT-PCR. The results showed that almost all investigated genes encoding chitinases from subgroups A, B and C responded to mycoparasitic conditions and were upregulated before contact or/and in contact with the host.
A qPCR approach is in progress to evaluate the effect of the selected *Trichoderma* isolates on pathogens' growth in straw and rice and further we are investigating the gene expression of ABC transporters in *Trichoderma* to evaluate their role in DON resistance.

[17]
COMPETITION IN ARTIFICIAL PLANT GROWTH MEDIA BY *TRICHODERMA* SPP.

Sarrocco S.[1], Lübeck M.[2], Vannacci G.[1]

[1] Department of Tree Science, Entomology and Plant Pathology "G. Scaramuzzi", Plant Pathology Section, Faculty of Agriculture - University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy. [2] Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering - Copenhagen Institute of Technology, Aalborg University, Lautrupvang 15, DK-2750 Ballerup, Denmark.
E.mail: sarrocco@agr.unipi.it

The key to achieve successful, reproducible biological control is the gradual appreciation that knowledge of the ecological interactions taking place in soil and root environments is required to predict the condition under which biological control can be achieved and, indeed, it may be part of the reason why more biocontrol agents are reaching the market place. A comparative evaluation of life strategies of both the pathogen and its antagonists is required to predict the fate of a biopesticide in agricultural systems. The objectives of this work have been: 1) to screen a collection of *Trichoderma* isolates in a natural pot mix in order to select potential fungal antagonists to be employed in the biocontrol of *Rhizoctonia solani* damping-off of radish, and 2) to verify the hypothesis that competition for a food base plays a role in reducing pathogen activity. Fifteen *Trichoderma* spp., selected among 150 isolates according to their growth rate, were evaluated as potential biocontrol agents against the soil-borne plant pathogen *Rhizoctonia solani* on radish *in vivo* in a natural peat based growth medium usually employed in commercial production. Two different temporal antagonist-pathogen soil inoculation procedures were employed. Four potential biocontrol agents, all identified as *T. harzianum* according to the ITS sequences, were selected according to their ability of reducing radish damping off, and were employed in a Competitive Saprophytic Ability (CSA) test, in order to investigate the role of competition for a food base as a possible mechanism of biocontrol. Three of these *T. harzianum* isolates were able to both significantly reduce haulm fragments possession by the pathogen and damping off of radish. Reduction of possession spans for a limited period of time, varying according to the antagonists, but even after *R. solani* was able to re-colonize haulm fragments, the positive effects on damping off were still evident, demonstrating a "residual" effect of antagonists. On the whole, competition for the food base explains a large part of the recorded effects.
Work supported by the Project "Messa a punto di substrati artificiali innovativi per il florovivaismo", MIPAAF.

[18]

PASTURE SEED ADDITIVE - A PROTOTYPE *TRICHODERMA*-BASED BIO-INOCULANT FOR ENHANCED SEEDLING EMERGENCE AND PASTURE GROWTH IN NEW ZEALAND

Wadia D.R. Kandula[1], Alison Stewart[1], Janet McDermid[2] and John S. Hunt[2]

[1]Bio-Protection Research Centre, PO Box 84, Lincoln University, Lincoln, New Zealand
[2]Agrimm Technologies Ltd, PO Box 35, Lincoln, New Zealand
Email: Diwakar.Kandula@lincoln.ac.nz

Soil-borne fungal diseases are a major constraint affecting seedling emergence, pasture establishment and subsequent productivity/plant persistence in New Zealand pastures. Extensive laboratory and glasshouse screening of more than one hundred isolates of *Trichoderma* spp. against several pathogens (*Rhizoctonia solani*, *Sclerotinia trifoliorum*, *Pythium ultimum*, *Fusarium culmorum*) identified a cohort of isolates which suppressed damping-off diseases and promoted plant growth of different pasture species. Ten *Trichoderma* isolates (*T. atroviride* LU132, LU140, LU584, LU633, LU634; *T. virens* LU540, LU547; *T. koningii* LU713; *T. hamatum* LU740; *T. viride* LU644) were tested further as granule and seed-coat formulations in small-scale field trials. Evaluation of a prototype pasture seed additive (PSA) product formulated as a prill containing four isolates (LU132, LU140, LU584, LU633) of *T. atroviride* in several large scale research and on-farm trials significantly improved seedling emergence by 20-44% and increased pasture dry matter (in repeated assessments) by 10-40% relative to untreated controls. Pasture and feed forage quality analysis indicated some increase in soluble sugars in PSA-treated pastures and no difference in metabolisable energy. *In vitro* bioassays revealed the compatibility of PSA with several plant protection chemicals and fertilisers. On-going experiments are aimed at integrating PSA with other farming practices and developing full technical specifications for a new commercial product.

