IIIp-1

THE FUMONISIN GENE CLUSTER AND THE MATING TYPE REGION AS EXAMPLES OF SYNTENY IN TOXIGENIC *FUSARIUM* SPECIES

Cees Waalwijk, Theo van der Lee, Ineke de Vries, Thamara Hesselink, Joop Arts[*] and Gert H.J. Kema
Plant Research International BV, P.O. Box 16, 6700 AA Wageningen, The Netherlands.
[*]Current address: Wageningen University, Department of Cell Biology and Immunology, Wageningen, The Netherlands.
Email: cees.waalwijk@wur.nl

Fusarium proliferatum is a pathogen with a wide host range and a complex toxin profile. We have used a comparative genomic approach to study toxin production in *F. proliferatum,* and its phylogenetic relationship to other ascomycetes. A BAC library, generated from *F. proliferatum* isolate ITEM 2287, was used to identify and sequence chromosomal regions flanking the mating type locus and the gene cluster involved in the biosynthesis of fumonisin. Comparison of these sequences with corresponding sequences in other ascomycetes showed that the level of synteny between ascomycetes varied strongly for the different regions and that the level of similarity of genes within a single region can also fluctuate. We found synteny in the regions flanking the mating type idiomorph among ascomycetes that supposedly diverged 280 million years ago. The fumonisin gene clusters of *F. proliferatum* and *Fusarium verticillioides* were found to be completely syntenic but the flanking regions are highly dissimilar. These results indicate that the fumonisin gene cluster was probably acquired by horizontal gene transfer. The cluster has been inserted at different genome locations in both species. Surprisingly low similarity was found between the corresponding genes within the fumonisin cluster between *F. proliferatum* and *F. verticillioides*, as compared to other sequences. This indicates that horizontal gene transfer may have occurred from distinct genetic sources. The results exemplify the power of comparative genomics for gene annotation and for studies on the evolution of genes, gene-clusters and species.

IIIp-2

THERE IS MORE THAN ONE HMG-BOX TRANSCRIPTION FACTOR REGULATING MATING IN USTILAGO MAYDIS

Thomas Brefort, Philip Müller, Regine Kahmann

Max-Planck-Institute for terrestrial Microbiology, Karl-von-Frisch-Str., D-35043 Marburg, Germany; Phone: +496421-178510; FAX: +496421-178509; brefort@staff.uni-marburg.de; mueller4@staff.uni-marburg.de; kahmann@staff.uni-marburg.de

In the phytopathogenic fungus Ustilago maydis fusion of compatible haploid cells is a prerequisite for infection. This process is controlled by the biallelic a locus encoding pheromone precursors and receptors. Binding of pheromone to its cognate receptor triggers the pheromone response leading to an activation of the HMG-domain transcription factor Prf1. Prf1 binds to the PRE-boxes located in the promoters of the a- and b-genes, the latter encoding the key regulators of pathogenic development. As a result, stimulated wild type cells show elevated transcription of these genes as well as conjugation tube formation, while prf1 mutants do not. After fusion, prf1 is required for the expression of the b-genes and is therefore essential for pathogenic development. Here, we present the characterization of rop1 and hmg3 encoding two additional sequence-specific HMG-domain proteins. While hmg3 mutants are slighly impaired in mating and do form conjugation hyphae, rop1 deletion strains display a severe mating and filamentation defect and do not respond to pheromone stimulation. In particular, rop1, but not hmg3, is essential for pheromone induced gene expression in axenic culture. Since constitutive expression of prf1 fully complements the mating defect of rop1 mutants, rop1 seems to be required for prf1 gene expression. Indeed, we could show that Rop1 directly binds to specific fragments of the prf1 promoter in vitro. In addition, cells overexpressing rop1 show induced prf1 gene expression and display morphological defects that are not observed upon prf1 overexpression. Surprisingly, on the plant rop1 deletion strains do form conjugation tubes and express sufficient prf1 to cause full pathogenicity, while hmg3 deletion strains only produce reduced disease symptoms. This indicates the involvement of additional components in the regulation of prf1 gene expression during pathogenic growth. Currently, we are applying DNA array technology to examine the prf1-independent regulatory network governed by Rop1 and the processes controlled by Hmg3.

