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Improved Method for Producing Secreted Proteins in Fungi

Optimal approach to DNA sequences, promoters, and fungal hosts

This invention is based on the novel finding that the expression level of genes encoding secreted proteins in filamentous fungi is decreased in conditions, in which the protein synthesis, folding or transport is impaired. This regulation mechanism has been demonstrated to function in cultures treated with chemical agents interfering with protein synthesis, folding or transport or in strains with functionally incomplete protein folding system.

In addition, strains producing heterologous proteins, such as tPA (tissue plasminogen activator) have been shown to display activated UPR as well as lower expression levels of endogenous genes coding for secreted proteins. VTT scientists found that this type of feed-back regulation occurs in the production of secreted proteins in filamentous fungi.

CHALLENGE

Developing a method for producing a promoter for protein production in a fungal host

Certain species of fungi are commonly used in biotechnological industry for protein production. The impairment of protein folding and further transport, likely to occur during production of a heterologous protein, is known to induce stress responses in the cell. In recent years, two feedback mechanisms have been reported allowing the cell to sense the state of the lumen of the ER. The cell also responds to perturbations in the normal functioning of this specialized environment for protein folding and processing.

These mechanisms constitute the Unfolded Protein Response (UPR), which increases the transcriptional activity of genes encoding chaperones and folding catalysts. This is in response to the presence of unfolded proteins in the lumen of the ER and phosphorylation of the eukaryotic initiation factor 2 which down-regulates translation activity in the cells.

SOLUTION

Modification of a homologous or heterologous promoter used for production of a protein

Very little is known about the transcriptional regulation of genes coding for endogenous secreted proteins in these stress conditions. No reported data is especially available on the feedback regulation of genes encoding the secreted proteins in response to limitations in the capacity of the cell to fold and transport the proteins. Accumulation of unfolded proteins and exposure of hydrophobic regions especially is known to activate the unfolded protein response (UPR) that is mediated by the transcription factor hac1.

Scientific reference: Pakula, T. M., Uusitalo, J., Saloheimo, M., Salonen, K., Aarts, J., and Penttilä, M. (2000) Monitoring the kinetics of glycoprotein synthesis and secretion in the filamentous fungus *Trichoderma reesei*: cellobiohydrolase I (CBHI) as a model protein. *Microbiology* 146, 223-232.

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Technology Summary

Optimized method for the production of secreted proteins in fungi, related to DNA sequences, promoters and fungal hosts

FI20010272, Patent in Finland

This invention can be used for modification of a homologous or heterologous promoter used for production of a protein, either homologous or heterologous, in a way that the expression is not subject to the down-regulation in a similar manner as the unmodified promoter. In particular, the invention is useful for producing heterologous proteins but can be applied also to production of homologous proteins.

Competitive Advantage

- Can be used for inactivation or reducing the activity or expression of the regulatory factor(s) mediating the down regulation of the promoter to improve protein production under the promoter.
- Can be used in over expression of a regulatory factor to decrease production of homologous secreted proteins during expression of homologous or heterologous proteins under a promoter that is not subjected to the down-regulation.

Available for Licensing

VTT offers exclusive and non-exclusive license depending on the technology partner.

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