[19]

EFFECT OF TREHALOSE ON THE BIOLOGICAL ACTIVITY OF *TRICHODERMA ATROVIRIDE*, LU132

Janaki Kandula[1], Mark Braithwaite[1], Amanda Hay[1] & Alison Stewart[1]
[1]Bio-Protection Research Centre, PO Box 84, Lincoln University, Lincoln, New Zealand
Email: Janaki.Kandula@lincoln.ac.nz

Trichoderma species are fungi that commonly occur in nearly all agricultural soils and selected strains can suppress plant diseases. However, their level of efficacy and their reliability as a biocontrol agent depends upon their ability to tolerate adverse environmental conditions. The incorporation of trehalose into spores can protect them from injury due to freezing, desiccation or excess heating. *Trichoderma atroviride* (LU132) spores with increased trehalose levels were compared to spores with normal levels for their tolerance to various environmental stress factors. Increased trehalose content improved germination by 15.28%. No measurable effect was observed on colony growth at lower temperatures (15, 25 and 30°C), but spores were better able to tolerate heat stress (35 and 40°C). Trehalose improved the tolerance of *Trichoderma* spores to UV-B radiation. Growth at various pH values (2-6) was not affected.

[20]

ECOLOGICAL STUDIES OF *TRICHODERMA*-PLANT INTERACTIONS USING FLUORESCENT REPORTER SYSTEMS

Johanna Steyaert[1], Claudia Lange[1], Richard Weld[2], Alison Stewart[1]

[1]Bio-Protection Research Centre, Lincoln University, PO Box 84, Lincoln 7647, New Zealand
[2]Lincoln Ventures Limited, PO Box 133, Lincoln University, Lincoln 7647, New Zealand
Email: Johanna.Steyaert@lincoln.ac.nz

Trichoderma spp. are ubiquitous soil-borne ascomycetous fungi with a high capacity for biocontrol of fungal phytopathogens. This capability has led to substantial exploitation and currently *Trichoderma* spp. represent one third of all commercial fungal biocontrol agents (BCAs) sold globally. Within our research group we have developed a commercial *T. atroviride* BCA (LU132) for control of *Botrytis cinerea* in grapevines and *Sclerotium cepivorum* in onions. To facilitate rhizosphere studies, a GFP-marked LU132 strain was created and used for root-interaction experiments, however autofluorescence of the onion root interfered with our ability to visualise the interaction. In this study we have transformed LU132 with two additional reporter genes: DsRed (encoding red fluorescent protein) and BFP (encoding blue fluorescent protein). The aim of this work is to develop and evaluate three different biocontrol reporter systems for *in situ* monitoring of fungal biocontrol inoculants. In particular we will evaluate the potential of each reporter system for use in interactions involving autofluorescence. Experiments are currently under way to investigate how well these systems work and if there are any changes in growth, morphology, biocontrol efficiency and colonisation of onion roots compared to the wild type. These biocontrol reporter strains will enable us to gain insight into fungal growth and activity and into important abiotic and biotic factors affecting biocontrol efficacy.

