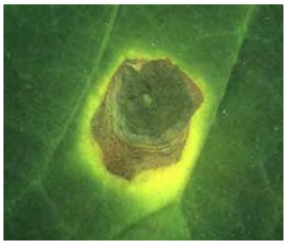
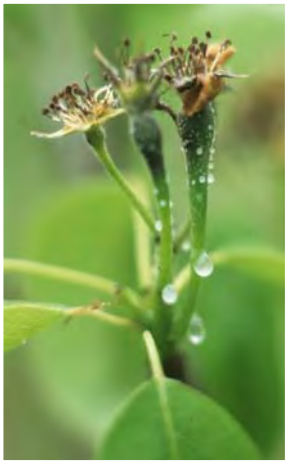
Berichte

aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft

Reports

from the Federal Biological Research Centre for Agriculture and Forestry





Heft 128

2005

1st International Symposium on Biological Control of Bacterial Diseases Darmstadt, Germany 23rd - 26th October 2005

Organizing and Scientific Committee
Programme
Abstracts of Papers and Posters
Participants and Authors

Under the Auspices of the
German Phytomedical Society and the
President of the Darmstadt University of Technology

Darmstadt University of Technology

Federal Biological Research Centre for Agriculture and Forestry
Institute for Biological Control

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1st International Symposium on Biological Control of Bacterial Diseases Darmstadt, Germany, 23rd - 26th October 2005

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Programme

Sunday, October 23

14:00 Beginning of Registration

18:00 Dinner

20:00 Reception and Opening by **Prof. Dr. Fred Klingauf**. Braunschweig (Former President of BBA): "General Status of Biological Control"

Monday, October 24

8:30 Opening address by **Prof. Dr. Georg Backhaus**, President of BBA

Welcome and opening remarks by **Prof. Dr. Jürg Huber** (Director of BBA Institute, Darmstadt) and by **Prof. Dr. W. Zeller** (Convenor)

SESSION 1: PRINCIPLES OF BIOLOGICAL CONTROL: ANTAGONISM, INDUCED RESISTANCE, PLANT GROWTH PROMOTION (ANTAGONISTIC MICROORGANISMS AND NATURAL COMPOUNDS)

Chair: Heinrich Buchenauer, University Hohenheim, Stuttgart, Germany

9:00 Introductory lectures:

Heinrich Buchenauer, University Hohenheim, Stuttgart, Germany:
"Mechanisms of biological control in host pathogen systems"

Zoltán Klement, P.G. Ott, Á. Szatmári, E. Besenyei, A. Czelleng, E. Szabó and Z. Bozsó. Academy of Science, Budapest, Hungary:
"Basic resistance of plants to bacterial infection as a possible means for biological control"

Contributed papers:

9:45 **Sophia Kürkcüoglu**, Markus Piotrowski, Juliana Degenhardt, Abdul Nasser Al-Masri, Achim E. Gau. Universität Hannover:
"Biological control of plant diseases by application of natural, epiphytically living non-pathogenic anatagonists to the plant phyllosphere"

10:00 Tea/Coffee break

10:30 **Kamal Abo-Elyousr**, Assiut University, Egypt:
"Studies on induced resistance against common blight *Xanthomonas campestris* pv. *phaseoli* by Bion®"

10:45 **Jinyun Li**, Fan Chen, Yanbing Guo, Jianhui Wang, Huimin Wang. China Agricultural University, Beijing, China:
"Biological control of grape crown gall in China with non-tumorigenic *Agrobacterium vitis* E26: possible mechanisms involved"

11:00 **Manuel Filotet Guerra**. Sugar Cane National Research Institute of Cuba:
"Sensibility of isolates of *Acidovorax avenae* subsp. *avenae* and *Xanthomonas campestris* pv. *vasculorum* against a bacteriocin"

11:15 **Nicola Sante Iacobellis**. Pietro Lo Cantore, Universitá degli Studi delle Basilicata, Potenza, Italy:
"Potential use of essential oils for plant bacterial disease control"

11:30 **Dilfuza Egamberdiyeva**. Tashkent State University of Agriculture, Tashkent, Uzbekistan:
"Production of Hydrogen Cyanide (HCN) and lytic enzymes by rhizobacteria isolated from different plants and soils"

11:45 **Peter Ott**, G. J. Varga, E. Szabó, A. Szatmári, Z. Bozsó, É. Klement, K.F. Medzihradszky, A. Czelleng, E. Besenyei, Z. Klement. Hungarian Academy of Sciences, Budapest, Hungary:
"Molecular markers of basal resistance, an inducible defense mechanism of plants against bacteria"

12:00 Lunch

SESSION 2: BIOLOGICAL CONTROL OF BACTERIAL DISEASES IN FIELD CROPS

Chair: Samuel Gnanamanikam, University Madras, Chennai, India

13:30 Introductory lectures:

Samuel Gnanamanikam, J. Ebenezar Immanuel. University Madras, Chennai, India:
"Bacterial inoculants for the suppression of rice bacterial blight in India: Role of 2,4 Diacetyl Phloroglucinol in disease suppression"

Brion Duffy. Agroscope FAW, Wädenswil, Switzerland:
"Biocontrol of soft rot erwinias on field crops with an emphasis on the mechanisms"

Contributed papers:

14:15 **Doan Thi Than**. Vietnam Agricultural Science Institute, Hanoi, Vietnam:
"Status of research on biological control of tomato and groundnut bacterial wilt in Vietnam"

14:30 **Kerstin Wydra**. Jörg Semrau, University of Hannover, Germany:
"Phenotypic and molecular characterization of the interaction of antagonistic bacteria with *Ralstonia solanacearum*, causing tomato bacterial wilt"

14:45 **Svetlana Lapa**, O. N. Reva. Inst. of Microbiology and Virology of the National Academy of Sciences of Ukraine, Kyiv, Ukraine:
"Antagonistic activity of the *Bacillus* strains against the phytopathogenic *Erwinia*"

15:00 Tea/Coffee break

15:30 **Amusa N. Adetunji**. Obafemi Awolowo University, Ibadan, Nigeria:
"Biological control of bacterial diseases of plants in Nigeria: Problems and prospects"

15:45 **Olinda Maria Martins**, C. A. Lopes, A. Quezado Duval. Embrapa Recursos Genéticos e Biotecnologia, Embrapa Hotalicas, Brasilia, Brazil:
"Fighting *Ralstonia solanacearum* in Brazil"

16:00 V. Shanmugaiah, S. Ramesh, M. Jayaprakashvel **N. Mathivanan**: University of Madras, Chennai, India:
"Biocontrol and plant growth promoting potential of *Pseudomonas sp.* MML2212 from the rice rhizosphere"

SESSION 3: BIOLOGICAL CONTROL OF BACTERIAL DISEASES IN HORTICULTURE

Chair: Maria López, I.V.I.A, Moncada, Spain

16:15 Introductory lectures:

María López, Ramón Penyalver. I.V.I.A, Moncada, Spain:
"Strategies for biological control of soilborne pathogenic bacteria and practical efficacy of different methods"

Thomas Burr Guixia Hao, Jodi Creasap. Cornell University, Geneva, NY, USA:
"Identification of genetic mechanisms that affect *Agrobacterium vitis*-plant interactions including biological control"

Contributed papers:

17:00 **Jodi E. Creasap**, G. Hao, H. Zhang, S. Zäuner, C.I. Ullrich, Th. J. Burr.
Cornell University, NY, USA:
"Mode of action and genes involved in biological control of grape crown gall by *Agrobacterium vitis* strain F2/5"

17:15 **Steven Roberts**, E. Koch, A. Schmitt, T. Amein, S. Wright. HDRA, Ryton Organic Gardens, Coventry, UK:
"Screening biocontrol agents for control of seedborne bacterial pathogens of carrots and brassicas"

17:30 E. Wulff, S. M. S. Massomo, C. M. Mguni, C. N. Mortensen, **John Hockenhull**: The Royal Veterinary and Agricultural University, Frederiksberg C., Denmark:
"Biological control of black rot of vegetable brassicas, caused by *Xanthomonas campestris*, with endophytic *Bacillus* strains"

17:45 **Giorgio Mariano Balestra**. A. Quattrucci, Universitá degli Studi della Tuscia, Viterbo, Italy:
"Biological control of bacterial pathogens on tomato plants"

18:00 **Sharanaia Umesha**. University of Mysore, Mysore, India:
"Biocontrol of bacterial canker of tomato using *Pseudomonas fluorescens*"

18:15 **Fatmi M'Barek**. Institut Agronomique et Véterinaire Hassan II, Complexe Horticile d'Agadir, Agadir, Morocco:
"Biological control of bacterial canker of tomato (*Clavibacter michiganensis* Ssp. *michiganensis*)

18:30 **Subramanian Gomthinayagam**, S.P. Kamalanalini, J. Lalithakumari. University of Guyana, Tain, Corentyne:
"A novel bacterium for biocontrol of broad spectrum of pathogens"

18:45 **Hatice Özaktan**, Tayyar Bora, Emek Aslan, Ahmet Uslu. University of Ege, Bornova-Izmir, Turkey:
"A study on the biological control potentials of fluorescent pseudomonad strains against bacterial blotch disease (*Pseudomonas tolaasii*) of cultivated mushroom"

19:00 **Rosa L.R. Mariano**, E. B. Silveira, F. H. V. Medeiros, A. Oliveira, E. R. Santos, E. R. Gouveia, A. M. Souto-Maior. Federal Rural University of Pernambuco, Recife, Brazil:
"State of art of the biological control of bacterial fruit blotch of melon in Pernambuco"

19:30 Dinner

20:30 **Poster session**

Tuesday, October 25

8:30 Opening address by **Prof. Dr. Johann-Dietrich Wörner**, President of Darmstadt University of Technology

SESSION 4: IMPLEMENTATION OF BIOLOGICAL STRATEGIES IN INTEGRATE DISEASE MANAGEMENT SYSTEMS

Chair: Klaus Rudolph, University Göttingen, Germany

8:45 Introductory lectures:

Damodaran Lalithakumari. University of Guyana, Tain, Corentyne, Guyana:
"Biological control of plant diseases is a fascinating promise for eco-friendly environment"

Ajit Varma. Amity Institute of Herbal and Microbial Studies, Delhi, India:
"State of registered biological control agents (BCA's) in the field of phytobacteriology"

Contributed papers:

9:30 **Aleksa Obradovic**, J. B. Jones, M. T. Momol, S. M. Olson, B. Balogh, L. E. Jackson. University of Belgrade, Belgrade, Serbia and Montenegro;
University of Florida; OmniLytics, UT, USA:
"Integrated management of tomato bacterial spot in Florida"

9:45 **Jeffrey B. Jones**, F. Iriarte, A. Obradovic, B. Balogh, M.T. Momol, L.E. Jackson University of Florida, Gainesville, USA:
"Management of bacterial spot on tomatoes with bacteriophages"

10:00 Tea/Coffee break

10:30 **Timur Momol**, P. Ji, S. M. Olson, J. B. Jones, University of Florida, USA:
"Integrated management of bacterial wilt on field-grown tomatoes"

10:45 **Tugba A. Koltuksuz**, E. Aslan, H. Özaktan, T. Bora, F. V. Sukan. Ege University, Bornova-Izmir, Turkey:
"Effects of different drying techniques on the inhibitory effect of *Pantoea agglomerans* strain Eh-24 bioformulation"

11:00 **Willem Ravensberg**. Koppert Biological Systems, The Netherlands:
"A new natural anti-microbial product for use as an agricultural bactericide and fungicide"

11:15 **Donald W. Edgecomb**, D. Manker. AgraQuest Inc. CA, USA:
"*Bacillus subtilis* strain QST 713 for bacterial disease control in fruit, vegetable and ornamental production"

11:30 **Yuri Gninenko**, A. M. Zhukov. Russian Research Institute of Silviculture and mechanization of Forestry, Pushkino, Russia:
"Bacterial disease of birch in forests of Russia"

SESSION 5: MOLECULAR APPROACHES IN BIOCONTROL OF BACTERIAL DISEASES

Chair: Matthias Ullrich, International University Bremen, Germany

11:45 Introductory lectures:

Matthias Ullrich. International University Bremen, Germany:
"Molecular Biology of plant associated bacteria involved in biocontrol"

Mikhail Chumakov. Russian Acad. Sc., Saratov, Russia:
"Application of non-pathogenic *Agrobacterium radiobacter* for preventing crown-gall disease in dicots, growth-promotion in monocots and molecular aspects of agrobacterial attachment to plant cell surfaces"

Contributed papers:

12:30 **Marta Pujol**, E. Badosa, C. Manceau, E. Montesinos. University of Girona, Girona, Spain:
"Monitoring the biocontrol agent *Pseudomonas fluorescens* EPS62e by means of real-time PCR"

12:45 **Sascha Braun**, Beate Völksch. Friedrich-Schiller-University Jena, Germany:
„Characterisation of an inhibitory strain of *Pseudomonas syringae* pv. *syringae* with potential as a biocontrol agent for bacterial blight on soybean"

13:00 **Sandor Süle**, E. Szegedi, O. Kovács, E. Novák, T.J. Burr. Hungarian Academy of Sciences, Budapest, Hungary:
"Sensitivity to biological control of *Agrobacterium vitis* is related to IST pTI"

13:15 David Devleesschauwer, Kris Audenaert, **Monika Höfte**. Gent University, Gent, Belgium:
"Suppression of bacterial blight on rice by inducing resistance and manipulation of endogenous abscisic acid levels"

13:30 Lunch

14:30 **Excursion to the State Research Institute of Viticulture and Horticulture Geisenheim, Rhine Valley; Dinner with Wine tasting**

Wednesday, October 26

SESSION 6: BIOLOGICAL CONTROL OF FIRE BLIGHT

Chair: Wolfgang Zeller, BBA, Darmstadt, Germany

8:30 Introductory lectures:

Herb S. Aldwinckle. Cornell University, Geneva, NY, USA:
"Current status of biocontrol of fire blight"

Virginia Stockwell. Oregon State University, Corvallis, OR, USA:
"Integrated control of fire blight with *Pseudomonas fluorescens* strain A506, an iron chelate, and antibiotics"

Contributed papers:

9:15 **Larry Pusey**. Tree Fruit Research Laboratory, Wenatchee, WA, USA:
"Chemistry of apple and pear stigma exudates related to bacterial antagonism toward *Erwinia amylovora*"

9:30 **Jordi Cabrefiga**, A. Bonaterra, E. Montesinos. University of Girona, Girona, Spain:
"Factors affecting efficiency of biological control of fire blight in *Pseudomonas fluorescens* EPS62e"

9:45 **Peter Laux**, Wolfgang Zeller. Nufarm GmbH, Köln:
"Mode of action of the bacterial antagonist *Rhanella aquatilis* against fire blight"

10:00 Tea/Coffee break

10:30 **Klaus Geider**, V. Jakovljevic, M. Mohammadi, S. Jock. Max Planck Institute, Ladenburg, BBA Dossenheim, Germany:
"Characterization of epiphytic bacteria from Australia and Europe as possible fire blight antagonists"

10.45 **Stefan Kunz**. Universität Konstanz, Konstanz, Germany:
"Fire blight control in organic fruit growing - Systematic investigation of the mode of action of potential control agents"

11:00 **Anja Seibold**, N. Giesen, W. Jelkmann. University Heidelberg; BBA Dossenheim, Germany:
"Antagonistic activity of different yeast spp. against *Erwinia amylovora*"

11.15 S.M. Lehman, W.-S. Kim, E. Barszcz, K. Schneider, A.J. Castle, **Antonet M. Svircev**. Agriculture and Agri-Food Canada, ON; Brook University, Canada:
"Control of the fire blight pathogen with bacteriophages"

11.30 **Maria Hevesi**, N. Boja, R. Bánatfy, P. Babulka, M. Tóth. Corvinus University of Budapest; Aromax Rt., Budapest, Hungary:
"*In vitro* inhibition of growth of *Erwinia amylovora* by plant oils"

11:45 **Joel Vanneste**. Ruakura Research Centre, Hamilton, New Zealand:
"Biological control of fire blight: why it works and how it works – registration of a biological control for fire blight in New Zealand"

12.00 **Poster session**

12:45 Lunch

SESSION 7: SAFETY AND REGULATORY ASPECTS OF BIOLOGICAL CONTROL AGENTS (BCA)

Chair: Sandra Wright, Göteborg University, Sweden

14:00 Introductory lectures:

Gabriele Berg. Graz University of Technology, Austria:
"Biocontrol agents and their potential risk for human health"

Klaus P. Schaal. University Bonn, Germany:
"Risk assessment when working with biological agents"

Contributed papers:

15:00 **Peter Hoffmann**. DSMZ, Braunschweig, Germany:
„Biosafety regulations in Germany"

14:45 **Sandra Wright**, Steven V. Beer. Göteborg University, Göteborg, Sweden:
"Antibiotics produced by strains of *Pantoea agglomerans*, biocontrol agents of Fire Blight"

15:15 Tea/Coffee break

15:45 **Panel discussion**

Moderation by **Jürg Huber:**
"On the way to biocontrol of bacterial plant diseases: Where are we?"

Closing

17:00 End of the Symposium

Poster Presentations

SESSION 1: PRINCIPLES OF BIOLOGICAL CONTROL: ANTAGONISM, INDUCED RESISTANCE, PLANT GROWTH PROMOTION (ANTAGONISTIC MICROORGANISMS AND NATURAL COMPOUNDS

1. **Kyungseok Park**, Ming-Shu Zeng, Yong-Chull Jeun, Ki-Woong Nam, National Institute of Agricultural Science and Technology, Suwon; Cheju National University, Jeju, Korea:
"*Rhizobacteria* mediated induced systemic resistance against various plant pathogens and its mechanisms of action"
2. **Reginaldo S. Romeiro**, J.R. Vieira Júnior, H.G.M. Ferraz, V.R.S. Barra, I.S. Melo, Universidade Federal de Vicosa, Vicosa; Embrapa Meio Ambiente, Jaguariúna, Brazil:
"A biocontrol agent for bacterial blight that induces systemic resistance as it restrains pathogen multiplication in bean leaf tissue"
3. **Ömür Baysal**, Y.Z. Gürsoy, H. Örnek, A. Duru, S. Soylu, M. Soylu, W. Zeller, West Mediterranean Agricultural Research Institute, Antalya, Turkey:
"Induction of oxidative burst in tomato leaves treated with unsaturated fatty acids of Tutle oil (*Caretta caretta*) against *Pseudomonas syringae* pv. *tomato*"
4. **Ömür Baysal**, S.B. Gölükcü, A. Ünlü, W. Zeller, West Mediterranean Agricultural Research Institute, Antalya, Turkey:
"An early oxidative burst in apple rootstocks treated with DL-ß-Amino butyric acid (BABA) against *Erwinia amylovora*"
5. **Leopold Fucikovski**, Colegio de Postgraduados, Texcoco, Mexico:
"Antagonistic coccus type bacteria producing death of a coryneform type bacteria, the pathogen of *Agave tequilana Weber* var. *Azul"*
6. **Kamal Abo-Elyousr**, Wolfgang Zeller, Oktay Yegen: University of Assiut, Egypt:
"Studies on induced resistance against fire blight (*Erwinia amylovora*) with different bioagents"
7. **Ömür Baysal**, Peter Laux, Wolfgang Zeller, Institute for Biological Control of BBA, Darmstadt, Germany:
"Studies on Systemic Acquired Resistance (SAR)-Effect of BTH (Bion®) against fire blight (*Erwinia amylovora*)"
8. **Karl Stich**, Technische Universität Wien, Wien Austria:
"Biosynthesis of dihydrochalcones and their influence on disease resistance in apple"
9. **Ewa Lojkowska**, A. Krolicka, E. Biskup, University of Gdansk and Medical University of Gdansk, Poland:
"Antibacterial activity of plant extracts against bacterial plant pathogens"
10. **Marusia Stefanova**, Sandra G. Rizo, C. Romeu, M. Francisca Coronado, M. Loreta Larrinaga, Instituto de Investigación de sanidad vegetal, Habana, Cuba:
"Bacterial activity in vitro of essential oils of *Hyptis suaveolens* (L.) poit and *Coleus amboinicus* (Lour)"
11. **Blanka Kokosková**, Roman Pavela, Research Institute of Crop Production, Prague, Czech Republic:
"Effectivity of essential oils against pectinolytic erwinias and pseudomonads"
12. **Blanka Kokosková**, Roman Pavela, Research Institute of Crop Production, Prague, Czech Republic:"
Effectivity of essential oils against *Xanthomonas hortorum* pv. *pelargonii*, the causal agent of bacterial blight on geraniums"

13. **Ömür Baysal**, S. Soylu, E.M. Soylu, W. Zeller, Mustafa Kemal University, Antakya-Hatay, Turkey:
"Antibacterial activities of essential oils from medicinal plants against the growth of *Clavibacter michiganensis* subsp. *michiganensis*"

SESSION 2: BIOLOGICAL CONTROL OF BACTERIAL DISEASES IN FIELD CROPS

14. **Fikre Lemessa**, Wolfgang Zeller, Institute for Biological Control of BBA, Darmstadt Germany:
"Biological Control of potato bacterial wilt caused by *Ralstonia solanacearum* in Ethiopia: I. Determination of biovars of *Ralstonia solanacearum* from Ethiopia"
15. **Miguel Obregon**, PNUD-MINAE, Costa Rica:
"*In vitro* and *in vivo* evaluation of *Streptomyces griseoviride* strains on pathogenic bacteria of some crops in Costa Rica"
16. **Renata Lebecka**, B. Flis, E. Zimmernoch-Guzowska, Plant Breeding and Acclimatization Institute, Mlochow, Poland:
"Resistance to *Erwinia carotovora* introduced to *Solanum tuberosum* from wild *Solanum* species"

SESSION 3: BIOLOGICAL CONTROL OF BACTERIAL DISEASES IN HORTICULTURE

17. **Aly E. Tawfik**, M.S. Mikhail, F.M. Barakat, R.M. Abd El-Aziz, Agric. Research Center, Giza; Cairo University, Cairo, Egypt:
"Reduction of crown-gall incidence on stone fruits with some antagonistic bacteria"
18. **Botond Balogh**, J.B. Jones, R.E. Stall, J.R.Dilley, H.D. Yonce, B.I. Canteroa, A.M. Gochez, University of florida, Gainesville, Florida; R.L. Dilley & Son Inc., Florida:
"Control of Asiatic citrus canker and citrus bacterial spot with bacteriophages in Florida"
19. **Sylwia Jafra**, J. Przysowa, J. Van Doorn, J.M. van der Wolf, University of Gdansk and Medical University of Gdansk, Poland:
"Possibility for biological control of pectinolytic erwinias in hyacinth using antagonistic bacterial isolates"
20. **Radwan Ftayeh**, K. Rudolph, Institute of Plant Pathology and Plant Protection, Göttingen, Germany:
"Multiplication and spread of the avirulent strain NCPPB 3123 of *Clavibacter michiganensis* spp. *michiganensis* (proposed resistance inductor) alone and concomitantly with a virulent strain in tomato plants"

SESSION 4: IMPLEMENTATION OF BIOLOGICAL STRATEGIES IN INTEGRATED DISEASE MANAGEMENT SYSTEMS

21. E. Biondi, F. Bini, P. Lancioni, A. Brunelli, **Carlo Bazzi**. Università di Bologna, Italy:
"Biological control agents as tools against some emerging bacterial plant diseases in Italy: a concrete perspective ?

