PURIFICATION AND CHARACTERIZATION OF INVA AND INVB INVERTASES FROM *ZYMOMONAS MOBILIS*. EXPRESSION IN *PICHIA PASTORIS* BY INDUCTION WITH METHANOL

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Introduction. *Zymomonas mobilis* produce two invertases: an intracellular (INVA) and an extracellular (INVB). These enzymes have attractive features for the food industry, *e.g.*, they have less susceptibility to inhibition by sucrose than the commercial invertase from *Saccharomyces cerevisie.*

The use of modified enzymes in industry bioprocess has increased in recent years. *Pichia pastoris* has been widely used for obtaining recombinant enzyme by means of different expression systems such as the methanol-regulated using the strong promoter AOX1. *P. pastoris* has been used for the expression of proteins for both basic research and industrial processes.

The aim of this study was to express the INVA and INVB from *Z. mobilis* in *P. pastoris* under the control of the promoter AOX1 and evaluate the biochemical properties of the recombinant enzymes

Methods. *invA* and *invB* genes from *Z. mobilis* were amplified from the bacterial genomic DNA and cloned into the pPICZαB expression vector. Constructions obtained (pPICZαB-invA and pPICZαB-INVB) were transferred to *P. pastoris* X-33 cells by electroporation. Transformants clones were selected for Zeocin resistance, functional bioassays (1-2), PCR amplification and protein expression in *P. pastoris* cells by small scale fermentation and induction with 1% methanol (3). Clones with the highest invertase activity in the culture supernatant were selected for the purification of the recombinant INVA (INVA-r) and INVB (INVB-r) invertases. Culture supernatant was concentrated by ultrafiltration (30 kDa membranes) or precipitation ($(NH_4)_2SO_4$). Recombinant enzymes were purified by ion exchange chromatography, and the purified enzymes were biochemically characterized.

Results. The *invA* and *invB* genes from *Z. mobilis* were expressed in *P. pastoris* and the recombinant enzymes were purified from the culture supernatant of clones with the highest extracellular invertase activity.

SDS-PAGE analysis of purified INVA-r and INVB-r showed a single band of 65 kDa and 48 kDa, respectively (**Fig. 1**).

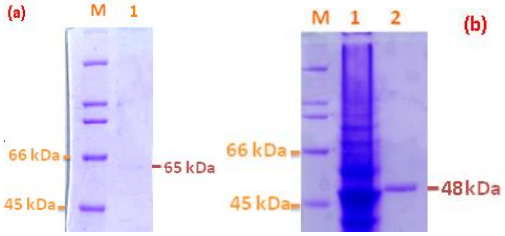

Fig.1 SDS-PAGE analysis of purified INVA-r (a) and INVB-r (b) invertases. *P. pastoris* cells were induced with 1% methanol. (a) Line M: MW marker, Line 1: INVA-r . (b) Line M: MW marker, Line 1: Culture supernatant, Line 2: INVB-r.

Purified enzymes were biochemically characterized and a summary of results is shown in Table 1.

Table 1. Biochemical properties of INVA and INVB from *Z. mobilis*, native (-n) and recombinant (-r) forms

	Optimum T (°C)	V_{max} (µmol/mg/min)	K_m (mM)
INVA-n (*Z. mobilis*)	30	n/d	42
INVA-r (*P. pastoris*)	35	833	0,042
INVB-n (*Z. mobilis*)	45	34	208
INVB-r (*P. pastoris*)	40	5000	0,05

Conclusions. The recombinant INVA and INVB invertases from *Z. mobilis*, expressed in *P. pastoris*, showed both higher catalytical activity and substrate affinity with sucrose as the substrate than those reported for native INVA and INVB invertases from *Z. mobilis*.

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