effect with various drugs and treatments are also reported. In recent years, there has been an increasing interest in isolation and use of β-glucan from brewer's yeast, which is a byproduct of beer production. All samples of insoluble β-glucans, isolated in our experiments from brewer's yeast using three different procedures and dried using three different methods, showed biological activity. β-Glucan preparations, obtained in modified drying conditions, showed noticeably higher ability of producing TNF-α. Use of spent brewer's yeast as a raw material for β-glucan production is technologically and economically justified.

http://dx.doi.org/10.1016/j.jbiotec.2017.06.052

Carbohydrate composition and site-occupancy determination in pituitary and recombinant preparations of human thyrotropin

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Human thyrotropin (hTSH) is a glycoprotein with three potential glycosylation sites: two in the α-subunit and one in the β-subunit. Carbohydrate site-occupancy is frequently neglected in glycoprotein characterization, even if related to folding, trafficking, initiation of inflammation, host defence and congenital disorders of glycosylation (CDG). For the first-time N-glycoprofiling analysis was applied to site-occupancy determination of two native pituitary hTSH, in comparison with three CHO-derived preparations of hTSH, a widely used biopharmaceutical. A single methodology provided: (i) average N-glycan mass; (ii) mass fraction of each monosaccharide and of sulfate; (iii) percent carbohydrate. The results indicate that occupancy (65–87%) and carbohydrate mass (12–19%) can be 34–57% higher in recombinant hormones. The average glycan mass is 24% lower in pituitary hTSH and contains ~3-fold fewer moles of galactose ($P < 0.005$) and sialic acid ($P < 0.01$). The number of moles of fucose per mole of hTSH was found 2.5-fold higher in the pituitary preparations. One of these native preparations, presenting the smallest glycan mass, lowest occupancy, GalNAc, sulfate, Gal and sialic acid contents, also presented the lowest in vivo bioactivity and circulatory half-life. This methodology, extremely important for comparing a recombinant biopharmaceutical to its native equivalent, can be applied to any physiologically or clinical relevant glycoprotein.

http://dx.doi.org/10.1016/j.jbiotec.2017.06.053

Construction of novel *Saccharomyces cerevisiae* hybrid strains resistant to growth and fermentation inhibitors

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Bioethanol production from raw lignocellulosic material requires a producer strain which tolerates both the presence of growth and fermentation inhibitors and high ethanol concentrations. Therefore, we constructed heterozygous (hybrid) diploid *S. cerevisiae* strains by mating two haploid isolates having those desirable traits. In comparison to parental haploids, hybrid diploids were more resistant to several inhibitors frequently found in different lignocellulosic hydrolysates, such as acetic and levulinic acid and 2-furaldehyde. Interestingly, hybrids showed different fermentation ability in a CO_2 production test. Our results suggest that construction of intraspecies hybrids coupled with the use of different genetic engineering techniques is a promising approach for the improvement or development of new biotechnologically relevant strains of *S. cerevisiae*. Additionally, we also show that in two biotechnologically interesting natural isolates percentage of successful gene targeting (gene targeting fidelity) is much lower than in widely used laboratory strains. Furthermore, the most frequent off-targeted event was duplication of a targeted chromosome yielding aneuploids having two copies of targeted chromosome, one containing transformed and the other containing untransformed allele. These results warn us that it could be more difficult to make precise genetic modifications of natural *S. cerevisiae* strains than expected.

http://dx.doi.org/10.1016/j.jbiotec.2017.06.054

Application of mathematical modelling in biorefinery processes design

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A biorefinery is a facility that integrates biomass conversion processes and equipment to produce bio-products from biomass. Biorefineries will have to employ the best possible processes (for biomass cultivation, harvesting, storage and transport, pretreatment, fermentation and bio-chemical conversion) to ensure efficient bio-based production. Nevertheless, numerous technical, strategic and commercial challenges have to be overcome to