192. Bulk segregant analysis followed by high-throughput sequencing identifies a novel gene required for acid production in Aspergillus niger. Jing Niu[1], Peter J. Punt[1,2], Arthur F. J. Ram[1]. 1) Leiden University, Institute of Biology Leiden, Molecular Microbiology and Biotechnology, Sylviusweg 72, 2333 BE Leiden, The Netherlands; 2) TNO Microbiology and Systems Biology, PO Box 360, 3700 AJ Zeist, The Netherlands.

We have combined high-throughput sequencing (Illumina) with bulk segregant analysis to identify the mutation in a previously isolated non-acidifying mutant in Aspergillus niger. Because of the lack of a sexual cycle for A. niger, the parasexual cycle was used to generate a pool of segregants. A set of defined color mutants with auxotrofic markers was constructed by targeted gene deletion to facilitate the construction of diploid strains in A. niger. In the bulk-segregant analysis approach, the mutant of interest is crossed to a wild-type strain and haploid segregants displaying the phenotype of interest are pooled and DNA from this pool of segregants is sequenced using a deep sequencing technology (e.g. Illumina). The mutation causing the non-acidifying phenotype was found to be recessive and since about 50% of the segregants (78 of the 140 segregants analysed) showed the non-acidifying phenotype, the phenotype was likely to be caused by a single mutation. In addition to sequencing the genomic DNA pool of segregants, the parental strains were also sequenced and single nucleotide polymorphisms (SNPs) between the strains were identified. SNPs between the parental strains not related with the phenotype, have a 50% chance to be present in the pool; SNPs responsible for the phenotype or closely linked to the mutation responsible for the phenotype, are conserved in the pool. In total, 52 SNPs were identified between the two parental strains and three SNPs were 100% conserved in the pool of segregants. All three SNPs mapped to the right arm of Chromosome II, indicating that this region contains the genetic locus affecting the phenotype related to acid production. It is currently determined which of the three SNP is responsible for the non-acidifying phenotype of the mutant by complementation and targeted deletion studies.

193. *Aspergillus niger* regulatory mutant strains for improved protein production: strain selection and mutant gene identification. Peter Punt[1], Jing Niu[2], Deepa Nair[1,2], Ellen Lagendijk[2], Mark Arendhorst[2], Arthur Ram[2]. 1) Microbiology, TNO, Zeist, Netherlands; 2) Leiden University, Institute of Biology Leiden, Molecular Microbiology and Biotechnology, Sylviusweg 72, 2333 BE Leiden, The Netherlands.

Optimized protein production in filamentous fungi requires the availability of fungal strains with low levels of secreted protease activity. Already for several decades research has been carried out to obtain these type of mutants, leading to the isolation of mutants with very favourable characteristics. Complementation studies have allowed identification of several of these mutants, one being a mutation in a transcriptional regulatory gene, prtT (e.g. Punt et al;., 2008).

Based on this mutant strain further improved strains have been selected using positive selection approaches. Controlled fermentation experiments with these strains revealed different protease profiles, whereas full genome sequencing was carried out in an attempt to identify the genetic basis of the mutant phenotypes.

Another method to identify regulatory protease deficient mutants, was based on the use of collections of regulatory gene knock-out strains in N. crassa and A. niger. Based on positive selection approaches and classical milkhalo screening novel mutant strains with modified protease production profiles were obtained.

194. Comparative genomics and gene cluster identification in 28 species of *Aspergillus* section *Nigri*. Tammi Vesth[1], Jane Nybo[1], Sebastian Theobald[1], Ellen K. Lyhne[1], Martin E. Kogle[1], Igor Grigoriev[3], Uffe H. Mortensen[1], Scott E. Baker[2], Mikael R. Andersen[1]. 1) Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark; 2) Joint BioEnergy Institute, Emeryville, CA and Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA; 3) Joint Genome Institute, Walnut Creek, CA, USA.

The filamentous fungus *Aspergillus niger* and its close relatives in *Aspergillus* section *Nigri* are of broad interest to the scientific community including applied, medical and basic research. The fungi are prolific producers of native and heterologous proteins, organic acids (in particular citrate), and secondary metabolites (including bioactives and toxins such as ochratoxin A). Because of these abilities they represent a substantial economic interests in bioenergy applications. While 8 individual species from this group has been whole-genome sequenced, the genetic basis for these diverse phenotypes remains largely unidentified.
In this study, we have de novo sequenced the genomes of 20 additional species of the section *Nigri*, thus allowing the genome comparison of all members of this important section of fungal species. Here we present the results of this large-scale genomic analysis where we have examined the core genome of these 28 species and identified variations in the genetic makeup of individual species and groups of species. In particular, we have found genes unique to *Aspergillus* section *Nigri*, as well as genes which are only found in subgroups of the section. Our analysis here correlates these genes to the phenotypes of the fungi.
Furthermore, we have predicted secondary metabolite gene clusters in all 28 species. We present here an overview of these gene clusters and how they are shared and vary between species. We also correlate the presence of gene clusters to presence of known fungal metabolites.

195. Comparison of a commercial *Aspergillus oryzae* strain and its degenerated strain by genome re-sequencing. Yiyi Zhong, Wenyan Nong, Hoishan Kwan. CUHK, Shatin, NT, HK.

The filamentous fungus *Aspergillus oryzae* plays a critical role in the koji-making process for soy sauce fermentation. It can produces many enzymes to extensively digest macronutrients which gives soy sauce a pleasant combination of flavor and appearance. The strain RD2 is commercially used in Chinese soy sauce industry. A degenerated strain TS2 obtained during production would yield poor-quality soy sauce when used in koji making. We aimed to explain the phenotypic differences of two strains at the molecular level by genomic