IIIp-3

AN ORTHOLOG OF THE SACCHAROMYCES CEREVISIAE PROTEIN PRM1 IS INVOLVED IN MATING OF BOTH HETEROTHALLIC AND HOMOTHALLIC COCHLIOBOLUS SPECIES

Scott E. Baker, Shun-Wen Lu**, Shinichi Oide**, B.Gillian Turgeon***
**Pacific Northwest National Laboratory, 902 Battelle Blvd., Richland, WA 99352, USA. Phone: (509) 372-4759 Fax: (509) 372-4732 email: scott.baker@pnl.gov; **Cornell Univ. Department of Plant Pathology, Ithaca, NY 14853, USA*

Mating of heterothallic ascomycetes involves both cell-cell recognition and fusion between cells of opposite mating type and nucleus-nucleus recognition and fusion, once cells have fused. Cell fusion is required initially when the male cell fuses with the female trichogyne and subsequently when cells in the crozier merge. Are all steps also a requirement for sexual reproduction of homothallic species? The process of cell fusion is complex, requiring participation of many proteins, including, ultimately, proteins mediating the actual fusion of cell membranes. Whole genome expression profiling has been used to identify these so-called 'fusase' proteins (Heiman and Walter, 2000). We have found orthologs of one of the S. cerevisiae fusase genes (PRM1), encoding a putative fusase protein, Prm1p, in the filamentous heterothallic ascomycete Cochliobolus heterostrophus and in C. luttrellii, a homothallic species closely related to C. heterostrophus. When PRM1 is deleted in C. heterostrophus and mutants crossed to wild type testers of opposite pigmentation to the mutants (to monitor the female parent), wild type pseudothecia are formed only when the tester strain acts as the female. Pseudothecia the color of the mutant parent are also found but these are smaller than wild type and barren. Mutant by mutant crosses yield small, barren 'pseudothecia'. Deletion of PRM1 in C. luttrellii and selfing of transformants yields very small pseudothecia with greatly reduced numbers of mature asci (less than 5-10% of wild type). The asci and ascospores that are made, appear to be wild type. Thus, Prm1p is required by both heterothallic and homothallic fungi.

IIIp-4

MOLECULAR ANALYSIS OF BREEDING BEHAVIOUR IN THE GENUS AGARICUS

Yonghua Li[1], Shirley Burrow[2], Mike Challen[2], Tim Elliott[2], Lorna Casselton[1, 2].*
[1]University of Oxford, Department of Plant Sciences, Oxford, OX1 3RB, UK; [2]Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK
*(*Email: mike.challen@hri.ac.uk)*

Agaricus species exhibit a range of breeding life-styles. Within the A. bisporus group are individuals that are typically heterothallic (non-self-fertile) as well as those that are truly homothallic (self-fertile). We are characterizing mating type genes from isolates within A. bisporus and amongst other Agaricus species to study the role and function of these genes in the evolution of different breeding behaviors. In the 2-spored cultivated A. bisporus var. bisporus, the unifactorial A mating type locus determines mating compatibility. Haploid nuclei of opposite mating type are partitioned into each of the two spores that germinate, to form a fertile heterokaryon (secondarily homothallism). Wild, 4-spored varieties of A. bisporus exhibit more conventional heterothallic behaviour where meiotic nuclei are individually compartmentalized; non-self-fertile homokaryons must fuse with compatible partners to generate fertile heterokaryons. Other Agaricus species may be truly self-fertile and do not need to mate. We have identified and sequence characterised a pair of divergently transcribed homeobox genes within the mating type locus of A. bisporus. PCR strategies have been used to identify allelic variants in different A specificities. These analyses should reveal whether a single pair of genes is sufficient to generate the allelic variation displayed in the wild collections of A. bisporus. Progress in the characterisation of mating type genes from the heterothallic A. bitorquis and homothallic A. subfloccosus will be described.

REPELLENTS OF USTILAGO MAYDIS DO FUNCTION DIFFERENTLY THAN HYDROPHOBINS.

Heine J Deelstra[1], Jan Dijksterhuis[2], John Kruijtzer[3], Rob MJ Liskamp[3], Han AB Wösten[1]

1. Microbiology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands. Phone: +31 302533854, fax: +31 302513655, email: h.j.deelstra@bio.uu.nl; h.a.b.wosten@bio.uu.nl 2. Fungal Biodiversity Centre, Uppsalalaan, Utrecht, The Netherlands. Phone: 31 302122600, fax: +31 302512097, email: dijksterhuis@cbs.knaw.nl 3. Medicinal Chemistry, Utrecht University, Sorbonnelaan 14-16, 3584 CA Utrecht, The Netherlands. Phone: +31 302537396, fax: +31 302536655, email: r.m.j.liskamp@pharm.uu.nl

Repellents of Ustilago maydis are cell wall located peptides of 35-53 amino acids in length that results from cleavage of the precursor protein Rep1 in the endoplasmic reticulum and were proposed to be analogous to the SC3 hydrophobin of Schizophyllum commune (Wösten et al., 1996). The phenotype of the ΔSC3 strain is similar to that of crosses of compatible ΔRep1 strains. No aerial hyphae are formed under conditions of high humidity but ΔRep1 strains did produce aerial hyphae under dry conditions. 80% of these hyphae were clustered in bundles of 2-6 hyphae. Wetting of these aerial hyphae resulted in their collapse. In contrast to the SC3 hydrophobin in S. commune, the repellents do not affect the cell wall contents of water and alkali soluble glucan.
Hyphae of ΔRep1 strains are not able to grow out of a droplet onto a hydrophobic solid. Addition of the synthetic peptide Rep1 partially complemented outgrowth. However, the SC3 hydrophobin of S. commune appeared to be more potent to mediate escape of hyphae of ΔRep1 strains from the water droplet. Interestingly, even more hyphae escaped in case of the wild-type strain. This suggests that the mode of action of synthetic repellents is different from that of the SC3 hydrophobin. Furthermore, ΔRep1 strains were transformed with C-terminally truncated forms of Rep1. Only the full-length gene could complement the mutant strain, suggesting a crucial role for the C-terminal part of Rep1.

