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EC MICROBIOLOGY
Conceptual Paper

Production of Recombinant Proteins via Their Over-Expression in Microbial Host Cells: Advancements and Challenges

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Received: August 27, 2018; **Published:** December 13, 2018

An industrial route for production of several important pharmaceutical recombinant proteins (such as insulin, interferons, streptokinase, growth hormone, etc.) has been via their over-expression in microbial cell host. Although this way of recombinant protein production has been attractive, mainly due to high quantity of target protein accumulation in the host cell, it has been encountered with serious problems caused by the creation of misfolded-protein aggregates-termed inclusion bodies (IBs)- in "high cell density cultivation" process [1]. These insoluble protein aggregates are usually solubilized by high concentration of denaturants (e.g. urea and guanidinium chloride), the process which generates completely-denatured (unfolded) proteins [2]. Recovery of highly-bioactive target proteins via *in vitro* refolding of such denatured proteins is normally a very challenging task in industry.

Many investigations have been carried out to tackle this issue. Directing of the target recombinant protein into the periplasmic space (the region between the cytoplasmic and outer membrane in gram-negative bacteria) has been done by many scientists in order to express the protein as correctly-folded soluble form in the host. Although this approach has led to promising results in lab scale (e.g. shake-flask cultivations), it has not been often victorious in large-scale high cell density cultivation in bioreactor because of stress applied to the cells and as a result formation of both soluble and insoluble forms of proteins plus target protein distribution inside (i.e. cytoplasm and periplasm) and outside the cells [3]. With the occurrence of such phenomena throughout large-scale high cell density cultivation in bioreactor, the process robustness and cell viability are obviously main parameters which are endangered; therefore, stress minimization strategies for optimization of these parameters are required [4]. Eventually, "periplasmic production of recombinant protein" approach has been proved very challenging and rather unfeasible at large-scale.

Several attempts have been also made on the "conventional IBs production route" to increase the recovery of "highly-bioactive" recombinant protein from IBs, following their separation from microbial host. For example, use of synergistic effect of combined chemicals at low and very low concentrations for solubilization of proteins from IBs, which reduce the chance of protein denaturation during protein-solubilization process, has been demonstrated very successful in recovery of "highly-bioactive" recombinant proteins from inclusion bodies derived from cells harvested from high cell density culture in large-scale bioreactor [5]. Therefore, with development of such chemical-solubilization methods, the challenges with recombinant protein production in "traditional inclusion body formation route" can be overcome in biotechnology industry.

Overall, it appears that attempts for expression of recombinant (pharmaceutical) proteins as soluble and correctly-folded form have not been usually successful at large-scale. It seems that there is still space for much more work to be carried out on certain steps (such as solubilization and refolding) of conventional route of recombinant protein production (i.e. protein production as IBs) in order to enhance the recovery of highly-potent target protein. It is inevitable that continuous success for such enhancements will make "the inclusion body protein production route" much more attractive than before.

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Volume 15 Issue 1 January 2019
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