MICRO BIOTEC'11

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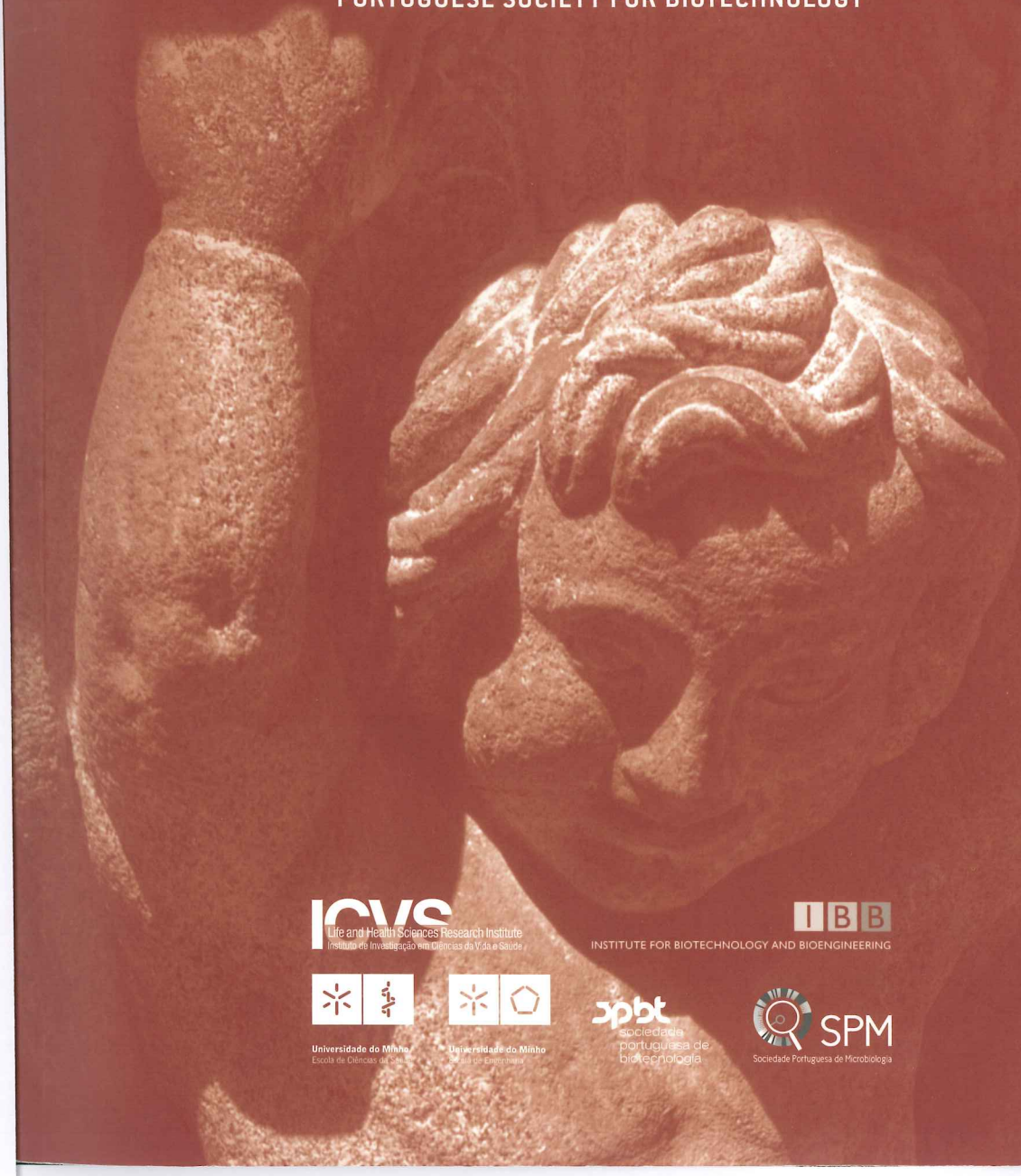

