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Oral Presentation

High level *Aspergillus* production of proteins

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Background

The *Aspergillus oryzae* expression system has been developed by Novozymes and used for recombinant protein production since 1988. Later on *Aspergillus niger* and *Fusarium venenatum* expression systems have been also developed.

The rationale for entering development of the *Aspergillus oryzae* expression system was that we needed an efficient expression system that could secrete large amounts of protein at high product purity for production of industrial enzymes.

The choice of *Aspergillus oryzae* as the preferred expression system was made from several different criteria. First of all *Aspergillus oryzae* is a well characterized organism that has been used in the food and fermentation industry in Japan for several hundred years. This means that the products of the fungus have been recognized as "Generally Regarded As Safe" (GRAS). The system was also used by producers of industrial enzymes using submerged fermentation for production for decades as *Aspergillus oryzae* has been used for production of amylases and proteases since the dawn of modern enzyme manufacturing. Another important advantage of *Aspergillus oryzae* was that it could be genetically manipulated. Finally there were no patents blocking the use of *Aspergillus oryzae* at the time.

Results

The early recombinant strains were essentially wild type strains transformed with plasmids based on a promoter and a selection marker that was cloned directly from different *Aspergillus* strains. Several shortcomings of these strains were quickly realized and work to improve the system was undertaken.

The host strain produced a lot of unwanted proteins such as amylases, amyloglycosidases and proteases.

In addition to improving the host strain, the expression vectors have also been improved. In particular the promoter has been the subject of optimization.

The promoter used in the first recombinant *Aspergillus oryzae* production strains was the TAKA promoter, the promoter driving the expression of the abundant TAKA amylase. We have characterized this promoter and found a specific transcription factor called *amyR* and binding sites for that transcription factor in the TAKA promoter [1]. The detailed understanding of the promoter has enabled us to design new synthetic promoters that are substantially improved compared to the wild type TAKA promoter.

The improvements of the *Aspergillus oryzae* expression system have resulted in a versatile, clean and high yielding expression system for enzyme production [2]. With these improvements the system is suitable for other uses than just expression of enzymes, including production of bioparmaceuticals. So recently we have initiated a number of projects aiming at developing biopharmaceutical products such as monoclonal antibodies and antimicrobial peptides [3] using *Aspergillus oryzae* as expression host.

References

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