GLYCOSYLATION IN SECRETED PROTEINS FROM YEAST *KLUYVEROMYCES LACTIS*

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The nutritional status of a cell culture affects either the expression or the traffic of a number of proteins. The identification of the physiological conditions which favor protein secretion has important biotechnological consequences in designing systems for recombinant extracellular protein industrial production. Yeast *Kluyvromyces lactis* has been cultured in a continuous stirring tank bioreactor (CSTR) under nitrogen limitation at growth rates ($0.03h^{-1}$ and $0.09h^{-1}$) close to either exponential or stationary batch growth phases, respectively the objective was to investigate the extracellular glycoproteins at these two level of nitrogen limitation. Proteins from free cell extracts were separated by gradient SDS-PAGE (5-15%) and two-dimensional chromatography, and were analyzed by mass spectrometry (MALDI-TOF-TOF-MS). In SDS-PAGE analysis, differences in extracellular proteome were visualized: diferent proteins profiles at these two growth rates. The $0.09h^{-1}$ growth rate showed larger number of bands using colloidal Comassie Blue staining. Different bands were detected at these two growth rates when the PAS assay for glycoprotein detection in polyacrilamide gel was used. The two-dimensional chromatogram profiles were comparatively distinguished between the $0.03h^{-1}$ and $0.09h^{-1}$ growth rate samples. Protein peaks from the second dimension, were subjected to mass spectrometry. The mass spectrums visualized showed glycosilated proteins with *N*-acetylglucosamine molecules and 8, 9 or 15 hexoses molecules. Comparisons between the proteins averaged mass values with the deduced proteins masses from *K. lactis* secreted proteins database indicated possible post-translational modifications, such as post-translational proteolysis, acetylation, deamidation and myristoylation. Supported by CNPq and FAPEMIG.