Expression of Cu, Zn superoxide dismutase recombinant protein from *Talaromyces marneffe* in *Pichia pastoris* and its resistance to oxidative stress

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INTRODUCTION

Talaromyces marneffe is an intracellular dimorphic fungus that causes a fatal disseminated disease in human immune-deficient virus-infected patients. The factors that affect the pathogenicity of this fungus remain unclear. Superoxide dismutase has been shown to contribute to the virulence of many human-pathogenic fungi through its ability to neutralize toxic levels of reactive oxygen species generated by the host. In this study, we constructed the recombinant Cu, Zn superoxide dismutase clone from *T. marneffe* in *Pichia pastoris* to determine for specific enzyme activity and Cu, Zn SOD overexpressed yeast was tested for resistance to the oxidative stress conditions.

METHODOLOGY

Fig 1. Full-length *sodA* gene was amplified from cDNA clone by specific primers with tag sequences. The PCR fragment was cut with *XhoI* and *NotI* restriction enzyme and clone in to pPICZαB plasmid. The ligated plasmid was transformed into TOP10 *E. coli* and selected with LB-zeocin.

Yeast expression

- Yeast cloning
- Linearized plasmid was transformed into *P. pastoris* strain X33 by electroporation.
- Protein expression
- The selected clone was cultured for 3 days in BMMY and induced expression by adding 0.5% methanol.

Protein analysis

- Protein detection
- The supernatant was collected and checked for protein expression by SDS-PAGE.
- His-tag detection
- Western blot analysis with His-tag antibody was performed and then protein was purified.

Enzyme activity and stress response

- Enzyme activity
- Activity staining with NBT was done according to Beauchamp and Fridovich (1971).
- Oxidative stress conditions
- Recombinant yeast and control were incubated in various stress conditions.

RESULTS

An approximate molecular mass 20 kDa of secreted recombinant Cu, Zn SOD was detected by Coomassie Brilliant blue staining-SDS-PAGE and his-tag was detected by western blot with Ni-NTA (Fig 2A and B). The results from Native-PAGE revealed that the purified Cu, Zn SOD recombinant protein had an enzymatic activity that was similar to the standard enzyme (Fig 3). The *sodA*-pPICZαB recombinant yeast appeared to be more resistance to oxidative stress conditions than pPICZαB recombinant yeast (Fig 4).

Fig 2. (A) Expression of Cu, Zn SOD recombinant protein by *P. pastoris*. M: marker, X33: secreted protein from X33 and D0-D3: secreted proteins from recombinant clone in day0 to day3. (B) Western blot for his-tag detection of secreted protein from positive clone (S) and X33.

Fig 3. Cu, Zn SOD enzymatic activity in native PAGE. M: marker, rSOD: purified recombinant Cu, Zn SOD, crude protein extracted from mold and yeast of *T. marneffe*, 1u, 5u and 10u of standard bovine SOD (sigma).

Fig 4. The oxidative stress resistance tests. The recombinant of *sodA*-pPICZαB (S19) and pPICZαB control (P) were grown for expression at 30°C for 24 h, adjusted to the same absorbance (OD600), incubated with heat at 48°C for 45min, 5mM menadione, paraquat or H₂O₂ for 1h and then serially ten fold diluted to 10⁻¹, 10⁻² and 10⁻³ in PBS. Samples, 10 µl, of each dilutions were spotted on YPD plates and incubated for 2 days at 30 °C.

CONCLUSION

We have successfully cloned and expressed the recombinant *T. marneffe* Cu, Zn SOD in *P. pastoris* system. This protein has an efficient enzymatic activity, moreover the recombinant yeast exhibited higher stress resistance than the control yeast. The further study will focus on characterization of the immunogenic and virulence properties of this protein.

Selected references

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