Evaluation of Different Methods for Ascomycetous Yeasts' DNA Extraction

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Background & Objectives: Many molecular genetics techniques have been developed for accurate and rapid identification of yeasts, in conjunction with laborious and time consuming biochemical Methods. In PCR-based systems for yeast identification, cell wall breakage and DNA isolation are vital steps to avoid shearing and breakage of DNA. Although there are many references representing the protocols, some modifications are necessary to be applied for gaining better results.Finding the best methods for mechanical shearing and chemical lytic disruption of yeast cell wall for DNA extraction was the purpose of this article.

Methods: Yeasts were grown for 24h at 30°ç in YM broth. Several mechanical Methods were used to fracture the yeast cell wall including grinding cells in liquid nitrogen with mortar and pestle, using of glass beads and repeated freeze-thawing cycles. Then different lysis buffers which were mainly consisted of EDTA, SDS and Tris-HCl were tested to disrupt the cell wall and release of DNA with lowest damage. Different ratios of phenol-chloroform or phenol-chloroform-isoamylalcohol were tested for DNA extraction. Finally DNA was analysed by 1% agarose gel electrophoresis and stained with ethidium bromide.

Results: DNA gel electrophoresis showed that the Methods in which glass beads were used beside lysis buffer and extraction with phenol-chloroform were the most appropriate ones.

Conclusion: Regarding the status of the extracted DNA, this methods could be used for amplifying isolated DNA by PCR in order to identify yeasts and analysis of phylogenetic relationships.

Keywords: Yeast; DNA; Extraction