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[1] and their permanence in nature induced genetic changes that were not found among a control group of isolates that derived from clonal expansion of the commercial "mother" strain [2]. The objective of the present study was to evaluate genome variations among four isolates of the commercial strain *S. cerevisiae* Zymaflore VL1 that were re-isolated from vineyards surrounding the wineries where this strain was applied, in comparison to the commercial "mother" strain, by the use of comparative genome hybridization on array. These approaches were carried out as described [3]. Data analysis showed genetic differences among the recovered isolates in comparison with the "mother" strain. Amplification (between 1 and 2 fold changes) of 14 genes were detected, related with mitosis (*SHE1*) or meiosis (*HFM1*), lysine biosynthesis (*LYS14*), galactose (*GAL1*) and asparagine catabolism (*ASP3-2*). *ASP3-2* amplification is in agreement with the previously shown increased expression during nitrogen starvation. This might occur as an adaptation to natural environments with poor yeast-utilizable nitrogen sources. Eight Ty elements were also amplified, whereas each of the recovered strains had a unique pattern of amplifications. Phenotypic screening was performed considering 28 physiological tests. Seven phenotypic traits distinguished recovered strains from the "mother" strain which was unable to grow at 18 ºC, but evidenced some growth in the presence of $CuSO_4$ 5 mM and SDS 0.01%. Variable growth patterns were found for NaCl 1.5 M, $KHSO_3$ (300 Mg/L) and wine supplemented with glucose (0.5% and 1% w/v). We hypothesize that the transition from nutrient-rich musts to nutritionally scarce natural environments induces adaptive responses and microevolutionary changes promoted by Ty elements. These changes (and possibly others as well) may contribute to intra-strain phenotypic variability.

[1] Schuller et al., *2007. Yeast 24:625-636 [2] Valero* et al., *2005.FEMS Yeast Research 5:959-969 [3] Carreto* et al., *2008. BMC Genomics 9:524.* *This work was funded by the fellowship SFRH/BD/48591/2008 and by the projects PTDC/BIA BCM/64745/2006, PTDC/AGR-ALI/103392/2008 and FP7/2007-2013 (nº 232454).*

S1: 2

Adaptive response to acetic acid in the highly resistant yeast species *Zygosaccharomyces bailii*, revealed by quantitative proteomics

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Zygosaccharomyces bailii is the most tolerant yeast species to acetic acid-induced toxicity, being able to grow in the presence of concentrations of this food preservative close to the legal limits. For this reason, *Z. bailii* is the most important microbial contaminant of acidic food products. However, the mechanisms behind this intrinsic resistance to acetic acid are very poorly understood. The experimental model *Saccharomyces cerevisiae* is also a contaminant of acidic foods and genome-wide approaches have been explored to unveil global mechanisms underlying yeast response and adaptation to acetic acid [1-3]. An expression proteomic approach, based on quantitative two-dimensional gel electrophoresis (2-DE), was explored to gain mechanistic insights into the adaptive response and intrinsic tolerance to acetic acid in *Z. bailii.* The experimental strategy detailed in [4] was used to identify alterations occurring in cellular protein content in response to sudden exposure to an inhibitory, but sub-lethal, concentration of acetic acid, following the supplementation of a minimal medium containing glucose with the acid or during the growth curve in this supplemented medium. The response to acetic acid was found to involve an increased activity of carbohydrate metabolic processes, in particular, the TCA cycle when glucose is present and gluconeogenic and pentose phosphate pathways when acetic acid is the only carbon source. Increased ATP production, required to assure acetate catabolism and cell detoxification, is also suggested, as well as the activation of oxidative and general stress responses. All these indications may be useful to guide the rational design of more effective strategies to control food spoilage by this highly acetic acid resistant yeast species.

[1] Teixeira, M.C. et al. *(2011)* Curr Opin Biotechnol 22, *150-156. [2] Mira, N.P.* et al. *(2010)* Microb Cell Fact 9, *79. [3] Mira, N. P.* et al. *(2010)* OMICS 14, *587-601. [4] Sá-Correia, I. and Teixeira, M. C. (2010)* Expert Rev Proteomics 7, *943-953.*

S1: 3

Enhancement of rate and extension of enzymatic hydrolysis of cellulose by high pressure pre-treatments

