ECFG12
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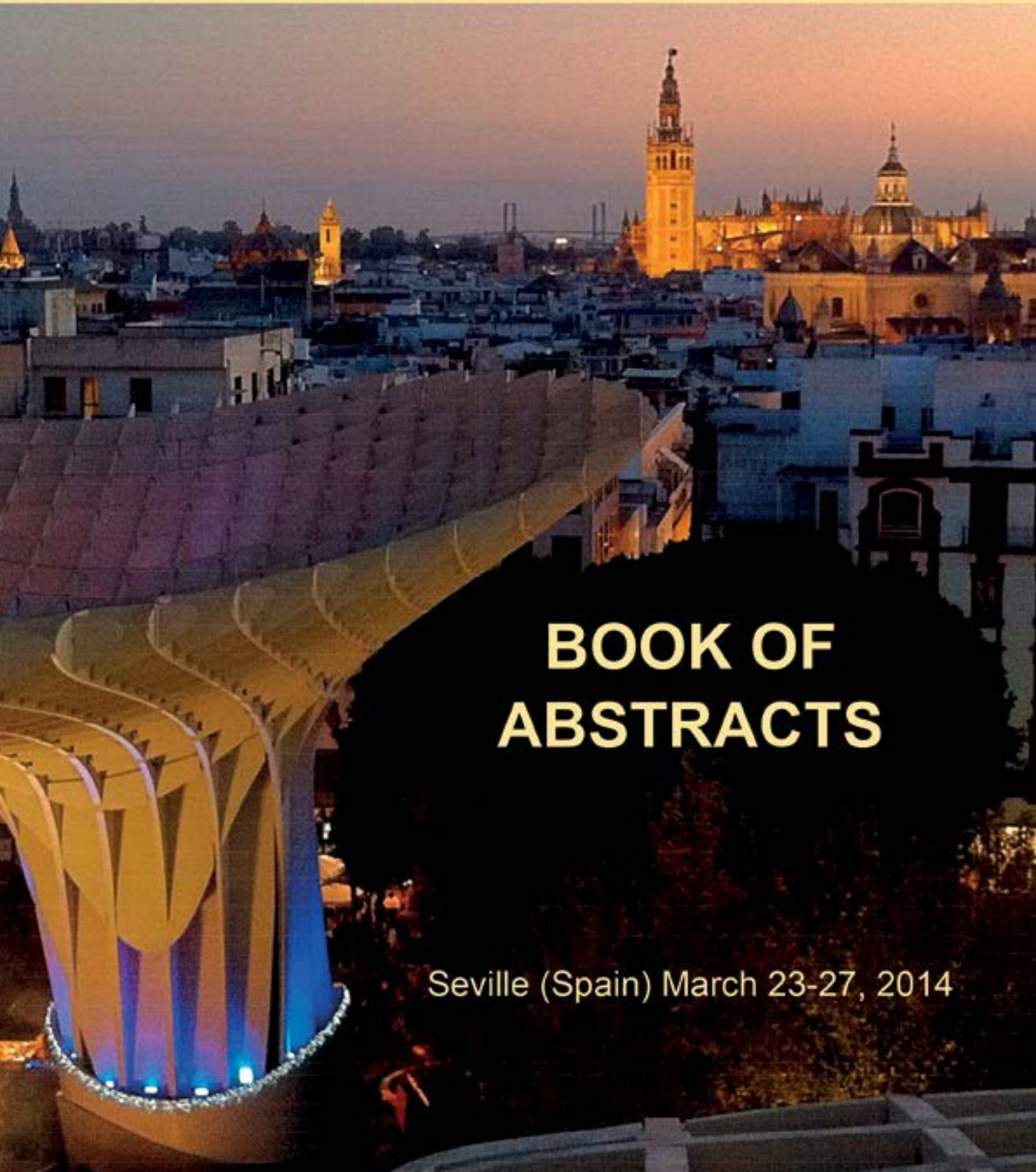

