[SPII-1]

Characterization and biotechnological application of multiple yapsin-deficient strains of *Saccharomyces cerevisiae*

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The yapsin family of aspartic proteases has a common specificity for paired or single basic reside cleavage sites of proproteins. The genome sequence of *Saccharomyce cerevisiae* has identified five *YPS* genes, *YPS1*, *YPS2*, *YPS3*, *YPS6* and *YPS7*, coding for the yapsin family. We have constructed a series of multiple yapsin-deficient mutants through disruption of the *YPS* genes and characterized the effect of multiple disruptions on the growth phenotype and the expression of recombinant proteins in *S. cerevisiae*. Up to the quadruple disruption of *YPS1*, *YPS2*, *YPS3* ,*and YPS6*, no detectable growth defect was detected, but the quintuple *yps1Δ/yps2Δ/yps3Δ/yps6Δ/yps7Δ* disruptant showed a slightly retarded growth rate under normal growth conditions. However, the double *yps1Δ/yps2Δ* disruptant showed hypersensitivity to hygromycin B and growth defect at acid (pH3.5) and alkali (pH8.0) conditions. The triple *yps1Δ/yps2Δ/yps3Δ* disruptant displayed a temperature sensitive growth defect, which could be completely recovered by the presence of 1M sorbitol. All the yapsin genes were highly transcribed at early growth stages, but the levels of yapsin transcripts decreased significantly as the culture entered into the stationary phase. Heat-shock stress appeared to induce remarkably the expression of the yapsin genes, especially *YPS3*, at the level of transcription, implying that yapsins might have important physiological functions, not yet assigned, under heat stress conditions. The multiple yapsin deficient strains were evaluated as useful host strains particularly at high cell density cultivations, for the production of the recombinant human lipocortin I and human parathyroid hormone proteins, which were highly susceptible to aberrant proteolytic cleavages by yapsins in the wild type background.

[SPII-2]

Structural and Regulatory Characteristics of the *putAP* Operon Encoding Proline Dehydrogenase and Proline Permease of *Vibrio vulnificus*

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The *Vibrio vulnificus putAP* genes encode proline dehydrogenase and proline permease involved in proline catabolism and proline uptake, respectively. The gene product of *putP* also contributes to the osmotic tolerance of *V. vulnificus*. In this study, we demonstrated that proline dehydrogenase activity of *V. vulnificus* was highly dependent on growth phase, reached a maximum during the exponential phase and then decreased when cells enter the stationary phase. This growth phase variation of proline dehydrogenase occurred at the level of transcription. Northern blot and primer extension analyses revealed that *putA* and *putP* are organized as one transcriptional unit, and the transcription of *putAP* operon begins at two different sites, consisting of putative promoter P1*put* and P2*put*. A null mutation of *crp* decreased proline dehydrogenase production and the cellular level of *put* transcript, indicating transcription of *put* is under the positive control of CRP. This decrease in the level of *put* transcript in the *crp* mutant appeared to be mediated by the reduced activity of P1*put* and P2*put* . Proline dehydrogenase was induced in the presence of proline and NaCl, and the induction of proline dehydrogenase was regulated at the level of transcription. A transcriptional attenuation was observed after the *putA* gene, so *putP* expression was reduced presumably to modulate the production of the proline permease according to the available concentration of proline. These results establish that *putAP* expression is directed by P1*put* and P2*put* in a growth phase-dependent manner, and induced by the gene product of *crp* in the presence of proline and osmotic stress.