22. **Svetlana Milijasevic**, A. Obradovic, Agricultural Research Institute, Serbia:
"Control of bacterial speck and bacterial spot of tomato using alternative strategies under greenhouse conditions"

22a **Hatice Özaktan** University of Ege, Bornova-Izmir, Turkey:
"Investigations on integrated management of bacterial speck of tomato (*Pseudomonas syringae* pv. *tomato*) focused on biological control"

23. **Aaron Hert**, T. momol, G.V. Minsavage, J.B. Jones, University of Florida, Gainesville; North Florida Research and Education Center, Quincy, USA:
"Evaluation of a bacteriocin-producing attenuated strain (opgH-) of *Xanthomonas perforans* to control *Xanthomonas euvesicatoria*"

24. **Giorgio Mariano Balestra**, E. Di Mattia, M. Muganu, Università degli Studi della Tuscia, Viterbo, Italy:
"Biocontrol of phytopathogenic bacteria by using organic compost"

25. **Aida Raio**, R. Peluso, G. Puopolo, A. Zoina, Via Università, Portici, Italy:
"Efficiacy of different control strategies for preventing crown gall in the nurseries"

26. **Daiva Burokiene**, Z. Luksiene, M. Vasinauskiene, Vilnius University, Vilnius, Lithuania:
"Biophotonic technology for inactivation of tomato pathogen *Clavibacter michiganensis* subsp. *michiganensis*"

27. S. Prashant, **V.R. Prabavathy**, K. Malarvizhi, K. Srinivasan and A. Mathivanan, University of Madras, Chennai, India:
"Biocontrol potential of a growth promoting soil bacterium against phytopathogenic fungi"

28. **Sunday Agbeniyi**, Cocoa Research Institute of Nigeria, Ibadan, Nigeria:
"Current status of biological control of cacao black pod disease in Nigeria"

29. **Ivan K. Kurdish**, A.A. Roy, N.V. Chuyko, L.V. Bulavenko, I.Y.Tsarenko, Zabolotny Institute of Microbiology and Virology Nas of Ukrain, Kiev, Ukraine:
"Microbial preparations of complex action on plants"

30. **Nancy Contreras**, Universidad Centroccidental Lisandro Alvarado, Cabudare, Venezuela:
"Experimental results on biological control of bacterial plant diseases in Venezuela"

31. **Anania Fessehaie**, R.R. Walcott, University of Georgia, Athens, USA:
"Biological control to protect watermelon blossoms and seeds from infection by *Acidovorax avenae* subsp. *citrulli*"

SESSION 5: MOLECULAR APPROACHES IN BIOCONTROL OF BACTERIAL DISEASES

32. **Annette Wensing**, H. Weingart, M. Ullrich, International University Bremen, Bremen, Germany:
„Influence of siderophore production on biological control among *Pseudomonas syringae* strains"

33. **Beate Völksch**, Friedrich Schiller Universität Jena, Jena, Germany:
"Characterization of an inhibitory strain of *Pantoea sp*. with potential as a biocontrol agent for bacterial plant pathogens"

34. **Bernd Schneider**, C. Berwarth, V. Hanke, W. Jelkmann, Institute for Plant Protection in Fruit Crops of BBA, Dossenheim, Germany:
„Engineering of transgenic apple cultivars by expression of human lactoferrin to study effects on *Erwinia amylovora*"

SESSION 6: BIOLOGICAL CONTROL OF FIRE BLIGHT

35. **Veljko Gavriloviæ**, S. Milijasevic, S. Zivkovic, Institute for Plant Protection and Environment, Belgrade, Serbia:
"Characterization of epiphytic bacteria originated from Quince and Medlar trees and ist antagonistic effect against *Erwinia amylovora in vitro*"

36. **Josef Korba**, J. Sillerová, Research Institute of Plant Production, Praha-Ruzyne, Czech Republic:
"Evaluation of epiphytic bacteria for potential control of the fire blight pathogen"

37. **Nicole A. Werner**, H.S. Aldwinckle, Cornell University, Geneva, NY, USA:
"A comparison of two years of research on biological control of fire blight in New York"

38. **Wolfgang Zeller**, Institute for Biological Control of BBA, Darmstadt, Germany:
"Recent status on the Biocontrol of fire blight in Germany"

39. **Marcel Wennecker**, B. Heijne, T. Deckers, I. Holb, S. Kunz, P. Oosterkamp, I. Grondona, B. Eilers, H. Junge, Applied Plant Research (PPO), The Netherlands; Royal Research Station Gorsem, Belgium, The Netherlands:
"BACTOFRUCT - Development of a biological pesticide against fire blight"

40. A. Fried, **Anja Seibold**, E. Moltmann, W. Jelkmann, Landratsamt Karlsruhe; University Heidelberg; Landesanstalt für Pflanzenschutz, Stuttgart; Institute for Plant Protection in Fruit Crops of BBA, Dossenheim, Germany:
„Field experiments for fire blight control by artificial and non-artificial infection of apple- trees in 2005"

41. **Birgit Heissenberger**, A. Spornberger, M. Keck, I. Loncaric, H. Foißy, Austrian Agency for Health and Food Safety; University of Agricultural Sciences; University of Veterinary Medicine, Vienna, Austria:
"*In vitro* studies on fire blight control by bacterial antagonists"

42. **Birgit Heissenberger**, B. Schaffer, I. Loncaric, R. Moosbeckhofer, Austrian Agency for Health and Food Safety, Vienna, Austria:
"Fire Blight Control by application of fungal antagonists"

43. **Igor Loncaric**, B. Heissenberger, R. Moosbeckhofer, Austrian Agency for Health and Food Safety, Vienna, Austria:
"Dispersal of the biocontrol agent *Aureobasidium pullulans* for fire blight control using honeybees (*Apis mellifera carnica*)"

44. **Susan Lehman**, W.-S. Kim, A.J. Castle, A.M. Svircev, Brock University, St. Catharines, ON, Canada:
"Using bacteriophages to prevent fire blight in the orchard"

45. **Nader Hassanzadeh**, Islamic Azad University, Teheran, Iran:
"Thyme essential oil as a natural plant extract for fire blight control"

46. **Iliana Atanasova**, Z. Urshev, J. Dimitrov, N. Bogatzevska, P. Kabadjova, P. Moncheva, Sofia University; LB Bulgaricum, Sofia, Bulgaria:
"Evaluation of diversity of *Erwinia amylovora* in Bulgaria"

47. **Klaus Richter**, A. Peil, M. Höfer, C. Fischer, Federal Centre for Breeding Research on Cultivated Plants, Aschersleben, Dresden, Germany:
"Identification of resistance donors for fire blight in *Malus*"

48. **Piotr Sobiczewski**, S. Berczynski, H. Bryk, Research Institute of Pomology and Floriculture, Skierniewice, Poland:
"Control of fire blight (*Erwinia amylovora*) using epiphytic bacteria with known activity against postharvest diseases of apples"

49. **Tamás Bubán**, T. Lakatos, T. Tóth, L. Dorgai, I. Hudák, M. Hevesi, Research and Extension Centre fir Fruit Growing, Újfehértó; Bay Zoltán Institute for Biotechnology, Szeged; Hungary:
"*Pantoea agglomerans* strain HIP32: a potential new antagonist of *Erwinia amylovora*"

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Table of contents
Abstracts of Papers and Posters (sorted by first author)

Biological control of bacterial diseases of plants in Nigeria: problems and prospects 20
N. A. Amusa

Biological control of bacterial pathogens on tomato plants 20
Balestra, G.M. and A. Quattrucci

Biocontrol agents and their potential risk for human health 21
Gabriele Berg

Characterisation of an inhibitory strain of *Pseudomonas syringae* pv. *syringae* with potential as a biocontrol agent for bacterial blight on soybean 21
Sascha D. Braun and Beate Völksch

Principles in biological control: colonization, antagonism, plant growth promotion and induced resistance 22
H. Buchenauer

Identification of genetic mechanisms that affect *Agrobacterium vitis*-plant interactions including biological control 23
Thomas J. Burr, Guixia Hao and Jodi Creasap

Factors affecting efficiency of biological control of fire blight in *Pseudomonas fluorescens* EPS62e 24
Cabrefiga, J.; Bonaterra, A.; Montesinos, E.

Application of non-pathogenic *Agrobacterium radiobacter* for preventing crown gall disease in dicots, growth-promotion in monocots and molecular aspects of agrobacterial attachment to plant cell surfaces 24
Chumakov, M.I.

Mode of action and genes involved in biological control of grape crown gall by *Agrobacterium vitis* strain F2/5 25
Jodi E. Creasap, Guixia Hao, Hongsheng Zhang, Simone Zäuner, Cornelia I. Ullrich, and Thomas J. Burr

Suppression of bacterial blight on rice by induced resistance and manipulation of endogenous abscisic acid levels 26
David Devleesschauwer, Kris Audenaert and Monica Höfte

Bacillus subtilis strain QST 713, bacterial disease control in fruit, vegetable and ornamental production 26
D. W. Edgecomb and D. Manker

Production of hydrogen cyanide (HCN) and lytic enzymes by rhizobacteria isolated from different plants and soils 27
Egamberdiyeva, D.

Biological Control of Fire Blight Disease in Egypt, (I) Usage of Different Strains of Bacteria 28
M. K. Abo El-Dahab, A.A. Shoieb and S. A. El-Kazzaz, A. K. Mohamed

Biological Control of Fire Blight Disease in Egypt, (II) in Comparison with Chemical Control 28
M. K. Abo El-Dahab, A.A. Shoieb and S. A. El-Kazzaz, A. K. Mohamed

Biological control of bacterial canker of tomato (*Clavibacter michiganensis* subsp. *michiganensis*) 28
M'barek Fatmi

Characterization of epiphytic bacteria from Australia and Europe as possible fire blight antagonists 29
K. Geider, V. Jakovljevic, M. Mohammadi and S. Jock

Bacterial inoculants for the suppression of rice bacterial blight in India: role of 2,4 diacetyl phloroglucinol in disease suppression 30
S. S. Gnanamanickam and J. Ebenezar Immanuel

Bacterial disease of birch in forests of Russia 31
U.I. Gninenko, A.M. Zhukov

A novel bacterium for biocontrol of broad spectrum of pathogens 31
Gomathinayagam, S., Kamalanalini, S.P. and Lalithakumari, J.

Sensibility of isolates of *Acidovorax avenae* subsp. *avenae* and *Xanthomonas campestris* pv. *vasculorum* against a bacteriocin 32
Manuel Filotet Guerra

In vitro inhibition of growth of *Erwinia amylovora* by plant oils 32
Hevesi, M., Boja, N., Bánatfy, R., Babulka, P., Tóth, M.

Biosafety regulations in Germany 33
P. Hoffmann

Potential use of essential oils for plant bacterial disease control 33
Nicola Sante Iacobellis and Pietro Lo Cantore

Management of bacterial spot on tomatoes with bacteriophages 34
J. B. Jones, F. Iriarte, A. Obradovic, B. Balogh, M. T. Momol and L. E. Jackson

Basal resistance of plants to bacterial infection as a possible means for biological control 35
Klement, Z., Ott, P.G., Szatmári, Á., Besenyei, E., Czelleng, A., Szabó, E. and Bozsó, Z.

Effects of different drying techniques on the inhibitory effect of *Pantoea agglomerans* strain Eh-24 bioformulation 35
T.A. Koltuksuz, E. Aslan, H. Özaktan, T. Bora, F.V. Sukan

Fire blight control in organic fruit growing - Systematic investigation of the mode of action of potential control agents 36
Kunz, S.

Biological control of plant diseases by application of natural, epiphytic living non-pathogenic antagonists to the plant phyllosphere 36
Kürkcüoglu, Sophia; Piotrowski, Markus; Degenhardt, Juliana; Al-Masri, Abdul Nasser; Gau., Achim E.

Antagonistic activity of the *Bacillus* strains against the phytopathogenic *Erwinia* 37
S.V. Lapa , O.N. Reva

Mode of action of the bacterial antagonist *Rahnella aquatilis* against *Erwinia amylovora* 38
P. Laux, W. Zeller

Control of the fire blight pathogen with bacteriophages 38
S.M. Lehman, W.-S. Kim, E. Barszcz, K. Schneider, A.J. Castle and A.M. Svircev

Biological control of grape crown gall in China with non-tumorigenic *Agrobacterium vitis* E26: possible mechanisms involved 39
Jiinyun Li, Fan Chen, Yanbing Guo, Jianhui Wang and Huimin Wang

Strategies for biological control of soilborne pathogenic bacteria and practical efficacy of different methods 39
María M. López and Ramón Penalver

State of art of the biological control of bacterial fruit blotch of melon in Penambuco 40
Rosa L.R. Mariano; Elineide B. Silveira; Flávio H. V. Medeiros; Aldenir Oliveira; Elizama R. Santos; Ester R. Gouveia; Ana M. Souto-Maior

Fighting *Ralstonia solanacearum* in Brazil 40
Martins, O. M.; Lopes, C. A.; Quezado Duval, A.

Integrated management of bacterial wilt on field-grown tomatoes 41
M.T. Momol, P. Ji, S.M. Olson, and J.B. Jones

Integrated management of tomato bacterial spot in Florida 41
A. Obradovic, J. B. Jones, M. T. Momol, S. M. Olson, B. Balogh, and L. E. Jackson

A study on the biological control potentials of fluorescent pseudomonad strains against bacterial blotch disease (*Pseudomonas tolaasii*) of cultivated mushroom 42
Hatice Özaktan, Tayyar Bora, Emek Aslan, Ahmet Uslu

Molecular markers of basal resistance, an inducible defense mechanism of plants against bacteria 42
Ott, P.G., Varga, G.J., Szabó, E., Szatmári, Á., Bozsó, Z., Klement, É., Medzihradszky, K.F., Czelleng, A., Besenyei, E. , Klement, Z.

Monitoring the biocontrol agent *Pseudomonas fluorescens* EPS62e by means of Real-Time PCR 43
Pujol, M.; Badosa, E.; Manceau, C. and Montesinos, E.

Chemistry of apple and pear stigma exudates related to bacterial antagonism toward *Erwinia amylovora* 43
P.L. Pusey

A new natural anti-microbial product for use as an agricultural bactericide and fungicide 44
Willem Ravensberg

Screening biocontrol agents for control of seed-borne bacterial pathogens of carrots and brassicas 44
Roberts, S.J., Koch, E., Schmitt, A., Amein, T., Wright, S.

Tomato polygalacturonase inhibiting protein (PGIP) is not induced by the pathogen but increased with ageing 45
Ali Salehzadeh, Bahram Tafaghodinia, Mostafa Motallebi and Mohammad Reza Zamani

Risk assessment when working with biological agents 45
Klaus P. Schaal

Antagonistic activity of different yeast *spp*. against *Erwinia amylovora* 46
Seibold, A., Giesen, N. and Jelkmann, W.

Biocontrol and plant growth promoting potential of a *Pseudomonas* sp. MML2212 from the rice rhizosphere 47
V. Shanmugaiah, S. Ramesh, M. Jayaprakashvel and N. Mathivanan

Optimized mixtures of biological control agents for suppression of fire blight 47
Virginia O. Stockwell

Sensitivity to biological control of *Agrobacterium vitis* is related to its pTi 48
S. Süle, E. Szegedi, O. Kovács, E. Novákand, T.J. Burr

Status of research on biological control of tomato and groundnut bacterial wilt in Vietnam 48
Doan Thi Thanh

Molecular biology of plant-associated bacteria involved in biocontrol 49
Matthias Ullrich

Biocontrol of bacterial canker disease of tomato using *Pseudomonas fluorescens* 50
S. Umesha

Antibiotics produced by strains of *Pantoea agglomerans*, biocontrol agents of fire blight 50
Sandra A. I. Wright and Steven V. Beer

Biological control of black rot of vegetable brassicas, caused by *Xanthomonas campestris* pv. *campestris*, with endophytic *Bacillus* strains 51
Wulff, E.G., Massomo, S.M.S., Mguni, C.M., Mortensen, C.N., and Hockenhull, J.

Phenotypic and molecular characterization of the interaction of antagonistic bacteria with *Ralstonia solanacearum*, causing tomato bacterial wilt 51
Kerstin Wydra and Jörg Semrau

Poster Presentations

Studies on induced resistance against fire blight (*Erwinia amylovora*) with different bioagents 52
Abo-Elyousr, K., Zeller, W., Yegen, O.

Evaluation of the diversity of *Erwinia amylovora* in Bulgaria 52
I. Atanasova, Z. Urshev, J. Dimitrov, N. Bogatzevska, P. Kabadjova, P. Moncheva

Biocontrol of phytopathogenic bacteria by using organic compost 53
Balestra, G.M., Di Mattia, E. and Muganu, M.

Control of Asiatic Citrus Canker and Citrus Bacterial Spot with bacteriophages in Florida 54
B. Balogh, J.B. Jones, R.E. Stall, J.R. Dilley, H.D. Yonce, B.I. Canteros and A.M. Gochez

An early oxidative burst in apple rootstocks treated with DL-β-Amino butyric acid (BABA) against *Erwinia amylovora* 54
Ö. Baysal, Ş. B. Gölükcüand A. Ünlü and W. Zeller

Induction of oxidative burst in tomato leaves treated with unsaturated fatty acids of turtle oil (*Caretta caretta*) against *Pseudomonas syringae pv. tomato* 55
Ö. Baysal, Y.Z. Gürsoy, Hakan Örnek, Ahmet Duru, S. Soylu, E. M. Soylu and W. Zeller

Antibacterial activities of essential oils from medicinal plants against the growth of *Clavibacter michiganensis* subsp. *michiganensis* 55
Soner Soylu, Emine Mine Soylu, Ömür Baysal and W. Zeller

An early oxidative burst in apple rootstocks treated with DL-β-Amino butyric acid (BABA) against *Erwinia amylovora* 56
Ö. Baysal, Ş. B. Gölükcü and A. Ünlü and W. Zeller

Biological control agents as tools against some emerging bacterial plant diseases in Italy: a concrete perspective ? 56
Biondi, E., F. Bini, P. Lancioni, A. Brunelli, C. Bazzi

Pantoea agglomerans strain *HIP32*: A potential new antagonist of *Erwinia amylovora* 57
Bubán, T., Lakatos, T., Tóth, T., Dorgai, L., Hudák, I., and Hevesi, M.

Experimental results on biological control of bacterial plant diseases in Venezuela 58
Nancy Contreras

Biological control to protect watermelon blossoms and seeds from infection by Acidovorax ssp. citrulli 59
Fessehaie, A. and Walcott, R.R.

Field experiments for fire blight control by artificial and non-artificial infection of apple trees in 2005 59
Fried, A., Seibold, A., Moltmann, E. and Jelkmann, W.

Multiplication and spread of the avirulent strain NCPPB 3123 of *Clavibacter michiganensis* spp. *michiganensis* (proposed resistance inductor) alone and concomitantly witha virulent strain in tomato plants 60
Ftayeh, R., Rudolph, K.

Antagonistic coccus type bacteria causing death of a coryneform type of bacteria, the pathogen of *Agave tequilana* Weber var. *Azul* 61
L. Fucikovsky

Characterization of epiphytic bacteria originated from quince and medlar trees and its antagonistic effect against *Erwinia amylovora* »in vitro« 61
Gavrilović, V., Milijašević, S. and Živković, S.

Thyme essential oil as a natural plant extract for fire blight control 62
N. Hassanzadeh

Fire blight control by application of fungal antagonists 62
Heissenberger, B., Schaffer, J., Loncaric, I., Moosbeckhofer, R.

In vitro-studies on fire blight control by bacterial antagonists 63
Heissenberger, B., Spornberger, A., Keck, M., Loncaric, I., Foißy, H.

Evaluation of a bacteriocin-producing attenuated strain (opgH-) of *Xanthomonas perforans* to control *Xanthomonas euvesicatoria* 63
Aaron P. Hert, M. Timur Momol, Gerald V. Minsavage, and Jeffrey B. Jones

Possibility for biological control of pectinolytic erwinias in hyacinth using antagonistic bacterial isolates 64
S. Jafra, J. Przysowa, J. van Doorn and J. M. van der Wolf

Effectivity of essential oils against *Xanthomonas hortorum* pv. *pelargonii*, the causal agent of bacterial blight on geraniums 64
Kokošková, Blanka, and Pavela, Roman

Effectivity of essential oils against pectinolytic erwinias and pseudomonads 65
Kokošková, Blanka, Pavela, Roman

Evaluation of epiphytic bacteria for potential control of the fire blight pathogen 65
Josef Korba, Jana Šillerová

Microbial preparations of complex action on plants 66
I.K.Kurdish, A.A.Roy, N.V.Chuyko, L.V.Bulavenko, I.Y.Tsarenko

Resistance to *Erwinia carotovora* introduced to *Solanum tuberosum* from wild *Solanum* species 67
R. Lebecka, B. Flis and E. Zimnoch-Guzowska

Using bacteriophages to prevent fire blight in the orchard 67
S. M. Lehman, W. -S. Kim, A. J. Castle, A. M. Svircev

Biological control of potato bacterial wilt caused by *Ralstonia solanacearum* in Ethiopia: I. determination of biovars of *Ralstonia solanacearum* from Ethiopia 68
F. Lemessa and W. Zeller

Antibacterial activity of plant extracts against bacterial plant pathogens 68
E. Lojkowska, A. Krolicka, E. Biskup

Dispersal of the biocontrol agent *Aureobasidium pullulans* for fire blight control using honeybees (*Apis mellifera carnica*) 69
Loncaric, I., Heissenberger, B., Moosbeckhofer, R.*

Biophotonic technology for inactivation of tomato pathogen *Clavibacter michiganensis* subsp. *michiganensis* 69
Z. Luksiene, M. Vasinauskiene, D. Burokiene

Bactericidal activity *in vitro* of essential oils of H*yptis suaveolens* (L.) Poit and C*oleus amboinicus* (Lour) 70
Marusia Stefanova, Sandra G. Rizo, C. Romeu, Ma. Francisca Coronado, Ma. Loreta Larrinaga.

Control of bacterial speck and bacterial spot of tomato using alternative strategies under greenhouse conditions 70
Svetlana Milijašević and Aleksa Obradovic

In vitro and *in vivo* evaluation of *Streptomyces griseoviride* strains on pathogenic bacteria of some crops in Costa Rica 71
Obregon, M.

Efficacy of different control strategies for preventing crown gall in the nurseries 71
A. Raio, R. Peluso, G. Puopolo, A. Zoina

Rhizobacteria-mediated induced systemic resistance against various plant pathogens and its mechanisms of action 72
Kyungseok Park, Ming-Shu Zeng, Yong-Chull Jeun and Ki-Woong Nam

Biocontrol potential of a growth promoting soil bacterium against phytopathogenic fungi 73
S. Prashanth, V.R. Prabavathy, K. Malarvizhi, K. Srinivasan and N. Mathivanan

Identification of resistance donors for fire blight in *Malus* 74
K. Richter, A. Peil, M. Höfer and C. Fischer

A biocontrol agent for bacterial blight that induces systemic resistance as it restrains pathogen multiplication in bean leaf tissue 74
Romeiro, R.S.; Vieira Júnior, J.R.; Ferraz, H.G.M.; Barra, V.R.S. and Melo, I.S.

Engineering of transgenic apple cultivars by expression of human lactoferrin to study effects on *Erwinia amylovora* 75
B. Schneider, C. Berwarth, V. Hanke and W. Jelkmann

Control of fire blight (*Erwinia amylovora*) using epiphytic bacteria with known activity against postharvest diseases of apples 75
Piotr Sobiczewski, Stanisław Berczyński, Hanna Bryk

Antibacterial activities of essential oils from medicinal plants against the growth of *Clavibacter michiganensis* subsp. *michiganensis* 76
Soner Soylu, Emine Mine Soylu, Ömür Baysal and W. Zeller

Reduction of Crown gall incidence on stone fruits with some antagonistic bacteria 76
A.E. Tawfik , M.S. Mikhail, F.M. Barakat, and Rabab M. Abd–el-Aziz

Characterisation of an inhibitory strain of *Pantoea sp*. with potential as a biocontrol agent for bacterial plant pathogens 77
Beate Völksch

BACTOFRUCT – Development of a biological pesticide against fire blight 78
M. Wenneker, B. Heijne, T. Deckers, I. Holb, S. Kunz, P. Oosterkamp, I. Grondona, B. Eilers, H. Junge

Influence of siderophore production on biological control among *Pseudomonas syringae* strains 78
Annette Wensing, Helge Weingart and Matthias Ullrich

A comparison of two years of research on biological control of fire blight in New York 79
Werner, Nicole A. and Herb S. Aldwinckle

Recent status of the biocontrol of fire blight in Germany 79
W. Zeller

List of Authors 80

Biological control of bacterial diseases of plants in Nigeria: problems and prospects

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Plant pathogenic bacteria cause serious problems to farmers in Nigeria, and because they tend to be thermophilic in nature, they affect virtually all economic crops cultivated in Nigeria. Several crops including vegetables, root and tuber crops, tree crops and other industrial crops are highly susceptible to bacterial infection. In fact as of now, tomato cultivation in the humid forest has been abandoned due to the activities of tomato wilt caused by *Ralstonia (Pseudomonas) solanacearum*. While cassava bacterial blight caused by *Xanthomonas campestris* pv *manihotis* still remains a problem in the cassava growing region of the country. The cultivation of Kenaf in a large scale for industrial uses has not been visible because of the activities of the seed borne pathogen *Pseudomonas syringae* pv *syringae*, which contributed to the low viability of its seeds. The cultivation of cowpea which is the only source of cheap protein for the farming population in Africa, is now under a serious threat by the activities of *Xanthomonas campestris* pv. *vignicola* the causal agents of bacterial blight

Of all the control measures available to farmers, biological control is about the most appropriate because it is the highly environmentally friendly. Several efforts have been made by scientists to provide suitable biological control measures for the managements of bacterial plant pathogens. However, several factors limit the research activities in biological control and the adoption of the research findings. The paper reports on the problems and prospects of biological control of bacterial diseases of plants in Nigeria.

Biological control of bacterial pathogens on tomato plants

Balestra, G.M. and A. Quattrucci

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Bacterial pathogens are a serious problem almost everywhere tomato crops are cultivated. Amongst them, *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), *Pseudomonas syringae* pv. *tomato* (Pst) and *Xanthomonas vesicatoria* (Xv) are the most dangerous. To control these phytopathogenic bacteria, cupric treatments and good agronomical practices are suggested. At present no valid alternatives with respect to the use of copper compounds are available. Moreover, considering the recent EU restrictions on copper uses, the development of effective organic compounds to control these pathogens appears to be very important, especially in organic agriculture. As potential bactericidal agents, vegetal extracts from *Liliaceae* and *Moraceae* plants were utilised. *In vitro* and *in vivo* tests were performed. Bacterial strains were tested at 10^6 and 10^8 cfu/ml. The extract of *Liliaceae* plants was utilised at a concentration of 1%; those of *Moraceae* plants at 10% and 30%. *In vitro* tests of both vegetal extracts showed the best results particularly on Cmm and on Pst growth inhibition. *In vivo* tests of natural substances confirmed their antimicrobial activity. Both, *Liliaceae* and *Moraceae* vegetal extracts were effective with respect to the Cmm and Pst bacterial strains tested; moreover, good results on Xv strains were obtained when the concentration of the pathogen was reduced.

Biocontrol agents and their potential risk for human health

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Biological control using naturally occurring antagonists offers an environmentally friendly and sustainable possibility to control fungal plant pathogens. One natural reservoir of antagonists is the rhizosphere, the zone around roots that is influenced by the plant. Due to a high content of nutrients, this habitat is a "microbial hot-spot", where bacterial abundance including a microflora with strong antagonistic traits is enhanced (1). Various bacterial genera, including *Burkholderia, Enterobacter, Herbaspirillum, Ochrobactrum, Pseudomonas, Ralstonia, Staphylococcus,* and *Stenotrophomonas* contain root-associated strains that can encounter bivalent interactions with both plant and human hosts. In general, antagonistic bacteria show a beneficial interaction with their host plants. They are able to promote plant growth and/or to suppress pathogens. Unfortunately, some of them belong to the group of facultative human pathogenic bacteria which cause diseases but only in patients with a strong predisposition to illness, particularly in those who are severely debilitated, immunocompromised or suffering from cystic fibrosis or HIV-infections. During the last years, the number of human infections caused by opportunistic pathogens has increased dramatically.

