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HIGH-LEVEL EXPRESSION OF INDUSTRIAL ENZYMES ORIGINATED FROM PLANTS IN FUNGAL HOSTS

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Enzymes employed as biocatalysts in technical processes have to be available in large quantities and at reasonable costs. This implies that highly efficient production systems have to be established in order to prove the basis for a competitive biocatalytic process.

Hydroxynitrillyases are a very interesting group of enzymes as they can catalyze the stereoselective synthesis of cyanohydrins by the addition of hydrogen cyanide to aldehydes or ketones [1]. These enzymes are found in various plants and for industrial production heterologous microbial systems have to be developed.

Fungi have been successfully employed for heterologous expression of various proteins from different origin including higher eukaryotes [2, 3]. Looking for suitable expression hosts for the production of plant derived hydroxynitrillyases (Hnl) of *Hevea brasiliensis* (S-selective Hnl) and of *Prunus amygdalus* (R-selective Hnl), we cloned the structural genes into various bacterial and fungal expression systems. *E. coli* host-vector systems allowed high-level Hnl protein production but mostly as inactive inclusion bodies. Expression in the yeast *Saccharomyces cerevisiae* could be achieved at medium levels using a constitutive *pgk* promoter based autonomously replicating vector. Expression experiments in filamentous ascomycetes such as *Aspergillus niger* or *Penicillium chysogenum* using standard expression vectors resalted in rather poor levels. However, we were able to show that expression of *H. brasiliensis* Hnl from a regulated fungal promoter was highly dependent on proper 3 terminator elements.

The methylotrophic yeast *Pichia pastoris* is a well-developed expression system suitable for large-scale production of proteins [4]. We were able to construct a highly

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efficient expression strain for *H. brasiliensis* Hnl based on the inducible promoter of the alcohol oxidase gene (*aox1*). We could achieve about 25 grams of soluble and active Hnl enzyme produced intracellularly per litre of culture in a high cell density fermentation process. This is the highest value of heterologous protein expression reported so far [5]. However, detailed analysis revealed this strain to be the result of a special event that occurred in the fungal host.

It was also possible to establish secretory expression of *P. amygdalus* Hnl in *P. pastoris* which represents the first successful functional heterologous production of this enzyme.

References

1. Griengl,H., Schwab,H., Fechter,D.R.: The synthesis of chiral cyanohydrins by oxynitrilases. Trends Biotechnol **18**, 252–256 (2000).
2. Gellissen,G.: Heterologous protein production in methylotrophic yeasts. Appl Microbiol Biotechnol **54**,741–50 (2000).
3. Gouka,R.J., Punt,P.J., van den Hondel,C.A.: Efficient production of secreted proteins by Aspergillus: progress, limitations and prospects. Appl Microbiol Biotechnol **47**, 1–11 (1997).
4. Cereghino,J.L., Cregg,J.M.: Heterologous protein expression in the methylotrophic yeast Pichia pastoris. FEMS Microbiol Rev **24**, 45–66 (2000).
5. Hasslacher,M., Schall,M., Hayn,M., Bona,R., Rumbold,K., Lückl,J., Griengl,H., Kohlwein,S.D., Schwab,H.: High level intracellular expression of hydroxynitrillyase from the tropical rubber tree Hevea brasiliensis in microbial hosts. Protein Expression and Purification **11**, 61–71 (1997).