Biology of Plant-Microbe Interactions, Volume 6

Edited by

Matteo Lorito
Sheridan Lois Woo
Felice Scala

Università Degli Studi Di Napoli
Portici, Italy

Proceedings of the 13th International Congress
on Molecular Plant-Microbe Interactions
Sorrento (Naples), Italy
July 21–27, 2007

Published by the
International Society for Molecular Plant-Microbe Interactions
St. Paul, Minnesota, USA

Articles may be referenced as follows:

[Authors' names]. 2008. [Paper title]. Paper [number] in: Biology of Plant-Microbe Interactions, Volume 6. M. Lorito, S. L. Woo, and F. Scala, eds. International Society for Molecular Plant-Microbe Interactions, St. Paul, MN.

Cover photo by Gianfranco Capodilupo; logo design by Antonino Balsamo

This CD-Rom has been reproduced directly from edited, computer-generated copy submitted by the authors to the editors of this volume. The editors have verified text formatting, references, and quality of text and figures. No editing or proofreading has been done by the publisher.

ISBN: 978-0-9654625-5-6

International Society for Molecular Plant-Microbe Interactions
3340 Pilot Knob Road
St. Paul, Minnesota 55121 U.S.A.

Preface

The XIII International Congress of the IS-MPMI began in Sorrento, Italy, on July 21, lasted for five full days packed with an abundance of scientific, social, cultural, and gastronomic activity, and then ended in a flurry on July 27 with the bustle of people, luggage, and buses. Approximately 1,250 participants registered, originating from about 60 countries worldwide! This turnout reflects a good response from the MPMI scientific community to this event, even though only 1.5 years had passed since the last congress in Mexico. Furthermore, the policy for meeting registration elicited an increase of about 45% in the number of IS-MPMI memberships versus the December 2006 count.

The scientific program proved to be comprehensive, covering a wide range of topics. The opening lecture by James C. Carrington gave us a novel insight into the role of small RNAs in plants, an exciting, topical, growing field of research. The IS-MPMI Award Lecture by Thomas Boller presented an inside view into the development of his exceptionally innovative research career. The regular congress day started every morning with talks in the plenary sessions, followed by informal lunch discussions. The afternoon activities included concurrent sessions and poster sessions, which immediately rolled into the evening special-interest workshops that, in some cases, proceeded until after 23:00. Then, if 15 hours of listening to the organized presentations weren't enough, nightcap sessions continued into the wee hours for those who were not yet satiated and wanted to discuss more science and organize collaboration! A heartfelt thank you is extended to all the speakers. Their participation formed a strong scientific foundation for the congress. Hearing about their "hot" findings was exciting, and their interpretations of the subject were stimulating and thought-provoking.

The visual impact of the about 1,000 posters throughout the congress venue was impressive. These scientific presentations were equally attractive and instigated much discussion thanks to the availability of the authors during the two sessions. Needless to say, the poster committees had a daunting task in selecting the award recipients.

The congress venue was put to a rigorous test in hosting so many congress delegates! Its strategic location overlooking the sea of the sirens was indeed

breathtaking. We had the fortune of having the Congress Centre renovated for the start of the congress – the paint was still wet the day before the opening! Every possible conference space was utilized and optimized! Every problem was dealt with and resolved. The organization of the community lunches together worked well! All participants must agree that it was indeed an extraordinary sight to see, not to mention notable to hear, about 1,200 delegates seated all together for lunch! The lunches, normally consisting of several courses, were served efficiently in order to accommodate the schedule of the scientific program. The little cakes, tarts, or cookies served during the coffee breaks were delicious and so colourful and beautifully arranged.