Mechanisms responsible for colonisation of the rhizosphere and antagonistic activity against plant pathogens are similar to those responsible for colonisation of human organs and tissues, and pathogenicity. Resistance to multiple antibiotics are not only found in clinical strains but also in strains isolated from the rhizosphere. High levels of competition, the occurrence of diverse antibiotics in the rhizosphere, and elevated roles of horizontal gene transfer in this microenvironment appear to contribute to the high levels of natural resistance. The features of members of the genus *Stenotrophomonas* will be discussed more in detail (2,3).

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Characterisation of an inhibitory strain of *Pseudomonas syringae* pv. *syringae* with potential as a biocontrol agent for bacterial blight on soybean

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The soybean epiphyte, *Pseudomonas syringae* pv. *syringae* 22d/93 (Pss22d), exhibits strong potential to control *Pseudomonas syringae* pv. *glycinea* (Psg), the causal agent of bacterial blight. Under greenhouse and field conditions the antagonism has been proven, but the underlying mechanisms are unclear up to

now. The secondary metabolites are of particular interest. In our workgroup we investigate toxin production as a potential mechanism in the antagonism between the strains. Simultaneously, the siderophores production is analysed in cooperation with the group of Prof. Ullrich at the International University of Bremen as a second possibility for this antagonism.

We know that Pss22d produces syringomycin, syringopeptin and at least one unknown toxin, which inhibits Psg in vitro. The unknown toxin was detected by the Psg growth inhibition assay, since growth inhibition was reversed by arginine and not by argininosuccinate, suggesting that the unknown toxin could interfere with this step of citrulline/arginine biosynthesis. In order to identify genes responsible for unknown toxin production a random Tn5-mutagenesis was conducted. After screening via inhibition assays we found 20 phenotypes of Pss22d: 10 Tn5-mutants do not produce the unknown toxin, 8 produce less than the wild type and 2 produce more than the wild type. Furthermore, the kind of antibiotic toxin produced by Pss22d should be analysed.

Via marker exchange mutagenesis in genes coding for non-ribosomal protein synthesis responsible for initial steps in biosynthesis of syringomycin (syrE) and syringopeptin (sypA), we construct different toxin-negative mutants. Subsequently a double mutant will be constructed as well. The aim is to determine the role of toxins in the antagonism in vitro as well as in planta.

Principles in biological control: colonization, antagonism, plant growth promotion and induced resistance

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Biological control comprises the decrease of inoculum or of the disease producing activity of a pathogen through one or more mechanisms. Interest in biological control of plant pathogens has increased considerably in the last few decades, partly to reduce the use of chemical disease control agents that adversely may affect the environment, but also because it may provide control of diseases that can not or only partly be managed by other control strategies.

Recent advances in microbial and molecular techniques have significantly contributed to new insights in underlying mechanisms by which introduced bacteria function.

Colonization of plant roots is an essential step for both soil-borne pathogenic and beneficial rhizobacteria. Visualization technologies using laser scanning microscopy in combination with the use of marked strains have increased our knowledge on rhizosphere microbiology. Colonization patterns showed that rhizobacteria acting as biocontrol agents or as growth promoting bacteria form microcolonies or biofilms at preferred sites of root exudation. Such microcolonies are sites for bacteria to communicate with each other (quorum sensing) and to act in a coordinated manner. Mutants defective in root colonization traits were impaired in their ability to control root diseases.

Biocontrol mechanisms by which rhizosphere bacteria may protect plants against soil-borne pathogens are multifarious.

They may compete for space and nutrients in the rhizosphere. Evidence has been accumulated that antibiotics produced by rhizobacteria constitute an important factor in suppression of root diseases. The degree of disease control by the antibiotic producing rhizobacteria depends on the sensitivity of the pathogen population to the antibiotics and the quantities produced. Fluorescent pseudomonad strains produce siderophores

that sequester iron, thereby depriving the pathogen from this essential element during its deleterious activities in the rhizosphere / phyllosphere. Mechanisms by which plant growth promoting rhizobacteria (PGPR) may stimulate plant growth are either by suppressing deleterious microorganisms or pathogens or by producing plant growth regulators (such as auxins, cytokinins and gibberellins) as well as by lowering ethylene levels in plants.

In addition selected strains of rhizobacteria reduce disease through activating resistance mechanisms in plants. Rhizobacteria mediated induced systemic resistance is expressed toward a broad spectrum of plant pathogens. Certain strains of rhizobacteria produce salicylic acid (SA) at the root surface and trigger the SA-dependent systemic acquired resistance (SAR)-pathway. Other rhizobacteria trigger a different signalling pathway named induced systemic resistance (ISR). The ISR is dependent on the plant hormones jasmonic acid (JA) and ethylene. Simultaneous activation of both the JA/ethylene dependent ISR pathway and the SA-dependent SAR pathway may result in enhanced levels of protection.

Identification of genetic mechanisms that affect *Agrobacterium vitis*-plant interactions including biological control

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Crown gall disease of grapevines occurs in major viticulture regions worldwide causing reduced vine growth and possible death. In addition to causing crown gall, *Agrobacterium vitis* causes a grape-specific necrosis and a hypersensitive response (HR) on tobacco. One non-tumorigenic strain, F2/5, is able to prevent crown gall infection when applied to wounds on grape prior to application of tumorigenic strains. Single gene mutations in F2/5 result in altered necrosis, HR and biological phenotypes suggesting that the underlying mechanisms of all three responses are related. We have identified three *luxR*-like genes, *aviR*, *avhR* and *avsR* that when knocked out give altered phenotypes of interest providing strong evidence that these grape/*A. vitis* interactions are regulated by a complex quorum-sensing system. At least six *N*-acyl-homoserine lactone autoinducers were found to be produced by F2/5. Other genes found to be associated with the HR, necrosis and biological control phenotypes include other regulatory genes, a gene cluster that is associated with production of long-chain polyunsaturated fatty acids and a gene that resembles a ClpA protease. Other ORFs associated with these responses on grape and tobacco have also been identified.

This research will greatly broaden our basic knowledge of how bacteria interact with plants. Another significant outcome will be determining how F2/5 controls grape crown gall and the implementation of this process in commercial agriculture. The recently completed sequencing of *A. tumefaciens* and other Rhizobiaceae allows comparisons to *A. vitis* providing clues to the evolutionary development of these bacteria.

Factors affecting efficiency of biological control of fire blight in *Pseudomonas fluorescens* EPS62e

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A bioassay based on detached immature fruits and flowers of pear consisting of treatment with the biocontrol agent (*P. fluorescens* ESP62e) and inoculation with the pathogen (*E. amylovora*) was used for partitioning the effect of host and strain of pathogen on efficacy of biological control of fire blight of rosaceous plants. The effect of cultivar type and plant material was studied in dose-response experiments with the same strain of pathogen. The median effective dose ED_{50} of the pathogen (K_x) and biocontrol agent (K_z) were estimated by fitting data to a hyperbolic saturation model. Host changes at the level of cultivar or type of plant material had a significant effect in biocontrol efficacy of fire blight and the more susceptible cultivars to *E. amylovora* were the more active in supporting biological control. The effect of the virulence of eight strains of *E. amylovora* on biocontrol efficacy was studied in one of the cultivars and plant material. The efficacy of biological control was inversely related to the virulence of the strain of *E. amylovora*, measured as the Gompertz rate of disease progression. An analysis of K_x and K_z was performed with five pear plant cultivars and two types of plant material. The K_x for the *E. amylovora* EPS101 covered a range of 10^3-fold (from 1 to 1.49×10^3 CFU per site of inoculation), K_z for the *P. fluorescens* EPS62e varied 10^5-fold (from 6.9 to 3.4×10^5 CFU per site of inoculation) and the efficiency of the biocontrol agent as the ratio K_z/K_x varied 10^3-fold (from 2 to 3.5×10^3). Relationships between K_z, K_x and the ratio K_z/K_x were observed and the significance will be discussed.

Application of non-pathogenic *Agrobacterium radiobacter* for preventing crown gall disease in dicots, growth-promotion in monocots and molecular aspects of agrobacterial attachment to plant cell surfaces

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The *Agrobacterium radiobacter* strain 5D-1 was reisolated from surface sterilized wheat roots and gave a positive reaction with 5D-1 strain antibody [1]. Wheat seed inoculation led to 50% increase of stem dry weight in field conditions. The introduction of the strain 5D-1 to sunflower rhizosphere lead to a two times increase of above-ground plant biomass in greenhouse conditions [2]. The pretreatment of tomato stems by strain 5D-1 can protect from *A. tumefaciens* gall formation. We worked out and applied in field conditions the monitoring method for root-associated bacteria based on combination of genetic and immunological methods. We showed that the attachment behaviour of *A. radiobacter* 5D-1, its attachment-mechanism and over-attachment mutations, defined in laboratory conditions and colonization capacity in field conditions, are directly correlated.

Bacterial adsorption and attachment to the plant cell surface is the first real contact in the interactions and real background for competition on plant cell surface [2,3]. I will present qualitative and quantitative analyses of the attachment of *A. radiobacter* 5D-1 to mono- and dicot plant root and root hairs cell surfaces using electronic (transmission, scanning), light microscopy, radioisotopic, and blocking chemical methods. Strain 5D-1 was able to attach to wheat and rice roots in higher numbers than did pathogenic (*A. tumefaciens*, *A. rhizogenes*) agrobacteria, but in the same mode as to dicotyledonous plants.

The agrobacterial surface molecules (*vir*- and *tra*-depending pilus, flagella, cellulose fibrils, LPS) were evaluated as attachment-mediating molecules. We have strong genetic evidence for the involvement of the *A. radiobacter* 5D-1 calcofluore-binding polysaccharides and flagelles in the attachment process to wheat root hair tips. It seems, the rhizadhesine-like protein *vir*- and *tra*-depending pilus, and wheat lectin are not involved in the agrobacterial attachment process to monocot plant cell surface.

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Mode of action and genes involved in biological control of grape crown gall by *Agrobacterium vitis* strain F2/5

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A. vitis causes crown gall disease in grape, which can be severe in grape growing regions worldwide. *Vitis vinifera* L. cultivars are highly susceptible to freeze injury, providing wounds important for infection. Phytohormone concentration within the plant appears to play a role in tumor development, as does natural wound healing. Via an unknown mechanism, the nontumorigenic strain, F2/5, prevents crown gall in grape tissue. M852, a Tn5 mutant of F2/5, lacks the biological control ability, does not induce an HR on tobacco, and is reduced in its ability to cause grape necrosis. The disrupted gene has been identified as homologous to an ATP-dependent Clp protease, *clpA*, with similarities to *A. tumefaciens* (86%), and *Sinorhizobium meliloti* Rm1021 (83%) *clpA* genes. With primers designed from the genome sequence of *A. vitis* strain S4, the ORF of *clpA* was amplified from F2/5 DNA, sequenced, and confirmed similar to *clpA* in S4 (97%). Site-directed mutagenesis has shown this gene is involved in biological control, and this *clpA* homolog will be cloned into an expression vector and used to complement M852 and the site-directed mutant µ*clpA*. Additionally, to determine whether *clpA* is solely responsible for the lack of biological control, the genes immediately upstream and downstream have been sequenced and identified as a *clpS* homolog and ORFD, respectively. Site-directed mutants of these genes have been tested for biological control, HR, and necrosis. Additional work in *Ricinus* determined that F2/5 prevented tumor development by tumorigenic strain K306. This indicates that the biocontrol activity is not grape specific and that F2/5 may act directly on tumorigenic strains. *Ricinus* stems were inoculated with either the tumorigenic

K306 *A. vitis* strain carrying p35SGUSINT or K306(p35SGUSINT) and F2/5. Isolation of mRNA from inoculated *Ricinus* stems indicated that F2/5 prevents expression of the GUS mRNA.

Suppression of bacterial blight on rice by induced resistance and manipulation of endogenous abscisic acid levels

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Bacterial blight disease, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious diseases of rice worldwide and is found in both tropical and temperate regions. The disease is mainly controlled by the use of resistant cultivars carrying major resistance genes, but the pathogen is highly variable and easily overcomes the deployed resistance. In our laboratory we are interested in alternative strategies to control plant pathogens such as induced systemic resistance and in the role of plant hormones in plant-pathogen interactions. Induced disease resistance is the phenomenon by which a plant exhibits an increased level of resistance to pathogen infection after appropriate stimulation by chemicals, incompatible pathogens or plant-growth promoting rhizobacteria (PGPR). We found that pre-inoculation of rice plants with incompatible isolates of *Magnaporthe grisea* (the causal agent of rice blast) or *Xoo* resulted in increased resistance to bacterial blight. In addition we tested whether we could induce resistance to bacterial blight by root inoculation with PGPRs. Well-known inducers of systemic resistance in dicots such as *Pseudomonas aeruginosa* 7NSK2, *P. fluorescens* WCS417 and *P. putida* WCS358 were unable to induce resistance to blight in rice; only *Serratia plymuthica* strain IC14 was very effective. We are currently studying mechanisms involved in *S. plymuthica* induced resistance to *Xoo*.

The plant hormone abscisic acid (ABA) plays a role in seed dormancy, suppression of vivipary and desiccation tolerance and is also involved in plant-pathogen interactions. Previously, we have shown that tomato mutants with reduced ABA levels (*sitiens*) are much more resistant to various fungal and bacterial pathogens than wild type plants. We investigated the role of ABA in the interaction of rice with *Xoo* using the ABA biosynthesis inhibitor fluridon. Root application of fluridon clearly lowers endogenous ABA levels and renders rice more resistant to *Xoo*. Preliminary experiments indicate that fluridon induced resistance to *Xoo* shares common feature with a classical gene-for-gene incompatibility.

Bacillus subtilis strain QST 713, bacterial disease control in fruit, vegetable and ornamental production

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Bacillus subtilis QST 713, a naturally occurring bacterial strain, discovered and commercialized by AgraQuest, Inc., USA, has been shown to possess significant efficacy against a broad spectrum of eco-

nomically important diseases in fruit, vegetable and ornamental production. In addition to activity against fungal pathogens, *B. subtilis* QST 713 also provides control of key bacterial pathogens such as *Erwinia amylovora* (fireblight of pome fruit), *Xanthomonas campestris* (bacterial spot of tomato and pepper) and *Pseudomonas* and *Xanthomonas* spp. (bacterial spots of ornamentals). *B. subtilis* QST 713 works through novel, multiple modes of action that involve the biological action of *B. subtilis* competing for nutrients on the host surface in addition to the antimicrobial activity of lipopeptide metabolites produced by *B. subtilis* causing permeability changes of the cytoplasmic membrane and subsequent disintegration of the pathogen cells. As determined by US-Environmental Protection Agency and international regulatory authorities, *B. subtilis* QST 713 is exempt from the requirement of a tolerance because there are no synthetic chemical residues, and it is safe to workers and the environment. As a result, treated fruit and vegetables can be exported throughout the world without restrictions. *B. subtilis* QST 713 is also safe to non target, beneficial organisms and has been shown to be an effective tool for disease control in organic crop production and in integrated disease control programs contributing to resistance management and reduction in the use of synthetic fungicides.

Production of hydrogen cyanide (HCN) and lytic enzymes by rhizobacteria isolated from different plants and soils

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Mechanisms of biological control of plant pathogens by plant growth promoting bacteria involve production of bacterial metabolites such as iron chelating siderophores, hydrogen cyanide (HCN) or extracellular lytic enzymes. The objective of the present study was to test in vitro production of extracellular lytic enzymes and hydrogen cyanide (HCN) by rhizobacteria associated with different plants and soils. We also studied their antagonistic effects on various plant pathogens. Bacterial strains isolated from wheat, maize, cotton, pea, tomato, melon, alfalfa grown on serozem arid soils from Uzbekistan and from wheat, pea, grown on loamy sand in Germany, and also from mycorrhiza hyphae associated with Silver birch, Finland. The results showed that bacterial strains isolated from different crops produce HCN and lytic enzymes such as cellulose and chitinase comparable with those isolated from mycrorrhiza associated plants. They were antagonist to various plant pathogens in vitro. In the present study among the various species isolated from different plants the *Pseudomonas* spp. and *Bacillus* spp. strains were positive for production of hydrogen cyanide (HCN) and also some cell wall lytic enzymes. Some bacterial strains with HCN production abilities where used for biological control of wheat root disease in pot experiments. The number of diseased plants was decreased after inoculation with those bacterial strains.

Biological Control of Fire Blight Disease in Egypt, (I) Usage of Different Strains of Bacteria

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Isolation trials made in 3 different Governorates in Egypt resulted in 98 strains of *Erwinia amylovora* which showed varying levels of virulence on immature pear fruits. Sensitivity tests *in vitro* of isolated *E. amylovora* against streptomycin showed that virulent or avirulent strains were sensitive or resistant to streptomycin. Tested strains (98 strains) were sensitive to all *Bacillus spp* with visible differences. While 3 isolates of *Pseudomonas fluorescens* directly inhibited *E. amylovora*, whereas *Rahnella aquatilis* showed weak inhibition. The inoculum of two antagonistic bacteria (*Bacillus* and *Pseudomonas*) established on blossoms greater populations than did *Rahnella aquatilis.* The secondary spread of *Bacillus* and *Pseudomonas* from treated to non-treated trees under natural conditions could be a diffusion process. Biological control agents (*Bacillus* and *Pseudomonas*) may provide an effective control, better than *Rahnella aquatillis.*

Biological Control of Fire Blight Disease in Egypt, (II) in Comparison with Chemical Control

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The biocontrol agents *Bacillus spp* and *Pseudomonas* could provide higher protection from fire blight infection than chemical compounds (streptomycin alone or with tetracycline and copper oxychloride except Bion which was the least). Results showed the ability of Dormex + mineral oil to encourage early blooming, which allows early fruit setting during unfavorable temperature for the pathogen to grow and invade blossoms. This treatment gave protection from fire blight infection greater than biological or chemical control treatments.

Biological control of bacterial canker of tomato (*Clavibacter michiganensis* subsp. *michiganensis*)

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Bacterial canker of tomato, caused by *Clavibacter michiganensis* ssp *michiganensis* (*Cmm*) is one of the world's major diseases of tomato (*Lycopersicon esculentum* Mill). *Cmm* is a seedborne and soilborne pathogen. The control of this disease is mainly based on the use of *Cmm* free seeds. Although this method is necessary, it is not sufficient. Therefore an integrated management is needed to control this disease. Biological control could be an important component of this management.

A procedure of biological control of bacterial canker of tomato using antagonistic bacteria was developed. One hundred and seventy-eight bacterial strains were isolated from the rhizosphere and the roots of tomato plants growing in different sites in the Souss-Mass Valley, Agadir, Morocco. The strains were screened *in vitro* for toxin production using the bacteriocin assay. Sixty nine percent of these strains reduced significantly the growth of *Cmm*. The inhibition zone diameter ranged from 5 mm to more than 90 mm. Fourteen percent of the strains induced total growth inhibition of *Cmm* on 90 mm-diameter plates. Twenty two antibiotic resistant mutants were obtained from the selected wild-type *Cmm* antagonists. These mutants showed bacteriocin production as compared to their respective wild types. A method for applying *Cmm* antagonists to tomato seeds was also developed. Good seed bacterization was obtained through dipping tomato seeds in a bacterial suspension containing 0.33% of xanthan gum.

The selected antagonistic strains were therefore evaluated for their ability to migrate from the bacterized seed along the radicle and then colonize the root system of tomato seedling. More than half of the tested strains colonized more than 50% of the root system. Twenty nine percent colonized the whole tomato seedling root system. Five antagonistic strains, among the selected ones, were used to compare two application methods (Seed bacterization alone and Seed bacteriazation followed by tomato seedling bactertization just prior to transplanting). Tomato seeds were treated separately with the antagonistic strains and sown for seedling production. Half of the seedlings were planted directly in pot containing *Cmm* infested substrate (2/3 peat and 1/3 sand). The rest were dipped in an antagonistic suspension for 3 min before transplanting in the remaining infested pots. The trial was carried out in a greenhouse. Seed bacterization followed by root bacterization prior to transplanting reduced significantly the disease incidence than seed bacterization alone. The incidence of the disease ranged from 75% to 50% with a mean of 63.4% for seed bacterization and from 8% to 0% with a mean of 3.2% for seed and root seedling bacterization. Wile in the case of the control (unbacterized seeds), disease incidence ranged from 75% to 100%. Seventeen qualified antagonistic strains were subsequently screened in pots using a *Cmm* infested substrate. The antagonists were applied to tomato seeds then to tomato seedling roots just before transplantation. In the case of the control, 80% of tomato plants were infected. The use of the antagonists reduced significantly the disease incidence. Six strains gave total control of the disease. Seven strains induced 80 to 94% disease control. The disease reduction for the last four strains ranged from 56 to 75%.

Characterization of epiphytic bacteria from Australia and Europe as possible fire blight antagonists

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In Victoria and Tasmania, apple and pear flowers were screened for bacteria, which form white colonies on nutrient agar and produce levan on sucrose containing media. Similar strains were isolated in Queensland from apple bark. They do not secrete bactericidal compounds on plates, and they are non-pathogenic on apple seedlings nor do they produce ooze when inoculated on sliced immature pears. Microbiological assays including API20E and API50CH (with B. Völksch, Jena) and nucleotide sequence analyses including 16S rRNA and the house keeping genes *gapDH* and *recA*, place these bacteria into the genus *Erwinia*. DNA/DNA hybridization kinetics (with G. Auling, Hannover) have suggested their classification into a novel species that we propose to name *Erwinia tasmaniensis*. Microbiological assays and

nucleotide sequence analyses further showed a relationship to *Erwinia amylovora* and the Asian pear pathogen *Erwinia pyrifoliae* from Korea and Japan. *E. tasmaniensis* strains were used in growth competition assays with *E. amylovora* on apple flowers and on slices of immature pears and suppressed pathogen growth, when applied in excess to *E. amylovora*. Field trials in an experimental orchard confirmed the ability of the epiphytes to reduce symptom formation of fire blight. *Erwinia billingiae* strains, which were isolated in England as "white herbicolas" from the surface of apple trees, did not produce levan, but were also antagonistic for *E. amylovora* on pear slices and in field assays. Both species produced autoinducers of type II (AI-2), detected with a *Vibrio harveyi* strain and *E. billingiae* additionally synthesized acyl-homoserine lactone, detected with a *Chromobacterium violaceum* mutant. Autoinducer 2 may interfere with gene regulation of *E. amylovora*, which also synthesizes AI-2. In contrast to *E. billingiae*, *E. tasmaniensis* caused a hypersensitive response (HR) on tobacco after induction under starvation conditions.

Bacterial inoculants for the suppression of rice bacterial blight in India: role of 2,4 diacetyl phloroglucinol in disease suppression

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We describe the characterization and evaluation of native strains of *Pseudomonas fluorescens* and *Bacillus* spp. from rice, the world's most widely grown cereal crop and report substantial benefits in terms of bacterial blight (BB) suppression, enhanced number of tillers, and grain yield from their application. In a recent study, we evaluated 600 strains of *P. fluorescens* and found that a small sub-set of 27 strains was more promising among 278 strains that suppressed the growth of *X. o.* pv. *oryzae* in plate assays, net-house and field experiments. All 27 strains produced 2,4-diacetylphloroglucinol (DAPG)(identified for the first time in India). DAPG production was confirmed by a PCR-based method, which uses *Phl*2a and *Phl*2b primers for *PhlD* gene developed by Raaijmakers et al. A 745-bp DNA fragment characteristic of 2,4-DAPG was amplified in all these strains. The most efficient of our strains, *P. fluorescens* PTB9 suppressed bacterial blight by 64.5% in the field. Isolation and characterization of DAPG from the culture fluids of the strain and biological activity of such purified preparations in *Xoo* inhibition afforded further confirmation that DAPG mediates bacterial blight suppression. *Tn*5-mutants defective in DAPG production (*Phl*$^-$) were also much less effective in their suppression of rice bacterial blight. In another separate study, five *Bacillus* spp. strains, which included a strain each of *B. cereus, B. circulans,* and *B. lentus* and two strains of *Bacillus* spp., were selected from more than 500 rice-associated bacteria. In laboratory assays, two net-house trials, and a field test in Kerala (southern India), the application of these *Bacillus* strains suppressed the incidence of bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* by more than 50% in popular indica rice cultivars, IR24 and Jyothi. When used as mixtures of either 3 or 4 strains, the bacteria caused 3- to 4-fold increases in tiller numbers of rice plants. Also, corresponding increases in grain yield were recorded. *Bacillus lentus* (strain Alp 18), which suppressed bacterial blight in the field by 55% in IR24 rice, produced a heat-stable aminoglycoside antibacterial antibiotic, whose physical properties (mp=172.8) and spectral (IR, ^{1}H-NMR, and ^{13}C-NMR) characteristics suggested that it is kanosamine.

Bacterial disease of birch in forests of Russia

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In some years in many regions of Russia birch stands are severely damaged by a bacterial disease of birch (pathogen *Erwinia multivora*). Over the last years focuses of this disease have been identified in Kaluga, Bryansk and some regions in western Russia as well as in Bashkiria, Orenburg region in the eastern part of European Russia.

