News & Analysis
Interview

Pharmaceutical
BIOPROCESSING

Pharm. Bioprocess.
(2013) 1(4) 329–331

Innovative cell factories for pharmaceutical production: focus on fungi

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Han AB Wösten studied biology at Groningen University where he also received his PhD in 1994. He undertook a post-doc at the University of Munich (EMBO long-term fellowship) and was appointed assistant professor in the Microbial Physiology group of the University of Groningen in 1998. Han Wösten was appointed full professor at the University of Utrecht in 2001. He is the president of the Royal Dutch Society for Microbiology (since 2009), is head of the Department of Biology of Utrecht University (since 2011), and is vice-flagship manager of the public–private partnership BE-BASIC (since 2013). Han Wösten has filed nine patent applications, published more than 100 papers, has a H-index of 39 (Google Scholar) and his articles have been cited more than 5600-times. Han Wösten received three research prices, among which is the STW Simon Stevin Meester prize 2008 (€500,000). Professor Wösten spoke to *Pharmaceutical Bioprocessing* for the first in a series of interviews on innovative cell factories for pharmaceutical production.

Interview conducted by Alice O'Hare, Commissioning Editor.

» What is the role of fungi in bioprocessing?

Filamentous fungi are used for biodegradation of waste products, for solid state fermentation of raw materials and as enzyme producers. Moreover, they are cell factories for the production of compounds from primary metabolism (e.g., organic acids) and secondary metabolism (e.g., antibiotics). *Aspergilli* are often used as a fungal cell factory. They have a high secretion capacity, which is illustrated by strains of *Aspergillus niger* that secrete up to 30 g/l of glucoamylase, 140 g/l of citric acid or 0.9 g/l IgG (for a review see [1]).

» What led you to your current work on fungi as cell factories in bioprocessing?

As a masters student I became interested in microbiology. I worked on mechanisms underlying conjugation in lactic acid bacteria during my first internship at the University of Groningen. I decided to do my second internship in a company and chose to work on penicillin production in *Penicillium chrysogenum* at DSM, Delft, The Netherlands. This was the start of 24 years of research on fungi.

» What would you say are the main advantages of a fungi-based production system, when compared with mammalian (e.g., CHO) or bacterial (e.g., *Escherichia coli*) systems, for pharmaceutical bioprocessing?

The use of mammalian cells has several drawbacks. Operational costs are high for instance because of the requirement to test for viral contaminants. These contaminants are absent in fungal cell factories. Moreover, filamentous fungi have been extensively used for industrial protein production. As mentioned, production levels can be very high. Since fungi are eukaryotes, they have post-translational modification machinery similar to that in humans and

FUTURE SCIENCE

10.4155/PBP.13.36 ISSN 2048-9145

animals. This is a main advantage when compared with bacterial cell factories. However, the post-translational modification machinery (e.g., that for glycosylation of proteins) is not identical. This makes fungi not yet the preferred host for production of complex therapeutic proteins such as antibodies. However, we recently found that the N-glycosylation machinery of mushroom-forming fungi is much more similar to that of humans when compared with that of a traditional fungal cell factory such as *Aspergillus* [2,3]. It is tempting to speculate that nature may be home for more 'human-like' fungi.

» Can you briefly outline the research your group undertakes?

We study growth and development in fungi. We focus on secretion of proteins, the regulation of their production and their role in growth and development

» What would you say has been the biggest development in your field of research ?

One of the main questions is why fungi are so enormously successful in the secretion of proteins and other metabolites. By sequencing genomes and studying expression profiles the research community had hoped to find the answers but they have remained elusive. We think that heterogeneity may explain, at least in part, why we still do not understand the success of filamentous fungi. We have shown that only part of the hyphae in a culture secrete proteins [4,5]. Yet, the research community routinely extracts RNA and proteins from whole cultures. As a result, the expression and protein profiles of the actively secreting hyphae are diluted up to 100-fold by the RNA and protein of the inactive hyphae. In this way, genes that play a crucial role in efficient protein production are easily missed.

» What would you say is the biggest challenge faced by this field at the moment and how is this challenge being overcome?

We have started to set up single cell expression profiling [6]. Actively secreting hyphae have to be identified, isolated and the RNA profiles determined. We can identify secreting hyphae and we can determine whole genome expression of single cells. However, we still have to work on a protocol that combines these technologies. For instance, fixation of hyphae for fluorescence microscopy is not yet compatible with our RNA extraction procedures.

» Is heterogeneity a problem unique to fungi, or do other cell systems experience this issue?

Heterogeneity is also observed in bacterial and yeast cultures as well as in mammalian cell cultures. I think it is a really interesting phenomenon and a major challenge to overcome this.

» Looking at novel research into fungi as cell factories in bioprocessing, what advances do you believe hold most promise?

As mentioned above, we should understand mechanisms underlying heterogeneity and try to reduce heterogeneity in a culture. Of course, we aim to engineer strains that consist purely of highly active secreting hyphae. The question is whether this can be realized. It may be that the nonactive hyphae in some way support the active hyphae.

Another area of interest would be synthetic biology. Novel cloning procedures allow the introduction of whole gene clusters in microbial cells. It would be highly interesting to humanize fungi by introducing sets of human genes for instance involved in post-translational modification.

» What other research is your team working on, within the field of pharmaceutical bioprocessing?

We have studied the role of hydrophobins in growth and development in fungi for a long time. These proteins form very stable, highly surface active, protein films. We have recently shown that these proteins can be used to increase bioavailability of hydrophobic drugs and have antitumor activity [7,8]. Clearly, a lot can still be learned about these fungal proteins that are so abundant in nature.

» How do you anticipate the field of pharmaceutical bioprocessing using fungi will progress in the next 5–10 years?

So far, industries have invested most of their budget in their pet organisms. I hope that new fungal cell factories will be introduced because of their natural capability to perform for instance human-like. At the same time I expect that synthetic biology will be used more and more to improve industrial strains. And, yes, I think that production of pharmaceuticals by fungi will be studied using single cells rather than whole cultures.

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

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