[21]

IDENTIFICATION OF STAGE-SPECIFIC MARKERS IN *TRICHODERMA ATROVIRIDE* AND THEIR USE IN ECOLOGICAL STUDIES

Artemio Mendoza-Mendoza[1], Maria Fernanda Nieto-Jacobo[1], Johanna Steyaert[1], Richard Weld[2] & Alison Stewart[1]

[1] Bio-Protection Research Centre, PO Box 84, Lincoln University, Lincoln 7647, New Zealand.
[2] Lincoln Ventures Ltd, PO Box 133, Lincoln, Christchurch 7640, New Zealand
Email: Artemio.Mendoza@lincoln.ac.nz

Several species of *Trichoderma* are fungal biocontrol agents that are active against a wide range of soil-borne, plant-pathogenic fungi in the glasshouse and field. However, the ecological fitness and hence efficacy of *Trichoderma* spp. as biocontrol agents in natural soils can be limited by the variable influence of biotic and abiotic factors. In the soil, fungi are present as both actively growing organisms and as dormant propagules. Thus, the use of stage-specific molecular markers to determine development and differentiation in *Trichoderma* provides a particularly useful tool for studying the influence of ecological factors on the life cycle of the fungus. Here we present a subtractive/suppressive hybridization analysis of genes regulated during critical morphological stages in *T. atroviride* LU132. The validation of the cDNA libraries was performed by Northern blot and by quantitative real-time reverse transcription-PCR (qRT-PCR). In addition, conidia of *Trichoderma* LU132 were inoculated in sterile soil, maintained under glasshouse conditions and collected at different time points. The influence of *Trichoderma* on maize plant development will be discussed. We consider that the use of stage specific markers will significantly increase our understanding of the ecological niche of *Trichoderma*.

[22]

Genetic stability and resistance to fungal diseases of transgenic rice plants with CWDE's genes from *Trichoderma atroviride*

Mei Liu[1,2], Zong-Xiu Sun[2], Jie Zhu[1], Tong Xu[1]

[1] College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310029, China; [2] China National Rice Research Institute, Hangzhou 310006, China.

Email: Tong Xu: xutong@zju.edu.cn

In our previous work, rice plants were transformed with a single or multiple of transgenes encoding CWDEs *ech42* (endochitinase), *nag70* (exochitinase) and *gluc78* (exo-1, 3-β-glucanase) from the biocontrol fungus *Trichoderma atroviride* via the binary vector pCAMBIA1305.2. The aim of the present work was to investigate the genetic stability of transgenic rice plants with CWDE's genes.

T_1 transgenic rice plants were detected with PCR and analyzed with hygromycin B resistance. Several lines showing resistance to hygromycin B were also analyzed by Southern blotting. About 70 percent of the T_1 lines with *ech* showed the segregation ratio of 3:1 (positive : negative), indicating that these lines had single integration locus, and about 20 percent of the T_1 lines displayed the segregation ratio of 15:1, indicating that these lines had two integration loci. But 80 percent of the T_1 lines with *nag* and 57 percent of the T_1 lines with *ech*＋*nag* showed the segregation ratio of 3:1 (positive : negative), no segregation ratio of 15:1 was observed. The results further confirmed that the transgenes were stably inherited to T_1 and generation.

In greenhouse experiment, the lesion height of sheath blight measured 30 d after heading of T_1 lines with *ech, nag and nag* was 16.3 cm, 12.7 cm and 14.8 cm, respectively, showing the significantly reduced symptoms in comparing with that of control (33.5 cm).

Comparing with the controls, most of the transgenic rice offspring lines showed enhanced resistance to *R. solani* Kühn in greenhouse or nursery at different level. Among them the transgenic line T64 (T_4) with endochitinase gene was tested for its resistance to 22 races of *Magnaporthe grisea* from Zhejiang Province in China, showing Immune to 21 races and moderate resistance to one (97-23-2D1).

[23]

Trichoderma a beneficial microorganism used in integrated production systems of Costa Rica

Miguel Obregón Gómez
Dr. Obregón Laboratories Costa Rica

m.obregon @doctor-obregon.com

The industrialized or conventional agriculture procures the yield increase per area unit, as a main goal. However, it has increased deterioration in agroecosystems, biodiversity, water resources, environment and human health.

During the last 30 years Costa Rica, as part of the mentioned process, has had a sustained pesticide importation; outstanding mancozeb and chlorothalonil, as the most common fungicides used in the crop production.