BOOK OF ABSTRACTS

Seville (Spain) March 23-27, 2014

005 ASPERGILLUS NIDULANS SIRTUIN A REGULATES SECONDARY METABOLISM PRODUCTIONS

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Sirtuins are member of the NAD(+)-dependent histone deacetylase (HDAC) family and are ubiquitous in eukaryotes although their physiological role in fungi is unknown especially that in gene regulation. We found Aspergillus nidulans gene encoding a sirtuin isozyme (SirA) with an amino acid identity of 47% to yeast Sir2p. Recombinant SirA exhibited nicotinamide sensitive NAD(+)-dependent HDAC activity, and deacetylated lysine 16 residue of histone H4 (H4K16), indicating that SirA is a fungal sirtuin counterpart. Gene disruptant of sirA (⊿SirA) accumulated more acetylated H4K16 at the gene promoters of ipnA and aflR and their transcripts and produced more penicillin G and sterigmatocystin, indicating that SirA removes H4K16 acetylation and represses the expression of these secondary metabolite genes. The increased cellular NAD(+) and the decreased secondary metabolite production induced by adding nicotinamide riboside accompanied decreased levels of H4K16 acetylation at the ipnA and aflR gene promoters, which was not evident in ⊿SirA. These results indicate that cellular NAD(+) modulates the HDAC reaction by SirA. DNA microarray analyses indicated that genes for synthesizing emericellamide, aspernidine A, xanthone, austinol, and siderophores are up-regulated in ⊿SirA, in addition to those for penicillin G and sterigmatocystin. HPLC analyses showed more peaks of secondary metabolites in mycelial extracts of ⊿SirA comparing to wild type, indicating that SirA repressed those secondary metabolite production. Adding nicotinamide to the wild-type and ⊿SirA cultures increased production of sterigmatocystin and other compounds, indicating that other sirtuin isozyme is involved in the secondary metabolite production.

006 BIOCHEMICAL CHARACTERIZATION AND CLONING OF A B-XYLOSIDASE FROM TALAROMYCES AMESTOLKIAE

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The main carbon sources of biosphere are cellulose and hemicelluloses, two plant cell wall components. Xylan is the predominant hemicellulosic material in hardwood and grass, and hence industrial 2G bioethanol production strongly depends of its explotation to reach profitable yields. In nature, xylan conversion to xylose (fermentable sugar) is catalyzed by xylanases, members of glycosyl hydrolase superfamily. Endo-β-1,4-xylanases and β-xylosidases are responsible for the main hydrolytic steps. The first enzymes cut the xylan polymer into oligosaccharides that are quickly converted to xylose by the action of β-xylosidases. A huge interest in the identification and characterization of new β-xylosidases is rising since robust enzymes are needed in commercial cocktails for lignocellulose biomass applications. In addition, fungal β-xylosidases usually show transxylosylation capacity, attaching xylose units to alcohols, monosaccharides and disaccharides. By this mechanism new xylooligosaccharides can be synthesized with prebiotic potential and interest for pharmacological uses. In the present work, a β-xylosidase of the ascomycete Talaromyces amestolkiae has been purified and named as BxyTW. BxyTW was biochemically characterized: physicochemical properties, kinetic parameters and substrate specificity were determined. BxTW had a molecular mass of 102.275 kDa and a a basic pI (7.6). Maximal activity was found at pH 3 and 70 °C. Its kinetic study using 4-Nitrophenyl β-D-xylopyranoside as the substrate, gave values of Km and Vmax around 0.17 mM and 19.8 mU/μg, respectively. The enzyme was also active against xylobiose and 4-Nitrophenyl α-L-arabinofuranoside. Preliminary results show that it is able to carry out transxylosylation reaction successfully. The transxylosylation capacity of BxyTW combined with good values of substrate affinity and stability comparing to other similar fungal β-xylosidases, suggest that it could be an attractive biotechnological tool. The coding sequence of BxyTW has been amplified by using PCR primers designed according to its peptide mass fingerprinting. Its comparison with other β-xylosidases suggests that it belongs to Glycoside Hydrolase Family 3. Currently BxyTW has been successfully expressed in Pichia pastoris and the recombinant protein is being studied.

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