Earlier in mid 70's of the last century big locus of the disease covered birch stands in trans-Urals, south of west Siberia, North Kazakhstan (Gninenko, Bezrutchenko, 1983). The disease was spotted in spring 1976 by available bark swellings. It spread widely by autumn the following year and covered around 100000 ha in Kurgan region and about 670000 ha in Cheljabinsk region. At the same time the disease was identified in birch stands in the south of Sverdlovsk region as well as in Omsk and Novosibirsk regions and in the forest-steppe zone in Altay region. In Kazakhstan focuses of the disease were in Kustanay, North-Kazakhstan and Pavlodar regions.

In 2003 bacteriosis was spotted in forests in 12 regions of Russia. Apart from birch bacteriosis damaged oak and Caucasian fir. In 300 ha bacteriosis of birch was found in Kaluga region in 2003. In these spots birch trees damaged by the disease range from 4 to 74% of the total number of trees in damaged stands. In Bashkiria the total area of focuses of this disease slightly decreased and in 2003 was 1400 ha.

Thus the bacteriosis affects birch forests in many regions in the European part of Russia sometimes building-up rather big areas.

A novel bacterium for biocontrol of broad spectrum of pathogens

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Strains of fluorescent Peudomonads are introduced on seed and planting materials to control plant diseases caused by soil-born pathogens or promote plant growth by suppressing deleterious rhizosphere microorganisms. Beneficial Pseudomonads antagonize pathogens by producing one or more of a variety of metabolites that induce antibiotics, siderophores and other substances such as cyanide. In the present study a novel *Pseudomonas chlororaphis*, isolated from paddy field was characterized. The bacteria suppressed the growth of many important plant pathogens. This bacterium was also an efficient spermosphere and rhizosphere colonizer, an added advantage for potential biocontrol agent. We studied the genetic basis of antagonistic potential and finally its *in vivo* efficiency by field experiments using paddy plants as model system.

It was observed, that paddy plants (ADT.36 and IR 20) treated with *P. chlororaphis* as seed treatment followed by foliar application protected paddy plants against all the pathogens. Intense colonization is one of the reasons for the reduction in blast, sheath blight, bacterial blight and brown spot of paddy. Present results confirm the colonization of chilli, tomato and bring additional support to the current concept that

rhizosphere bacteria are active inducers of plant disease resistance. The inhibition of pathogens *in vitro* and *in vivo* is due to plasmid (pKL 1) mediated antibiotic production.

In the present study inoculation of paddy with *P. chlororaphis* significantly promoted plant growth and this attribute of promoting plant growth is associated with suppression of deleterious micro flora by the introduced bacterium. The experimental results showed that *P. chlororaphis* is very good root colonizer and a foliar antagonist with its broad spectrum of antifungal activity, offering a holistic biocontrol system that works against the pathogens of both root and foliar tissues. Formulation and mass production of the organism is under progress and this will definitely open up a challenging control in the disease management.

Sensibility of isolates of *Acidovorax avenae* subsp. *avenae* and *Xanthomonas campestris* pv. *vasculorum* against a bacteriocin

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The bacteriocin produced by a coryneform Gram positive bacterium was effective against isolates of *Acidovorax avenae* subsp. *avenae* causal agent of the red stripe, top rot and spindle rot diseases and *Xanthomonas campestris* pv. *vasculorum* causal agent of gumming disease of sugarcane, respectively. The best activity of the bacteriocin against *Acidovorax avenae* subsp. *avenae* was obtained after 24 hours incubation at 30 °C and against *Xanthomonas campestris* pv. *vasculorum* after 48 hours incubation at the same temperature.

In vitro inhibition of growth of *Erwinia amylovora* by plant oils

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Previous studies have demonstrated the antimicrobial properties of essential oils and their constituents from a wide variety of plants effective against different microorganisms. The aim of the present investigation was to assess the antibacterial activities of test oils towards *E. amylovora.* Thirty essential oils from medicinal plants grown in Hungary were investigated by application of the agar diffusion method.

Experiments were carried out *in vitro* to determine the magnitude of antibacterial spectra of essential oils (prepared by Aromax Rt., Budapest) against different phytopathogenic species/strains of *E. amylovora;* strain Ea1 [Strep^{S} Hungary (H), apple] and resistant Ea 110 (Rif^{R} USA, apple), Ea 88 (Strept ^{R} USA, pear) and Ca 11 (Strept ^{R} USA, apple). Other species such as *Pseudomonas syringae* pv. *syringae* P53 (H, pepper), *P. savastanoi* pv. *phaseolicola* E1356 (H, bean), *Xanthomonas vesicatoria* XV73 (H, pepper), XVS03 (H, tomato) served as test bacteria.

In the first selection ten different oils displayed a very effective inhibition of bacteria. From this group four oils came out on top, which displayed the most comprehensive inhibitory properties even in a dilution of 1:4 (*Origanum*-, *Mentha*, *Thymus*, and *Tagetes* spp.). Gas chromatography analysis showed that the most effective components are carvone, dihydrocarvone, thymol and carvacrol. There was no difference found between the antibiotic sensitive (Hungarian) and resistant (USA) *E. amylovora* strains in their reaction to essential oils.

The potential utility of these essential plant oils as biopesticides should be confirmed *in vivo* by future experiments.

Biosafety regulations in Germany

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The minimal standards to maintain occupational health and safety during work with biological agents (microorganisms, viri, cell cultures, etc) are defined in the EU directive 2000/54/EG. In Germany, this has been given legal status with the 'Biostoff-Verordnung' (Ordinance on safety and health at work involving biological agents) which came into force in April 1999 and was last modified in Dec. 2004. As an aid for the obligatory risk assessment before beginning work with biological agents, all have been classified according to the 4 risk group concept. These lists are published and updated separately as 'TRBA's (Technical Rules) by the Federal Ministry of Labour in cooperation with the Trade Association of the Chemical Industry (BG Chemie). A scientific committee (ABAS) was established to give advice to the Ministry and edit the 'Technical Rules'; its secretary is with the BAuA (Federal Institute for Occupational Health), which also maintains a web site with all information regarding biological safety at work (www.baua.de/praxis). There may also be found detailed information on the characteristics employed (TRBA 450), and a general scheme for carrying out the necessary risk assessment (TRBA 400) as well as guidelines for special working situations and places (e.g. TRBA 200 – 250). These regulations are valid only for biological agents of risk group 2 or more; it is one of the peculiarities of both EU directive and national ordinance that it defines a risk group 1, but excludes these organisms from coverage.

Potential use of essential oils for plant bacterial disease control

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The control of bacterial diseases of plants is a considerable problem in the agriculture practice because of the limited availability of bactericides and the ability of a large number of phytopathogenic bacteria to spread, even at long distances, by contaminated and/or infected seeds. The use of healthy seeds as well as

other propagation material is one of the main pre-requisites for a successful crop. Besides the antibiotic and copper compounds no other active principles are available for the bacterial plant disease control. Furthermore, antibiotics are actually forbidden in the agricultural practices in many countries and the use of copper compounds, because of their general toxicity and mainly of the impact on the environment, is on the way to be restricted and controlled in the European Union.

The above consideration and, in general, the requirement of reduction in the use of the pesticides in agriculture prompts the need for the development of alternative active compounds and/or methods for the control of plant bacterial diseases to be used in integrated crop management as well as in bio-organic agriculture.

Many studies have pointed out the possibility to use the essential oils and/or their components in medical and plant pathology as well as in the food industry for the control of micro-organisms pathogenic to consumers and/or responsible for food spoilage.

Recently our studies showed the significant antibacterial activity of essential oils of coriander, cumin, and caraway against Gram positive and Gram negative bacteria belonging to *Clavibacter, Curtobacterium, Rhodococcus, Erwinia, Xanthomonas, Ralstonia* and *Agrobacterium* genera, responsible for several plant and mushroom diseases. A much weaker effect was observed for the wild fennel oil. Furthermore, in the same assays a considerable antibacterial activity was shown by some of the above oil components such as carvacrol, eugenol, geraniol, nerol and linalool. A lower activity was observed in the case of other components. Preliminary findings suggested the potential use of some of the above oils and/or components for seed sanitation procedures. In fact, their use significantly reduced the bacterial population on bean seeds artificially contaminated by *Xanthomonas campestris pv. phaseoli* var. *fuscans* with no or negligible effect on seed germination.

Management of bacterial spot on tomatoes with bacteriophages

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Bacterial spot of tomato, caused by *Xanthomonas euvesicatoria*, *X. perforans*, and *X. vesicatoria*, is a serious disease in tropical and subtropical climates as well as in certain temperate climates. For many years, only tank mixes of copper bactericides and mancozeb were used for controlling bacterial spot. Recently, bacteriophages have been used quite extensively in Florida to control this disease. The strategy used with bacteriophages for bacterial spot control differed from those used previously. The bacteriophages were applied as a mixture of several different phages so that resistant bacterial strains do not build up. We also determined that the timing of bacteriophage application was a critical factor. Applications made in the mid-morning resulted in poor control, whereas applications made prior to sunrise or close to sunset resulted in a significant reduction in disease and increase in yield compared to the standard copper-mancozeb treatment. Further improvement of bacteriophage efficacy resulted from modifications in the formulation and timing of application. We have determined that phage populations plummet during the sunlight hours in non-formulated phage suspensions, but are affected to a much lesser extent when powdered milk is added or if the phage suspension is applied in the evening.

Basal resistance of plants to bacterial infection as a possible means for biological control

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Surface receptors of plant cells are able to recognize the surface molecules (e.g. flagellin, LPS, LPS-protein complex) of saprophytic and pathogenic bacteria and to develop a rapid early, non-specific defence mechanism, so-called basal resistance (BR) (syn. innate-, general-, basic-, local induced resistance). The BR develops symptomless and independent of the hypersensitive response (HR) or of the systemic acquired resistance (SAR). The BR inhibits bacterial activity and the induction of HR. When the temperature drops below 10 ^{O}C the BR is delayed or does not develop enabling pathogens like *P. syringae* pv. *syringae* to cause serious diseases of various plants. We isolated two chitinases which are good markers for the detection of BR. Gene activation or supression in *Medicago truncatula* during the development of BR was detected by a microarray. Our final goal is to produce transgenic plants in which the overproduction or regulation of key genes of BR leads to a nonspecific wide-spectrum control against pathogens.

Effects of different drying techniques on the inhibitory effect of *Pantoea agglomerans* strain Eh-24 bioformulation

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Fire Blight caused by *Erwinia amylovora* has threatened pear cultures in Turkey since 1985. Due to the destructive nature of this disease and the difficulties in controlling it by chemicals, the antagonistic bacterium (*Pantoea agglomerans*) has been extensively studied as a potential biological control agent of fire blight in the last decade in Turkey. The strain of *P. agglomerans* Eh-24 was isolated from blossoms collected from healthy pear orchards in the Aegean Region. Talc-based formulations of *P. agglomerans* were applied at 30 % and 100 % blooming on two pear orchards which were selected from different locations in the Aegean Region for two years (1999 and 2000). Bioformulations were sprayed on pear trees which were naturally infected with *E. amylovora*. In orchard trials conducted in 1999 and 2000, talc-based formulation of *P. agglomerans* strain Eh-24 reduced the percentage of blighted blossoms on pear orchards by 63% and 76%, approximately.

In this study, the effectiveness of drying techniques on the inhibitory effect of *Pantoea agglomerans* Eh -24 bioformulation was determined by using laminar air flow, spray dryer and fluidized bed dryer as drying methods. Before bioformulation, *Pantoea agglomerans* strain Eh-24 was grown in a stirred tank bioreactor using either pretreated molasses and heat-disintegrated baker's yeast (HDBY) or sucrose and HDBY as medium. A stirring rate of 500 rpm was used. The temperature and pH during fermentation were kept at 24 ^{o}C and 6.8, respectively. Each batch was centrifuged at 6000 rpm for 20 minutes and biomass was used for talc based formulation.

Fire blight control in organic fruit growing - Systematic investigation of the mode of action of potential control agents

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In organic fruit growing effective control agents are needed to prevent blossom infections by the fire blight pathogen *Erwinia amylovora*. Many potential control agents are under discussion for use against fire blight. In a research project, funded in the "Bundesprogramm ökologischer Landbau" by the BMVEL, the efficiency of 18 control agents against fire blight was investigated with two different test systems.

In shaken cultures 13 control agents reduced the growth of *E. amylovora* by more than 90%. In this system different mechanisms of action were found. Six control agents shifted the pH of the cultures to values not suitable for bacterial growth (<5 or >8). Three agents act by copper, a known bactericide. Elot Vis has a high content of ethanol, which was responsible for the high efficiency in vitro. But these effects are not sufficient for a good performance on detached blossoms or in the field.

On detached apple blossoms [1] only four control agents led to a symptom reduction by more than 50%. And only three of them (Blossom-Protect fb, Myco-sin and Serenade WPO) reached a high efficiency in field trials [2]. The possibility to combine different control agents with different modes of action to enhance the efficiency will be discussed.

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Biological control of plant diseases by application of natural, epiphytic living non-pathogenic antagonists to the plant phyllosphere

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The biological control of plant diseases by application of antagonistic microorganisms to the plant phyllosphere is an alternative strategy to prevent the frequent treatment of plants with pesticides. Microbiological antagonists can firstly interact directly with the pathogen by releasing antimicrobial compounds *and/or* secondly induce the plant resistance of the host plant by expression of pathogenesis-related proteins (PR proteins). The focus of our study is on the interaction of the non-pathogenic *Pseudomonas fluorescens* Bk3 with the plant phyllosphere of *Malus domestica* cv. Holsteiner Cox. After spraying *P. fluorescens* Bk3 to the phyllosphere of *M. domestica* cv. Holsteiner Cox we observed dramatic changes in the protein composition of the apoplast of the host plant. Sequencing of the induced proteins by ESI-Q-ToF mass spectrometry

and homology search identified these additional proteins as PR proteins like β-1,3- glucanase, thaumatin-like protein, chitinase and hevein-like protein. In addition we found the decline of a non-specific lipid transfer protein to a non-detectable level within five days. To confirm these findings a suppressive subtractive hybridization with total RNA from leaves before and after inoculation of *P. fluorescens* Bk3 to the leaves of the host plant was performed and revealed an increased expression level of many PR and stress related genes e.g. ribonuclease-like PR10b, germin-like protein, proteasome subunit alpha type 4, endochitinase class III, heatshock protein 90 and glutathion-S-transferase. The induction of PR proteins and plant defence genes in host plants after application of non-pathogenic bacterial antagonists to the plant phyllosphere can presumably prevent or reduce successful infections by plant pathogens.

Antagonistic activity of the *Bacillus* strains against the phytopathogenic *Erwinia*

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Bacteria of the genus *Erwinia* are known as causative agents of sporadic and epiphytotic plant rotting, withering and deterioration. A number of phytopathogenic isolates identified as *E. atroseptica* (6 strains) and *E. carotovora* (12 strains) were collected and used in a trial of *Bacillus* antagonists aimed at the isolation of the strains amenable to be used to control pests caused by *Erwinia* and other bacterial phytopathogens. Among tested *Bacillus* there were recently isolated soil-borne and rhizosphere associated isolates and the strains from the Ukrainian Collection of the Microorganisms (www.imv.kiev.ua/catalog/catalog.html). Laboratory *in vitro* investigations by the delayed suppression of the tested pathogens around the colonies of the strains-antagonists on the Petri dishes (Yegorov, 1983) showed that the isolated *Bacillus* spp. strains strongly inhibited growth of bacteria of both tested species, *E. atroseptica* and *E. carotovora*. A significant level of protection of the model plants (*Brassica napus*) was observed in the greenhouse trial, however, not all antagonists were as effective *in vivo* as in the *in vitro* trial. The strains active against *Erwinia* pathogens were further studied for their ability to survive on roots and colonize the model plants. It was shown that ability to colonize the plants was crucial for the biocontrol activity of *Bacillus* against *Erwinia* pathogens. By comparative analysis of the sequences of 16S rRNA, *cheA* and *gyrA* the top 10 biocontrol strains were identified as *B. subtilis* and *B. amyloliquefaciens*. Some new biocontrol preparations based on the selected *Bacillus* strains are under construction now.

Mode of action of the bacterial antagonist *Rahnella aquatilis* against *Erwinia amylovora*

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The mode of action of bacteria antagonistic against bacterial plant pathogens include antibiosis, competition for nutrients, trace elements and binding sites and also the induction of resistance by lipopolysaccharides (LPS). The isolate *Rahnella aquatilis* Ra 39, which has shown protective effects against fire blight in the field over several years, was analyzed for its mechanism against the pathogen. *In vitro* studies revealed that Ra 39 had only limited direct effect against *Erwinia amylovora.* The LPS of Ra 39 was purified, analyzed, and tested on its resistance inducing activity. It has been observed that the purified substance induces the generation of superoxide in leaves of apple rootstocks. Furthermore, the LPS application reduced multiplication of *Erwinia amylovora in planta.* The details of the influence of bacterial LPS on changes of defence related enzyme activities are discussed.

Control of the fire blight pathogen with bacteriophages

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Rosaceous spp. infected with *Erwinia amylovora* served as a source of wild type bacteriophages. Soil samples were collected below the canopy of diseased *Malus*, *Pyrus* and *Sorbus* species. Fifty bacteriophages were isolated from the collected soil samples. The phages were enriched using a six host system, purified and characterised by PCR and restriction endonuclease digestion. The molecular characterisation profiles allowed for the creation of six distinct *Erwinia* spp. bacteriophage groups. The phages from each group were further characterised by host range and the ability to infect the orchard epiphyte, *Pantoea agglomerans*. *In vitro* forced pear blossom assays tested the efficacy of various combinations of bacteriophages and bacteriophages + carrier system (phages with *P. agglomerans*). In separate experiments, 100 wild type isolates of *P. agglomerans* were screened for ability to be infected with the lytic bacteriophages. Field trials in pear and apple orchards tested the efficacy of 4 phage isolates and associated carrier bacteria. The biological activity of the phages was compared to BlightBan A506 and C9-1 formulations and streptomycin. The carrier-phage and the carrier alone treatments decreased fire blight incidence ($p<0.05$) in the field. The phage-carrier system needs to be optimised in order to improve the efficacy of the phages *in planta*. Multiplex real-time PCR protocol was developed to simultaneously follow the pathogen, carrier and phage distribution in the orchard. Unique probes and primers were designed to detect each microorganism on the hypanthial surface. The technique can detect introduced bacteria to the minimum level of 20 cfu/ml. Work continues on the optimisation of the phage-carrier system *in planta* and the development of technologies essential for the scale up production of phages and carrier bacteria.

Biological control of grape crown gall in China with non-tumorigenic *Agrobacterium vitis* E26: possible mechanisms involved

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Previous studies revealed that non-pathogenic *Agrobacterium vitis* E26 was a potential biocontrol agent of grape crown gall. This work was aimed to explore the biocontrol mechanisms. The suppression of tumor formation was more effective when E26 was inoculated either prior to the pathogenic strain or at a higher concentration in greenhouse. In field tests, E26 strongly reduced the tumor index when co- inoculated with the pathogen at the same concentration. The dynamics of E26 in grape rhizosphere were studied under field conditions, using green fluorescence protein gene (*gfp*)-marked strains. Root colonization and survival in the rhizosphere of E26(*gfp*) was observed during 5 months experiment. The interaction between E26(*gfp*) and the pathogen on grapevine wound were examined in vitro. E26(*gfp*) and the pathogen attached at a similar level to stem explants. Attachments of pathogen were blocked by E26(*gfp*). Scanning electronic microscope showed that E26 could attach to grape wound with the same manner as that of pathogen. E26(*gfp*) and the pathogen grew competitively in grape wound exudates, and they were identical in utilizing carbon and nitrogen nutrients reported to present in grape tissues in vitro. Agrocin E26 was purified and showed broad activity against tumorigenic bacteria. Its mode of action was to inhibit DNA and RNA syntheses in bacterium cells. Two mini-Tn5 induced minus agrocin-producing mutants grew at a rate similar to that of the wild type E26 and were active in suppressing tumor formation, but, neither of them controlled crown gall as effective as E26 did. PCR –based genomic subtraction technology is being used to screen and identify genes of E26 that suppress tumor development.

Strategies for biological control of soilborne pathogenic bacteria and practical efficacy of different methods

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Biological control of the most important soilborne bacterial diseases of fruits and vegetables has been assayed by using different bacterial agents and strategies with a large spectrum of mechanisms. Three models of biocontrol will be discussed based on their practical efficacy and studied mechanisms: the first is the successful use of the non-pathogenic *Agrobacterium* strain K84 and its genetically modified strain K1026 for the control of pathogenic *Agrobacterium* strains all around the world. The second is the biocontrol of *Ralstonia solanacearum* applying different strategies that have shown some success, but they are still at the developmental step. The third is the most recently developed biocontrol system of soft rot *Erwinia* based on the interference of the expression of genes involved in quorum sensing and pathogenicity.

The experience of more than thirty years in the use of strains K84-K1026 for biocontrol of *Agrobacterium* has shown the need of looking at, not only the short term practical efficacy, but to study the interactions among the pathogen, the biocontrol agent and the soil and rhizosphere microbiota, to know in deep the complexity of the system and to prevent possible failures in the control.

State of art of the biological control of bacterial fruit blotch of melon in Penambuco

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Bacterial fruit blotch (BFB) of melon is responsible for 40 to 50% yield losses in Northeastern Brazil. BFB is caused by *Acidovorax avenae* subsp. *citrulli* (Aac) and disseminated mainly through contaminated seeds. In Pernambuco, Brazil, efforts have been made to select microorganisms for BFB biocontrol, by (i) treating infected seeds; (ii) treating healthy seeds or (iii) spraying seedlings with antagonists in order to protect the plant lately challenged by Aac. Evaluations of disease incidence (INC) and severity (SEV) were performed, and the incubation period (IP), area under disease progress curve (AUDPC), disease index (DI) and disease severity reduction were calculated. Our first work showed that strain RAB9 (*Bacillus* sp.) increased IP and reduced AUDPC while MEN2 (*Paenibacillus lentimorbus*) reduced INC, AUDPC and DI. Therefore they could be applied respectively as seed treatment and plant spraying, individually or sequentially for BFB biocontrol. In a second work, ENM13 (*Bacillus* sp.) and ENM9 (*B. cereus*) were effective to reduce IP, SEV, DI and AUDPC. Both isolates solubilized phosphate, but did not produce HCN or present antibiosis against Aac. In our third work, infected melon seeds were treated with *Bacillus* spp. fermented broths with and without bacterial cells. Bioactive compounds produced during fermentations, partially characterized as lipopeptides, achieved the control. Strain RAB7 (*B. megaterium* pv. *cerealis*) reduced INC, DI, AUDPC, and elevated IP.

Fighting *Ralstonia solanacearum* in Brazil

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Bacterial wilt (BW) caused by *Ralstonia solanacearum* is one of the most important diseases of many hosts, especially in the tropics and subtropics, where the pathogen can be indigenous. BW´s control has been a hard task because of the pathogen´s variability and unusual ability to survive in different kinds of soil. Cultural practices such as crop rotation, avoiding warm planting seasons and use of "health" propagating material are about the only recommendations for fighting the disease in Brazil. The old method of grafting to resistant rootstocks is again promising in high-value crops, such as hydroponic or protected tomato. Although biological control has been regarded as an alternative strategy to be used in the integrated control of BW, assays carried out in Brasilia, Brazil, in field and greenhouse, using avirulent mutants and fluorescent pseudomonads, completely failed to control the disease. Some expectation now rely on promising laboratory and greenhouse preliminary tests with actinomycetes. The recent sequencing of *R. solanacearum* genome and novel ecological and epidemiological studies are expected to cause impacts on new ways to fight BW, including using biological control.

Integrated management of bacterial wilt on field-grown tomatoes

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New tactics are developed for the management of bacterial wilt on tomatoes caused by *Ralstonia solanacearum* (Rs). Thymol used for soil fumigation consistently provided significant reductions in bacterial wilt incidence and resulted in significantly higher tomato yield in repeated field trials. To develop practical application methods, thymol was applied through drip irrigation lines and it reduced significantly the disease incidence, indicating application of thymol through drip irrigation is a feasible method for bacterial wilt control. Application of Acibenzolar-S-methyl (ASM) significantly reduced bacterial wilt incidence on moderately resistant tomato cultivars, such as Neptune, BHN 466 and FL 7514. While ASM was not effective on susceptible cultivars when high concentrations of Rs were used, ASM in conjunction with pre-plant soil fumigation by thymol significantly improved disease reduction on a susceptible cultivar Solar Fire compared with either thymol or ASM applied alone. Other biorational products reduced disease incidence significantly in greenhouse experiments when used alone or in combinations with other compounds. Sorghum-sudan and rye as cover crops reduced Rs population in artificially infested fields to undetectable levels in a two-year study. These studies indicate that thymol and ASM have the feasibility to be used as a fumigant and a plant activator, respectively, in an integrated management program of bacterial wilt of tomatoes.

Integrated management of tomato bacterial spot in Florida

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In order to develop more sustainable and integrated strategies for reducing tomato bacterial spot severity, we investigated various combinations of biological control agents, including bacterial antagonists, plant growth promoting rhizobacteria (PGPR), host-specific bacteriophages, and systemic acquired resistance (SAR) inducers in greenhouse and field experiments. Copper hydroxide was applied as a standard bactericide treatment, while untreated plants served as untreated control. Results from the greenhouse experiments showed insignificant effect of antagonistic and PGPR strains on the disease severity. Therefore, we further investigated combinations of SAR compounds (harpin protein, acibenzolar-*S*-methyl), and formulated bacteriophages for controlling tomato bacterial spot in the north and central Florida fields. In three consecutive seasons, the combination of formulated phages and acibenzolar-*S*-methyl (ASM) provided reduction in disease pressure and resulted in more efficient foliar disease control than the other treatments. Although there was no significant increase in yield by this combination, there appeared to be a trend of higher yield harvested from plots treated with phage, either alone or in combination with SAR compounds, than in plots receiving no phage applications. When results of phage- and corresponding non-phage treatments were grouped and compared by using SAS CONTRAST analysis, it was shown that phage-treated plants produced significantly more marketable fruits than plants not receiving phage. Based on these experiments, the

combination of ASM and bacteriophage may be an effective new tool for tomato growers to manage bacterial spot.

