CLONING OF STREPTAVIDIN CORE GENE INTO A *KLUYVEROMYCES LACTIS* EXPRESSION SYSTEM IN ORDER TO PRODUCE PROTEINS WITH AFFINITY TO BIOTIN

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The high affinity interaction streptavidin-biotin provides an excellent system for industrial enzyme separation or immobilization involving a fused streptavidin-enzyme and a biotinylated support. We have modified the commercial *K. lactis* system, pKLAC1 (New England Biolabs), a LAC4 promoter-driven integration vector for protein expression, with the gene coding streptavidin. The codified sequence for biotin binding domain of streptavidin (cSVA) was amplified by PCR from pSTP4, purified and subcloned to the pCR2.1TOPO (Invitrogen). The fragment revealed 100% identity with streptavidin gene according to Gene Bank was inserted into *Xho* I *Bgl* II pKLAC1 in frame with mating-a-factor signal peptide. The resulting pKLAC1cSVA/AhdI was used to transform the strains *K. lactis* MW 98.8C and CBS 2359. The transformants selected in Yeast Carbon Base (YCB) containing 5mM acetamide and confirmed by PCR were mitotically stable. A high cell biomass (OD_{600} = 18), obtained in shake-flask 50 mL YPD medium, was centrifuged and transferred to an induction medium, containing 2% of lactose. The cell free medium was qualitatively analyzed for streptavidin and proteins by SDS PAGE. The biotin binding activity of streptavidin in the culture supernatant determined by HABA test revealed that YPL and YNB supplemented with 0,5% of yeast extract have resulted the highest yields for streptavidin and extracellular protein production.