GRAM-POSITIVE BACTERIA AS HOST CELLS FOR HETEROLOGOUS PRODUCTION OF BIOPHARMACEUTICALS

Gram-positive bacteria as cell factories

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Abstract

The increasing demand of recombinant compounds in bioscience and bioindustries requires the further exploration and improvement of production systems including bacteria, fungi, insect and human cells. For compounds that do not require glycosylation for biological activity, microbial systems are most favourable hosts because of high level expression and relatively inexpensive culture systems. Traditionally, Escherichia coli was and still is most often the host of choice. However, the major drawbacks of this Gram-negative organism are the periplasmic location of the secreted proteins of interest due to the presence of an outer membrane and the frequent occurrence of cytoplasmically or periplasmically located inclusion bodies consisting of denaturated recombinant proteins. Gram-positive bacteria secrete the protein of interest directly into the culture media, thereby greatly facilitating downstream processing and protein recovery. As a consequence, they are being extensively explored for recombinant protein production.

This report reviews the possible applications of Gram-positive bacteria as host cells for the production of proteins of biopharmaceutical interest. It will also highlight eventual advantages of Gram-positive bacteria compared to Gram-negative organisms. Although successful in some cases, several bottlenecks in the secretion of heterologous proteins remain. Approaches undertaken to improve the yield of secreted recombinant proteins in these Gram-positive bacteria will be summarised. Finally, results obtained so far regarding the production of biopharmaceutical compounds in a soluble active form and with respect to the cell surface display of recombinant proteins by Gram-positive bacteria will be discussed.
1. Introduction

Much research has already been carried out to produce eukaryotic proteins of biopharmaceutical interest via heterologous expression systems at reasonable cost. In spite of its drawbacks, *Escherichia coli* is the most employed host, so far, for the production of recombinant proteins. This is largely because of its well-defined genetics and physiology and owing to the availability of a wide range of expression vectors. The membranaceous outer layer of Gram-negative bacteria, however, represents a barrier to the secretion of recombinant proteins into the culture medium. Recombinant polypeptides that are overproduced in *E. coli* often accumulate in the cytoplasm or periplasm as inclusion bodies, insoluble aggregates of denaturated proteins. Under these conditions protein recovery from *E. coli* requires breaking the cells and solubilising the aggregates with strong chaotropic agents followed by refolding of the polypeptide under appropriate conditions. An additional problem is the presence of endotoxin, a toxic lipopolysaccharide in the cell wall of Gram-negative bacteria, which is very difficult to remove during purification processes. Because of these problems encountered with *E. coli*, other systems are currently under investigation. Systems looked for are those that secrete the proteins in the culture medium at high yield in a soluble, biologically active form, thus avoiding inclusion bodies and accompanying problems of refolding. Gram-positive bacteria such as *Streptomyces* and *Bacillus* are promising hosts. They are widely exploited in industry for the production of homologous proteins extracellularly secreted in large amounts. However, the exploration of these bacteria for the production of heterologous (eukaryotic) proteins has shown a number of bottlenecks. These include impaired translation, inefficient translocation across the plasma membrane, retarded release of the secreted protein from the cell wall, incorrect folding and degradation of the recombinant protein by extracellular proteases. In recent years, much effort has been invested to characterise at the molecular level the secretion process in these organisms in order to be able to rationally modulate these bacterial cell factories for yield improvement of heterologous proteins. Other Gram-positive bacteria including lactic acid bacteria, *Staphylococcus* and others are also being tested as host for heterologous protein production, but with variable success.

In addition, bacteria are becoming attractive tools for the production of cell surface displayed heterologous proteins. Applications of this cell surface display technique are the use of bacteria as live vaccine delivery vectors, an alternative for the phage display technique to select peptides or recombinant antibody fragments from large libraries, and the use of enzyme-coated bacteria as novel biocatalysts. Gram-negative bacteria such as *E. coli* and *Salmonella* spp. were used first for this purpose as a consequence of the more extensive knowledge of their genetics and the wealth of available genetic tools for manipulating these organisms. However, both translocation through the cytoplasmic membrane and correct integration into the outer membrane are required for surface display in Gram-negative bacteria. In Gram-positive bacteria, the translocation through the cytoplasmic membrane is sufficient to achieve proper surface exposure of the heterologous protein. Therefore, *Mycobacterium, Listeria, Lactococcus, Lactobacillus, Staphylococcus* and *Streptococcus* are currently extensively investigated for their possible use as live antigen delivery system.