A study on the biological control potentials of fluorescent pseudomonad strains against bacterial blotch disease (*Pseudomonas tolaasii*) of cultivated mushroom

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Mushroom growing is subjected to some serious diseases in Turkey because of insufficient growing techniques. Bacterial Blotch disease caused by *Pseudomonas tolaasii* is the most serious problem of cultivated mushroom (*Agaricus bisporus*) growing. The objective of this study is to find out some effective antagonistic fluorescent pseudomonads (FP) for the biological control of this disease. In this study, 38 fluorescent pseudomonad (FP) strains were tested for biological control of Bacterial Blotch disease of cultivated mushroom caused by *P. tolaasii*. FP strains such as 12, 13, 37, 50, 51, 74, M4/2, M5/3, and 39A, which were sprayed on freshly harvested detached mushroom caps decreased the disease severity of Bacterial Blotch by 70-100%. The selected candidate FP strains (M4/2, M5/3, and 39A) effectively inhibited the disease development on detached mushroom caps and were tested for biological control of *P. tolaasii* under mushroom growing house conditions. The suspensions of FP strains (10^{11}CFU/ml) were applied at three different growing stage of mushroom: five days after casing soil, at the stage of button, and before the harvest. A suspension of 10^8 cfu/ml of the pathogen was sprayed one day after the first application of the antagonists. A randomized complete block design was applied with five replications. Disease severity was classified by a 0-3 scale: (0): healthy caps, (1): small spots up to 3, (2): numerous small spots, (3): extended blackening and rotting. FP strains M-4/2 and M-5/3 decreased the severity of disease caused by *P. tolaasii*, by 88% and 85 %, respectively, when FP strains were applied at the stage of button.

Molecular markers of basal resistance, an inducible defense mechanism of plants against bacteria

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Basal resistance (BR) of plants has gained a burst of attention in recent years due to its molecular relations to innate immunity in animals. Most studies concentrated on relatively late (24-48 h) events after BR elicitation that affect only challenge bacteria. We have pointed to a possibly significant natural role of BR, which depends on its response velocity and affects the very bacteria used as elicitors. Using genomics and proteomics we have identified several molecular markers of early BR, some of which may prove candidates for improving resistance of plants against a broad range of pathogens. The early BR- specific transcriptional changes encompass an astonishing large portion (12%) of genes. Outlines of several pathways leading to

this complex response emerge, ranging from basic metabolism to stress responses and cell wall modifications. In the apoplast where bacteria and plants actually encounter, novel chitinases and other putative effector proteins appear. The chitinases may directly affect general bacterial metabolism and/or potentiate plant signal transduction towards more effective resistance.

Monitoring the biocontrol agent *Pseudomonas fluorescens* EPS62e by means of Real-Time PCR

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Real-Time PCR has been developed in order to detect and quantify the biological control agent of fire blight *Pseudomonas fluorescens* EPS62e after field release. RAPD and U-PCR were used to find natural genomic markers within EPS62e genome which differentiate the biocontrol strain against other *P. fluorescens* strains. Two differential amplified fragments were sequence characterized as SCAR markers, and no homologies against known sequences from the GenBank Database were found. Two SCAR primer pairs were designed, the 450 SCAR primer pair which amplified a 177 bp fragment and the 900 SCAR primer pair which amplified a 392 bp fragment. Both primers were selected for their specificity against EPS62e, as they amplified neither the 162 strains of *P. fluorescens* tested, nor the 71 strains of other closely related species analysed. Then, a Real-Time PCR was designed within each SCAR sequence developed, minimising the length of the amplification product and designing a TaqMan® probe for each fragment. The specificity of both designs was verified. Finally, the new molecular monitoring method was validated against a classical microbiological monitoring method based on dilution plating on selective media and colony forming units counting. The experiment was developed on blooming Golden trees that were sprayed with an EPS62eNal mutant suspension and were periodically sampled during 55 days. Each sample was doubly evaluated by means of the Real-Time PCR and the plating method. There were no significant differences between both techniques regarding the EPS62e population level estimated. Moreover, the biocontrol agent colonized and survived well on apple flowers in field conditions, reaching population values of 10^8 CFU/blossom and remaining stable at this level in immature fruits for 55 days after inoculation.

Chemistry of apple and pear stigma exudates related to bacterial antagonism toward *Erwinia amylovora*

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Fire blight of apple and pear is most commonly initiated by epiphytic populations of *Erwinia amylovora* that first become established on flower stigmas. Since microbial activity on the stigma is largely supported by the presence of fluidal exudate, knowing the chemistry of this substance could lead to advancements in biocontrol. When stigma exudates from apple were analyzed, identifiable components by weight were 48%

complex carbohydrates, 46% protein, 6% free sugars, and <0.1% free amino acids. Predominant free sugars in exudates from apple and pear were glucose and fructose in near-equal proportions; predominant free amino acids were asparagine, glutamine, proline and serine. These sugars and amino acids were incorporated in a liquid stigma-based medium (SBM), to which selected bacterial antagonists were added in advance of *E. amylovora*. Differences in antagonist capacity to suppress growth of *E. amylovora* in SBM were comparable to results of inoculations performed with detached crab apple flowers. Also, SBM results mimicked those of flowers better than did other synthetic media or altered forms of SBM. For instance, replacement of the glucose-fructose combination in SBM with the weight equivalent of glucose alone had an outcome less similar to that of flowers. Efforts to identify modes of antagonism in SBM point to competitive exclusion and antibiosis; however, pathogen suppression correlated best with the capacity of antagonists to increase medium acidity to a level unfavorable for *E. amylovora*, a phenomenon observed in vitro by early workers (Goodman, 1965; Riggle and Klos, 1972). Preliminary investigations with inoculated apple flowers also indicate pH modification on the stigma. Further investigations of stigma chemistry related to microbial interactions should prove advantageous in maximizing the use of biocontrol agents for fire blight.

A new natural anti-microbial product for use as an agricultural bactericide and fungicide

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KOPPERT Biological Systems; R&D Microbials

A new natural fungicide/bactericide has been developed on the basis of the so-called LP-system, a anti-microbial system active in bovine milk. The activity of the LP-system (lactoperoxidase system) is the enzymatic formation of reactive oxygen molecules which react with proteins in micro-organisms, such as bacteria and fungi. Those micro-organisms are killed as a result of this. The currently developed formulation is applied as a curative contact fungicide and initially targeted to control powdery mildew in greenhouse vegetables and ornamentals. Results will be presented from various crops. The product can be used also to control bacterial diseases. Initial test results on fire blight in apple will be given.

The registration in the EU of a product based on naturally occurring substances will be discussed, as well as possibilities to control other fungal and bacterial plant diseases.

Screening biocontrol agents for control of seed-borne bacterial pathogens of carrots and brassicas

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As part of an EC co-funded project to identify and develop seed treatments for organic vegetable production (STOVE), a number of potential biocontrol agents (BCAs) were examined for their efficacy in controlling *Xanthomonas hortorum* pv. *carotae* and *X. campestris* pv. *campestris*, the causal agents of bacterial blight of carrot and black rot of brassicas, respectively. Seed-borne bacterial pathogens present particular experimental difficulties due the relatively low (but epidemiologically significant) levels of infestation found in naturally infested seed lots. Potential BCAs were initially screened in vitro for inhibition/antagonism against the target pathogens. The best potential BCAs from the first screening were then applied to naturally infested seed and their effects on pathogen transmission (from seed to seedling) were assessed in glasshouse experiments. Finally the most effective was screened in a field trial (carrot) or further glasshouse transmission experiments (brassicas). The results obtained to date will be presented.

Tomato polygalacturonase inhibiting protein (PGIP) is not induced by the pathogen but increased with ageing

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In this research study the inhibiting effect of PGIP on the polygalacturonase (PG) enzyme from highly virulent isolates of *Fusarium oxysporum f. sp. lycopersici* by protein extraction was carried out with various organs (stem, leaf and root) of 40, 60 and 80 days old tomato *plants (Lycopersicon esculentum* cv. FDT 202). The study of PGIP-PG interaction showed a correlation between the plant age and an increasing inhibitory effect of PGIP with higher effect of stern PGIP. There were some differences in protein patterns of induced and non-induced tomato plants by *F. oxysporum.* Tomato PGIP showed different inhibitory activity on PG extracted from different phytopathogenic isolates of *F. oxysporum.* Also, for pgip gene detection in different tomato, an expected 1174bp fragment was amplified and confirmed by Hind III and EcoRI restriction enzymes.

Risk assessment when working with biological agents

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It has been known since the early days of microbiology that working with micro-organisms may present various degrees of health hazards not only to the workers involved, but also to the community and possibly to the environment. Considering the yet continuously growing importance of biotechnology and gene technology it is thus understandable that both international and national authorities have developed regulations which are aimed at minimizing any hazard to workers' health that may be related to the exposure to micro-organisms or other biological agents at work. For Germany, these regulations include Directive

2000/54/EC, Biostoff-Verordnung (BioStoffV), Gentechnik-Gesetz (GenTG), and Gentechnik-Sicherheitsverordnung (GenTSV). Directive 2000/54/EC provides in Article 2 the definition of four risk groups into which biological agents shall be classified. Although this classification is rather artificial than natural it provides a feasible means of assigning adequate safety measures to work with various biological agents in that for each risk group a specific containment or biosafety level (Schutzstufe) is defined under which work with the respective agents has to be performed.

Although classification into risk groups according to Directive 2000/54/EC is only based upon the level of risk of human infection originating from a given microbial species or variety, it is a highly complex task which needs to be based upon well-defined criteria as they are provided by the German TRBA 450 (**T**echnical **G**uideline for **B**iological **A**gents) in order to be scientifically sound and reproducible. Risk groups 1 and 4 organisms are usually easy to allocate while members of risk groups 2 and 3 are more difficult to define. In particular risk group 2 agents represent a broad spectrum of microbes which still differ considerably in their ability to cause human infective processes. The border-line between risk groups 1 and 2 is especially difficult to draw although it is of utmost practical importance.

On the basis of typical examples, the rationale of risk grouping and risk assessment in Germany will be given and various problems and peculiarities will be discussed.

Antagonistic activity of different yeast *spp*. against *Erwinia amylovora*

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Epiphytic yeast strains of *Aureobasidium pullulans* and *Metschnikowia pulcherrima* have shown their antagonistic activity against *Erwinia amylovora* in field-, greenhouse- and laboratory experiments. Colonisation of stigma and hypanthium of apple blossoms and suppression of the fire blight pathogen on the stigma was observed. The mode of action may be a combination of effects resulting in the suppression of *E. amylovora*. Furthermore yeast isolates of the apple phyllosphere and strains from culture collections, antagonistic against postharvest diseases on apples, were investigated for their ability to control fire blight. First results of agar diffusion assays, co-culture experiments and the suppression of the fire blight pathogen on detached apple blossoms indicated various effects of individual yeast *spp*. against *E. amylovora*. The investigated yeasts seem to provide different modes of action. Further investigations will show whether these yeasts are suitable to control fire blight in the field.

Biocontrol and plant growth promoting potential of a *Pseudomonas* sp. MML2212 from the rice rhizosphere

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In recent years fluorescent pseudomonads have drawn much attention as plant growth promoters and biocontrol agents. They effectively control plant diseases by secretion of secondary metabolites and improve plant growth by production of phytohormones or plant growth promoting substances. Considering their potential, we have initiated research to identify efficient fluorescent pseudomonads. A total of 460 fluorescent pseudomonads were isolated from the rice rhizosphere soils collected from Tamil Nadu State of India. They were screened for their antagonistic activity against important phytopathogens *viz., Rhizoctonia solani, Pyricularia oryzae, Macrophomina phaseolina, Fusarium udum, Alternaria alternata, Bipolaris oryzae* and *Curvularia lunata* by dual culture assay. Owing to superior antagonistic performance against the fugal phytopathogens over other strains, an efficient bacterial strain (MML2212) was selected for further studies. Later this new strain was identified as *Pseudomonas* sp. by various morphological, physicochemical analyses mentioned in the Bergey's manual.

The growth pattern of *Pseudomonas* sp. MM2212 in different media, its tolerance to antibiotics, heavy metals and fungicides were studied. The mechanisms of biological control exerted by the *Pseudomonas* sp. MML2212 were investigated. The culture filtrate of the new strain significantly inhibited the mycelial growth, conidial and sclerotial germination of fungal phytopathogens. Comparison of PCR product of *Pseudomonas* sp. MML2212 with other fluorescent pseudomonads strains indicated that the new strain does not produce major antifungal metabolites such as 2,4-diacetylphloroglucinol, pyrrolnitrin and pyoluterin. However, it was producing hydrogen cyanide and siderophores. In addition, the strain *Pseudomonas* sp. MML2212 produces two antifungal compounds with the Rf values of 0.67 and 0.36. Development of fermentation process and separation and purification of these antifungal compounds are in progress.

Besides the biocontrol potential, the *Pseudomonas* sp. MML2212 significantly improves the plant growth. Seed treatment and soil application of *Pseudomonas* sp. MML increased the root and shoot lengths in rice as compared to control. This strain was multiplied in fermentor and formulated in talc powder. The shelf life of the bacterium in the formulation and its efficacy in controlling sheath blight of rice under field conditions are to be investigated in order to recommend this bacterium as a biocontrol agent.

Optimized mixtures of biological control agents for suppression of fire blight

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Bacterial antagonists *Pseudomonas fluorescens* A506 and *Pantoea agglomerans* C9-1 decrease the incidence of fire blight on pear and apple by an average of 30% and 45%, respectively, compared to water-treated controls in our pathogen-inoculated orchard trials. Disease control is primarily due to suppression of epiphytic growth of the pathogen *Erwinia amylovora* on stigmas of flowers. A506 suppresses floral colonization by the pathogen by competitive exclusion. Strains of *P. agglomerans*, such as C9-1, suppress pathogen growth by competition and the production of peptide antibiotics. When A506 and C9-1 were applied as

mixtures, establishment and growth of antagonists was enhanced compared to single strain inoculations. Biocontrol efficacy, however, did not increase; a mixture of A506 and C9-1 provided an average of 48% disease control, which was not significantly different from that of individual antagonist strains. In laboratory assays, we found that A506 produced an extracellular metalloprotease that detoxified an antibiotic of C9-1. We attributed the lack of synergism of the two strains for disease control to 'mechanistic incompatibility' or the inactivation of an antibiotic of C9-1 by the protease of A506. We derived mutants of A506 that were deficient in production of the extracellular protease. The protease-deficient mutants colonized flowers and provided similar levels of disease control as wild-type A506. Combining a protease-deficient derivative of A506 with C9-1 increased the level of disease control to an average of 60%, which was significantly greater than single strain inoculants. Altering A506 to be mechanistically compatible with its co-inoculant C9-1 improved disease suppression and decreased variability in biological control of fire blight of pear and apple.

Sensitivity to biological control of *Agrobacterium vitis* is related to its pTi

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Crown gall of grape caused by *Agrobacterium vitis* can be controlled in model experiments in greenhouse by a nontumorigenic strain of *A. vitis* (F2/5). The mechanism by which F2/5 inhibits crown gall of grape is not fully understood. Previous studies have shown that agrocin produced by this strain is not a major factor in the mechanism of biological control. However, transfer of T-DNA of *A. vitis* to grape was greatly inhibited in the presence of F2/5.

In this study, hybrid strains prepared from *A. tumefaciens* C58 chromosomal background (NT1,UBAPF2) and *A. vitis* Ti plasmids (Ag162, AB4) were controlled by F2/5 on grape shoots. The hybrid strains produced normal galls on grape shoots, were resistant to agrocin, and transferred their T-DNA to grape in gus-assays. However, when they were mixed with F2/5, their gall formation and T-DNA transfer to grape tissues were perfectly inhibited. In the control experiment wildtype *A. tumefaciens* strain C58 was not inhibited in gall formation. As expected, the wildtype *A. vitis* strains carrying the pTis that were used to generate the hybrid strains were perfectly inhibited by F2/5. We conclude that biological control on grape shoots by F2/5 depends on pTi of *A.vitis*.

Status of research on biological control of tomato and groundnut bacterial wilt in Vietnam

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Bacterial wilt caused by *Ralstonia solanacearum* is one of the most destructive bacterial diseases on many crops in Vietnam. The disease regularly causes significant damage to tomato and groundnut. In the past,

research on bacterial wilt in Vietnam mainly concentrated on host-plant resistance. Only in very recent years, some research efforts have been initiated on biological control of bacterial wilt of tomato and groundnut.

Two strains of *Pseudomonas fluorescens* (B16 and VK58) and one strain of *Bacillus subtilis* (B1) were identified to be promising for biological control of bacterial wilt. They were tested and showed high effectiveness in control of bacterial wilt on tomato in both greenhouse and field conditions of Northern Vietnam.

In a field trial conducted in Bac Giang, disease percentage was reduced by 56%, 51% and 46% by VK58, B16 and B1, respectively. In Bac Ninh, disease was reduced by 58%, 52% and 47% by VK58, B16, and B1, respectively. In Ha noi, VK58, B16, and B1 reduced bacterial wilt severity by 54, 50 and 45%, respectively. In these field trials tomato yield increased by 35-46%.

On groundnut, using *Pseudomonas fluorescens* strains for seed treatment also showed relatively high effectiveness in control of bacterial wilt (the percentage of diseased plant was reduced by 69 % compared to control).

Some efforts have also initiated to study the possibility of application of induced systemic resistance in control of bacterial wilt on tomato and groundnut, using salicylic acid and rhizobacteria. However, this method still shows low effectiveness in field conditions. Research in this direction now continues extensively in Vietnam.

Initial results indicated that research on biological control of bacterial wilt should be encouraged in coming years.

Molecular biology of plant-associated bacteria involved in biocontrol

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In this introductory lecture, our recent progress on the molecular biology of plant-associated bacteria involved in biocontrol mechanisms will be dealt with. Genetic tools for fluorescent pseudomonads, *Erwinia* and *Agrobacterium* species, as well as gram-positive biocontrol organisms have been developed and are now available in many laboratories. More importantly, the complete and, in part, fully annotated genome sequences of many relevant biocontrol organisms have been published and are easily accessible via the internet. A systemic approach to combine these sequence information with transcriptome analyses of various biocontrol systems is the desirable major point for the current and future research. Recent advances in the molecular biology of biocontrol bacteria are discussed. In addition, an example for a resistance mechanism of a plant-pathogen, *Erwinia amylovora*, towards a biocontrol organim, *Panthoea agglomerans*, will be introduced and general consequences will be presented.

Biocontrol of bacterial canker disease of tomato using *Pseudomonas fluorescens*

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Use of plant growth promoting rhizobacteria in managing bacterial canker disease of (*Clavibacter michiganensis* ssp. *michiganensis*) tomato was studied in the present work. Tomato seeds were treated with five strains of *Pseudomonas fluorescens* and were selected for seed germination and seedling vigor. Among the *P. fluorescens* strains tested only three strains (*Pf*3, *Pf*7a and *Pf*11) which showed enhancement in the seed quality parameters like seed germination, seedling vigor, were further subjected to estimation of one of the defense related enzyme phenylalanine ammonia lyase (PAL) with total phenol contents. The same three strains were recorded for maximum disease protection under greenhouse conditions. The level of PAL and total phenol contents increased significantly upon the PGPR treatment. The rate of reduction in the bacterial canker disease incidence was directly proportional to the increase in the level of PAL and total phenol content. The possible use of these *P. fluorescens* strains in effective management of bacterial canker of tomato is discussed in the present work.

Antibiotics produced by strains of *Pantoea agglomerans*, biocontrol agents of fire blight

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Strains of *Pantoea agglomerans* (syn. *Erwinia herbicola* and *Enterobacter agglomerans*) are ubiquitous in nature, inhabiting diverse environments, such as plants, water, soil, humans and animals. It is considered to be an opportunistic pathogen of animals and humans, a direct cause of septicemia and an allergen. *P. agglomerans* has been isolated from internal organs of deer, from human throats and from stool specimens in patients with typhoid fever. In fire blight lesions on rosaceous plants, these bacteria occur as saprophytes. Often they are isolated from lesions in conjunction with the fire blight pathogen, *Erwinia amylovora*. They are Gram-negative, rod-shaped and form yellow colonies on growth media. Strains of *P. agglomerans* have been used as protectants for fire blight in the laboratory and in orchards. Many strains produce antibiotics belonging to diverse chemical classes. However, several that have been studied are peptide-based compounds. Their antibiotic activity against *E. amylovora* is apparent on minimal media, and their activity is inhibited by the presence of specific amino acids. Enzymes involved in the biosynthetic pathway for the inhibiting amino acid often are the cellular targets of the compounds. The role of these antibiotics in nature is not fully understood. For instance, if specific amino acids are present in the nectar of blossoms of the pomaceous plants that *P. agglomerans* inhabits, these amino acids presumably would inhibit the activity of such peptide-based antibiotics. Therefore, it does not seem likely that these antibiotics would be inhibitory to *E. amylovora* in blossoms. The current literature on *P. agglomerans* as a biocontrol agent and an opportunistic pathogen, as well as the literature on its antibiotics and the role of antibiosis in biocontrol will be reviewed.

Biological control of black rot of vegetable brassicas, caused by *Xanthomonas campestris* pv. *campestris*, with endophytic *Bacillus* strains

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The Plant Pathology Section at the Royal Veterinary and Agricultural University in Denmark has successfully used *Bacillus* strains in the control of black rot of brassicas in both Tanzania and Zimbabwe. One of the promising *Bacillus* strains (strain BB) showed endophytic ability and could be re-isolated from plants growing under greenhouse and field conditions. Preliminary results suggested that induced resistance seems to be one of the mechanisms involved in the control of black rot by the tested strain BB.

Phenotypic and molecular characterization of the interaction of antagonistic bacteria with *Ralstonia solanacearum*, causing tomato bacterial wilt

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Bacterial wilt caused by *Ralstonia solanacearum* is a devastating disease attacking over 200 plant species in the tropics and subtropics. Control of bacterial wilt in tomato is difficult, if not impossible. Therefore, biological control methods as part of integrated approaches appear promising to reduce the disease. Among 44 strains of rhizosphere bacteria screened *in vitro* for antagonism against *R. solanacearum* and for production of antimicrobial compounds and rhizosphere competence, five strains (*Bacillus pumilus* A8, *B. atropheus* A9, *B. subtilis* CH6, *Pseudomonas fluorescens* CMR03 and *P. fluorescens* CRS02) were analysed for their efficiency against tomato bacterial wilt *in planta* in pot and split root experiments. Disease severity and wilt incidence were reduced by up to 41% and 68%, respectively, while increasing fresh weight by over 170% compared to infected control plants. Antagonist-triggered induced resistance with reduced pathogen populations by more than two magnitudes from 10^5 to 10^2 CFU/g were observed in split-root experiments of genotype King Kong2 treated with antagonist *B. pumilus* A8. Immunofluorescence microscopy and immuno-tissue prints revealed a decrease in number of fluorescent vessels indicating reduced production of phenolic compounds after A9 treatment in the infected genotype NHG3, while the fluorescence in A9-treated NHG167 was caused by an increase of arabinogalactan-protein epitopes in midstems, suggesting an induced resistance expressed by increased synthesis of hydroxyproline-rich glycoproteins (HRGPs). Additionally, the tolerance to high bacterial numbers was increased after treatment with *B. atropheus* A9.

P O S T E R - Presentations

Studies on induced resistance against fire blight (*Erwinia amylovora*) with different bioagents

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Induced systemic resistance (ISR) as a general phenomenon in plants was already described very early in plant pathology. In compatible host parasite interaction bacterial pathogens, abiotic agents and stress have been largely used to induce systemic resistance against bacterial diseases (Goodman et al. 1986). In the following study the three bioagents Bion®, BioZell-2000B and the antagonistic strain Ra39 were assayed on their ISR- potential effect against fire blight on the highly susceptible apple rootstock M26 in pathological and physiological investigations.

The disease index of fire blight in shoots of M26 rootstock plants was suppressed by BION®, Bio Zell-2000B and Ra39 up to 64, 31 and 59% respectively. This was correlated with a decreasing effect on the growth of bacteria up to 64, 50 and 64% respectively during the course of infection.

Increase in ß-glucosidase and chitinase activity were found after treatments with BION®, BioZell-2000B and Ra39 in both non inoculated and inoculated shoots after 6 and 4 days, respectively. In uninoculated shoots, β-glucosidase activity increased by 133, 33 and 100%, respectively, after 6 days application. Also chitinase activity enhanced after the same period by 156, 108 and 174%. Moreover in inoculated shoots β-glucosidase activity increased by all treatments by 100, 67 and 83 % after 4 days application respectively. Also chitinase activity was enhanced after the same period by 98, 32 and 63% 6 d. p. i., respectively.

- all 3 bioagents were able to induce a defense reaction against fire blight in the tested host plant,
- a reduction in symptom development was correlated with a decrease in the bacterial multiplication,
- enzymatic changes indicated an induced resistance reaction by all bioagents.

