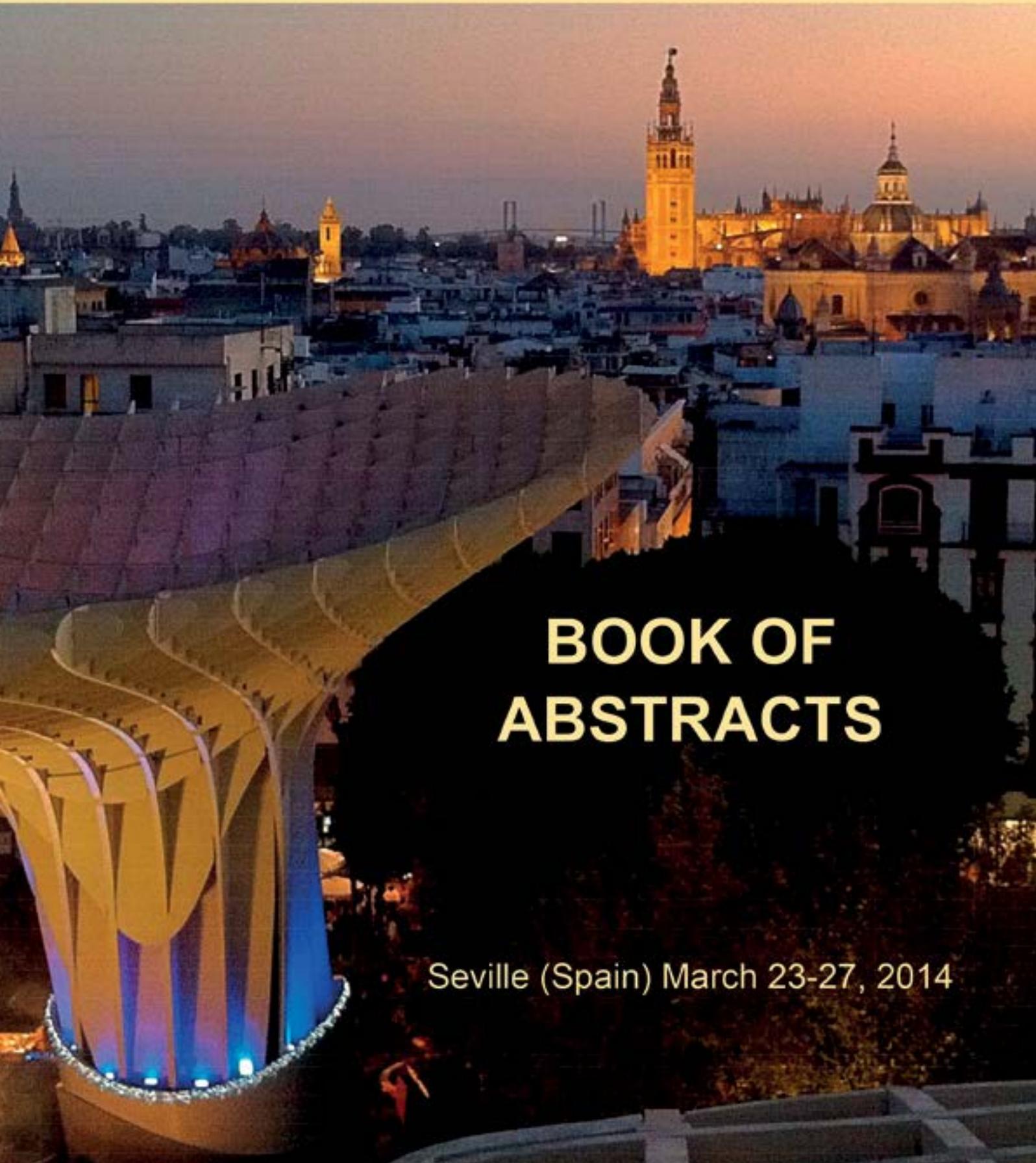




12th European Conference on Fungal Genetics



BOOK OF ABSTRACTS

Seville (Spain) March 23-27, 2014

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DEVELOPMENT OF A HIGH THROUGHPUT GENE EXPRESSION PLATFORM FOR EXPRESSION OF TRANSCRIPTION FACTORS IN ASPERGILLUS NIDULANSDIANA ANYAOGU⁽¹⁾, DORTE K. HOLM⁽²⁾, ALI ALTINTAS⁽¹⁾, CHRIS WORKMAN⁽¹⁾, UFFE HASBRO MORTENSEN⁽¹⁾⁽¹⁾ DTU SYSTEMS BIOLOGY, DENMARK, ⁽²⁾ NOVOZYMES A/S, DENMARK

