

Recombinant expression of *Pleurotus ostreatus* laccases in yeasts: a basis for directed evolution

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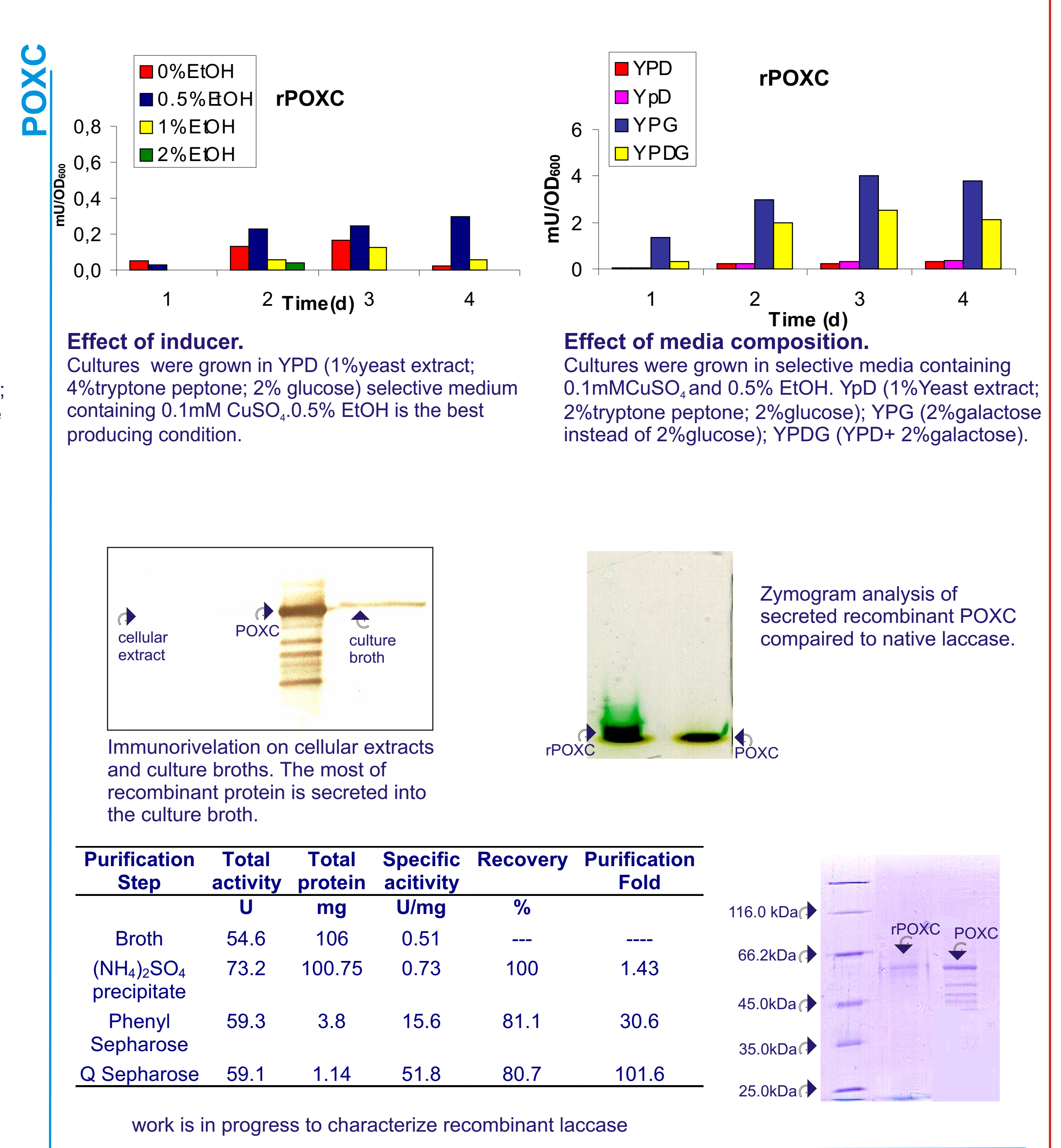
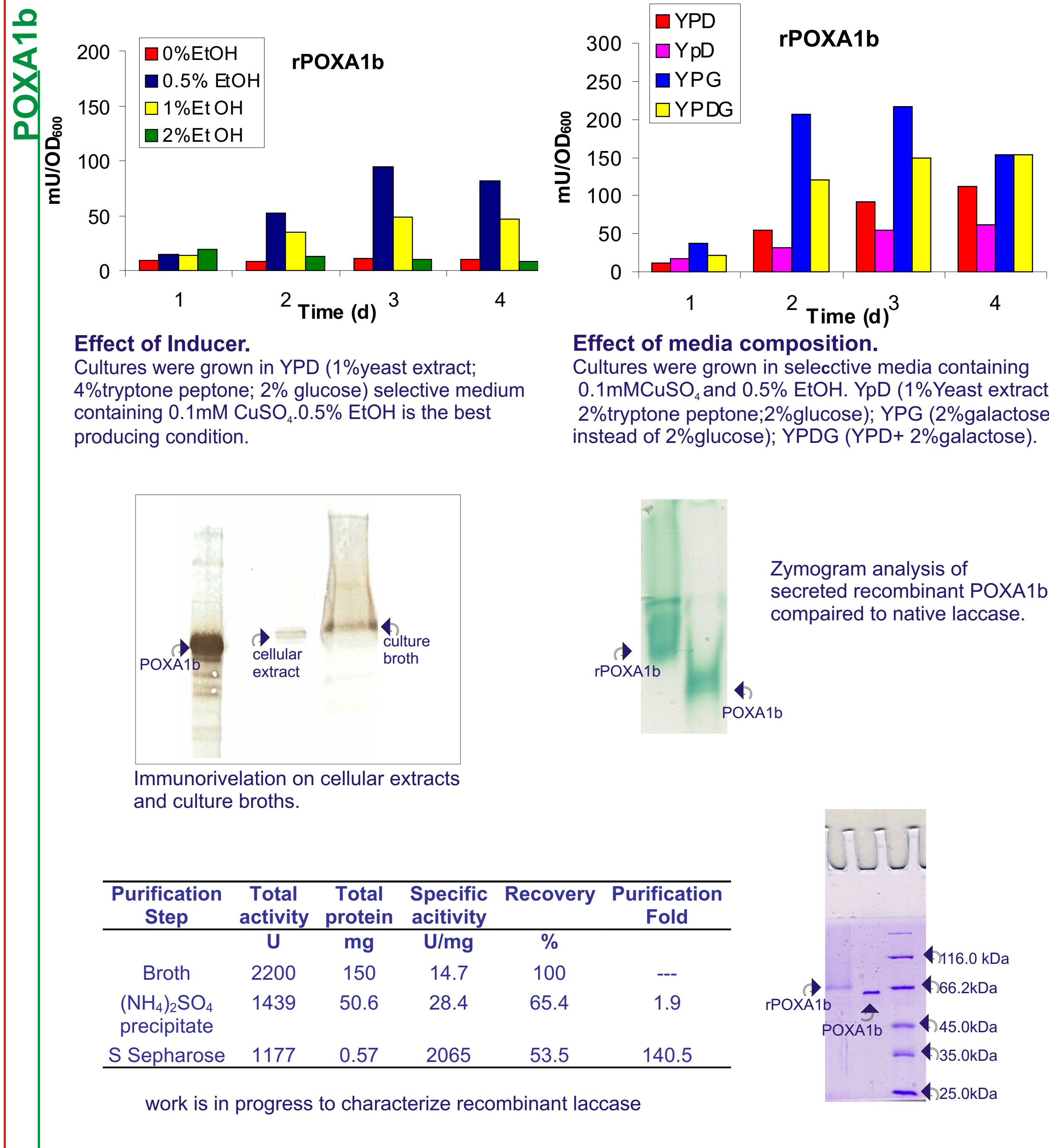
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The white-rot fungus *Pleurotus ostreatus* is able to express multiple laccase genes encoding isoenzymes with different and particularly interesting physico-chemical characteristics: POXC, POXA1w, POXA1b, POXA3a and POXA3b. In order to get a new insight into the structure/function/stability relationships of these laccases, and with the aim to use directional evolution to select for improved laccases with desirable physico-chemical characters, we focused on the recombinant expression of *P. ostreatus* laccases in yeast systems.

