<b>MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF THE NcPHO85 CYCLIN-DEPENDENT PROTEIN KINASE FROM <i>NEUROSPORA CRASSA<i><b>

Avaca, J. S.; Bertolini, M. C.
Departamento de Bioquímica e Tecnologia Química, Instituto de Química, Universidade Estadual Paulista, Araraquara, São Paulo, Brazil

In <i>Saccharomyces cerevisiae<i>, <i>PHO85<i> encodes the cyclin-dependent protein kinase with multiple regulatory roles depending on its association with different cyclin partners. The cDNA (<i>Ncpho85<i>) encoding the homologous protein in <i>Neurospora crassa<i> was isolated (1,1 kb). Gene expression analysis during the vegetative growth was performed by <i>Northern blot<i> using either the whole cDNA sequence or a 380 bp cDNA fragment as probes. The fungus was cultivated in two different media: Vogel´s minimum medium (VM) and the low phosphate Fries medium. Two transcripts were observed in both media. Gene expression was analyzed at protein level using anti-NcPHO85 antibody raised in rabbits. Only one protein with approximately 40 kDa, the expected size of the protein NcPHO85 was observed by Western blot. In order to investigate the role of the protein in the fungal metabolism and cell growth, the gene <i>Ncpho85<i> was inactivated by RIP (Repeat Induced Point-Mutation). The resulting strain (<i>pho<sup>rip<sup><i>) produced a truncated protein of 165 amino acids and showed severe morphologic changes in solid culture medium. The growth capacity of the mutant and wild type strains was compared in experiments using race tubes. Mutant strain presented only 30% of the wild type strain growth in VM medium at 25<sup>o<sup>C. In addition, the mutant strain was unable to grow at 37<sup>o<sup>C.

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