The social program began with the welcome reception, which was held outdoors in the refreshing lemon orchard against the backdrop of the Gulf of Naples and the background music and dance of the classic Neapolitan "tarantella". The full attendance made it difficult to move about and locate old acquaintances in the crowd, but with patience, circulation was possible and encounters were successful. The week of the congress was substantially sunny and hot, hot, hot. Many participants, corresponding to about 20 busloads, braved the climate to participate in the congress tour to the Pompeii excavation site! It was striking to see so many bright yellow congress hats moving around the extensive archaeological complex. Fortunately, no PAMP (Pompei-Associated Missing People) were registered. In spite of the heat, seeing the ancient town of Pompeii below the shadow of the volcano Vesuvius provided a thrill and merited the visit, no matter what meteorological conditions prevailed.

In the closing ceremony, the presidency of the IS-MPMI was passed from Pierre de Wit to the new elected president, Federico Sanchez, who addressed the crowd, presented the poster awards, and introduced the site for the XIV IS-MPMI Congress in 2009.

The congress dinner was also hosted in the lemon orchard. A sumptuous meal was served, accompanied by an interactive Neapolitan music spectacle that was capable of drawing in all attendees. A highlight to the evening of entertainment involved the participation of the past and present presidents of IS-MPMI and the congress chair at centre stage.

The ultimate goals for these IS-MPMI congresses are to reinforce and expand the community, to share new and exciting scientific findings, as well to initiate and sustain personal contacts during these biennial encounters. Consensus is that these objectives have been achieved.

Thank you for joining us in Sorrento and see you in Quebec City !

Matteo Lorito and the Local Organizers

Plant carbohydrate scavenging through TonB-dependent receptors by the phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris*

Servane Blanvillain[1#], Damien Meyer[1], Guillaume Déjean[1], Alice Boulanger[1], Martine Lautier[1,2], Catherine Guynet[1], Nicolas Denancé[1], Jacques Vasse[1], Emmanuelle Lauber[1#], and Matthieu Arlat[1,2#]

[1] Laboratoire des Interactions Plantes-Microorganismes, CNRS/INRA UMR2594/441, Chemin de Borde Rouge BP52627, 31326 Castanet-Tolosan, France ; [2] Université Paul Sabatier, Toulouse III, 118 Route de Narbonne, 31062 Toulouse, France ;

[#] *These authors contributed equally to this work.*

arlat@toulouse.inra.fr

Phytopathogenic bacteria have in general a dual life: they are able to infect and colonize their host plants but they also have the ability to survive outside their hosts in various environments. This remarkable feature likely reflects a high degree of adaptability and the presence of specific genetic programs devoted to the exploitation of nutrients present in these diverse habitats. With the aim to study molecular mechanisms controlling adaptation of phytopathogenic bacteria to their host-plants, we undertook a global analysis of receptors and regulators of *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causal agent of black rot of crucifers. This pathogen infects a wide range of *Brassicaceae* plants of economic interest, including cabbage, cauliflower and radish as well as the model plant *Arabidopsis thaliana* (Meyer et al. 2005).

Our analysis of the *Xcc* (ATCC33913) genome revealed an overrepresentation of a particular family of receptors, named TonB-dependent receptors (TBDRs) (Blanvillain et al. 2007). These proteins are located in the outer membrane of Gram-negative bacteria and are mainly known to transport iron-siderophore complexes and vitamin B12 into the periplasm (Postle and Kadner 2003). In most cases, the expression of genes encoding these receptors is under the control of the Fur repressor (ferric

uptake regulator) and activated under conditions of iron starvation (Bagg and Neilands 1987). In *Xcc*, we showed that only 9 TBDRs out of 72 are directly associated with iron uptake: these nine genes are indeed regulated by the iron status, have a Fur-Box in their promoter region, and are repressed by the Fur protein.

The sucrose utilization locus

A systematic study of *Xcc* TBDRs, based on mutagenesis and pathogenicity tests, identified one *Xcc* TBDR (XCC3358) controlling pathogenicity on *A. thaliana*. In the genome, this TBDR gene, named *suxA* (sucrose utilization in *Xanthomonas*), is associated with genes related to sucrose metabolism: *XCC3359* (*suxB*) encodes an amylosucrase, *XCC3356* (*suxR*) encodes a transcriptional repressor of the LacI family and *XCC3357* (*suxC*) encodes a sugar transporter of the inner membrane (Fig. 1).

