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Oral Presentation

Pseudomonas fluorescens – a robust expression platform for pharmaceutical protein production

Diane Retallack*, J Carrie Schneider, Lawrence Chew, Tom Ramseier, Jeffrey Allen, Anant Patkar, Charles Squires, Henry Talbot and Jon Mitchell

Address: Biotechnology R&D, Dowpharma, The Dow Chemical Company, 5501 Oberlin Dr. San Diego, CA 92121, USA
* Corresponding author

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Background

A bottleneck to protein pharmaceutical production can be efficient expression of the target protein. A *Pseudomonas fluorescens*-based manufacturing platform for high yield production of non-glycosylated protein pharmaceuticals has been developed. This platform is derived from *P. fluorescens* biovar I strain MB101 [1]. The system's performance is due to the combination of a robust host strain, the availability of extensive molecular biology and bioinformatics tools, and a well optimized high cell density fermentation process. The Systems Biology tools include a genomics and functional genomics capability, a range of stable plasmid vectors of various copy numbers, non-antibiotic-dependent plasmid maintenance [2], multiple expression cassettes [3] and engineered host strains for stringent control of gene expression, and the ability to export proteins to the cell's periplasmic space.

Results

The ability to export proteins to the periplasmic space enables the formation of a precise N-terminus and formation of disulfide bonds. Moreover, export to the periplasm can simplify downstream processing. Multiple native *P. fluorescens* secretion leader sequences have been evaluated for the ability to direct heterologous proteins to the periplasm. Several secretion leaders were shown to effectively enable secretion of recombinant proteins with precise N-terminal processing at the expected amino acid. Yields of up to 18 g/L of secreted protein have been observed at the 20 L fermentation scale.

Conclusion

P. fluorescens is a robust expression host for high yield expression of secreted proteins. The identification of multiple, effective secretion leaders that can direct secretion of high levels of protein to the periplasm offers flexibility to identify the best secretion leader for each recombinant protein.

References

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