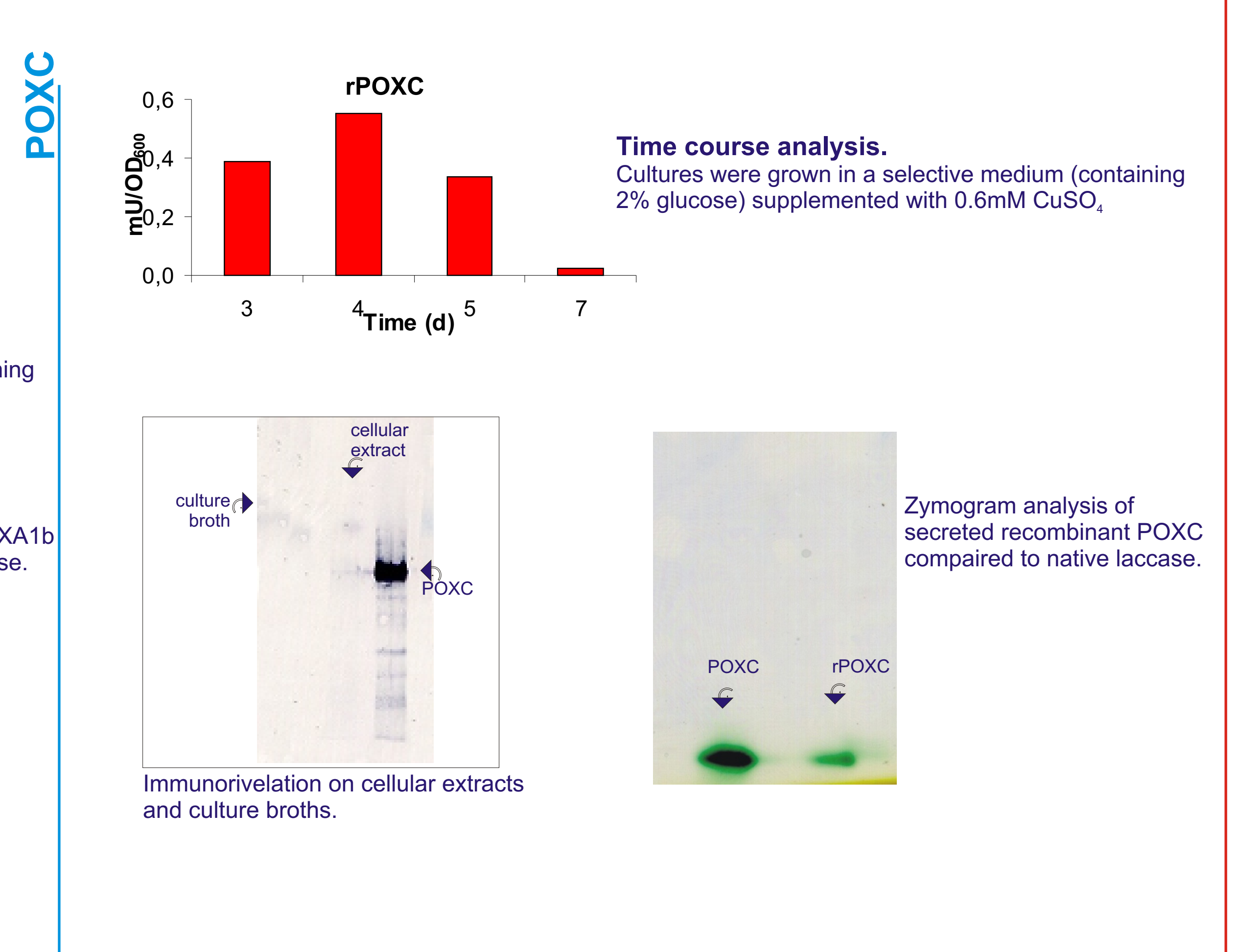
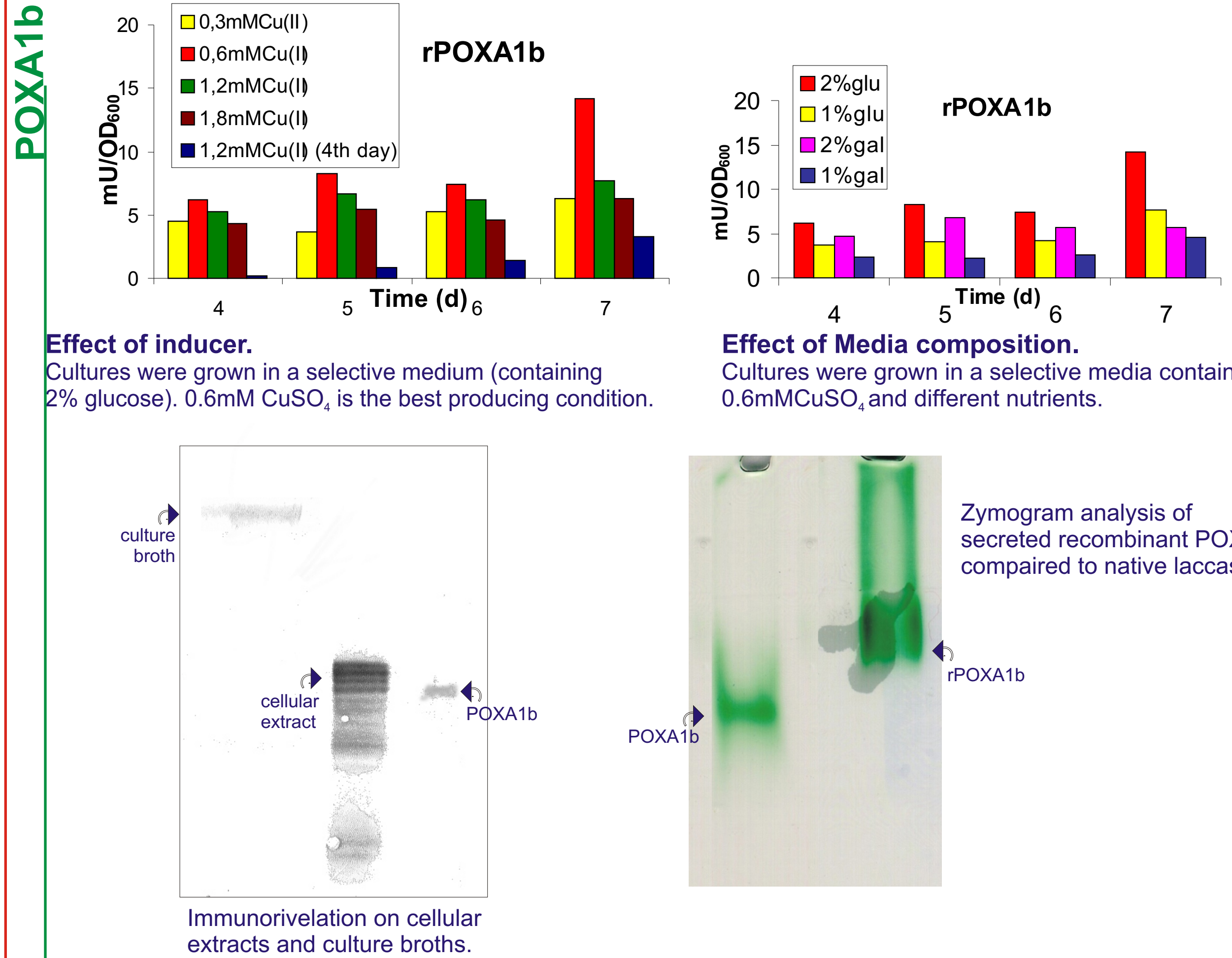
HOST *Kluyveromyces lactis*: a non-conventional yeast offering significant advantages, such as high-level secretion of non-hyperglycosylated recombinant proteins.

HOST *Saccharomyces cerevisiae*: proven to be success-full in directed-evolution experiments.

K. lactis



S. cerevisiae



P. ostreatus laccases are successfully expressed in two yeasts.

The best yields of production are:

Laccase	Yield (U/ml)	Host
POXA1b	4.2	<i>K. lactis</i>
POXC	0.1	<i>K. lactis</i>
POXA1b	0.2	<i>S. cerevisiae</i>
POXC	0.01	<i>S. cerevisiae</i>

Laccase signal peptide proved to be more effective to drive the secretion of recombinant laccase proteins.

K. lactis is a more efficient host to express *P. ostreatus* laccases.

rPOXA1b secreted activity is higher than rPOXC in both hosts.