

SCREENING UNCHARACTERIZED HYDROSTATIC PRESSURE STRESS
PROTEINS FROM *SACCHAROMYCES CEREVISIAE* FOR STRUCTURE
DETERMINATION BY SOLUTION NMR

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High hydrostatic pressure (HHP) stress leads to transcriptional modifications in *S. cerevisiae*, however, many of the highly expressed genes are uncharacterized. Therefore, we screened for targets for structure determination by NMR of putative proteins which genes were up-regulated by HHP. The cDNA coding for 8 ORFs, *YER067W*, *YFL014W*, *YDR070C*, *YLR327C*, *YMR107W*, *YDL110C*, *YPR096C*, *YNL266*, was inserted in pET28a and several conditions for recombinant protein expression in *E. coli* were tested. Inspection of the cell lysates by SDS-PAGE revealed that 7 proteins were efficiently expressed. The solubility and the aggregation state of these gene products were analyzed by gel filtration chromatography. 4 soluble proteins that eluted as a single peak in the expected retention time, according to the predicted molecular weight, were selected for structural analysis using NMR. Yer067wp presented the best 1D ¹H-NMR spectrum with sharp lines and large chemical shift dispersion, characteristic of a well-structured protein. 2D [¹H-¹⁵N] HSQC spectrum was recorded using uniformly labeled ¹⁵N-Yer067wp and presented all the expected HN peaks, which strongly indicates that this 20 kDa protein is suitable for structure determination using 3D NMR spectroscopy. Yer067w seems to be well conserved in fungi, however there is no close homologous with known fold or function, making this protein an interesting NMR structure proteomic target Support: CNPq