The pesticide problem in the monoculture systems that mainly supports the national economy, has encouraged people to look for control alternatives trying to restore agroecosystems and change the production to sustainable systems, as well. In order to contribute to the sustainable production of this country, the author started in 1998, an applied research program to promote and increase the use of biological control practices by the farmers.

The work started prospecting the soil biodiversity looking for potencial biocontrol agents. As part of the results, isolates of different species of *Trichoderma* like *T. harzianum, T. viride, , T. asperellum,* were obtained. All isolates were evaluated *in vitro* against different plant pathogens as: *Botrytis cinerea, Fusarium oxysporum, Sclerotium rolfsii, Sclerotium cepivorum, Rhizoctonia* sp., *Sphaerotheca panosa, Sclerotinia sclerotiorum* and *Rosellinia* sp. The results showed colonization percentages up to 90% in all cases, according to the findings, massive production protocols on solid media and quality control standardization were done before use in field evaluations.

Field trials showed interesting results in the control of pathogens like *S. sclerotiorum* that presented 95.5 % of effectiveness when *T. asperellum* was used, compared with the control. Based on these results, companies like DOLE, BANACOL, CORBANA and others involved in pineapple, strawberry and flower exportation, have included in their production systems the use of *Trichoderma* spp.

[24]

Biological efficiency of Nematophagous fungi to control the nematodes *Globodera pallida* (stone) on potatoes.

1. Ricardo Piedra Naranjo Instituto Nacional de Innovación y Transferencia de Tecnología Agropecuaria. INTA. Costa Rica
2. Miguel Obregón Gómez Fitopatólogo, Profesor de Doctorado en Ciencia Naturales Para el Desarrollo (DOCINAE). Costa Rica.

The study was carried out at San Juan Chicuá, province of Cartago, Costa Rica, the place is located 5 kilometres from the Irazu volcano at 2800 m above the sea level, 26 kilometres northeast from the city of Cartago, in a wet tropical forest, the soil has a volcanic origin (andisol order). The temperature, precipitation and annual relative humidity average are 15 º C, 2,100 mm and 85% respectively. The experiment had an unrestricted randomly design, with 6 treatments and 4 repetitions. Treatments were: *Beauveria* sp (JV), *Paecilomyces lilacinus* (IFC), *Pochonia* spp. (Mog 08 H), *Lecanicillium lecanii*, (BOS), *Trichoderma* spp (native isolate from Carlos Duran experimental station) and an absolute control without any treatment. 700 grams of sterilized soil were used in each treatment and an infection viability average of 180 larvae and eggs by cyst were used. For the cyst inoculation a muslin type fabric was used to determine the hatching of cysts and soil recuperation at the end of the study. The work objective was to assess the effectiveness of treatments. The statistical analysis showed differences among treatments; *Trichoderma* sp and *Beauveria* sp (JV), in contrast to the fungus *Lecanicillium lecanii* (BOS), *Pochonia* sp., *Paecilomyces* sp. and the absolute control in the variable cysts recovery on soil, but the treatment *Trichoderma* sp. was the best, in both the non cyst recovery from soil and the greater weight of potato tubers at harvest. Further studies can validate and provide follow-up to these results under field conditions. The results are important, as an element within an integrated management of the pest in agronomic practices of cultivation of potatoes in Costa Rica.

Key words: viability, hatching, cysts recovery, efficiency

[25]

Determination of Extracellular Siderophores of *Trichoderma atroviride* by High Resolution Liquid Chromatography - Mass Spectrometry

Rainer Schuhmacher[1], Sylvia Lehner[1], Susanne Zeilinger[2], Norbert Stoppacher[1], Rudolf Krska[1]

[1]Center for Analytical Chemistry, Department for Agrobiotechnology, IFA-Tulln, University of Natural Resources and Life Sciences Vienna, Konrad Lorenz Strasse 20, 3430 Tulln, Austria. [2]Institute of Chemical Engineering, Vienna University of Technology, Getreidemarkt 9/1665A, 1060 Vienna, Austria.
E-mail: rainer.schuhmacher@boku.ac.at