Evaluation of the diversity of *Erwinia amylovora* in Bulgaria

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Fifty one strains of *Erwinia amylovora* were isolated from various host plants (pear, apple, quince, chokeberry, strawberry, hawthorn, *Cotoneaster* and *Pyracantha*) growing in distant regions of Bulgaria. The

strains were characterized and identified using API 20E, BIOLOG and PCR-amplification of specific regions in chromosome and pEA29 plasmid. The diversity among the strains was studied by different approaches – determination of antibiotic resistance, total cellular proteins, metabolic activity, macrorestriction analysis of chromosome DNA. The strains showed very similar reaction to antibiotics tested and all were sensitive to the streptomycin. The protein profiles obtained after SDS-PAGE revealed high similarity among the strains studied. At 75 % similarity they were grouped in four clusters. Cluster I included 24 % of the strains, cluster II - the type strain of *E. amylovora* and 61 % of Bulgarian isolates, cluster III – 13 % of the isolates, and cluster IV consisted of only one strain. The determination of the metabolic fingerprints of the isolates was performed by BIOLOG system. At 75 % similarity the strains were grouped in three clusters. The patterns obtained by *Xba*I digest after PFGE allowed us to distinguish five groups of bulgarian *E. amylovora* strains. The major group included 79 % of them and their profile is type Pt2. We established a new PFGE profile unknown in the literature referred to the strawberry strains. The PFGE patterns obtained by *Spe*I digest were similar.

Biocontrol of phytopathogenic bacteria by using organic compost

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Bacterial strains were isolated from soil samples obtained from field areas amended with organic compost. Their potential biocontrol activity with respect to several bacterial pathogens was verified by using different *in vitro* tests. Potential biocontrol activity of bacterial strains isolated from compost were carried with respect to phytopathogenic bacteria (*Agrobacterium tumefaciens*, *Brenneria* spp., *Clavibacter michiganensis* subsp. *michiganensis*, *P. avellanae*, *P. viridiflava*, *P. s.* pv. *actinidiae*, *P. s.* pv. *syringae*, *P. s.* pv. *tomato*, *P. savastanoi*, *Xanthomonas vesicatoria*) able to cause severe damages at rizhosphere and at phyllosphere level on different host plants. The biocontrol activity of bacterial strains isolated from organic compost has been revealed. In particular, Gram negative strain IF25 showed a wider control activity of different bacterial plant pathogens. It was characterised by using BIOLOG Identification System and the results obtained suggest that IF25 may be referred to as *Burkholderia pyrrocinia*, a species that is included in the *Burkholderia cepacia* complex (Bcc). Bcc currently comprises a cluster of nine species that are an object of growing interest because of their capacity to act as plant growth promoting, biocontrol and bioremediation agents.

Control of Asiatic Citrus Canker and Citrus Bacterial Spot with bacteriophages in Florida

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Asiatic citrus canker, caused by *X. axonopodis* pv. *citri* (*Xac*), is a serious threat to Florida's $8.5 billion citrus industry. The pathogen was introduced to Florida several times during the 1990's, and the disease is currently under eradication. However, recent hurricane events hindered the eradication efforts and distributed the pathogen over wide areas. Several control measures of the disease are under development including the use of bacteriophages.

A collection of 81 phages was established from the following sources: 13 *Xac* phages from existing collections, 4 phages of other Xanthomonads that are also pathogenic on *Xac*, and 64 phages isolated from diseased plant tissue in Florida and Argentina. In greenhouse experiments a single foliar application of a mixture of four phages (2×10^8 PFU/ml) significantly reduced the disease severity caused by a "Miami" strain of *Xac* (10^6 PFU/ml). Field trials were conducted with citrus bacterial spot (CBS), incited by *Xanthomonas axonopodis* pv. *citrumelo* (*Xacm*), since the current regulations do not allow field research with citrus canker in Florida. In trials conducted in a commercial citrus nursery, twice-weekly application of a mixture of three phages at 2×10^8 PFU/ml significantly reduced the CBS disease severity.

An early oxidative burst in apple rootstocks treated with DL-β-Amino butyric acid (BABA) against *Erwinia amylovora*

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Systemic acquired resistance (SAR) was induced by pretreatment with a chemical inducer DL-β-Amino butyric acid against fire blight disease caused by *Erwinia amylovora* (Ea 7/74). Different doses of DL-β-Amino butyric acid (250-1000 µg/ml doses) were tested on apple rootstock M9. The plants were inoculated with 10^8 CFU/ ml bacterial suspension, and disease development was evaluated up to 10 days post inoculation. Although *in vitro* growth of the bacteria was not affected by DL-β-amino butyric acid treatment, its spray (500 µg/ ml dose) significantly reduced disease severity up to 42 % and bacterial population (up to 57 %) by 96 h post inoculation. DL-β-Amino butyric acid treated plants showed significantly higher H_2O_2 generation, compared to untreated plants. These findings indicate that pre-treatment with the chemical inducer DL-β-Amino butyric acid activated H_2O_2 generation *in planta* more strongly when the plants challenged with pathogen, and these cases may be associated with induction of plant resistance to bacterial pathogen and affect of BABA in modulation of pathogen defense pathways.

Induction of oxidative burst in tomato leaves treated with unsaturated fatty acids of turtle oil (*Caretta caretta*) against *Pseudomonas syringae pv. tomato*

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Tomato bacterial speck, caused by bacteria *Pseudomonas syringae* pv. *tomato*, is effectively controlled on tomato (*Lycopersicon esculentum* Mill.) by a foliar spray of unsaturated fatty acids. Induced resistance effect of different UFAs on plant resistance was investigated on three-week old plants. The plants were inoculated with 10^8 CFU/ ml bacterial suspension and, bacterial growth were evaluated by day 7 after inoculation. Resistance induced by unsaturated fatty acids (UFAs) oil showed a suppressive effect against *Pseudomonas syringae* pv. *tomato*, and was correlated with rapidly resistance response of treated leaves, challenged with pathogen by day 1 after treatment. Bacterial multiplication 120 fold in linoleic acid, 150 fold in decosohexaenoic acid and 180 fold reduced in eicosapentaenoic acid treated plants by 2 day post inoculation. These values were significantly lower than control plants after inoculation. In order to understand the reason of suppressive effect of UFAs H_2O_2, NADPH oxidase generation were investigated in the early phase of inoculation. In these studies, the plants sprayed with linoleic acid, decosohexaenoic acid and eicosapentaenoic acid accumulated H_2O_2 and had elevated the levels of NADP(H) oxidase production. However, oleic acid failed to show higher NADP(H) oxidase increased by linoleic acid, decosohexaenoic acid and eicosapentaenoic acid treatment. These findings indicated to fail of suppressive effect of exogenously oleic acid application in plant defense pathways. No considerable changes were observed in water-treated plants. These findings suggest that linoleic acid decosohexaenoic acid and eicosopantaenoic acid activates plant defense mechanism, and the treatments of UFAs leads to induction of active oxygen species, acting as mediates of plant immunity against bacterial pathogen.

Antibacterial activities of essential oils from medicinal plants against the growth of *Clavibacter michiganensis* subsp. *michiganensis*

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In the present study, antibacterial activities of essential oils obtained from aerial parts of aromatic plants such as thyme (*Thymbra spicata* subsp. *spicata*), oregano (*Origanum syriacum* var. *bevanii*), mint (*Mentha spicata*) and lavender (*Lavandula stoechas* subsp. *stoechas*) were investigated against seed-borne plant pathogenic bacterium *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm* ICP7200), causal agent of bacterial canker or wilt of tomato. By using paper disc diffusion assay, all essential oils have shown anti-

bacterial activity against bacterial strain used. Essential oils used in the paper disc diffusion assay varied in their antibacterial activity. Essential oil from thyme was the most effective in inhibiting the growth of the *Cmm.* This was followed by essential oils obtained from of oregano and lavender respectively. By using micro agar broth dilution assay, minimum bactericidal concentrations of the essential oil of thyme, oregano and lavender were 10, 10, 25 and 50 µg/ml, respectively.

An early oxidative burst in apple rootstocks treated with DL-β-Amino butyric acid (BABA) against *Erwinia amylovora*

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Systemic acquired resistance (SAR) was induced by pre–treatment with a chemical inducer DL-β-Amino butyric acid against fire blight disease caused by *Erwinia amylovora* (Ea 7/74). Different doses of DL-β-Amino butyric acid (250-1000 µg/ml doses) were tested on apple rootstock M9. The plants were inoculated with 10^8 CFU/ ml bacterial suspension, and disease development was evaluated up to 10 days post inoculation. Although *in vitro* growth of the bacteria was not affected by DL-β-amino butyric acid treatment, its spray (500 ug/ ml dose) significantly reduced disease severity up to 42 % and bacterial population (up to 57 %) by 96 h post inoculation. DL-β-Amino butyric acid treated plants showed significantly higher H_2O_2 generation, compared to untreated plants. These findings indicate that pre-treatment with the chemical inducer DL- β- Amino butyric acid activated H_2O_2 generation *in planta* more strongly when the plants challenged with pathogen, and these cases may be associated with induction of plant resistance to bacterial pathogen and affect of BABA in modulation of pathogen defense pathways.

Biological control agents as tools against some emerging bacterial plant diseases in Italy: a concrete perspective ?

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A modern management of crop protection should be based on integrated control programmes, including the use of environmentally safe products. Antagonistic/beneficial bacteria may have a great potential in the prophylaxis of diseases caused by common and quarantine pathogens. This work was - and is being carried out - to confirm the ability of the known strains IPV-BO G19 (*Pseudomonas putida*) and IPV-BO 4027C (a non fluorescent *Pseudomonas* sp.) against fire blight (*Erwinia amylovora*), as well as to evaluate their efficacy against southern bacterial wilt of tomato (*Ralstonia solanacearum*), bacterial spot/canker of stone fruits (*Xanthomonas arboricola* pv. *pruni*) and grapevine crown gall (*Agrobacterium vitis*). In the labora-

tory, there was a trend toward a higher colonisation of apple and pear flowers when Na-alginate (0.5%) was added to suspensions of freeze-dried cells (approx. 10^7 cfu/ml) of wild type and rifampicin resistant mutants of both strains; *E. amylovora* populations on apple flowers were significantly reduced by strain IPV-BO 4027C. In a field experiment, performed on actively growing shoots of pear scions cv. Abbé Fétel during spring-summer 2005, different treatments were also tested, including copper compounds, the resistance inducers prohexadione-Ca and acybenzolar-S-methyl, Serenade and BS-F4 (*Bacillus subtilis*), etc. The strain IPV-BO G19 plus Na-alginate gave the highest level of relative protection (65%), six weeks after wound inoculation with *E. amylovora*. The enzymatic activity of catalase (CAT), superoxide dismutase (SOD) and guaiacol peroxidase (POD) was also estimated as markers of resistance in pear leaf tissues. A virulent strain of *R. solanacearum* race 3 was inhibited by both antagonists on plate. When the pathogen was inoculated 48 h after their application (approx. 10^8 cfu/ml) to the root apparatus of tomato plants grown in a climatic chamber, bacterial wilt progression rate was clearly reduced; in particular, IPV-BO G19 gave the highest relative protection (84%), twelve days after pathogen's inoculation. An average of approx. 55% of the plants treated with the antagonists did not show any typical symptom: nevertheless, *R. solanacearum* was detected by direct isolation and PCR in bulk samples of disks from the basal part (8-10 cm) of stems. The strain IPV-BO G19 was effective against *X. arboricola* pv. *pruni* both *in vitro* and *in planta*: when sprayed on plum fruitlets cv. Angeleno it reduced bacterial spot incidence by 90%, eight days after inoculation with the pathogen.The anti-tumour efficacy of these bacteria and of other bioagents, able to inhibit the *in vitro* growth of virulent *A. vitis* strains, is being tested on grape-vines under glasshouse and vineyard conditions.

Pantoea agglomerans strain *HIP32*: A potential new antagonist of *Erwinia amylovora*

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Pantoea agglomerans strain HIP32 was isolated from the leaves of the apple cultivar 'Starking' by Hevesi and Al Arabi, in Hungary. Its efficacy was tested in detached apple flowers under controlled conditions: day/night temperature of 22/19°C, 85% relative humidity and diurnal lighting of 16/8 hours.

The first year of investigations (2004) detached flowers of 'Sampion' and 'Gala Must' apples were pre-treated with HIP32, incubated for 24 hours followed by the inoculation with *E. amylovora* strain Ea1 ($5x10^7$ cfu/ml). The 3rd day after inoculation the population size of *E. amylovora* was estimated by semi-quantitative PCR, and on the 5th day the remaining flowers were scored for disease development, using a modified rating scale of Pusey. The index of infection was calculated from the incidence and severity of symptoms. In 2005, 'Idared' apple trees were sprayed with HIP32. The initial population size of the antagonist was $>10^4$ cfu/flower. Detached flowers were treated and investigated as in 2004, except that *E. amylovora* Ea1 Kan^R ($5x10^4$ cfu/flower) was used for inoculation and its population size was measured by plating on selective medium.

HIP32 treatments resulted in a decrease of blighted blossoms' percentage by about 50% for 'Sampion' and even more for 'Gala Must' and 'Idared'. Both disease severity and the index of infection in flowers treated with HIP32 were reduced nearly to the half ('Sampion') and to one-third (Gala Must'). In the 'Idared'

flowers the disease severity and the index of infection was about 50% and 40% of the control values, respectively. The PCR-detectable population (involving both viable and dead cells) of *E. amylovora* was not decreased in 2004, however, while measuring viable cells only in 2005 the population size of the pathogen was reduced by about two orders of magnitude, due to the treatment with HIP32.

Experimental results on biological control of bacterial plant diseases in Venezuela

Nancy Contreras

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El uso de agroquímicos ha impactado de manera significativa la sostenibilidad de la agricultura, debido a la contaminación ambiental por el uso indiscriminado de estos productos los cuales han reducido la biodiversidad del ecosistema y ha n traído problemas a la salud pública. El control biológico basado en el antagonismo microbiano, la resistencia inducida en las plantas, el uso de plantas con propiedades antagónicas se vislumbra como una de las estrategias más prometedoras dentro del mar co del control integrado de enfermedades que afectan la producción de nuestros cultivos. Martinez y Colmenarez (2001) a nivel in vitro evaluaron el efecto de extractos de plantas con propiedades bactericidas, antiflamatoria, cicatrizante, astringente y de sinfectante, sobre el desarrollo de colonias de *Xanthomonas campestris* aislada del cultivo de la cebolla. Los extractos utilizados fueron *Caesalpinia corieriria* (fruto); *Allium sativum*, (bulbo); Bixa ore! llana (fruto); *Pterocarpus officinalis*m (savia) y *Zingiber officinalis* (raíz). Entre un 90 y 100%, de inhibición de crecimiento bacteriano se encontró en los primeros cuatro tratamientos y un 55% en el último. En otro expe rimento se evaluaron extractos vegetales de 12 familias de plantas por su efecto bactericida sobre bacterias patógenas del mango *(Mangifera indica)*, girasol (*Helianthus annuus*), lechoso (*Carica papaya*) y banano (< I>Musa sp.). Los mejores efectos bactericidas *In vitro* para el control del género *Erwinia* se consiguieron con *Eryngium foetidum*, *Ruellia*/ *Tuberosa* y cundeamor *Momordica charantia*. El extracto de *Melicocca bijuga* tuvo efecto bactericida sobre la bacteria *Pseudomonas* sp. que ataca el banano. Ningún extracto tuvo efecto sobre *Pseudomonas syringae* pv. *helianthi*.(Guevara, et al, ! 1999). Aplicaciones de biopreparados de *Trichoderma harzianum* dirigidos a hojas, vástagos, en plantón y sobre los hijos) bajaron los niveles de inóculo de la bacteria *Pectobacterium carotovorum*, se disminuyo la incidencia de la enfermedad hasta un 80%, y aumentó 40% el rendimiento por tallo (Bracho et al, 2001). De las alternativas de control biológico de bacterias fitopatógenas en Venezuela por los productores es la aplicación de biopreparados de *T. harzianum*. Esto se debe a la exis tencia a nivel comercial de diversas presentacionesdel producto. Asimismo, el uso de extractos naturales en un mediano plazo sería una excelente alternativa, ya que en nuestro país no se ha patentado la producción de estos productos. Por otra parte se ha identificado *P. agglomerans* en diferentes cultivos y a *P. fluorescens* en sustratos naturales para la siembra. El asunto que debe analizarse es que *P. agglomerans* está actuando como organismo fitop! atógenico y polífago en cultivos de Cereales, Ornamentales, Hortalizas, Musáceas y Zábila (*Aloe vera*) en Venezuela, demostrándonos esto la evolución de la bacteria que ha pasado de un estado epifitito a un estado patogénico en los rubros mencionados.

Biological control to protect watermelon blossoms and seeds from infection by Acidovorax ssp. citrulli

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Bacterial fruit blotch (BFB), caused by *Acidovorax avenae* ssp. *citrulli (Aac),* is a devastating disease of cucurbits that was first reported in commercial watermelon production fields in Florida, USA in 1989 (Somodi et al., 1991). Since then, the pathogen has been a significant threat to watern melon production worldwide. Infested cucurbit seeds represent the most importanl *Aac* inoculum source and there in zero tolerance for the pathogen in seed and transplant production systems. Despite attempts to exclude infested seeds, BFB outbreaks continue to occur sporadically with significant economic impact. In general, current seed treatments reduce, but fail to eliminate seedborne *Aac.* Since most commercial watermelons are produced via transplants under conditions that are highly favorable for disease development, even low levels of seedborne inoculum present a significant threat to BFB. Hence, to manage BFB it is critical to develop management strategies that prevent seed infection. Such strategies will depend on an accurate knowledge of the mechanisms by which seeds become infested. Recently, it was demonstrated that female watermelon blossoms could be a pathway of ingress for *Aac,* leading to seed infestation within symptomless fruits (Walcott et al., 2003). Based on this knowledge, it may be possible to prevent *Aac* seed infestation by biological or chemical protection to open female watermelon blossoms. If successful, this strategy could be easily incorporated into current hybrid watermelon seed production systems, in which hand pollination is employed. The objectives of this study were to:

1) identify biocontrol agents with antagonistic activity against *Aec*
2) evaluate the efficacy of blossom protection for preventing seed infestation

Field experiments for fire blight control by artificial and non-artificial infection of apple trees in 2005

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Until now there is no efficient procedure world wide to fight fire blight without using antibiotics. Field experiments were arranged at a site in Southern Germany (Kirschgartshausen) to test alternatives to control fire blight. The experimental design was well proven since eight years and follows EPPO standard PP 1/166(3). The guideline is based on artificial inoculation of single trees as source of high disease pressure for non-artificial infections of neighbouring trees. In 2005 results of non-artificial infected apple trees fulfilled the guideline of 5% infected blossoms in the untreated control but the demanded minimal number of blossom clusters of 200 was not reached in every plot. The results lack statistical confirmation but support the trend of previous years. However, the data support the results from previous experiments. The best re-

sults achieved Strepto (Streptomycin) with an efficacy of 81%. It was followed by Blossom Protect, a product based on two antagonistic yeast strains, applied after fire blight prediction (efficacy 59%) and in strategy with the yeast incompatible fungicide Dithianon (67%). The two antagonists *Erwinia tasmaniensis* and *Erwinia billingiae* showed with 52% and 48 % the third highest efficacies. The efficacy of two Serenade agents with *Bacillus subtilis* strains as active agents were 10% lower. Another yeast strain combined with a buffer (BPMSK), the resistance inducer Phytovital and FZB 42/FZB 24 with a *Bacillus subtilis* strain as active agent showed no effect. These results indicate, that there is still no alternative agent against fire blight, whose efficacy and reliability is comparable to streptomycin. Blossom Protect confirmed the good results of previous years while its applications were reduced from a maximum of four to a minimum of two. It was successfully applied in a strategy with the yeast incompatible fungicide Dithianon sprayed to control apple scab (*Venturia inaequalis*) during bloom. The effect of the yeast strains on fruit russetting is subject of further investigations. The two new antagonists showed promising effects. Serenade displayed a more or less constant effect around 50% efficacy. Further experiments are needed to confirm and improve the efficiencies of these agents.

Multiplication and spread of the avirulent strain NCPPB 3123 of *Clavibacter michiganensis* spp. *michiganensis* (proposed resistance inductor) alone and concomitantly witha virulent strain in tomato plants

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The avirulent bacterial strain NCPPB 3123 of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) was used by Griesbach *et al*. (2000) for resistance induction against the canker disease of tomatoes so that the appearance of wilt symptoms was delayed (Griesbach *et al*., 2000). We used a mutant of the resistance inductor with resistance against 600 ppm streptomycin ("GSPB 3134") and a mutant of a virulent Cmm-strain with resistance against 100 ppm rifampycin in order to exactly determine multiplication of both strains *in planta* (tomato cv. Lyconorma).

When inoculated alone, the virulent strain reached more than 10^{10} cfu/g fresh weight within 5 weeks, whereas the avirulent strain reached only 10^{7} very near to the inoculation point. However, when both strains were inoculated concomitantly, the avirulent strain reached 10^{9} cfu/g f.w.. Nevertheless, multiplication and spread of the virulent strain was not reduced by the avirulent strain. Also, symptom expression was not reduced by pre- or post-inoculation with the avirulent strain. It is concluded from our results that comparison of mutant strains differing in resistance against specific antibiotics allows a quantitative evaluation of the effect of resistance inductors.

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Antagonistic coccus type bacteria causing death of a coryneform type of bacteria, the pathogen of *Agave tequilana* Weber var. *Azul*

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Gram positive, Coryneform type pathogenic bacteria were isolated from *Agave tequilana* Weber var. *Azul* and as supposedly pure colonies were grown on King´s B medium at 28ºC. After one day of incubation it was observed that the colonies of this bacterium started to disappear as if they were dissolving. These "ghost" type colonies were barely visible and if transfered to the same medium, did not grow, evidencing that these bacteria died. After performing the Gram staining on these "ghost" type colonies it was found that another bacterium was associated with these colonies, namely a coccus type bacteria, which also stained Gram positive. On occasion, when the affected colonies did not dissapear completely and transferring them onto another King´s B medium, the Coryneform bacteria started to grow, but again almost dissapeared in approximately 24 hours. This has been repeated several times with the same results. This phenomenon has been observed for the first time and it could be a natural biological control system, because in the field it has been observed, that the rot produced by the Coryneform type bacterium sometimes stops without any possible explanation. In an electron microscope one can observe that the coccus type bacteria attaches to the Coryneform type bacteria and this one condenses or its cytoplasm shrinks. It is not known if a bacteriocin may be involved in this phenomenon.

Characterization of epiphytic bacteria originated from quince and medlar trees and its antagonistic effect against *Erwinia amylovora* »in vitro«

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Erwinia amylovora has been reported as the causal agent of fire blight in Serbia since 1989. Quince (*Cydonia oblonga*) and medlar (*Mespilus germanica*) appeared to be very susceptible to the pathogen. High incidence of fire blight symptoms was recorded on these fruit trees during the past few years. Although quince and medlar trees are not economically significant in fruit production in Serbia, the infected trees represent very important inoculum source for apple and pear commercial orchards. In order to detect the presence of epiphytic bacteria and to study their antagonistic effects against *Erwinia amylovora*, samples of symptomless quince and medlar flowers and leaves were collected from different regions in Serbia. Samples were taken three times during 2004-2005. First samples were collected during the flowering period, the second one month later and third two months later. During the flowering period in both years, gram-negative, anaerobic, yellow-pigmented bacteria were predominant. According to their phenotypic characteristics they belonged to *Pantoea agglomerans*, most common epiphytic bacteria in pome fruit trees. *Pseudomonas fluorescens*, *Bacillus* sp., and *Erwinia amylovora* strains were isolated later on. Some of these strains showed antagonistic effects against *Erwinia amylovora*, in vitro, especially few *Pantoea agglomerans* strains and one *Bacillus* sp. strain. Further investigation is in progress.

Thyme essential oil as a natural plant extract for fire blight control

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Plant extracts including essential oil of *Thymus vulgaris* were prepared from air-dried spring season plant samples and assessed against *Erwinia amylovora* both *in vitro* and *in vivo*. To extract the essential oil (EO), 60 g of aerial parts of thyme plant was added into 1200 ml of D.H_2O and boiled at 100º C for 4 h in Clevenger apparatus. Other extracts were obtained by mixing 20 g of the plant leaf and flower samples in flasks containing 600 ml of different organic solvents (methanol, ethanol, acetone and petrolether) and distilled water. The macerated samples at 10% concentrations were placed on a shaker at 150 rpm and Soxhlett organic extracts received low (70º C) temperature treatments.

The antibacterial effects of different extracts were screened in dual cultures against *E. amylovora* in agar-well diffusion method, immature pear fruit assay and spraying the extracts on apple, pear and quince blossoms. Of various extracts tested, thyme EO isolated by the hydro-distillation method showed high levels of bacteriostatic effects producing 20-26 mm inhibition zones on SNA medium and suppressing bacterial exudates on pear slices. Either of crude water /organic mixtures and soxhlett extracts could not exhibit any distinct antibacterial properties. It was only found that the alcoholic extracts obtained by soxhlett apparatus give some degree of bacterial growth (2mm inhibition zones) and exudation suppression without exerting the phytotoxicity effects on blossoms. This efficiency faded during the 48 h incubation period.

The main demerit with plant EOs including the thyme EO is their phytotoxic effect. This can be solved somehow by determining the appropriate MIC for the test EO. In the present study EO of thyme plant was diluted by 10^{-3} level using the mixture of ethylene glycol, soprophor FL and EO in the same ratio of 1:1:1. It was found that the reaction mixture at this level induces 1-1.5 mm inhibition zones with minor side effects on blossoms. This result seems to be promising when it was compared with efficacy of some other recommended chemicals such as 0.3% copper oxychloride, 1% Bordeaux mixture (1:3:100) and 1% serenade inducing trace, <1 mm and >1mm inhibition zones, respectively.

