

N°1276 / PC

TOPIC(s) : Enzyme production, immobilization

One-step production and immobilization approach for unspecific peroxygenases

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PURPOSE OF THE ABSTRACT

One-step production and immobilization approach for unspecific peroxygenases

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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 (PaDal) 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 PaDal 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 PaDal. 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 PaDal 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 PaDal 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

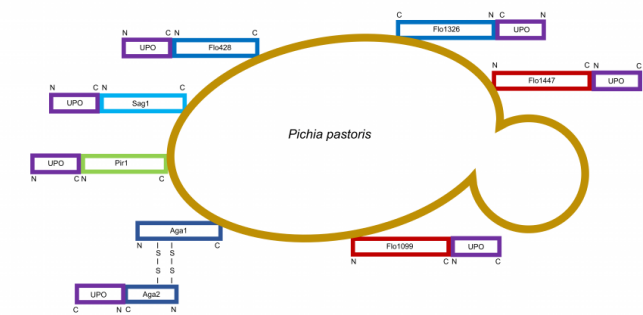


FIGURE 1
Scheme 1: Overview over the used cell surface display systems
N: N-terminus
C: C-terminus
S--S: disulfide bond
SAG1: alpha-agglutinin
AGA1: anchoring subunit of a-agglutinin
AGA2: receptor binding subunit of a-agglutinin
PIR1: protein containing internal repeats
FLO: protein involved in flocculation

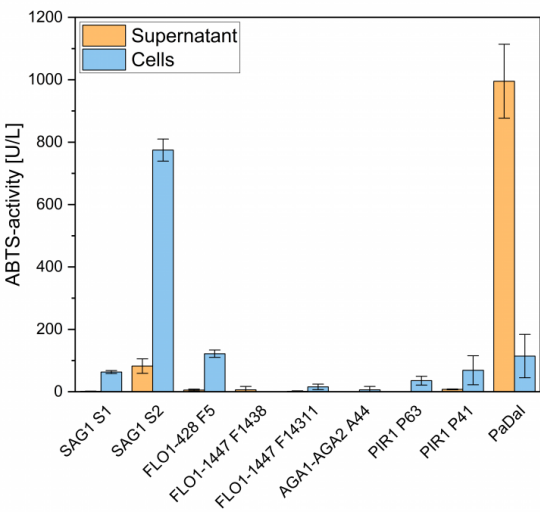


FIGURE 2
Comparison of ABTS-activity of different cell surface display systems and secreted PaDal
orange bars: activity in supernatant
blue bars: activity in cell pellet, resuspended in 100 mM KPi and normalized to initial culture volume

KEYWORDS

unspecific peroxxygenases | immobilization | cell surface display

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