Wösten, et al. Embo J, 1996. 15(16): p. 4274-81.

CYTOLOGICAL KARYOTYPING OF *FUSARIUM* SPP. BY THE GERM TUBE BURST METHOD

Cees Waalwijk, Masatoki Taga, Tsuyoshi Sato* and Gert H.J. Kema*

*Plant Research International B.V., P.O.Box 16, 6700 AA Wageningen, The Netherlands and *Department of Biology, Faculty of Science Okayama University, Okayama Japan. Email: cees.waalwijk@wur.nl*

Mitotic metaphase chromosomes were visualized using the germ tube burst method (GTBM) in different *Fusarium* spp., which enabled a precise determination of the cytological karyotypes of these fungi. Conditions suitable for specimen preparation were established for several *Fusarium* spp. These include cultivation of the isolates to produce standardized conidia and the incubation time of the conidia on the microscopic slides. The results were compared using pulsed field gel electrophoresis (PFGE) data and genetic maps available in the literature. In *F. graminearum* (teleomorph *Gibberella zeae*) the chromosome number (CN) deduced from the GTBM was four, which is in good with the combined physical and genetic map of the lineage 7 isolate PH-1 (NRRL 31084) sequenced at the Whitehead institute (http://www.broad.mit.edu/annotation/fungi/fusarium/maps.html). Lineage 7 is the predominant population of the wheat and barley scab fungus found in North America and Europe and is distributed worldwide. Provided the genome size of *F. graminearum* is 36 Mb, these chromosomes are apparently too large to be separated on PFGE. In *F. culmorum* we also identified four chromosomes and cytological karyotypes of related species will be presented.
F. proliferatum was studied as a representative of the *Fusarium* species in the *Liseola* section. We identified 12 chromosomes in this species which is in good agreement with the 12 linkage groups established in the closely related *G. moniliformis* as well as with results from PFGE of both *G. fujikuroi* and *G. moniliformis.* Chromosome numbers in all species analyzed, were supported by hybridization experiments using telomere specific oligonucleotides. The results presented here indicate that the GTBM is a useful tool, supplementary to PFGE and linkage mapping for genetical studies of various *Fusarium* species.

IIIp-7

REACTIVE OXYXGEN SPECIES IN THE INTERACTION OF CLAVICEPS PURPUREA AND RYE: THE ROLE OF AN NADPH OXIDASE

Sabine Giesbert and Paul Tudzynski
Institut für Botanik, Westf. Wilhelms-Universität, Schlossgarten 3, D-48149 Münster,
Tel. +49-251-8324997, Fax +49-251-8321601 e-mail: giesber@uni-muenster.de

Reactive oxygen species (ROS) are known to play a major role in host-pathogen interactions. Plants and animals react via an “oxidative burst” to pathogen attacks. Analyses of the interaction of the biotrophic fungus C. purpurea and its host Secale cereale have shown that (1) there is no obvious oxidative burst as response to wild type infection; (2) the fungus itself secretes H_2O_2 mediated by a cell wall associated SOD; (3) deletion of a transcription factor encoding gene (cptf) controlling all fungal catalase genes leads to an oxidative-burst-like plant reaction and impairs virulence[1]. As a possible source for generation of the fungal ROS a NADPH oxidase gene (cpnox1) was cloned and characterized. Cpnox1 shows significant homology to the recently isolated noxA gene of Aspergillus nidulans[2]. The expression of cpnox1 in planta and in axenic culture was studied and its role in the interaction is going to be analyzed by a gene replacement approach.