All Siderophores (from greek sideros: iron and phorein: to carry) are very strong low molecular mass chelators of ferric ions and are produced by almost all microorganisms. With few exceptions, fungal siderophores are derived from ornithine and can be classified into the structural classes of are hydroxamates, namely fusarinines, coprogens and ferrichromes [1].
Besides transporting and storage of iron, fungal siderophores have also been described to be involved in the interaction with plants and other fungi. As iron acquisition is an essential process fungal siderophores can act as virulence / pathogenicity factors by limiting the availability of iron to the plant (e.g. Eisendle et al., 2003) [2]. Moreover, fungal siderophores are involved in the suppression of the growth of plant pathogenic fungi by competition for iron in natural soils as has been demonstrated for the control of *Fusarium* wilt disease by *Trichoderma asperellum* [3].
Although more than 200 fungal siderophores have been discovered and characterized [1], only a few publications deal with the chemical structures of siderophores produced by biocontrol strains of *Trichoderma spp*..
Here we present results with respect of the siderophore production of *Trichoderma atroviride* ATCC 74058. The fungus was grown under iron deficient conditions for several days, using a liquid culture medium. After addition of iron to the culture filtrates aliquots of the filtrates were analyzed for the presence of siderophores by high resolution liquid chromatography - mass spectrometry. MS full scan spectra revelead the presence of several different siderophore structures and their putative molecular masses were assigned by accurate mass measurements. The presentation will also include further structural elucidation of the ferri-forms of the detected siderophores by the use of high resolution tandem mass spectrometry.

[1] Renshaw, J. et al., Mycol. Res. 2002, 106: 1123-1142.
[2] Eisendle, M. et al., 2003, Mol. Microbiol. 49: 359-75.
[3] Segarra, G. et al., Microb. Ecol. 2010, 59: 141-149.

[26]

The Peptaibiotics Database – a valuable tool for the research on Aib-peptides

Norbert Stoppacher[1], Martin Schwarzböck[1], Susanne Zeilinger[2], Rudolf Krska[1], Rainer Schuhmacher[1]

[1]Center for Analytical Chemistry, Department for Agrobiotechnology, IFA-Tulln, University of Natural Resources and Life Sciences Vienna, Konrad Lorenz Strasse 20, 3430 Tulln, Austria. [2]Institute of Chemical Engineering, Vienna University of Technology, Getreidemarkt 9/1665A, 1060 Vienna, Austria.
E-mail: rainer.schuhmacher@boku.ac.at

Peptaibiotics constitute a large group of secondary metabolites, produced by filamentous fungi of the genera *Trichoderma*, *Stilbella*, *Emericellopsis* and others. Peptaibiotics constitute non-ribosomal linear peptides, containing unusual amino acids such as alpha-aminoisobutyric acid (Aib) and exhibit antibiotic activity against e.g. fungi, bacteria or helminthes. Due to their manifold biological activities, peptaibiotics have increasingly become the target of extensive research in the agrobiological, pharmaceutical and medical sciences. Regularly, such peptides are detected in cultures of fungi, isolated from various terrestrial and marine sources [1].
In this report the authors present a comprehensive database for peptaibiotics. The exisiting “Peptaibol Database” [2], which has been published on the internet a few years ago, provides structural information on about 300 amino acid sequences of the peptaibiotic subclass of peptaibols, the members of which carry an acetyl moiety at the amino terminus and a carboxyterminal amino alcohol.
The present study will provide an update on the peptaibiotics sequences which have been published in the scientific literature and will also include the subclasses of lipopeptaibols and aminolipopeptides. So far, our MS-Excel based database contains more than 860 peptides of natural origin. Compound names, amino acid sequences, the producing microorganisms, literature references, etc. have been compiled and the database also provides search functions for the relevant data types, such as amino acid sequence motifs or entire sequences. The presented database will help to compare experimentally determined peptide sequences or motifs thereof with formerly found compounds and to identify them as known or novel peptaibiotics. Moreover, it is possible to extend the database by new peptaibiotics and other type of information on the peptides, according to the needs of researchers, thus rendering the database a valuable tool in the field of peptaibiotics research.