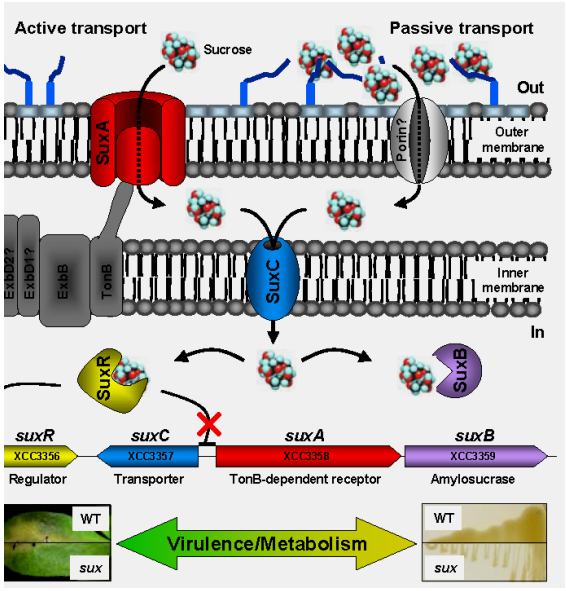

Fig. 1. Model of the *Xanthomonas campestris* pv. *campestris sux* locus functioning (reprinted by permission, from Blanvillain et al. 2007).This scheme shows sucrose outer membrane active transport *via* the SuxA TBDR or by passive diffusion through a putative porin. After crossing the inner membrane through the SuxC transporter, sucrose is proposed to interact with the SuxR repressor (thus allowing *sux* gene induction) and also to serve as a substrate for the SuxB amylosucrase. The large double headed arrow below the *sux* locus represents the balance between metabolic adaptation and virulence control putatively mediated by the *sux* locus.

Expression studies showed that *suxC*, *suxA* and *suxB* are specifically induced by sucrose, and that these three genes are repressed by the upstream regulator *suxR* in the absence of sucrose. Moreover, *suxC* and *suxB* mutants are affected in growth on sucrose and in pathogenicity on *Arabidopsis*, showing, like the *suxA* mutant, a clear delay in symptom development as compared to the wild-type strain.

The role of the *sux* locus in sucrose transport was confirmed by time course uptake experiments using radioactively labelled sucrose. We showed that *suxA* and *suxC* are necessary for sucrose entry into *Xcc*. However, even if SuxA is the predominant pathway for sucrose transport through the outer membrane, a second slower pathway exists, probably by passive diffusion through porins (Fig. 1). This hypothesis is reinforced by *suxA* expression studies which showed that this gene is required for its own induction only at low sucrose concentrations. Indeed, in the presence of 100 µM sucrose, *suxA* induction by sucrose was clearly slower in the mutant than in the wild-type background, whereas no significant difference was observed in the presence of 20 mM sucrose.

Concentration-dependent sucrose transport experiments showed a biphasic kinetic similar to those observed for vitamin B12 transport through the BtuB TBDR in *E. coli* and more recently for maltose transport through the MalA TBDR in *Caulobacter crescentus* (Neugebauer et al. 2005). As proposed for these two systems, we presume that the low K_d value (0.033 µM) mainly reflects binding to the SuxA TBDR whereas the higher K_d value (0.59 µM) reflects binding to the inner membrane transporter SuxC. Interestingly, the K_d value of sucrose binding to SuxA is 1500- to 3000-fold lower than that of the *E. coli* ScrY sucrose porin (Schulein et al. 1991; Van Gelder et al. 2001). Similar results were obtained for the *C. crescentus* MalA TBDR suggesting that both SuxA and MalA represent a new class of outer membrane carbohydrate transporters showing a much higher affinity for their substrate than porins.

