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Hyper-production and stability of human serum albumin in *Pichia pastoris* through a combination of medium design and genetic strategies

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Human serum albumin (HSA) is an important therapeutic recommended for treatment against trauma, burn injury, hypoproteinemia, hypoalbumenia as well as for maintenance of homeostasis, transportation of hormones and microelements in blood. In this study, we report medium design and genetic strategies that lead to high production of this protein in the culture spernatant of *Pichia pastoris*. The codon-optimized gene for HSA was cloned downstream of α–factor secretory signal sequence and the mature HSA was secreted in the culture supenatant of *P. pastoris* under the control of alcohol oxidase 1 promoter. Extracellular protein level of 0.12, 0.40, 1.2 g/L were obtained in the un-optimized medium for 1-copy, 2-copy and 3-copy expression casettes respectively at shake flask level. Factors affecting production were identified which included initial peptone concentration, methanol concentration and temperature, amongst many other (pH, aeration, sorbitol concentration, initial inoculum) investigated parameters. A three level factorial design named central composite design using Plackett Burman response surface methodology was used to optimize the medium which lead to levels of protein up to 0.075, 0.40 and 0.98 g/L total extracellular protein for 1,2 and 3-copy constructs respectively. Under these conditions, HSA produced was stable and free of other contaminating proteins in the culture supernatant. A detailed transcriptome analysis of the recombinant *P. pastoris*, cultivated on unoptimized and optimized medium lead to identification of several protein coding transcripts which were up-regulated and helped in efficient HSA production and secretion. These were mapped to biochemical activities linked (and not restricted to) to carbon, nitrogen metabolism, gene transcription, protein transport and secretion. Additional genetic strategies applied included modification of signal sequences. Application of optimized medium to these mutants lead to stable production of HSA with reduced proteolytic degradation of the synthesized protein. This illustrated the robustness of the designed medium with a production of over 2 g/L protein at shakeflask level. An understanding of the underlying mechanisms is likely to play significant role in use of *Pichia* system for production of heterologous proteins.

Biography

Saroj Mishra completed her PhD from City University of NewYork, USA followed by Post-doctoral research at Institute Pasteur, Paris, France, VTT Biotechnical Laboratory, Espoo, Finland and University of California, Davis, USA. She has published more than 87 papers in reputed journals and leads a large group of scientists working in the area of enviromental biotechnology, whole cell biotransformation and recombinant therapeutics.

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