Aspergillus nidulans is a well characterized model organism for which many genetic engineering tools have been developed. This makes it an ideal system to study and uncover the mechanisms that control fundamental physiological aspects of fungal life forms i.e. cell differentiation, signaling and metabolism. In order to address fungal physiology in a systems perspective we are in the process of making an overexpression library of all 490 putative and annotated transcription factors (TF) contained in the *Aspergillus* genome. To facilitate the construction of this overexpression library we have developed a high throughput (HTP) gene expression platform with background free cloning vectors and background free integration systems. Furthermore, to limit the manual work most of the construction of gene targeting substrates has been automated, including the validation of PCR fragments. Here we present the results of the first generation of the library, which is composed by all 52 TF on chromosome I expressed under the control of the inducible Tet-on promoter. The initial characterization identified several strains with an altered metabolite profile. Hence, some showed up-regulation, or down-regulation, of a few secondary metabolites indicating that the library contains both activators and repressors. Among the up-regulated compounds some are potentially novel. Interestingly, overexpression of some of the TFs had significant impact on the morphology, conidiation and growth rate. Together our results show that the high throughput gene expression platform is an efficient and suitable way to construct a TF library, which can be used to study the regulation of the secondary metabolism as well as various other aspects of fungal physiology like cell cycle regulation, cell differentiation and signaling.

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DIFFERENTIAL EXPRESSION OF LIGNINOLYTIC PEROXIDASE GENES BY PLEUROTUS OSTREATUS GROWING ON LIGNOCELLULOSE MEDIUM UNDER DIFFERENT ENVIRONMENTAL CONDITIONSELENA FERNÁNDEZ FUEYO⁽¹⁾, RAÚL CASTANERA⁽²⁾, FRANCISCO J RUIZ-DUEÑAS⁽¹⁾, MARÍA F LÓPEZ-LUCENDO⁽¹⁾, LUCÍA RAMÍREZ⁽²⁾, ANTONIO G PISABARRO⁽²⁾, ANGEL T MARTÍNEZ⁽¹⁾⁽¹⁾ CIB-CSIC, SPAIN, ⁽²⁾ UPN, SPAIN

Pleurotus ostreatus is an important edible mushroom and a model lignin degrading organism, whose genome contains nine genes of ligninolytic peroxidases, characteristic of white-rot fungi. These genes encode six manganese peroxidase (MnP) and three versatile peroxidase (VP) isoenzymes that differ in their catalytic and stability properties. Using liquid chromatography coupled to tandem mass spectrometry, secretion of four of these peroxidase isoenzymes (VP1, VP2, MnP2 and MnP6) was confirmed when *P. ostreatus* grows in a lignocellulose medium (pH 5.5) at 25 °C (three more were identified by only one unique peptide). Then, the effect of environmental parameters on the expression of the above nine genes was studied by reverse transcription-quantitative PCR (RT-qPCR) by changing the incubation temperature and medium pH of the *P. ostreatus* cultures pre-grown under the above conditions (using two reference genes for normalization of the RT-qPCR results). The cultures maintained at 25 °C provided the highest levels of peroxidase transcripts and the highest total activity on Mn²⁺ (a substrate of both MnP and VP) and Reactive Black 5 (a VP specific substrate). After global analysis of the expression patterns, peroxidase genes were divided into three main groups according to the level of expression at optimal conditions (vp1/mnp3 > vp2/vp3/mnp1/mnp2/mnp6 > mnp4/mnp5). Adjusting the culture pH to acidic or alkaline conditions (pH 3 and 8) or decreasing/increasing the incubation temperature (to 10 °C/37 °C) led to downregulation of most of the peroxidase genes (and decrease of the enzymatic activity) in most of the cases. The analysis also reveals differences in the transcription levels of the peroxidase genes when the culture temperature and pH parameters were changed, suggesting a possible adaptive expression according to environmental conditions. pH modification produced more dramatic effects than temperature modification, with vp expression resulting more affected than mnp expression. While mnp3 was the less affected gene under temperature modified conditions, mnp4 and mnp5 were the only peroxidase genes being slightly upregulated under alkaline pH conditions.



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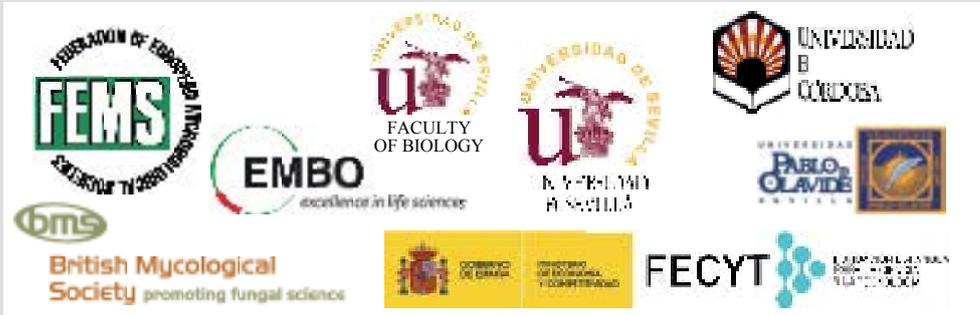
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