Fire blight control by application of fungal antagonists

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The yeast-like fungus *Aureobasidium pullulans* and the yeast *Metschnikowia pulcherrima* are potential antagonists of the fire blight pathogen *Erwinia amylovora* due to their ability to colonize apple and pear flowers. In greenhouse experiments the efficacy of the antagonistic products Blossom Protect™ (*Aureobasidium pullulans*) and $BPMC_P$ (*Metschnikowia pulcherrima* and *Aureobasidium pullulans*) were evaluated under controlled environmental conditions and artificial inoculation to reduce fire blight incidence on apple trees in comparison to Plantomycin™. Both products reduced the incidence of fire blight symptoms.

In vitro-studies on fire blight control by bacterial antagonists

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Four different agar diffusion tests were compared for their applicability to screen for antagonistic effects of bacteria against *E. amylovora* under laboratory conditions. *Pantoea agglomerans* strains, isolated from bees and bees forage (pollen loads, honeysacs, freshly stored nectar) were used as test organisms. In addition, some yellow pigmented bacterial isolates derived from fire blight host plants in Austria – some of them already confirmed as *P. agglomerans* - were tested for their antagonistic potential against the fire blight pathogen. 44,6% of 130 tested isolates were antagonistic to *E. amylovora* 295/93 in the agar diffusion test "Live Assay". Nine of twelve of the most effective antagonistic strains totally suppressed growth of the *E. amylovora* 295/93 in an immature apple slice assay.

Evaluation of a bacteriocin-producing attenuated strain (opgH-) of *Xanthomonas perforans* to control *Xanthomonas euvesicatoria*

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Xanthomonas perforans strain 91-118 produces at least three different bacteriocin-like compounds (BCN-A, BCN-B, BCN-C) of which two have been shown to give the strain a competitive advantage over *Xanthomonas euvesicatoria* strain on tomato in the field. Prior greenhouse and field experiments demonstrated that the bacteriocins conferred different levels of inhibition toward an *X. euvesicatoria* strain. In an *X. perforans* mutant 91-118Δ*opgH* that is attenuated in pathogenicity, the BCN-B activity was disrupted to create Mut-B:Δ*opgH*. Greenhouse and field experiments were conducted to evaluate the effect of the Δ*opgH* mutation on pathogenicity of the mutant on tomato as well as its ability to reduce *X. euvesicatoria* populations. In greenhouse experiments following infiltration of the strains into leaflets there was a 1 to 1.5 log reduction in bacterial populations *in planta* in the attuenuated mutant compared to the wild-type; however, the mutant strains still maintained antagonism toward the *X. euvesicatoria* strain 91-106. A field experiment in the Fall of 2004 has also shown the ability of the attenuated Mut-B *X. perforans* strains to suppress *X. euvesicatoria* in the field.

Possibility for biological control of pectinolytic erwinias in hyacinth using antagonistic bacterial isolates

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The pectinolytic *Erwinia* species, *Erwinia chrysanthemi* (Ech) and *Erwinia carotovora* (Ec), are plant pathogenic bacteria responsible for soft rot disease among many important crops including flower bulbs such as hyacinth, freesia and *Zantadeschia.* Spread and development of the disease can only be controlled by application of an integrated strategy comprising testing the propagation material, hygienic measures and use of chemical, physical and biological control agents. Within this study, the possibilities to control pectinolytic erwinias with antagonistic microorganisms have been explored.

The bacteria were isolated from hyacinth bulbs and antagonists were selected *in vitro* on the basis of the ability to inhibit growth of Ech and Ec strains on LB agar medium. Also the isolates capable of degrading of acyl-homoserine lactones (AHLs), the signalling molecules involved in quorum sensing of Ech or Ec, were chosen to analyse the possibility for interference with the production of pectinolytic enzymes by degradation of these molecules. Selected isolates were identified by 16S rDNA sequencing, characterised by BIOLOG system and their ability to degrade different AHLs. These isolates were classified into following genera: *Serratia, Pantoea, Pseudomonas, Erwinia* and *Bacillus.* The results of *in vitro* analysis provide a promising base for further studies. The best antagonists will be tested for reduction of soft rot in leaf- and bulb bioassay.

Effectivity of essential oils against *Xanthomonas hortorum* pv. *pelargonii*, the causal agent of bacterial blight on geraniums

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Xanthomonas hortorum pv. pelargonii [(Smith) Davis et al.] *(Xhp)* is a quarantine bacterium causing leaf spot and stem rot of plants resulting in serious losses in areas where geraniums (*Pelargonium* spp.) are planted. The pathogen is transmitted by seeds, disseminated by infected nursery plants and cuttings where it latently persists. A choice of chemicals effective against *Xhp* on the market is not sufficient. In our experiments, about 30 essential oils obtained from various plants were tested for antimicrobial effectivity against *Xhp*. The screening was conducted in laboratory conditions on agar plates, where average of inhibitory zones was measured. Each neat extract was dropped on the surface of agar plates in the dosage of 1 µl with 6 repetitions. Streptomycin and erythromycin in 0.02 – 0.2 % concentrations were used as controls (zones 2.5 - 7.0 mm). The medium inhibitory activity against *Xhp* was found in essential oils from *Citrus aurantifolia, Eugenia caryophyllata, Lavandula angustifolia, Mellisa officinalis, Mentha arvensis, Mentha citrate, Origanum majorana, Rosmarinus officinalis, Thuja occidentalis* and *Thymus mastichina* (zones 11 - 20

mm); strong inhibitory effectivity was shown in essential oils from *Artemisia absinthium, Nepeta cataria, Ocimum basilicum* and *Salvia officinalis* (zones 21 - 30 mm); the strongest inhibitory activity had essential oils from *Origanum compactum* and *Thymus vulgaris* (zones 31 - 50 mm).

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Effectivity of essential oils against pectinolytic erwinias and pseudomonads

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Soft rot bacteria infect many species of plants. They might cause high economical losses on vegetables and ornamental flowers planted in glasshouses and fields, because symptom-bearing plants are refused on the market. Chemical compounds have been used against soft rot bacteria, however resistance to them occurring in bacterial populations decreases their effectivity, and therefore new chemicals should be available on the market. In our experiments, about 30 essential oils obtained from various plants were tested if they inhibit pectinolytic erwinias and pseudomonads. The antimicrobial activity tests were conducted *in vitro.* The inhibitory zones were measured on agar plates where extracts were dropped. Streptomycin and erythromycin were used as controls. With *Pseudomonas marginalis* pv. *marginalis* - a weak inhibitory activity (but higher than by streptomycin) was found in essential oils from *Citrus aurantifolia, Lavandula latifolia, Origanum majorana, Rosmarinus officinalis* and *Thymus mastichina* (zones 6 - 10 mm); medium inhibitory effectivity was shown in essential oils from *Ocimum basilicum, Tagetes bipinnata* and *Tsuga canadensis* (zones 11-15 mm); strong inhibitory activity had essential oils from *Origanum compactum, Origanum vulgare* and *Thymus vulgaris* (zones 16 - 20 mm). Against *Erwinia chrysanthemi* - weak inhibitory activity (but higher than by erythromycin) was found in essential oils from *Juniperus communis, Lavandula latifolia, Mellisa officinalis, Mentha pulegium, Pelargonium graveolens, Pelargonium roseum, Rosmarinus officinalis* and *Salvia officinalis* (zones 6 - 10 mm); medium inhibitory effectivity was found with essential oils from *Ocimum basilicum* (zones 11-15 mm); strong inhibitory activity had essential oils from *Origanum compactum* and *Origanum vulgare* (zones 16 - 20 mm).

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Evaluation of epiphytic bacteria for potential control of the fire blight pathogen

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In the Czech Republic, fire blight of rosaceous plants, caused by *Erwinia amylovora* (*Ea*), was first observed in 1986. Nineteen years later, in 2005, the disease is occurring in all fruit-growing areas. The use of

antibiotics against the fire blight bacterium is not allowed for field sprays in the CR, similarly as in many other European countries. The use of copper compounds is restricted because of phytotoxicities. Therefore, biological control would be a welcome alternative to streptomycin and other antibiotics. The purpose of our investigation was to isolate micro-organisms from epiphytic microflora that might be antagonistic against *Ea*. Among isolated epiphytic micro-organisms, a total of 10 isolates of Gram-negative, endospore-forming bacteria (GNS bacteria) was obtained from blighted host plants (*Malus domestica, Pyrus communis and Cotoneaster monogyna*). The screening of their antagonistic behaviour against *Ea* included agar plate tests and pear fruit tests. Antagonistic effectiveness of 10 isolates of GNS bacteria was compared with effectiveness of copper hydroxide (Koside 2005, 0.3%), copper oxychloride (Kupricol, 0.6%) and streptomycin (500 ppm). In agar plate tests and pear fruit tests, three strains of GNS bacteria (K 3Y, K 11, L1) inhibited *Ea* comparable with copper compounds and one strain (K 11) inhibited *Ea* comparable with streptomycin. Although these GNS bacteria have shown promise in agar plate and pear fruit tests, they have to be tested further in green-house assays and under field conditions.

Microbial preparations of complex action on plants

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The development of microbial preparations of a complex action on plants and their application in the agriculture is one of the most perspective fields of research. On the basis of some strain of nitrogen-fixing bacteria (*Azotobacter, Bradyrhizobium* etc.), and a strain of *Bacillus subtilis* we created a number of highly effective granulated bacterial preparations for the agricultural use. These preparations increase productivity of plants by improvement of their nitric and phosphoric feeding, secretion by bacteria of the growth-stimulating substances, and also appreciable suppression of phytopatogenic microorganisms. Along with the ability to mobilize the phosphorus from organic and inorganic compounds, *Bacillus subtilis* IMV B-7023 suppresses growth of a wide range of phytopathogenic microorganisms, causative agents of diseases of many agricultural plants: tomato, cabbage, cucumber, sugar beet, barley, wheat, conifer and other plants. The rather high antagonistic activity of these bacilli was observed against the following bacteria: *Clavibacter michiganensis* subs. *michiganensis, Xanthomonas campestris* pv. campestris, *Pseudomonas syringae* pv. *syringae, Ervinia carotovora* subs. *carotovora, Pseudomonas fluorescens*. They also appreciably suppressed the development of the phytopatogenic fungi of the genus *Fusarium, Biopolaris, Alternaria, Gliocladium*.

Bacterial preparations of the complex action improve development of the floral, decorative, vegetable, grain and technical cultures both in field and greenhouse conditions. The application of these preparations for biocontrol allows a significant decrease of the plants, deterioration caused by the phytopathogens and an increase of the productivity of plants by 18-37 %.

Resistance to *Erwinia carotovora* introduced to *Solanum tuberosum* from wild *Solanum* species

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Resistance of potato tubers to soft rot, caused by bacteria *Erwinia carotovora* subsp. *atroseptica* (Eca), was introduced to tetraploid potatoes from diploid hybrids of *Solanum tuberosum*, *S. chacoense*, *S. yungasense* and *S. phureja* (Lebecka et al., 2004). Six clones were selected (out of 1353 individuals) during a breeding program, based on their good agronomical characteristics (like: yield, phenotypic appearance, starch content, chipping quality) and presence of additional resistances to *Synchytrium endobioticum*, PVY, PLRV, PVM and resistance to *Phytophthora infestans*. The high level of resistance to Eca found in these clones was confirmed in two consecutive years of evaluation. The five-year mean values of rotten tissue diameter ranged from 5.8 to 6.8 mm in resistant clones, while the average value for the susceptible cultivar Irys was 16.2 mm. Screening for resistance to *Erwinia carotovora* subsp. *carotovora* in one year indicated a high resistance of four clones (mean diameter of rotten tissue from 5.3 to 7.9 mm) and medium resistance of two other clones (9.9 and 10.5 mm) as compared with 14.0, 17.4 and 16.9 mm measured for susceptible cv. Irys and susceptible tetraploid parental clones PS 646 and PW 378, respectively. The source of resistance to potato soft rot demonstrated its value in a breeding program and some of these clones will be used in a SAFE FOODS project as parental lines.

Lebecka, R., E. Zimnoch-Guzowska, and Z. Kaczmarek. 2004. Resistance to soft rot (*Erwinia carotovora* subsp. *atroseptica*) in tetraploid potato families obtained from 4*x*-2*x* crosses. American Journal for Potato Research 82:107-114

Using bacteriophages to prevent fire blight in the orchard

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Fire blight, a disease affecting apples and pears, is a promising target for phage-based biocontrol since primary infection requires the bacterial pathogen, *E. amylovora*, to grow to a threshold population size before infection can occur. Our strategy uses *P. agglomerans* as a carrier for mixtures of phages that have been selected for their broad host range and ability to reduce symptom development. It is expected that the lytic activity of phages and the antagonistic effects of *P. agglomerans* will provide a more effective, stable control than either alone. Three orchards of apple or pear were used for experiments during the 2005 field season. Experiments in each orchard were carried out in a randomized complete block design, with one tree per treatment per block. Treatments included a water control, streptomycin, BlightBan® formulations, a bactericidal soap, and mixtures of phages and the carrier bacterium in varying concentrations. A significant reduction ($p<0.01$) in the incidence of fire blight was observed in trees treated with streptomycin and several of the biological control agents (BCAs), including phage-based BCAs. Throughout the experiment, the populations of the BCAs and the pathogen were monitored using multiplex real-time PCR. Increases in the carrier and phage populations confirmed BCA establishment. Pathogen populations varied between treatments.

Biological control of potato bacterial wilt caused by *Ralstonia solanacearum* in Ethiopia: I. determination of biovars of *Ralstonia solanacearum* from Ethiopia

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Ralstonia solanacearum is a very destructive pathogen that causes wilt in potato and many other solanaceae crops in Ethiopia. An increasing number of reports has indicated that biological control of potato bacterial wilt could be achieved using antagonistic micro-organisms. In order to select effective antagonistic biocontrol agents for the *R. solanacearum* strains, it is necessary to characterize the population of pathogenic strains. Therefore, sixty two strains collected from wilted potato, tomato and pepper plants and potato tubers from the major potato producing regions of Ethiopia were characterized culturally and classified physiologically according to Hayward's (1964) classification scheme based on their capacity to oxidize 3 disaccharides (lactose, maltose and cellobiose) and 3 hexose alcohols (mannitol, sorbitol and dulcitol). The results of this study indicated that all virulent strains from Ethiopia produce fluidal and irregular colonies with red centre and whitish periphery on triphenyl tetrazolium chloride (TZC) medium after 48 hours of incubation which is typical to *R. solanacearum* (Kelman, 1954). On another medium, casamino acids-pepton-glucose (CPG), the colonies were irregular, fluidal, and creamy white and produced a brown pigment after 48 hours. Based on Hayward's classification scheme 19 strains were grouped to biovar I and 43 strains to biovar II. Previous studies from Ethiopia reported the availability of only biovar II of *R. solanacearum.* Thus biovar I is herewith the first report from Ethiopian *R. solanacearum* population.

Antibacterial activity of plant extracts against bacterial plant pathogens

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Plant tissues are rich in secondary metabolites with potential use in phytomedicine e.g. furanocoumarins, alkaloids and flavonoids. Fresh tissue or dried plant material of *Ruta graveolens*, *Rhaponticum carthamoides* and *Drosera capensis* plants were extracted with petrol ether, chloroform and methanol using Soxhlet apparatus or sonication. Isolated secondary metabolites were analysed quantitatively by SPE and RP-HPLC in a Hewlett-Packard model 1050 LC. Antimicrobial activity of plant extracts against several bacterial pathogens: *Erwinia carotovora*, *Erwinia chrysanthemi* and *Agrobacterium tumefaciens* was checked using Minimal Bactericidal Concentrations (MBC) test (1). The bacterial suspensions were incubated overnight with equivalents of plant extracts and the aliquots of 0.1 ml were plated out on agar plates. After the overnight incubation the MBC of tested extracts were evaluated and compared to MBC of several antibiotics. The results indicate that methanol extracts of *R. graveolens* (contains coumarins and furanocoumarins) and *R. carthamoides* (contains ecdysteroids) of different tissues (stems, leaves, roots) have bactericidal activity; equivalent of 30 to 200 mg of DW inhibit 99% of bacterial growth. The bactericidal activity of chloroform extracts was lower; equivalent of about 500 mg of DW inhibit 99% of bacterial growth. The chloroform extracts of *in vitro* grown plants of *D. capensis* (contains naphthoquinones and flavonoids) indicate even higher bactericidal activity; 10 mg DW inhibit 99% of bacterial growth. Presented data indi-

cate that the tested plant extracts contain biologically active compounds with potential use for biological control of bacterial plant pathogens.

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Dispersal of the biocontrol agent *Aureobasidium pullulans* for fire blight control using honeybees (*Apis mellifera carnica*)

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Honeybees have previously been shown to disperse bacterial fire blight antagonists, such as *Pantoea agglomerans*, *Pseudomonas fluorescens* and *Bacillus subtilis*. In the present study we examined whether honeybees could disperse two strains of the yeast-like fungus *Aureobasidium pullulans*, the active ingredient of Blossom Protect™, from a hive mounted dispenser to apple and pear trees. In greenhouse trials we investigated the dispersal of *Aureobasidium pullulans* on apple trees and its potential to reduce fire blight incidence. Additionally the dispersal of the fungus on apple and pear trees in two orchards was monitored. Freshly stored nectar and honey were tested for the presence of distributed strains.

Biophotonic technology for inactivation of tomato pathogen *Clavibacter michiganensis* subsp. *michiganensis*

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Photosensitization is described as early as in the beginning of 20th century, when O. Raab observed destruction of *Paramecium caudatum* in the presence of acridine orange and light. Recently this phenomenon is widely used for different purposes, for instance, inactivation of microorganisms in human blood, wound healing or local infections. This study is focused on the possibility to increase the plant safety using photosensitization as novel biophotonic technology for inactivation of *Clavibacter michiganensis* subsp. *michiganensis*, a Gram-positive, coryneform bacterium, the most important bacterial pathogen of tomato, in an alternative, ecologically friendly and easy-to maintain way. For this purpose bacteria were grown on potato dextrose agar and transferred into nutrient broth media with addition of 1% glucose to a final volume of 25 ml. Photosensitizer was added to the culture medium at the exponential phase and treatment started upon illumination.

Illumination was performed with light source consisting of a tungsten lamp (500W), optical system for light focusing and optical filter for UV and infrared light elimination ($370nm<\lambda<680nm$). Hematoporphyrin di-

methyl ether (HPde) was used as photosensitizer at a concentration 10^{-4} M. Incubation time reached 20 min. Optical density at 670 nm was chosen as parameter to evaluate growth of bacteria. Control and treated samples were measured 24 hours after illumination. Data obtained indicate that under certain experimental conditions *Clavibacter michiganensis* subsp. *michiganensis* might be inactivated using photosensitization with HPde.

Bactericidal activity *in vitro* of essential oils of H*yptis suaveolens* (L.) Poit and C*oleus amboinicus* (Lour)

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In the last years the study of the fungicidal and bactericidal properties of plant extracts have extended towards phytopathogens in search of alternative solutions for their control. Extracts of essential oils of the Wild origanum (*Hyptis suaveolens* (L.), Poit) and the French origanum (*Coleus amboinicus* Lour) were tested for determining their bactericidal activity against isolates of *Xanthomonas axonopodis pv vesicatoria, Xanthomonas campestris pv campestris, Xanthomonas axonopodis pv malvacearum, Xanthomonas axonopodis pv manihotis, Xanthomonas sp., Erwinia carotovora* subsp. *carotovora, Erwinia chrysanthemi, Burkholderia glumae, Acidovorax avenae* and *Pseudomonas syringae pv tomato.* The disks impregnated with the essential oils were placed onto the surface of the dishes with nutrient agar previously inoculated with a bacterial suspension. Three replicates for each variant were carried out. The bactericidal effect was determined by the inhibition area after 24 hours incubation period. All bacterial isolates were sensitive to the extracts of the two origanum species at 1%, with inhibition areas between 14,3 - 28,0 mm for the Wild origanum, and of 14,3 - 25,6 mm for the French origanum. The extract at 0,5% of this latter species showed a bactericidal effect against all bacterial isolates tested. Eleven isolates were sensitive for the extracts at 0,25% with inhibition areas between 4,0 - 13,0 mm. These results encourage to continue the study of the effectiveness of plant extracts for the control of these bacterial species in their host plants.

Control of bacterial speck and bacterial spot of tomato using alternative strategies under greenhouse conditions

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Bacterial speck of tomato, caused by *Pseudomonas syringae* pv. *tomato* and bacterial spot, caused by *Xanthomonas vesicatoria*, continue to be a problem in tomato production worldwide. The efficacy of a plant resistance activator, acibenzolar-S-methyl, an antagonistic strain *P. syringae* Cit7, a formulated complex of hydrogen peroxide, silver ions (Ecocute), and electrolytically decomposed water (pH 5.5-6.5) was tested for control of bacterial speck and spot of tomato in greenhouse bioassays. Copper hydroxide was used as a

standard treatment, while untreated plants served as untreated control. Acibenzolar-S-methyl was applied twice: 12 and five days prior to inoculation whereas other treatments were applied once followed by inoculation. Experiments were conducted twice in four replications per treatment. In both experiments acibenzolar-S-methyl and a complex of hydrogen peroxide and silver ion were the most effective, significantly reducing foliar bacterial speck severity. However, in the first experiment, *P. syringae* strain Cit7 showed lower efficacy, providing a similar level of bacterial speck control to copper hydroxide and electrolytically decomposed water treatments and it was less effective then standard bactericide treatment in the second trial. Significant reduction of bacterial spot symptoms was achieved using acibenzolar-S-methyl and a complex of hydrogen peroxide and silver ions, showing similar efficacy as the standard bactericide treatment whereas electrolytically decomposed water treatment was less efficient in both experiments. In the first experiment *P. syringae* strain Cit7 was as effective in bacterial spot control as the standard treatment, whereas it was significantly less effective in the second one.

In vitro and *in vivo* evaluation of *Streptomyces griseoviride* strains on pathogenic bacteria of some crops in Costa Rica

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Several strains of *Streptomyces* spp. were isolated from soil and organic fertilizers obtained from different regions of Costa Rica. In the lab, purification and identification of the strains were done. The plant pathogenic bacteria *Xanthomonas* and *Erwinia* were tested against all isolates to study their efficacy *in vitro*. Then the best *Streptomyces* strains were reproduced by fermentation on solid substrate, in order to test them on field plots cultivated with pineapple and cucurbits.

Twenty isolates were identified as *S. griseoviride* strains, obtained mainly from organic fertilizers. Tests showed that only three of them efficiently inhibited the growth of *Erwinia* and *Xanthomonas.* The three strains codified as St1L.s, St2gza and St3TB, showed a similar or higher effect than synthetic antibiotics, when the pathogenic bacteria were tested on field plots. Also the establishment of *Streptomyces* in the treated soils was probed.

As part of this work, an efficient fermentation method for massive production of *Streptomyces,* using brown rice as solid substrate, was established.

According to the results of this work, we conclude that *S. griseoviride* is a good biological option to control plant pathogenic bacteria, in organic and conventional production systems, at low cost.

Efficacy of different control strategies for preventing crown gall in the nurseries

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Evaluation of the efficacy of different control strategies has been the main topic of our research on crown gall during the last years. The relevant results of our work are briefly illustrated below.

The screening for resistant genotypes showed that Mr.S. 2/5 was the last sensitive to crown gall among the most common peach rootstocks. This rootstock is a *Prunus cerasifera* x *Prunus spinosa* hybrid that has a low compatibility with some peach varieties and for this reason it is not widely used.

Solarization was performed both in naturally infected and in artificially inoculated soils during a four year experiment performed in different geographic areas. Results showed that this method strongly reduces but does not eliminate the agrobacteria in the treated soil and that this effect is more evident in sandy than in clay soil. Moreover, agrobacteria survive on the roots of weeds growing around the borders of solarized plots where they may represent a dangerous source of soil re-contamination.

A great number of non-pathogenic agrobacteria was isolated from peach tumors and was evaluated for antagonistic activity. Many were able to inhibit tumorigenic strains in vitro but not in vivo. The majority of the strains produced siderophores but no one produced agrocin-like compounds, thus showing that the ability to compete for iron is a mechanism that alone is not sufficient to prevent plant infection.

The efficacy of the biocontrol strain K84 in preventing peach crown gall was monitored for three years in several nurseries located in Southern Italy. In one single nursery K84 insensitive tumorigenic strains caused a disease outbreak. Molecular analyses showed that these strains originated by plasmid transfer (pAgK84) from K84 strain to autochtonous tumorigenic agrobacteria. Except for this single case, the use of the biocontrol strategy always was highly effective in peach nurseries where it still represents the most reliable way to prevent crown gall. Selection of dangerous transconjugant agrobacteria can be avoided by the use of a K84 derivative strain (named K1026) in which the region coding for pAgK84 transfer has been deleted. It is considered as a genetically modified microrganism and EU legislation does not allow its use in agriculture.