[1] Degenkolb, T., Brückner, H., 2008. Chem. & Biodivers. 5 (9):1817-1843.
[2] L. Whitmore, L. & Wallace, B.A., 2004. Nucleic Acids Res. 1, 32 (Database issue): D593-594. (www.cryst.bbk.ac.uk/peptaibol/home.shtml).

[27]
Identification of *Trichoderma* species - A polyphasic approach

Rajesh A. Marigowda, Prameela Devi Thokala and Malathi V. Ganesan, Deeba Kamil, Prabhakaran Narayanasamy and Pankaj Pandey
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi- 110012, India
Email: anprabha05@yahoo.com

A polyphasic approach was used for the authentic identification of *Trichoderma* isolates collected from different fields of Indian Agricultural Research Institute, New Delhi with morphology and molecular based techniques. Twelve isolates of *Trichoderma* were obtained from *Trichoderma* selective medium selected on the basis of genus characters. These twelve isolates were made into five different groups under two different sections on the basis of their colony characters and microscopic observations. The characters of these isolates were compared with the reported literature and confirmed them in to different species. PCR amplification and sequence analysis of r-DNA region (ITS 1-5.8s DNA –ITS 2) and a small fragment of translation elongation factor (*tef-1*) gene were used to characterize these twelve isolates of *Trichoderma*. The respective universal primers (for ITS and *tef-1* regions amplification) were successfully used to amplify genomic DNA extracted from mycelium of these isolates and sequence analysis showed clear difference among the isolates. Morphological grouping and speciation matched with molecular grouping of most of the isolates. The integration of morphology and molecular based techniques gave fair solution in easing some of the complex problems that are associated with either morphology or molecular based techniques alone.

[28]

A Combined morpho-genetic approach towards identification of *Trichoderma* species

Rajesh A. Marigowda, Prameela Devi Thokala and Malathi V. Ganesan, Deeba Kamil, Pankaj Pandey and Prabhakaran Narayanasamy
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi- 110012, India
Email: pankajpandey34@rediffmail.com

Twelve isolates of *Trichoderma* species obtained from rhizosphere soil of plantation crops and agricultural fields of Indian Agricultural Research Institute, New Delhi, India, were analyzed based on morphological and molecular characteristics and classified these isolates into phenotypic groups. Considering the morphological characters viz., colony, conidiophore, phialides, conidia and chlamydospore characters, the isolates were grouped into two different sections and five groups. This morphological identification was further revalidated by molecular approach. Therefore attempt was made to characterize twelve isolates of *Trichoderma* using ITS1-5.8s-ITS2 region. This region of rDNA was amplified using universal ITS-1 and ITS4 primers. Amplified products of size in the range of 500-600bp were produced by the primers and indicated the identification patterns of *Trichoderma* isolates. In the present study, it was confirmed that the ITS1-5.8s-ITS2 marker had much capacity to resolve taxa and a good correlation between morphological and molecular characteristics among isolates of *Trichoderma virens*. In the most of remaining isolates a good correlation between morphological and molecular characteristics was observed up to the sectional level classification.

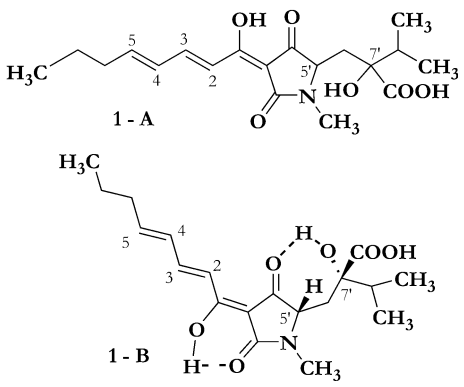
[29]

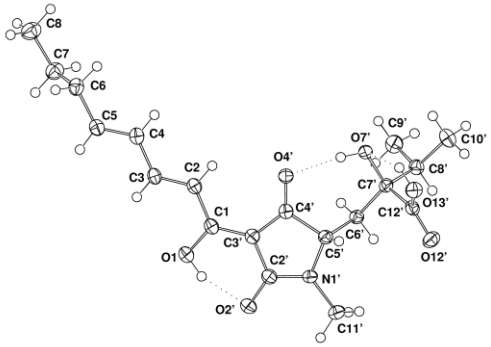
HARZIANIC ACID, A SIDEROPHORE FROM *TRICHODERMA HARZIANUM* WITH ANTIFUNGAL AND PLANT GROWTH PROMOTING ACTIVITY