[1] Nathues E, Joshi S, Tenberge KT, von den Driesch M, Oeser B, Bäumer N, Mihlan M, Tudzynski P (2004) CPTF1, a CREB-like transcription factor is involved in the oxidative stress response in the phytopathogen Claviceps purpurea and modulates ROS level in its host Secale cereale. Mol Plant Microbe-Interact (in press)

[2] Lara-Ortíz T, Riveros-Rosas H, Aguirre J (2003) Reactive oxygen species generated by microbial NADPH oxidase NoxA regulate sexual development in Aspergillus nidulans. Mol Microbiol (2203) 50(4), 1241-1255

IIIp-8

IDENTIFICATION OF THE TELEOMORPH OF *SEPTORIA PASSERINII*, THE BARLEY SPECKLED LEAF PATHOGEN

S.B. Ware, E.C.P. Verstappen*, J.R. Cavaletto**, S.B. Goodwin**, C. Waalwijk*, and G.H.J. Kema**

**Plant Research International B.V., Postbus 16, 6700 AA Wageningen, The Netherlands. Phone: +31-317-477001. Fax: +31-317-418094. E-mails: S.B. Ware: sarah.ware@wur.nl.*

***Purdue University, Department of Botany and Plant Pathology, USA.*

Septoria passerinii causes speckled leaf blotch on barley. Although *S. passerinii* is considered to be asexual, mating-type idiomorphs were recently identified (Goodwin *et al.*, 2003). In addition, both mating type idiomorphs were commonly found among isolates from single leaves, suggesting that sexual recombination of *S. passerinii* in field conditions may be common. We were therefore interested in discovering the possible sexual stage of *S. passerinii*. Using the *M. graminicola in vitro* crossing method, isolates of *S. passerinii* with opposite mating types were inoculated on the susceptible barley cultivar Topper 33 (Kema *et al.*, 1996). From one of these combinations (P71 x P83), approximately 80 ascospores were found that morphologically resembled ascospores of the very closely related species, *Mycosphaerella graminicola* (Goodwin and Zismann, 2001). Seventeen viable two-celled single ascospores could be isolated, and conidial growth of these isolates on PDA plates, as well as in liquid culture, was identical to the parental isolates. The 17 progeny isolates were inoculated onto the barley cv. Topper 33, and all 17 produced septoria speckled leaf blotch symptoms. RAPD and AFLP analyses of the progeny and parental isolates (P71 and P83) clearly indicated a segregating population of the hitherto unknown teleomorph of *S. passerinii*. Based on the pathogenicity tests and genotypic data, we conclude that *Septoria passerinii*, formerly known as an asexual pathogen, has an active teleomorph. An active teleomorph has important implications for resistance breeding and understanding the population dynamics of this pathogen.

References

Goodwin, S.B., Waalwijk, C., Kema, G.H.J., Cavaletto, J.R. and Zhang, G. (2003) Cloning and analysis of the mating-type idiomorphs from the barley pathogen *Septoria passerinii*. Molecular Genetics and Genomics **269**:1-12.
Goodwin, S.B., and Zismann. V.L. (2001) Phylogenetic analyses of the ITS region of ribosomal DNA reveal that *Septoria passerinii* from barley is closely related to the wheat pathogen *Mycosphaerella graminicola*. Mycologia **93**:934-946.
Kema, G.H.J., Verstappen, E.C.P., Todorova, M. and Waalwijk, C. (1996) Successful crosses and molecular tetrad progeny analyses demonstrate heterothallism in *Mycosphaerella graminicola*. Current Genetics **30**:251-258.

MATING BEHAVIOUR AND CLONALITY IN PLEUROTUS OSTREATUS NATURAL POPULATION

A.V. Shnyreva
Department of Mycology @ Algology, Moscow Lomonosov State University, Moscow, 119992, Russia.
Tel: +7 095 9395057;
fax: +7 095 9393970; e-mail: shnyreva@herba.msu.ru

The higher basidiomycetous fungi form panmictic populations resulting from the hybridization of haploid basidiospores that are heteroallelic at both of two mating type loci. However, mating is not only restricted to the interactions between haploid homokaryons (ho-ho pairings). Heterokaryon is also capable of contributing a fertilizing nucleus to the haploid homokaryon that results in a new heterokaryon formation (he-ho pairings).
While analyzing a population structure of the oyster mushroom, Pleurotus ostreatus in Moscow region, we have revealed rather rare mechanism of genetic recombination by he-ho mating. We have screened in detail the fruit bodies (basidiocarps) collected within a single cluster and within a single log/substrate. Heterokaryotic strains derived from the same cluster are shown to be somatically compatible clones reproduced on the same heterokaryotic mycelium. To examine the distribution of mating incompatibility factors among basidiocarps within a fruit body cluster, matings between basidiospores were carried out. Thus, some of the heterokaryotic strains are shown to be identically constituted with respect to A and B factors resulted in 25% of compatible pairings, while the others were distinguished at one of each two mating type loci (75% of compatible pairings). This phenomenon can be explained by the heterokaryon-homokaryon hybridization occurring in natural habitat. The heterokaryotic mycelium occupying a log can fuse with an alien homokaryotic germling that eventually leads to the association of three different nuclei within the same mycelium. The further random reassortment of nuclei results in different combinations of mating type alleles between the fruit bodies within a cluster that are shown to be somatically compatible individuals. Likewise, these somatically compatible clones, but differed at mating type loci are shown to be identical at 14 polymorphic isozyme loci and RAPD profiles. However, the most common type of mating event in the P.ostreatus natural population is confirmed to be a spore-mediated outcrossing reflected in spatial distribution of mating type factors. Population structure of the P.ostreatus will be discussed with a special reference to sexual reproduction, clonality, and the role of outcrossing. The research was supported by the RFBR grant 01-04-49447.

