

Expression of recombinant aprotinin in *Pichia pastoris*: A case study

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P*ichia pastoris*, a methylotrophic yeast has been used for heterologous protein expression. Fast growth, high biomass, simple media for growth and availability of strong inducible as well as constitutive promoters are some of the characteristics due to which the yeast is extensively used for expression of extracellular proteins as well as for intracellular VLP based vaccines. Expression of protein in *Pichia* depends on a number of factors such as nucleotide sequence of the protein, signal sequence and promoters used for the purpose. Alpha mating factor signal sequence (MAT α 1) of *Saccharomyces cerevisiae* is commonly used for secretion of protein in the culture supernatant. Since cloning downstream to Glu-Ala-Glu-Ala- (Ste 13 signal cleavage) leaves overhang of Glu-Ala-Glu-Ala on the secreted protein due to improper processivity, we prefer to clone protein sequences downstream to kex cleavage site (-Glu-Lys-Arg-). However, for aprotinin, cloning downstream to kex cleavage showed improper processivity. Hence, on the basis of N terminus data and known literature, 2 different constructs were prepared wherein aprotinin was cloned downstream to 2 different sites in pre-sequences of MAT α 1. The constructs were independently transformed into *P. pastoris*. Secretion of protein was observed in the clone wherein aprotinin was cloned downstream to Ser- Ala- Leu-Ala-. Further characterization of purified protein is by intact mass analysis, N terminus sequencing, RP-HPLC showed good match with bovine aprotinin. Though levels of protein secretion decrease in the new strategy employed, proper processivity of the protein was obtained which is one of the important factor in development of similar biologics.

Biography

Sachin R. Tendulkar has completed his Ph.D in Microbiology from M. S. University, Baroda under the guidance of Prof. B. B. Chattoo. Further he pursued his postdoctoral studies in McGill University, Montreal, Canada. He joined Reliance Life Sciences Pvt Ltd in 2007 and working on expression of proteins of therapeutics importance since then. He has published 5 papers and a review on therapeutic protein expression and presented his work in national as well as international conferences.

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