BIOTRANS
LA ROCHELLE 2023

N°765 / Pitch Talk
TOPIC(s) : Enzyme production, immobilization

One-step production and immobilization approach for unspecific peroxygenases

AUTHORS
Niklas TEETZ / UNIVERSITY OF APPLIED SCIENCES MITTELHESSEN, WIESENSTR. 14, GIESSEN
Selina LANG / UNIVERSITY OF APPLIED SCIENCES MITTELHESSEN, WIESENSTR. 14, GIESSEN
Dirk HOLTMANN / UNIVERSITY OF APPLIED SCIENCES MITTELHESSEN, WIESENSTR. 14, GIESSEN

PURPOSE OF THE ABSTRACT
One-step production and immobilization approach for unspecific peroxygenases

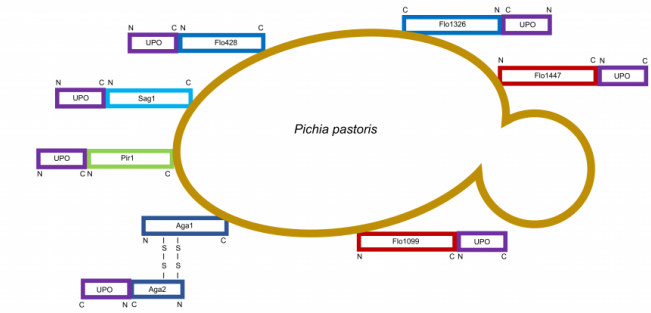
Niklas Teetz, Department of Bioprocess Intensification, THM Gießen, Department of Technical Biology, Karlsruhe Institute of Technology, Karlsruhe;
Selina Lang, Department of Bioprocess Intensification, THM Gießen;
Dirk Holtmann, Department of Bioprocess Intensification, THM Gießen, Department of Technical Biology, Karlsruhe Institute of Technology, Karlsruhe

Unspecific peroxygenases (UPOs) are regarded as a “dream catalyst” [1] for a variety of selective oxyfunctionalization reactions like hydroxylations, epoxidations and oxygenations [2]. The catalyzed reactions are similar to those of the well-known P450 monooxygenases [3] but UPOs offer independence from reduced nicotinamide cofactors and electron transport chains by using the easily produced hydrogen peroxide as only cofactor [1]. Here, we present the display of the model UPO rAaeUPO (PaDaI) on the cell surface of the commonly used heterologous production host Pichia pastoris (Komagataella phaffii) as a 1-step production and immobilization approach.

Multiple genes were cloned, combining the coding sequence for PaDaI with genes or part of genes coding for cell wall proteins from Saccharomyces cerevisiae. The genes were transformed into P. pastoris via an integration vector for production of the fusion proteins and subsequent comparison of the different systems among each other and with secreted, free PaDaI. All used systems yielded active UPOs, immobilized on the cell surface. Only minimal to no enzyme activity was detected in the supernatant, indicating a near complete surface display of the produced enzymes. One system in particular, a C-terminal fusion of PaDaI and SAG1, which is a glycoprotein involved in cell-cell contact during yeast mating, yielded identical ABTS activity per volume culture broth to the secreted PaDaI with ~90 % of the activity being in the cell pellet.

The presented cell surface system of UPOs offers even easier downstream processing than systems with secreted UPOs do and already includes immobilization on a cheap, easily retainable and replacable matrix, that is the cells of the production host themselves.

FIGURES



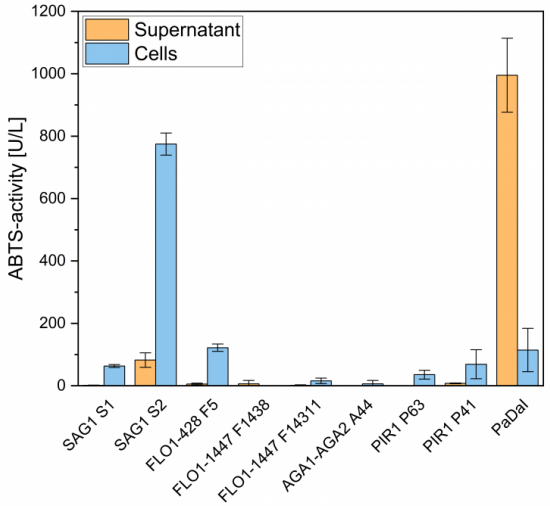


FIGURE 1

Scheme 1: Overview over the used cell surface display systems

N: N-terminus

C: C-terminus

S--S: disulfide bond

SAG1: alpha-agglutinin

AGA1: anchoring subunit of a-agglutinin

AGA2: receptor binding subunit of a-agglutinin

PIR1: protein containing internal repeats

FLO: protein involved in flocculation

FIGURE 2

Comparison of ABTS-activity of different cell surface display systems and secreted PaDal

orange bars: activity in supernatant

blue bars: activity in cell pellet, resuspended in 100 mM KPi and normalized to initial culture volume

KEYWORDS

unspecific peroxygenases | immobilization | cell surface display

BIBLIOGRAPHY

[1] Y. Wang et al, Current Opinion in Chemical Biology 2017, 37:1-9

[2] Hofrichter et al, Antioxidants 2022, 11, 163

[3] S. Bormann et al, Molecular Catalysis 2020, 492, 110999