PROMOTER ANALYSIS OF CGL2, A GALECTIN ENCODING GENE TRANSCRIBED DURING FRUITING BODY FORMATION IN COPRINOPSIS CINEREA (COPRINUS CINEREUS)

Rinaldo C. Bertossa[1], Ursula Kües[2], Markus Aebi[1], Markus Künzler[1]

[1] Institute of Microbiology, ETH Zurich, Schmelzbergstrasse 7, 8092 Zurich, Switzerland, Phone: +41 1 632 49 25, FAX: +41 1 632 11 48, e-mail: markus.kuenzler@micro.biol.ethz.ch; [2] Molecular Wood Biotechnology, Institute of Forest Botany, Georg-August-University Göttingen, Büsgenweg 2, 37077 Göttingen, Germany.

In the Homobasidiomycete Coprinopsis cinerea the two galectins (galactose binding lectins) CGL1 and CGL2 are specifically expressed during fruiting body development, from the first stages of hyphal aggregation till completion of tissue differentiation in the primordia. In particular, CGL2 expression starts in the dark when primary hyphal knots form within the mycelium. Onset of CGL1 expression correlates with the light induced formation of the more compact secondary hyphal knots. In mycelia cultivated in constant light, primary hyphal knot production and fruiting body formation is inhibited and so is galectin expression. In cultures kept in constant darkness, fruiting is also inhibited and CGL2 expression arrests with aging of the mycelium. In this study, we analyzed the promoter of the cgl2 gene by measuring transcript levels by quantitative real-time PCR (RT-qPCR) and show that regulation of CGL2 expression occurs at the transcriptional level. 627 nucleotides upstream of the start codon were sufficient to confer regulated expression of a GFP reporter gene. We identified a promoter element mediating induction of the cgl2 gene in constant darkness. Along with two (or three) direct repeats (TGGAAG/TGGAAG/GGAA), the sequence (bp positions 627 to 566 from ATG) contains a CRE consensus site (cAMP-responsive element, TGCGTCA) suggesting the involvement of cAMP in cgl2 activation. An element responsible for light repression was not found.

IIIp-11

4-DIHYDROMETHYLTRISPORATE DEHYDROGENASE : MATING-RELATED EXPRESSION AND PROTEIN ACTIVITY IN MUCOR

A. Petzold, A. Burmester, F. Wolschendorf, J. Wöstemeyer, C. Schimek
Institute of General Microbiology and Microbe Genetics, Neugasse 24, Friedrich-Schiller-University, D-07743 Jena, Germany. phone: +49-3641-949327; fax: +49-3641-949312; e-mail: b9sccr@nds.rz.uni-jena.de

4-dihydromethyltrisporate dehydrogenase (TDH) catalyses the conversion of 4-dihydromethyltrisporate (4-DHMT) into methyltrisporate, a key event in the complementary trisporic acid synthesis pathway of Mucorales and Mortierellales (Schimek et al., 2003). This reaction is mating-type specific, as conversion of the (+) specific trisporic acid precursor 4-DHMT occurs only in the (-) mating type, whereas the single copy TDH gene exists in both mating types (Czempinski et al., 1996). RT-PCR analysis revealed constitutive expression of the gene in both mating types. Similar responses were found in all cultures, either sexually stimulated or unstimulated. Separated by an intergenic region of only 92 bp, a gene encoding a putative acyl-CoA thioester hydrolase (ACT) is located downstream of TDH but oriented in opposite direction. That gene is also expressed constitutively, irrespective of the mating situation, but at a much lower level than TDH. Hints to antisense regulation were not found. Translation of the TDH gene product was examined using an antibody against purified Mucor mucedo TDH. Histochemical analysis revealed enzyme activity only in sexually stimulated (-) cultures. Activity increased with the duration of sexual stimulation. An activity plateau was reached after about 100 minutes of stimulation. In situ histochemical analysis confirmed that protein activity is restricted to one of the mating partners, presumably the (-) mating type, and depends on sexual activity. TDH activity is therefore proposed to be regulated post-transcriptionally but not via antisense ACT-mRNA.