Rhizobacteria-mediated induced systemic resistance against various plant pathogens and its mechanisms of action

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Treatment of *B. amyloliquefaciens* strain EXTN-1 showed a broad disease-controlling spectrum to the plant diseases caused by viral, bacterial, and fungal pathogens such as cucumber mosaic virus, tobacco mosaic virus, potato virus Y and *Pseudomonas syringae* pv. *lacrymans*, *Ralstonia solanacearum*, *Colletotrichum orbiculare*. Furthermore, soil drenching or seed priming (10^6cfu/ml) of *B. amyloliquefaciens* strain EXTN-1 stimulated seed germination and growth of about 20 crops used without any harmful effect. When *B. amyloliquefaciens* strain EXTN-1 was drenched to lettuce grown in hydroponic system, population of *B. amyloliquefaciens* strain EXTN-1 was similar or increased in the rhizosphere, while the population was gradually decreased up to 10 fold in the hydroponic solution 4 weeks after treatment compared with initially treated population of the bacterium. Treatment with EXTN-1 increased oxidative burst in early stage and induced the expression of resistance genes, PR-1a, PDF1.2. The mechanism involved in induced systemic resistance by EXTN-1 was revealed as simultaneous activation of SA and JA or ethylene metabolic pathways and pretreatment with EXTN-1 reduced germination and appressorium formation of conidia of *C. orbiculare* on the leaf of cucumber with increase of callus formation. Furthermore, treatment with EXTN-1 inhibited the bacterial wilt on tomato caused by *R. solanacearum* for 4 weeks after treatment. Treatment of *B. amyloliq-*

uefaciens strain EXTN-1 showed the increased plant height of the three barley varieties and shorter heading stage of two varieties compared with non-treated control. Taken together the above results, *B. amyloliquefaciens* strain EXTN-1 can be considered as a promising agent for practical application.

Biocontrol potential of a growth promoting soil bacterium against phytopathogenic fungi

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A number of bacterial strains was isolated from soil samples collected from different parts of Tamil Nadu State in South India and screened against fungal phytopathogens *viz., Rhizoctonia solani, Macrophomina phaesolina, Fusarium udum, Alternaria alternata* and *Phytopthora infestans* for their antagonistic potential. *In vitro* screening resulted in selecting a highly efficient bacterial isolate designated as MML2501, later identified as *Bacillus firmus* by phenotypic as well as biochemical tests carried out as suggested in Bergey's Manual of Determinative Bacteriology. This strain effectively inhibited the mycelial growth of a wide range of fungal phytopathogens in dual plate assay. The cell-free culture filtrate of *B. firmus* MML2501 inhibited spore germination and mycelial growth of most fungal pathogens but failed to exhibit antifungal activity against *R. solani* and *P. infestans*. The proteins from the culture filtrate of *B. firmus* MML2501 were precipitated by salting out, dialysed and concentrated by freeze-drying. The concentrated proteins of *B. firmus* MML2501 did not exhibit any antifungal activity against pathogenic fungi even at higher concentrations, which clearly indicated that the inhibitory principle in the culture filtrate of *B. firmus* 2501 might be non-proteinous in nature. Hence, the production of secondary metabolites in the culture filtrate was analyzed and the presence of a prominent compound with the R_f value of 0.73 was identified. The secondary metabolite was extracted from the culture filtrate with organic solvent and concentrated by vacuum evaporation. The crude metabolites significantly inhibited the spore germination and mycelial growth of many fungal phytopathogens.

The plant growth promoting activity of *B. firmus* MML2501 was assessed on rice, green gram and tomato. The seeds treated with whole culture of *B. firmus* MML2501 exhibited high growth parameters *viz.,* seed germination, root and shoot lengths and seedling vigour in all the crops as compared to seed treated with cell-free culture filtrate and sterile water. Analysis of culture filtrate indicated that the *B. firmus* MML2501 was able to produce indole acetic acid (IAA) in high quantity in the presence of L-tryptophan. The above findings suggested that the soil bacterium *B. firmus* MML2501 can be used as biocontrol agent for the management of a wide range of fungal pathogens and the growth promoting ability is an additional advantage of this bacterium.

Identification of resistance donors for fire blight in *Malus*

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Fire blight is a dangerous disease in pome fruit production. The cultivation of resistant apple varieties can prevent losses caused by *Erwinia amylovora* thereby avoiding spraying of chemicals. Some resistant varieties (eg. ´Reanda`, ´Remo`, ´Rewena`) were bred at the Institute of Fruit Breeding in Dresden-Pillnitz. The donor of resistance is *Malus x floribunda*. To enlarge the genetic basis of resistance to fire blight, a wide range of accessions of wild species, present in the gene bank in Dresden-Pillnitz, was tested for resistance to fire blight in the greenhouse. Some accessions were tested up to 14 times in the last 14 years to find durable resistance donors independent of the *E. amylovora* strain used.

Highly virulent strains of *E. amylovora* were inoculated into growing shoots grafted on rootstocks. Eight weeks p.i. the ratio between length of necrosis and shoot length was calculated. Accessions of *M. x atrosanguinea, M. x dawsoniana, M. fusca, M. x prunifolia, M. x robusta, M. sieboldii* and *M. x zumi* were found to be highly resistant to fire blight, whereas accessions of *M. tschonosky, M. arnoldiana* and *M. trilobata* were very susceptible to the pathogen. Accessions of *M. baccata* revealed large differences in the reaction to fire blight, some of them were very highly resistant and some highly susceptible.

Respective wild species showing stable resistance to fire blight provide a valuable tool for breeding of fire blight resistant cultuivars.

A biocontrol agent for bacterial blight that induces systemic resistance as it restrains pathogen multiplication in bean leaf tissue

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The UFV-172 isolate of *Bacillus cereus* was selected in *in vitro* and *in vivo* tests as the best among 501 prokaryotic residents, found on the phylloplane of healthy dry bean plants, for protection against several pathogens. The isolate was identified as *B. cereus* based on the sequence of its 16 S ribosomal gene. Although able to protect against all tested bean pathogens in greenhouse and field trials, UFV-172 was not at all antagonistic to them in *in vitro* assays. Known that *B. cereus* is $Pen^{-}Amp^{-}Amx^{-}$ and *Xcp* is $Pen^{+}Amp^{+}Amx^{+}$, a possible repression of *X. campestris pv. phaseoli* in bean leaf tissues, previously exposed to the antagonist, was investigated based on the differential sensitivity of each microbial component in their interaction with each of the antibiotics penicillin, ampicillin, and amoxacillin. Plants were exposed to a suspension of the antagonist propagules (OD_{540} = 0.4), and four days later the primary leaves were infiltrated with a pathogen cell suspension (OD_{540} = 0.05). Five leaf disks were removed at intervals from the infiltrated leaves, from a different leaf and at every time interval, weighed, and ground in PBS amended with 1% w/v PVP. Each homogenate was serially diluted and each dilution plated out in the antibiotic-containing culture medium. Population tendencies of the pathogen in leaf tissue exposed and not exposed to the antagonist were in sharp contrast to each other, a clear indication of pathogen repression. To account for

a systemic response, upper or lower bean leaf pairs were sprayed with antagonist propagules and each treated leaf wrapped inside a plastic bag. Four days later, the exposed leaves were set free from the bags, and the corresponding, untreated opposite leaves, either the below or the above ones, were sprayed with a pathogen suspension at O_{D540} = 0.2. Lesions on these leaves were counted seven days later. The antagonistic effect was evident in leaves below or above the inoculation sites, a clear indication that protection became systemic. Being also known that in *in vitro* bioassays the antagonist had exhibited no direct antibiosis against the pathogen, these three kinds of evidence support the hypothesis that control resulted from induced systemic resistance.

Engineering of transgenic apple cultivars by expression of human lactoferrin to study effects on *Erwinia amylovora*

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Several pathogenicity factors have been described enabling *Erwinia amylovora* to colonize apple plants and cause disease. Secreted siderophores sequestering iron are considered to be one important factor in this process. By engineering transgenic apple cultivars expressing human lactoferrin, an iron binding protein from breast milk, we are trying to challenge the iron uptake machinery of the bacterium and study the effects on the pathogen. We have transformed two different apple cultivars with the human lactoferrin gene. The transferred gene is preceded by the CaMV35S promoter and a signal sequence, directing the protein to the plant apoplast. Nineteen transgenic 'Pinova' lines and one 'Royal Gala' line were regenerated carrying the lactoferrin gene. The presence of the gene has been confirmed by PCR analysis. Between one and 17 offsprings originating from one line were rooted, potted and grown in the greenhouse. Western blot analysis performed with a few lines so far failed to show the presence of the recombinant protein. Different sampling and protein extraction protocols and more sensitive detection methods will be applied to detect the recombinant protein.

Control of fire blight (*Erwinia amylovora*) using epiphytic bacteria with known activity against postharvest diseases of apples

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Strains B90 of *Pantoea agglomerans* and B262 of *Pseudomonas syringae*, isolated from apple leaves, were used in study on biological protection of apple and pear tissue against fire blight. Our earlier experiments have shown that both strains were highly effective in protecting apples against gray mold (*Botrytis cinerea*) and blue mold (*Penicillium expansum*) during storage. In the present study B262 inhibited growth of *Erwinia amylovora* on PYGA medium, while B90 was not active in this test. Apple flowers on trees cv.

Idared/M9 growing in pots in the greenhouse, sprayed with water suspension of B90 and inoculated after 2 hours with a highly virulent strain of *E. amylovora* (Ea 659), were significantly less infected than those non treated. However, treatment of flowers with B262 was not effective. Both strains, applied as water suspension sprays on wounded apple terminal shoots of cv. Idared/M9 trees kept in a greenhouse, were moderately effective against fire blight up to 10 days after inoculation. On the other hand the disease symptoms on pear fruitlet slides cv. Conference dipped in water suspension of both strains and after 6 hours inoculated with *E. amylovora* were very mild during up to 7 days of experiment. The efficacy of both strains was comparable with copper oxychloride.

Antibacterial activities of essential oils from medicinal plants against the growth of *Clavibacter michiganensis* subsp. *michiganensis*

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In the present study, antibacterial activities of essential oils obtained from aerial parts of aromatic plants such as thyme (*Thymbra spicata* subsp. *spicata*), oregano (*Origanum syriacum* var. *bevanii*), mint (*Mentha spicata*) and lavender (*Lavandula stoechas* subsp. *stoechas*) were investigated against seed-borne plant pathogenic bacterium *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm* ICP7200), causal agent of bacterial canker or wilt of tomato. By using paper disc diffusion assay, all essential oils have shown antibacterial activity against bacterial strain used. Essential oils used in the paper disc diffusion assay varied in their antibacterial activity. Essential oil from thyme was the most effective in inhibiting the growth of the *Cmm*. This was followed by essential oils obtained from of oregano and lavender respectively. By using micro agar broth dilution assay, minimum bactericidal concentrations of the essential oil of thyme, oregano and lavender were 10, 10, 25 and 50 µg/ml, respectively.

Reduction of Crown gall incidence on stone fruits with some antagonistic bacteria

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Crown gall cause significant economic losses to stone fruits because galls can weaken or kill the host plant. Economic losses from the disease occur primarily in nurseries where galled plants should be discarded and damaged.

Biological control of crown gall by some antagonistic bacteria was evaluated *in vitro* by screening *Agrobacterium tumefaciens* strains for sensitivity to antagonistic bacteria and to determine the efficacy of antagonistic bacteria in biological control of crown gall in Egypt.

The tested antagonists could inhibit the growth of thirteen isolates of *A. tumefaciens* on NGA medium. *A. radiobacter* had higher inhibitory effect on the pathogen followed by *Pseudomonas putida*, moderate inhibition was recorded with Ps. *fluorescens and Bacillus subtilis.*

No gall formation was observed on both peach and apricot plants treated with *A. radiobacter* .The lowest number of galls was observed in plants treated with Ps. *fluorescens* . In general all antagonists significantly reduced disease severity. Formation of galls on the roots generally resulted in an increase in fresh and dry weight of roots compared with the control. The effect of gall formation on the weight of foliage showed an opposite trend.

Characterisation of an inhibitory strain of *Pantoea sp*. with potential as a biocontrol agent for bacterial plant pathogens

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The *Pantoea sp*. 48b/90 (former *Erwinia herbicola*) is a naturally occurring epiphyte which was isolated from a soybean leaf. 48b/90 inhibited the growth of a broad spectrum of bacterial species of distinct genera including plant-pathogenic bacteria (e. g. various *Pseudomonas syringae* pathovars, *Erwinia amylovora, Agrobacterium tumefaciens*) *in vitro* on minimal medium. It was also active against *Ps. syringae* pv. *glycinea*, the pathogen of bacterial blight of soybean, *in planta* by reducing the population size of the pathogen. Consequently, disease symptoms were suppressed. Therefore, the strain 48b/90 is interesting as a suitable candidate for biocontrol. Antibiosis, competition for limited resources, induction of host plant defence, and interference with the pathogen's quorum sensing have been determined as mechanisms involved in biological control.

The phenotypic characteristics of 48b/90 are typical of *Enterobacteriaceae.* Based on 16S rDNA sequences it belongs to the genera *Pantoea*, which includes the following two species: *P. agglomerans* and *P. dispersa.* In some characteristics, however, 48b/90 differs from these two species.

During growth under iron limitation the strain 48b/90 produced at least two different siderophores: the hydroxamate siderophore ferrioxamine E, the principle siderophore of the former *Erwinia herbicola* group, and a non-identified catechole siderophore. Interestingly, 48b/90 produced approximately twice as much siderophore at 18 °C as it produced at 28 °C at the same growth densities. Additionally, it produced antibiotic metabolites and quorum sensing signal molecules.

The aim of this study is to determine the biological traits which are responsible for this antagonism. The results will provide important information for the development of new biocontrol strategies.

BACTOFRUCT – Development of a biological pesticide against fire blight

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Fire blight is a major disease of apple and pear trees in many fruit producing areas of the world. The disease is most commonly initiated by epiphytic populations of the bacterium *Erwinia amylovora*. Microbial biocontrol of fire blight has been demonstrated as an alternative to antibiotics. In November 2004 the Bactofruct-project was started, funded by the European Community under the Sixth Framework Program. Aim of the project is the development of biocontrol agents for use in fire blight control. An extensive collection of *Bacillus subtilis* and related non-pathogenic *Bacillus*-species is available for the project. Screening by the University of Konstanz has shown that the bacterium *Bacillus subtilis* has antagonistic potentials to be used effectively against fire blight. In laboratory and field trials in Belgium, Germany, Hungary, the Netherlands and Spain the efficacy of selected *Bacillus subtilis* strains will be evaluated.

Influence of siderophore production on biological control among *Pseudomonas syringae* strains

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Suppression of a plant pathogen's growth *in planta* by naturally occurring antagonists offers a favorable alternative to classical methods of plant protection. *Pseudomonas syringae* pv. *syringae* strain 22d/93 (Pss22d/93), an epiphytical isolate from soybean plants is an efficient control agent against bacterial blight of soybean. While suppression of growth of *P. syringae* pv. *glycinea* by Pss22d/93 has been shown in greenhouse system as well as under field conditions, the mechanisms involved are not yet understood. Our current work aims at the influence of siderophore production on this biological control system. Although antagonist and pathogen do produce the same pyoverdin-type siderophore, they show clearly distinct iron-chelating phenotypes. Mutational analysis of siderophore production in Pss22d/93 led to the identification of an additional siderophore system with high sequence similarities towards the achromobactin biosynthesis genes of *Erwinia chrysanthemi.* Production of both siderophores was analyzed in detail. The relevance of siderophore production for biological control and for survival in planta is discussed.

A comparison of two years of research on biological control of fire blight in New York

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The blossom blight phase of fire blight, caused by *Erwinia amylovora*, is managed by applying control products to open blooms. Organisms such as *Pseudomonas fluorescens* A506, *Pantoea agglomerans* C9-1 and *P. agglomerans* E325 have been shown to be effective in preventing blossom infection via preemptive exclusion. Efficacy of such products has been variable on a yearly and regional basis. Temperature plays a major role on epiphytic pathogen and biological control agent populations. The three organisms mentioned above were included in orchard trials in New York in 2004 and 2005. The antagonists were applied at 20-30% and 70-80% bloom in both years. Additional applications of A506 and C9-1 were made 24 hr after inoculation in 2004 and A506 was applied with the surfactant Breakthru at 1-5% bloom in 2005. The maximum and minimum temperatures at 20-30% bloom were 28ºC and 14ºC in 2004, and 10ºC and 1ºC in 2005, respectively. The maximum and minimum temperatures at 70-80% bloom were 25ºC and 15ºC in 2004, and 23ºC and 11ºC in 2005, respectively. In 2005 a damaging frost event occurred 24 hr prior to the 70-80% application. In 2004 87% of blossom clusters were blighted on non-treated, inoculated trees, while the same treatment caused only 33% infection in 2005. This is most probably because the maximum and minimum temperatures on the day of inoculation were 30ºC and 18ºC in 2004, and 13ºC and 5ºC in 2005, respectively. C9-1 provided 34% control in 2004 and less than 1% control in 2005. E325 provided 17% control in 2004 and no control in 2005. A506 provided 8% control in 2004 and no control in 2005. The standard control material, Agrimycin (streptomycin), provided 44% and 80% control in 2004 and 2005, respectively. High populations of viable bacteria were recovered from all tank samples using dilution plating in 2005. In the same year, antagonistic bacteria were recovered from 26-85% of the blossoms collected using stigma imprints.

Recent status of the biocontrol of fire blight in Germany

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Research on alternatives to the antibiotic streptomycin for the control of fire blight in pome fruits has become of main interest in German and European fruit growing, as permission for the use of antibiotics in EEC-countries can be withdrawn in future time. Thus the development of biologicals on the basis of natural products and antagonism against the pathogen have been undertaken in Germany especially by the Federal Research Centre (BBA) in cooperation with plant protection service. Latest results with antagonistic bacteria and natural product based on an etheric oil of thyme, BioZell 2000B, which was developed together with Turkish colleagues, will be presented.

The antagonistic effect of *Rahnella aquatilis* against fire blight was increased through combination with other compounds (Na-benzoate or growth regulator).

The etheric compound BioZell – 2000B is able to induce a defence reaction against *E. amylovora* in the host plant and can be recommended as an biological preparation against fire blight with special emphasis for ecological farming.

List of Authors

Abd–el-Aziz, R.M. 76
Abo El-Dahab, M.K. 28
Abo-Elyousr, K. 52
Aldwinckle, H.S. 79
Al-Masri, A.N.....36
Amein, T.....44
Amusa, N.A. 20
Aslan, E. 35, 42
Atanasova, I.....52
Audenaert, K.t 26
Babulka, P. 32
Badosa, E.....43
Balestra, G.M. 20, 53
Balogh, B.....34, 41, 54
Bánatfy, R.....32
Barakat, F.M.....76
Barra, V.R.S. 74
Barszcz, E.....38
Baysal, Ö. 54, 55, 56, 76
Bazzi, C. 56
Beer, S.V. 50
Berczyński, S.....75
Berg, G. 21
Berwarth, C. 75
Besenyei, E.....35, 42
Bini, F.....56
Biondi, E.....56
Biskup, E. 68
Bogatzevska, N.....52
Boja, N.....32
Bonaterra, A. 24
Bora, T.....35, 42
Bozsó, Z.....35, 42
Braun, S.D. 21
Brunelli, A. 56
Bryk, H. 75
Bubán, T. 57
Buchenauer, H. 22
Bulavenko, L.V. 66
Burokiene, D. 69
Burr, T.J.....23, 25, 48
Cabrefiga, J.....24
Canteros, B.I.....54
Castle, A.J.....38, 67
Chumakov, M.I.....24
Chuyko, N.V.....66
Contreras, N.....58
Coronado, M.F.....70
Creasap, J.....23, 25
Czelleng, A. 35, 42
Deckers, T.....78
Degenhardt, J.....36
Devleesschauwer, D. 26
Di Mattia, E. 53
Dilley, J.R. 54
Dimitrov, J.....52
Doan Thi Thanh.....48
Dorgai, L.....57
Duru, A. 55
Ebenezar Immanuel, J.....30
Edgecomb, D.W. 26
Egamberdiyeva, D. 27
Eilers, B. 78
El-Kazzaz, S.A. 28
Fan Chen.....39
Ferraz, H.G.M.....74
Fessehaie, A.....59
Fischer, C.....74
Flis, B. 67
Foißy, H.....63
Fried, A.....59
Ftayeh, R.....60
Fucikovsky, L. 61
Gau, A.E. 36
Gavrilović, V. 61
Geider, K. 29
Giesen, N. 46
Gnanamanickam, S.S.....30
Gninenko, U.I. 31
Gochez, A.M. 54
Gölükcü, S.B. 54, 56
Gomathinayagam, S.....31
Gouveia, E.R. 40
Grondona, I.....78
Guerra, M.F. 32
Gürsoy, Y.Z. 55
Hanke, V.....75
Hao, G.....23, 25
Hassanzadeh, N..... 62
Heijne, B. 78
Heissenberger, B.... 62, 63, 69
Hert, A.P. 63
Hevesi, M..... 32, 57
Hockenhull, J. 51
Höfer, M. 74
Hoffmann, P..... 33
Höfte, M..... 26
Holb, I. 78
Hudák, I. 57
Huimin Wang..... 39
Iriarte, F. 34
Jackson, L.E..... 34, 41
Jafra, S. 64
Jakovljevic, V. 29
Jayaprakashvel, M. 47
Jelkmann, W. 46, 59, 75
Ji, P. 41
Jianhui Wang 39
Jiinyun Li 39
Jock, S..... 29
Jones, J.B. 34, 41, 54, 63
Junge, H. 78
Kabadjova, P..... 52
Kamalanalini, S.P. 31
Keck, M. 63
Kim, W.S. 38, 67
Ki-Woong Nam 72
Klement, É. 42
Klement, Z. 35, 42
Koch, E. 44
Kokošková, B. 64, 65
Koltuksuz, T.A..... 35
Korba, J..... 65
Kovács, O. 48
Krolicka, A. 68
Kunz, S. 36, 78
Kurdish, I.K. 66
Kürkcüoglu, S. 36
Kyungseok Park..... 72
Lakatos, T. 57
Lalithakumari, J. 31
Lancioni, P..... 56

Lapa, S.V. 37
Larrinaga, M.L. 70
Laux, P. 38
Lebecka, R. 67
Lehman, S.M. 38, 67
Lemessa, F. 68
Lo Cantore, P.L. 33
Lojkowska, E. 68
Loncaric, I. 62, 63, 69
Lopes, C. A. 40
López, M.M. 39
Luksiene, Z. 69
M'barek Fatmi 28
Malarvizhi, K. 73
Manceau, C. 43
Manker, D. 26
Mariano, R.L.R. 40
Martins, O. M. 40
Massomo, S.M.S. 51
Mathivanan, N. 47, 73
Medeiros, F.H.V. 40
Medzihradszky, K.F. 42
Melo, I.S. 74
Mguni, C.M. 51
Mikhail, M.S. 76
Milijašević, S. 61, 70
Ming-Shu Zeng 72
Minsavage, G.V. 63
Mohamed, A.K. 28
Mohammadi, M. 29
Moltmann, E. 59
Momol, M.T. 34, 41, 63
Moncheva, P. 52
Montesinos, E. 24, 43
Moosbeckhofer, R. 62, 69
Mortensen, C.N. 51
Motallebi, M. 45
Muganu, M. 53
Novák, E. 48
Obradovic, A. 34, 41, 70
Obregon, M. 71
Oliveira, A. 40
Olson, S.M. 41
Oosterkamp, P. 78
Örnek, H. 55
Ott, P.G. 35, 42
Özaktan, H. 35, 42
Pavela, R. 64, 65
Peil, A. 74
Peluso, R. 71
Penalver, R. 39
Piotrowski, M. 36
Prabavathy, V.R. 73
Prashanth, S. 73
Przysowa, J. 64
Pujol, M. 43
Puopolo, G. 71
Pusey, P.L. 43
Quattrucci, A. 20
Quezado Duval, A. 40
Raio, A. 71
Ramesh, S. 47
Ravensberg, W. 44
Reva, O.N. 37
Richter, K. 74
Rizo, S.G. 70
Roberts, S.J. 44
Romeiro, R.S. 74
Romeu, C. 70
Roy, A.A. 66
Rudolph, K. 60
Salehzadeh, A. 45
Sante Iacobellis, N. 33
Santos, E.R. 40
Schaal, K.P. 45
Schaffer, J. 62
Schmitt, A. 44
Schneider, B. 75
Schneider, K. 38
Seibold, A. 46, 59
Semrau, J. 51
Shanmugaiah, V. 47
Shoieb, A.A. 28
Šillerová, J. 65
Silveira, E.B. 40
Sobiczewski, P. 75
Souto-Maior, A.M. 40
Soylu, E.M. 55, 76
Soylu, S. 55, 76
Spornberger, A. 63
Srinivasan, K. 73
Stall, R.E. 54
Stefanova, M. 70
Stockwell, V.O. 47
Sukan, F.V. 35
Süle, S. 48
Svircev, A.M. 38, 67
Szabó, E. 35, 42
Szatmári, Á. 35, 42
Szegedi, E. 48
Tafaghodinia, B. 45
Tawfik, A.E. 76
Tóth, M. 32
Tóth, T. 57
Tsarenko, I.Y. 66
Ullrich, C.I. 25
Ullrich, M. 49, 78
Umesha, S. 50
Ünlü, A. 54, 56
Urshev, Z. 52
Uslu, A. 42
van der Wolf, J.M. 64
van Doorn, J. 64
Varga, G.J. 42
Vasinauskiene, M. 69
Vieira Júnior, J.R. 74
Völksch, B. 21, 77
Walcott, R.R. 59
Weingart, H. 78
Wenneker, M. 78
Wensing, A. 78
Werner, N.A. 79
Wright, S. 44, 50
Wulff, E.G. 51
Wydra, K. 51
Yanbing Guo 39
Yegen, O. 52
Yonce, H.D. 54
Yong-Chull Jeun 72
Zamani, M.R. 45
Zäuner, S. 25
Zeller, W. .. 38, 52, 54, 55, 56, 68, 76, 79
Zhang, Hongsheng 25
Zhukov, A.M. 31
Zimnoch-Guzowska, E. 67
Živković, S. 61
Zoina, A. 71