F. Vinale [1], G. Flematti [2], K. Sivasithamparam [3], E.L. Ghisalberti [2], R. Marra [1], M. Ruocco[4], S.L. Woo[1], S. Lanzuise[1], M. Nigro[1], M. Lorito [1,4]

[1] Dipartimento di Arboricoltura Botanica e Patologia Vegetale, Università di Napoli 'Federico II', Portici, 80055 Naples, Italy; [2] School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia; [3] School of Plant Biology, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia, 6009; [4]Istituto di Protezione delle Piante, Consiglio Nazionale delle Ricerche, via Università 133, 80055 Portici (ITALY).
E mail: frvinale@unina.it; lorito@unina.it.

Fungal strains of the genus *Trichoderma* are well known producers of secondary metabolites with antibiotic activity. The role that secondary metabolites could play in the complex interactions occurring between plants, pathogens and antagonistic fungi has recently been investigated.
A *T. harzianum* strain, isolated from composted hardwood bark in Western Australia, produces a metabolite that showed antifungal activity *in vitro* against *Pythium irregulare*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*. The structure and absolute configuration of the fungal metabolite, harzianic acid (**HA**), was determined by X-ray diffraction studies.
The effect of harzianic acid on plant growth promotion was evaluated by treating seeds of canola (*Brassica napus*) with different concentrations of the **HA** and measuring the stem length of developing seedlings. Applications of the compound at concentrations of 100, 10 and 1 ng per seed, stimulated plant growth as indicated by an increase of 42%, 44% and 52% of stem length respectively, compared to the untreated control (water). However, this metabolite also inhibited plant growth up to 45% and 33% (stem length) when augmented concentrations of 100 and 10 μg, respectively were applied to the seed. Additionally, spectrophotometric analysis showed that this tetramic acid is capable of binding essential metals such as Fe^{3+} with a good affinity. The complex was further characterized by using LC-MS, confirming the formation of a **HA**- Fe^{3+} complex.





[30]

Comparison of solid state and liquid fermentation derived formulations of *Trichoderma harzianum* with special reference to shelf-life

S. Sriram, M.J.Savitha, R.Rajeshwari and B.Ramanujam
National Bureau of Agriculturally Important Insects, (Formerly Project Directorate of Biological Control), HA Farm Po, Bangalore 560024, India.
Email: sriram1702@gmail.com

The shelf-life of formulations of *Trichoderma* is an important criterion in developing and promoting bioagents for commercial production and uptake by endusers at farm level. In general the biomass of Trichoderma spp. derived from liquid fermentation are sensitive to desiccation and other stress conditions compared to solid-state fermentation derived biomass. Few interventions like addition of colloidal chitin to production media, chitin to formulation, glycerol to production medium, heat shock at the end of log phase of fermentation and use of specific packing materials found to extend the shelf-life of Trichoderma formulations derived from liquid fermentation. Addition of glycerol as humectants reduced the water activity in the production medium. The formulations derived from glycerol amended medium was having shelf life of 15 months. Similarly heat shock at the end of log phase for 30 min at 40^{o}C also induced desiccation tolerance due to trehalose production. Low density polythene packing materials were found to be supporting shelf life compared to polypropylene and high density plastic materials. Talc based formulations of *Trichoderma harzianum* with 15 months shelf-life with real time data on viability and bioefficacy was developed with few of the above mentioned interventions. Similarly the shelf-life of solid state fermentation derived formulations could be extended by applying different drying methods. Vacuum drying with or without silica gel also resulted in the extension of desiccation tolerant propagules of *T. harzianum*. The sensitiveness of liquid fermentation derived biomass of *T. harzianum* to low water activity and low moisture content in formulations and the steps to overcome by different interventions are discussed. Comparison of formulations from solid state and liquid fermentation are discussed.

Notes