Schimek C, Kleppe K, Saleem A-R, Voigt K, Burmester A, Wöstemeyer J
(2003) Sexual reactions in Mortierellales are mediated by the trisporic acid system. Mycological Research 107, 736-747
Czempinski K, Kruft V, Wöstemeyer J, Burmester A (1996) 4-dihydromethyltrisporate dehydrogenase from Mucor mucedo, an enzyme of the sexual hormone pathway: purification, and cloning of the corresponding gene. Microbiology 142, 2647-2654

IIIp-12

CHARACTERIZATION OF THE MATING TYPE LOCI OF SPORISORIUM REILIANUM

Jan Schirawski, Bernadette Heinze and Regine Kahmann. Max-Planck-Institute for terrestrial Microbiology, Karl-von-Frisch Str., 35043 Marburg, Germany. phone: +49-6421-178510/ +49-6421-178501, fax: +49-6421-178509, email: schiraws@staff.uni-marburg.de, heinzeb@staff.uni-marburg.de, kahmann@staff.uni-marburg.de

Sporisorium reilianum, the causative agent of head smut disease of maize and sorghum, infects the host plant through the roots and proliferates asymptomatically within the plant. Disease symptoms become apparent in the male or female flowers only when spore formation occurs.
We have adapted molecular biological techniques to make S. reilianum amenable to genetic manipulation. These techniques in combination with newly developed mating assays have been used to characterize the mating type loci of S. reilianum. Screening of isolates from several countries revealed that S. reilianum contains two different unlinked mating type loci (a and b). The b locus is present in at least three alleles and codes for subunits of heterodimeric homeodomain transcription factors, as in the close relative Ustilago maydis. The a alleles encode pheromone/receptor systems. However, in contrast to U. maydis where the a locus is biallelic, S. reilianum contains at least three different a alleles.
A hybrid b locus was constructed that allowed the expression of an active heterodimeric transcription factor, and was introduced into haploid S. reilianum strains by homologous recombination. Such solopathogenic S. reilianum strains will be used to identify genes and signalling cascades involved in tissue specificity at the site of host plant entry as well as at the site of proliferation and development of disease symptoms.

MATING TYPES AND GENETIC DIVERSITY IN PODOSPHAERA FUSCA

E. Mingorance[1], M. E. Rivera[1], F. M. Cazorla[1], J. A. Torés[2], A. de Vicente[1] and A. Pérez-García[1]
[1]Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, E-29071 Málaga and [2]Estación Experimental "La Mayora" (CSIC), Algarrobo Costa, E-29750 Málaga, SPAIN. Phone: 34-952131890. Fax: 34-952132000. E-mail: aperez@uma.es

Powdery mildew is a devastating disease of cucurbits worldwide. Disease symptoms are characterised by the whitish talcum-like, powdery fungal growth developing on both leaf surfaces, petioles and stems. In Spain the main causal agent of cucurbit powdery mildew is Podosphaera fusca, an obligate biotrophic ectoparasite which is responsible for important yield losses in cucurbit crops under field and greenhouse conditions. In heterothallic fungi like P. fusca, sexual reproduction provides a means by which an increase of genetic diversity can be generated, an aspect that may be particularly important in allowing plant pathogenic fungi to respond to selection pressures such as the introduction of resistant cultivars and novel fungicide treatments. As part of a research program on cucurbit powdery mildew management in Spain, we are carrying out a detailed analysis on the biological and genetic diversity of P. fusca populations from different cucurbit production areas, in order to elucidate the possible impact of sexual reproduction on disease epidemiology.
Population genetics of P. fusca presents a serious challenge because, as the fungus is an obligate biotroph, the range of phenotypic characters that can be studied is limited. From a collection of isolates, frequencies of physiological races of the pathogen and mating types are being established by biological methods. To date, four races (1, 2, 4 and 5) and both mating types (MAT1-1 and MAT1-2) have been detected in all cucurbit production areas sampled. Because the current method to determine mating types in P. fusca is time consuming and labour intensive, we are trying to isolate P. fusca mating type genes to develop a PCR approach for mating type identification. In population genetics it is best to use molecular markers that are selectively neutral, highly informative, reproducible, and relatively easy to assay. For organisms that cannot be grown on artificial media like P. fusca, PCR-based techniques are the methods of choice due to limitations on the amount of tissue that can be used for DNA isolation. We are currently using RAPD and rDNA RFLP analyses to establish genetic variation within and among populations of P. fusca in Spain.

A NEW MEMBER OF RECQ HELICASE FAMILY FROM MUSHROOM

Shiho Katsukawa, Takashi Yamazaki, Susumu Kajiwara, and Kazuo Shishido
Department of Life Science, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8501, Japan; Phone: +81-45-924-5716;
Fax: +81-45-924-5773; E-mail: shihokat@bio.titech.ac.jp