The importance of the *sux* locus in *Xcc* is highlighted by its requirement for full virulence on *A. thaliana*. Thus, it appears that the ability to scavenge sucrose with a very high affinity plays a key role during the interaction with host plants. This study is the first one showing the functionality of a carbohydrate utilization locus containing a TBDR, or "CUT locus", which can be defined by high affinity uptake systems involved in the scavenging of plant molecules.

A CUT system involved in xylan utilization

The exploration of the *Xcc* genome revealed the existence of other putative CUT loci involved in carbohydrate utilization. Among these, the *XCC4115-XCC4122* locus was proposed to be involved in xylan degradation and uptake, as this locus contains genes coding for xylan degradation enzymes as well as inner membrane and outer membrane transporters. Interestingly, the *XCC4120* TBDR gene was found to be specifically induced by xylose and xylan. A similar pattern was also obtained for the *XCC2828* TBDR gene. *In silico* analyses revealed the presence of a perfectly conserved palindromic motif (X-box) in their promoter region, as well as upstream two other genes: *XCC4100* and *XCC4119*. Experiments based on mutagenesis and expression assays suggest that the X-box is recognized by XCC4101, a transcriptional repressor of the LacI family (Fig. 2).

These data suggest that the XCC4101 regulon encompasses 3 operons, one containing the major xylanase (XCC4118) detectable *in vitro*. Furthermore, direct and indirect transport assays suggest that inner membrane and outer membrane transporters of the studied CUT system are involved in xylo-oligosaccharides uptake rather than in xylose uptake. Thus, this *Xcc* CUT system defines a new pathway for the utilisation of xylan by bacteria.

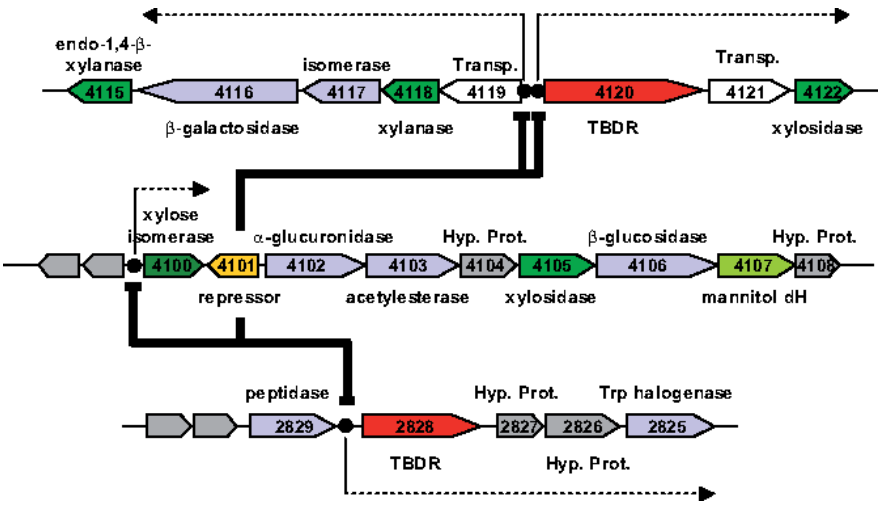

Fig. 2. Schematic representation of the *Xanthomonas campestris* pv. *campestris* CUT system involved in xylan utilization and its regulation by XCC4101 repressor. Dashed arrows indicate putative operons. Repression by XCC4101 is illustrated by bolded lines. Filled circles represent palindromic X-box motifs. Gene function description are from *Xcc* ATTCC33913 genome annotation (da Silva et al. 2002).

