TITLE: EXPRESSION OF HUMAN EPIDERMAL GROWTH FACTOR (HEGF) IN *PICHIA PASTORIS*

AUTHORS: ALFONSO, A.L.

INSTITUTION: UNIVERSITY OF BRASILIA, BRASILIA, DF (CAMPUS UNIVERSITARIO DARCY RIBEIRO CEP 70910-900, BRASILIA – DF, BRAZIL)

ABSTRACT:

Pichia pastoris is a methylotrophic yeast, meaning that it can utilize methanol as the sole carbon source. *P. pastoris* is an established system for the production of heterologous proteins, particularly biopharmaceuticals and industrial enzymes. It is a highly effective and versatile yeast for the extracellular expression of recombinant heterologous proteins. Signal peptides can differ widely in their efficiency to secrete recombinant proteins, so it is necessary to identify multiple available secretion signals and find the optimum secretion signal for a protein. Epidermal growth factor (EGF) is a 53-amino acid peptide that plays an important role in regulating cell growth, survival, migration, apoptosis, proliferation, and differentiation. In addition, EGF has been established to be an effective intestinal regulator protecting intestinal barrier integrity, which is essential for the absorption of nutrients in humans and animals. The aim of this study was to evaluate the human EGF (hEGF) expression with different signal peptides (SUC2, PHO1 and αF). The *P. pastoris* strain, Kex1Δ M12 (M12-K) was used for the expression of hEGF. Primers were designed to amplify the gene of the synthetic hEGF containing three signal peptides together with restriction enzyme sites. The hEGF gene was amplified from plasmid pkEGF-ld. The expression cassettes were subcloned into the vector pkGFP-ld between BamHI and NotI or XhoI and NotI sites, under the control of the PGK1 promoter. The expression construct pkSPEGF-ld was linearized with SacI restriction enzyme and transformed into leucine auxotrophic *P. pastoris* strain M12-K. The resulting transformants were screened on minimum medium plates and 30 clones of each system were selected. The presence of the hEGF gene was confirmed by colony PCR in 5 clones for each system. A growth curve experiment, in 100 µL, was performed with clones of each system and compared to the M12-K host to determine differences in the doubling time in minimum medium. To evaluate the expression of hEGF in *P. pastoris* the selected transformants were grown in 100 mL of BMGY at a concentration of 0.1 OD_{600}. This culture was incubated at 28 °C for 72 h in a shaking platform at 250 rpm. The cells were harvested by centrifugation at 5000 rpm for 5 min at room temperature. Culture supernatant samples were analyzed by HPLC to identify the best expressing clone compared to commercial EGF. The presence of hEGF (~6kDa) was observed in a Tris-Tricine SDS PAGE, in the cell free supernatant fraction. The presence of the hEGF was confirmed by western blotting analysis with an anti-EGF antibody. The clones with the highest yield in the expression of EGF will be used for large scale production. In summary, this study showed the efficient production of hEGF in the *P. pastoris* expression system, which in turn could be translated into large-scale production. The hEGF has other potential applications in cosmetics, burn and injury treatment.

Keywords: *Pichia pastoris*, epidermal growth factor, signal peptide.

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