Heterologous protein production of a His-tagged peroxidase in *Aspergillus niger*

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Introduction

Filamentous fungi have a very efficient protein-production capacity which make them suitable host organisms for the overproduction of commercially interesting homologous and heterologous proteins. The overall efficiency of an enzyme production process is influenced by the production yield (fermentation) and purification yield (Down Stream Processing). Unfortunately, since every protein is different, in many cases production and purification protocols and strategies must be developed for each individual protein.
In *E. coli* the fusion of proteins to oligo-histidine tags followed by affinity chromatography is a very common protein purification strategy. As far as we know, the use of His-tags in the extracellular production and purification of heterologous proteins in *Aspergillus* has not yet been demonstrated. Recently, we successfully produced *Arthromyces ramososus* peroxidase (ArP) in *Aspergillus niger* under control of the *inuE* (exo-inulinase) expression signals. To allow fast and easy purification we introduced N- and C-terminal His_6-tags, respectively. Extracellular peroxidase activity could be measured and was obtained with both, N- and C-terminal His_6-tagged ArP. The ability to purify the different His_6-tagged proteins by affinity chromatoraphy is under investigation.

Expression cassettes

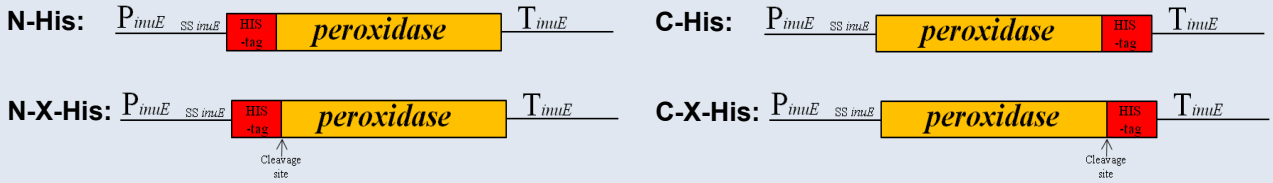

Figure 1: Expression cassettes of the *Aspergillus* expression vectors. PinuE: inducible *InuE* promoter; ssinuE: signalsequence of *inuE*; His-tag: 6xHis; Cleavage site: N-terminal enterokinase and C-terminal thrombine site, respectively; peroxidase: *Arthromyces ramosus* Peroxidase (ArP); TinuE: Termination signal of *inuE*.

Peroxidase production

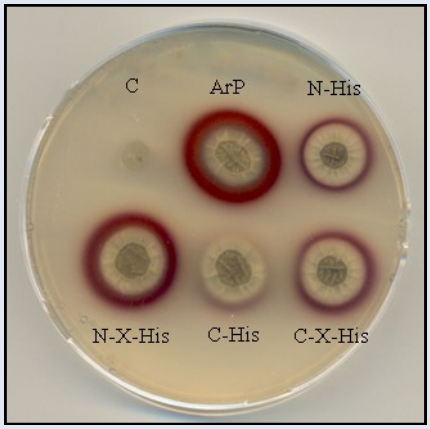

Figure 2: O-anisidine plate assay of peroxidase (ArP) producing *A. niger* transformants. Extracellular peroxidase activity could be measured and was obtained with both, N- and C-terminal His_6-tagged ArP. C: negative control; ArP: ArP producing transformant without His-tag; N-His, N-X-His, C-His, C-X-H: transformants harbouring the expression cassettes as indicated in figure 1.

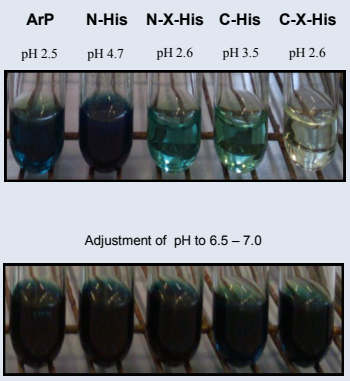

Figure 3: Peroxidase activity in culture medium. Peroxidase activity was demonstrated in an ABTS-assay on medium samples after 4 hours of induction in the presence of inuline. Activity was demonstrated in in both N– and C-terminal His transformants and was strongly depended on the pH.

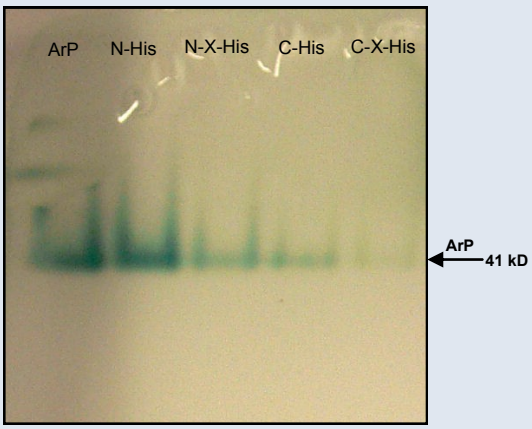

Figure 4: Native PAGE gel with ABTS overlay. ArP product could be demonstrated in medium samples after 4 hours of induction in the presence of inuline. Secreted active ArP could be detected in all transformants with an ABTS overlay.

Conclusion / Future Plans

- The presence of a His-tag, either N– or C-terminal, does not affect the production and activity of ArP. Differences in activity might be due to copynumber differences of the expression cassettes. Southernblot analyses of transformants are in progress.
- The presence and functionality of the His-tag will be established by Westernblot analysis and affinity chromatography.
- Low pH of the culture medium might be a problem for the stability of the fusion protein. For that reason *A. awamori* will be tested as host-strain.