CUT loci and carbohydrate scavenging in Gram negative bacteria

A survey of TBDR was performed in 226 eubacterial completely sequenced genomes (Blanvillain et al. 2007). Most bacteria have less than 20 TBDRs per proteome, but some possess more than 40 TBDRs, thus forming a particular class in which TBDRs seem to be over represented. Very interestingly, in addition to *Xanthomonas* species, this class contains several aquatic bacteria, that are either oligotrophic or well known for their ability to degrade complex carbohydrates from algae, mollusc or arthropods. This class also contains *Bacteroides thetaiotaomicron*, which is a prominent mutualist in the distal intestine of human adults that has the ability to scavenge complex plant carbohydrates (Backhed et al. 2005; Sonnenburg et al. 2005; Xu and Gordon 2003). Furthermore, phytopathogenic bacteria, such as *Pseudomonas syringae* pathovars and *Erwinia carotovora* subsp. *atroseptica*, belong to an intermediary class comprising bacteria having between 21 and 39 TBDRs. Therefore, it is likely that TBDR overrepresentation might be related to the ability to utilize or even to scavenge complex carbohydrates in various environments and in particular from plant origin. This hypothesis is strengthened by the observation that several *Xcc* putative CUT loci are partially conserved in some of the bacteria dispalying TBDR overrepresentation. TBDR might allow these bacteria to scavenge complex carbohydrates, too large to pass through porins, even if they are present at very low concentrations in their environment. Thus CUT loci, which seem to participate to the adaptation of phytopathogenic bacteria to their host plants, might also play a very important role in human health as well as in the biogeochemical cycling of organic carbon in the environment. Our work on *Xcc* TBDRs which opens a new research area in plant-microbe interactions might also have a broader impact in the study of bacterial adaptation and evolution.

Literature cited

Backhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., and Gordon, J.I. 2005. Host-bacterial mutualism in the human intestine. Science 307**:**1915-1920.

Bagg, A., and Neilands, J.B. 1987. Ferric uptake regulation protein acts as a repressor, employing iron (II) as a cofactor to bind the operator of an iron transport operon in *Escherichia coli*. Biochemistry 26**:**5471-5477.

Blanvillain, S., Meyer, D., Boulanger, A., Lautier, M., Guynet, C., Denance, N., Vasse, J., Lauber, E., and Arlat, M. 2007. Plant Carbohydrate Scavenging through TonB-Dependent Receptors: A Feature Shared by Phytopathogenic and Aquatic Bacteria. PLoS ONE 2**:**e224.

da Silva, A.C.R., Ferro, J.A., Reinach, F.C., et al. 2002. Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. Nature 417**:**459-463.

Meyer, D., Lauber, E., Roby, D., Arlat, M., and Kroj, T. 2005. Optimization of pathogenicity assays to study the *Arabidopsis thaliana-Xanthomonas campestris* pv. *campestris* pathosystem. Mol. Plant Pathol. 6**:**327-333.

Neugebauer, H., Herrmann, C., Kammer, W., Schwarz, G., Nordheim, A., and Braun, V. 2005. ExbBD-dependent transport of maltodextrins through the novel MalA protein across the outer membrane of *Caulobacter crescentus*. J. Bacteriol. 187**:**8300-8311.

Postle, K., and Kadner, R.J. 2003. Touch and go: tying TonB to transport. Mol. Microbiol. 49**:**869-882.

Schulein, K., Schmid, K., and Benzl, R. 1991. The sugar-specific outer membrane channel ScrY contains functional characteristics of general diffusion pores and substrate-specific porins. Mol. Microbiol. 5**:**2233-2241.

Sonnenburg, J.L., Xu, J., Leip, D.D., Chen, C.H., Westover, B.P., Weatherford, J., Buhler, J.D., and Gordon, J.I. 2005. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science 307**:**1955-1959.

Van Gelder, P., Dutzler, R., Dumas, F., Koebnik, R., and Schirmer, T. 2001. Sucrose transport through maltoporin mutants of *Escherichia coli*. Protein Eng 14**:**943-948.

Xu, J., and Gordon, J.I. 2003. Inaugural Article: Honor thy symbionts. Proc. Natl. Acad. Sci. U S A 100**:**10452-10459.