We cloned a RecQ-type DNA helicase gene from eubasidiomycete Lentinula edodes (Le.). This gene, named Le.recQ, was found to have coding capacity of 945 amino acids (aa) and be interrupted by eleven small introns. The deduced Le.RECQ protein was clearly smaller than other fungal RecQ proteins such as Neurospora crassa QDE3 (1955 aa), Schizosaccharomyces pombe Rqh1 (1328 aa), and Saccharomyces cerevisiae SGS1 (1447). The Le.RECQ protein has a helicase domain which shows about 50% identity in aa level to these fungal RecQ helicase.
Northern-blot analysis revealed that Le.recQ gene is constitutively transcribed during the course of fruiting body formation. But the amount of Le.recQ transcript in the vegetative growth phase was different between the compatible monokaryotic strains and the dikaryotic strain obtained by mating between two monokaryotic strains; the vegetatively growing dikaryotic cells contained the Le.recQ transcripts several times as much as those of the monokaryotic mycelial cells. Since the same amount of total cellular RNA was used for the experiment, the results suggest that the Le.recQ gene functions more actively in the growing dikayotic cells. Quantitative RT-PCR was done to analyze the contents of the Le.recQ transcripts in the same amount of total cellular RNA isolated from stipe, gill tissue, and gill-depleted pileus of mature fruiting body. The result showed the presence of similar contents of Le.recQ transcripts in the RNA preparations from all the parts of mature fruiting body. The in situ RNA staining experiment revealed that significantly large amount of RNA molecules are present in gill tissue. So the above results suggest that the Le.recQ gene may play a role most actively in gill tissue in which a large number of basidiospores are formed. The results, taken together, suggest the possibility that the Le.recQ gene is involved in DNA replication.

IIIp-15

GENETIC ANALYSIS OF SPORE KILLING IN THE FILAMENTOUS ASCOMYCETE PODOSPORA ANSERINA

Andrea Hamann, Jens Rabenstein, and Heinz D. Osiewacz

Botanisches Institut, J.W.Goethe-Universitaet, Marie-Curie-Str. 9, D-60439 Frankfurt, Germany
phone: +49 (0)69 798 29279
fax: +49 (0)69 798 29363
email: a.hamann@em.uni-frankfurt.de

In some filamentous fungi like Neurospora sitophila and Podospora anserina, a phenomenon named spore killing has been described. It is characterised by the degeneration of a proportion of the ascospores in crosses between killer and sensitive strains. In P. anserina, spore killing either leads to two-spored asci (first division segregation, FDS) or four-spored asci (second division segregation, SDS). The analysis of different P. anserina strains for their ability to induce spore killing resulted in the identification of three new killer strains. Test crosses of each of these strains with different sensitive strains led to different SDS ratios suggesting an influence of the sensitive strain on crossing-over frequency. Unexpectedly, in crosses of killer strain O, a highly variable frequency of two-spored asci is observed. Moreover, in specific crosses of this strain with some sensitive strains (e.g. Us5) an unexpected high number of four-spored asci is formed. Genetic analysis of these spores demonstrated that a siginificant proportion of them are not the consequence of SDS but appear to be the result of gene conversion leading to a preferential transmission of the killer allele. In addition, in a number of these spores the killer and the sensitive allele reside in one nucleus resulting in a sensitive killer strain. Such recombined alleles, which have not yet been described in any form of spore killing, confer reduced susceptibility to spore killing. The biological role and the impact of this recombined allele on the number of surviving spores will be discussed.

IIIp-16

THE COP9 SIGNALOSOME IS AN ESSENTIAL REGULATOR OF DEVELOPMENT IN ASPERGILLUS NIDULANS

Silke Busch[1], Elke U. Schwier[1], Oliver W. Draht[1], Krystyna Nahlik[1], Sabine E. Eckert[2], Gerhard H. Braus[1**]*

[1]Institut für Mikrobiologie und Genetik, Georg-August-Universität, Grisebachstrasse 8, D-37077 Göttingen (GER); e-mail: sbusch@gwdg.de,phone: +49-551-393820, fax: +49-551-393820 ** e-mail: gbraus@gwdg.de, phone: +49-551-393771, fax: +49-551-393820 [2]Department of Biosciences, Faculty of Science, Technology and Medical Studies, University of Kent at Canterbury, Canterbury, Kent CT2 7NJ (UK)*

The COP9 signalosome (CSN) is a conserved multiprotein complex involved in regulation of eukaryotic development. The deduced amino acid sequences of three Aspergillus nidulans genes, csnA, csnD and csnE, show high identities to the first, fourth and fifth CSN subunits of higher eukaryotes. At present, ongoing yeast two-hybrid experiments revealed protein-protein interactions of two subunits. The csnD transcript is abundant during vegetative growth as well as development and the corresponding protein accumulates in the nucleus.

Strains deleted for either of the csn genes are viable and show identical mutant phenotypes at conditions that allow development: Hyphae appear partly red and contain cells of reduced size. Additionally, light-dependence of propagation onset is affected, as shown for the csnD and csnE deletion strains. All three csn deletion strains are capable to initiate the sexual cycle and develop primordia, but maturation to sexual fruit bodies is blocked. This developmental arrest could not be overcome by overexpression of the sexual activator velvet (VEA).