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The inevitable reduction of dependence from fossil fuels for energy and chemicals production has increased attention to the use of renewable biomaterials for these processes, as is the case of the conversion of cellulose from plant resources into bioethanol and chemicals synthesis, via bioprocessing of fermentable sugars. However, the structural arrangement and tight packing of cellulose chains hinders its hydrolysis, being this an obstacle for a successful use of these naturally abundant renewable sources for

production of second-generation fuels and chemicals. High pressure (HP) is now an established technology for food preservation, with potential also to modify the properties of macromolecules, by action on non-covalent bonds. A previous work of our research team showed that HP treatments of cellulosic pulps are a promising tool for non-degradative modification of cellulose fibres properties, such as rearrangement of elementary fibrils in cellulosic fibres, in such a way that those become less aggregated and more hydrated (containing increased amounts of strongly bound water), leading to improvement of cellulose accessibility towards hydrolysis with diluted sulphuric acid. Based on these results and following the same rational strategy, the effect of HP, as a pre-treatment, on the subsequent enzymatic hydrolysis of bleached kraft *Eucalyptus globulus* cellulosic fibres by cellulase, from *Tricoderma viride*, was evaluated in the present work. Results showed that pressure pre-treatments of 300 and 400 MPa during 5-45 min, lead to both an increased rate and degree of hydrolysis, reaching values ranging from 1.5- to 1.9-fold, quantified by the formation of reducing sugars. Both the pressure and time under pressure influenced the enzymatic enhanced "hydrosability" of the cellulosic pulps, with the former being more important. A kinetic effect of HP treatment time on hydrosability of cellulose was verified. These results open promising possibilities, to contribute to overcome conventional limitations of enzymatic cellulose hydrolysis, by enhancement of both rate and yield of hydrolysis of cellulose. The results are also of interest for the preparation of "pressure engineered" cellulose, with respect to the desired hydrolysis extent and profile, with tailored hydrolysis patterns and possible different properties for new applications. Optic microscope images of the hydrolysed fibres corroborate this possibility.

S1: 4

Evaluating food safety management performance in an Irish milk pasteurising facility using a microbiological assessment scheme

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Milk and milk products are a heterogeneous group of food products. Depending on the heat treatment applied during production, different pathogens pose risks. The pathogens of concern are *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella* spp, *Staphylococcus aureus* and *Escherichia coli* since these may survive pasteurisation treatments. The performance of a food safety management system (FSMS) in a drinking milk pasteurisation establishment was measured using a microbiological assessment scheme (MAS). The MAS consisted of multiple sampling locations along the processing line consisting of high-risk raw materials, the processing environment, process water and end products. A total of 1268 samples were analysed over an 18-month-period. Nine microbial parameters (*Salmonella* spp., *Listeria* spp., *B. cereus, S. aureus*, Total Bacterial Counts (TBC), *Enterobacteriaceae*, *E. coli*, faecal enterococci and coliforms) were assessed using standard methods. Results were benchmarked against legal, industry and best practice norms. 100% (n_0=233) of raw milk samples met the EU TBC standard of < 10^5CFU/Ml, however, *Listeria innocua* was isolated in 3% (n_1=134) of raw milk samples. *Listeria* spp. (n_2=128), *Salmonella* spp. (n_3=118), *S. aureus* (n_4=118), *Enterobacteriaceae* (n_5=114), *B. cereus* (n_6=38) and *E. coli* (n_7=23) were not detected in any end products. *Listeria welshimeri* (a poor hygiene indicator) was identified in 2% (n_8=153) of environmental samples. *Salmonella* was not isolated in any of 63 environmental samples. 6% and 1% of operator hand swabs (n_9=100) had TBC and *Enterobacteriaceae* counts respectively in excess of best practice norms of 10^2 cm^{-2} and 10^1 cm^{-2} respectively. One (2.2%) water sample (n_{11}=46) had a coliform count of 201 CFU/Ml whereas five samples (11%) had TBC counts above acceptable norms. The results indicate that the FSMS is producing a safe product. The MAS is an effective risk assessment tool that is useful to assess the overall performance of the FSMS and allows a more targeted use of resources to implement improvement. Satisfactory end product microbiological results indicate that cold chain control, post pasteurisation contamination from dry ingredients (e.g. buttermilk cultures), packaging or unsanitary pipe work are not issues for this plant. However, the prerequisites of environmental sanitation, raw material supply and control, water treatment and storage and staff hygiene are the areas within the FSMS that pose the greatest risks.