P. 90

PROTEOME CHARACTERIZATION OF THE PENICILLIN-PRODUCER FUNGUS *PENICILLIUM CHRYSOGENUM*. ANALYSIS OF HIGH-PRODUCING STRAINS

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Penicillium chrysogenum is a filamentous fungus known by its ability to produce penicillins and related β-lactam antibiotics. Current industrial strains have derived after numerous rounds of mutagenesis and selection from a single natural isolate of *P. chrysogenum*, NRRL1951. Product titers and productivities have increased by at least three orders of magnitude in the past 60 years, representing an unprecedented success in classical strain improvement. Biochemical and genetic analysis of industrial strains led to the identification of several important mutations in high-producing strains, including amplification of penicillin biosynthesis gene clusters, but much of the molecular basis for improved productivity remained to be elucidated. More recently, sequencing of the complete genome of *P. chrysogenum* Wisconsin54-1255 have revealed that transcription of genes involved in biosynthesis of the amino acid precursors for penicillin biosynthesis, as well as of genes encoding microbody proteins, has been increased in the high-producing strains. However, full exploitation of the *P. chrysogenum* genome sequence requires the integration of the proteomic level, which may contribute to further improvement of this important cell factory, which serves as a model for the development of other products of secondary metabolism.

In this work we show an optimized protein-obtaining method for *P. chrysogenum* that allows the analysis of the cytoplasmic proteome. Different buffers, extracting protocols and culture conditions, as well as staining techniques have been tested. As a result, the reference map for this filamentous fungus has been developed.

In addition, we have stablished the differences existing between the proteome of the reference strain (Wisconsin54-1255) and one high-producing strain (AS-P-78) growing under the same conditions. The comparison among the existing microarray and the proteomic data allows an up-date in the understanding of the antibiotic production mediated by this fungus.