We conclude that the COP9 signalosome in A. nidulans is a key regulator of sexual development and several other cellular processes. In order to identify potential downstrem targets of CSN action, we started a proteomics approach. The proposed structural and functional conservation of the A. nidulans COP9 signalosome to the CSN of higher eukaryotes enables studies on this regulatory complex in a genetically amenable organism.

VCG ANALYSIS OF *FUSARIUM OXYSPORUM* F.SP. *LACTUCAE*

Matias Pasquali, Angelo Garibaldi, Maria Lodovica Gullino
AGROINNOVA- via L. da Vinci 44- 10095 Grugliasco TO ITALY
Tel:+3901167088696; fax +390116708541.

e-mail: matias.pasquali@unito.it

Session: Mating and Sexual Development

In the last ten years *Fusarium oxysporum* isolates pathogenic on lattuce (*Lactuca sativa* L.) spread all over the world (Taiwan, Japan, Italy, USA). Epidemics in Northern Italy were firstly reported in 2002. In order to define the origin of the isolates found in Italy and eventually to define genetic differences in world isolates, a VCG analysis was performed. Isolates from Italy, Usa, Japan and Taiwan were used in this study. Moreover some ATCC isolates attacking plants of C*ompositae* family were used as comparison.

Nit mutants were obtained and classified as *nit 1*, *nit 3* and *nit M*. Two self compatible mutants for each isolate were selected and paired in all possible combinations with each other. The experiment was performed twice. VCG assignation to the same group was decided when *nit* mutants from different isolates produced robust heterokaryon formation with each other before 10 days.

Results revealed that all isolates were self compatible and all the isolates proved to be pathogenic on lattuce have no compatibility with *Fusarium oxysporum* isolates attacking other plant species.

The isolates belonging to *forma specialis lactucae* tested in these experiments belong to two different VCGs. They are not compatible with any non-pathogenic isolates obtained from lettuce plants and any other *F. oxysporum* used in this study.

Results contribute to describe the evolution of the f.sp. *lactucae*. Isolates were considered with a clonal origin: by contrast there are genetic differences that will be further investigated with molecular analysis in order to verify if the different VCG populations are the effect of evolution of a single population or if they have a polyfiletic origin.

VCG proved to be useful for the identification of the population that caused the Italian epidemics in 2002. The most widespread isolate that has been identified in USA and Taiwan is also present in Italy. Its diffusion may probably be carried out by infected seeds, which were found in many seed samples.

SEXUALITY AND PARASITISM SHARE COMMON REGULATORY PATHWAYS IN THE FUNGUS PARASITELLA PARASITICA

Schultze Kornelia, Schimek Christine, Wöstemeyer Johannes and Burmester Anke

Institute for Microbiology, Friedrich-Schiller-Universität Jena, Chair of General Microbiology and Microbe Genetics, Neugasse 24, D-07743 Jena, Germany, E-mail: b5buan@rz.uni-jena.de,Tel: +49 3641 949310, Fax: +49 3641 949312

Parasitella parasitica, a facultative mycoparasite of zygomycetous fungi, forms cytoplasmic fusions with its hosts during infection. Thus, the organism is an efficient donor of genetic material in parasexual host-parasite interactions (Kellner et al. 1993, Wöstemeyer et al. 2002). Recognition between parasite and host is mediated by trisporoids, which are also responsible for sexual communication. The TDH gene for the key enzyme of trisporic acid biosynthesis, 4-dihydromethyl-trisporate dehydrogenase, was cloned and its transcription analysed. TDH was cloned on a 6175 bp insert and was found to map in a complex cluster of genes and ORFs that suggest post-transcriptional antisense regulation. Histochemical TDH analysis in developing parasitic or sexual structures shows high enzymatic activity in Parasitella. TDH is linked to a gene for acyl-CoA thioesterase (ACT). Two ORFs were identified in the 5'-region of the TDH gene, a third one, coding for 176 amino acids overlaps the ACT gene in antisense direction completely. Expression levels of ACT and ORF1 depend on parasitic or sexual interactions. A gene coding for a putative heat shock protein (HSP) was identified several kb upstream of the ACT gene. Expression analysis shows a low expression of the HSP gene in Parasitella parasitica (-) and a strong expression of this gene in mated cultures. In Parasitella parasitica (+) single cultures the HSP gene is not expressed.

Kellner, M., Burmester, A., Wöstemeyer, A., Wöstemeyer, J., 1993. Transfer of genetic information from the mycoparas te *Parasitella parasitica* to its host *Absidia glauca*. Curr. Genet. 23, 334-337.

Wöstemeyer, J., Burmester, A., Wöstemeyer, A., Schultze, K., Voigt, K., 2002. Gene transfer in the fungal host-parasite system *Absidia glauca-Parasitella parasitica* depends on infection. In Syvanen M., Kado C. (eds.) Horizontal gene transfer, 2nd edn. Academic Press, San Diego pp. 241-247.d