Purification and functional characterization of heme-dependent catalase in respirative strain \textit{Lactobacillus casei} N87

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Introduction

Home-dependent catalases are the largest group of H\textsubscript{2}O\textsubscript{2}-degrading enzymes. Although the genes encoding for heme-catalase are present in 34 species of lactic acid bacteria (LAB); Integrated Microbial Genomes database; IMG, https://img.jgi.doe.gov, to our knowledge, no studies optimised a chromatographic protocol for the purification of natural (without heterologous expression) heme-catalases in LAB. The genome of the respiratory-competent strain \textit{Lactobacillus casei} N87 (Zotta et al. 2014; Ianniello et al. 2015) contains sequences for both heme- and manganese-catalases (Zotta et al. 2016); the presence of both catalases are rare within LAB and may be relevant for several food and health-related applications.

AIM: a protocol for the purification of heme-catalase of \textit{L. casei} N87 was optimised and the functional properties of the purified enzyme were evaluated.

Protein sequence of heme-catalase of \textit{L. casei} N87

The amino acid sequence (486 aa length) of heme-catalase was retrieved from the genome of \textit{L. casei} N87 (GenBank accession n. LCUN00000000.1; Zotta et al. 2016). The estimated molecular weight (55.2 kDa) and the theoretical pI (5.74) were calculated and used to optimise the purification protocol.

Rapid assay for catalase detection

A rapid detection assay, based on the formation of potassium ferricyanide-ferrichloride complex, was optimised to select the chromatographic catalase-positive fractions (Fig. 1).

**Functional characterisation of purified heme-dependent catalase**

Catalase-containing fractions were pooled and used for functional characterization.

- The effect of pH (4-10), temperature (10-80°C), heme-inhibitors (potassium cyanide, 0-20 μM; sodium azide, 0-1 μM; hydroxylamine, 0-1 μM) and H\textsubscript{2}O\textsubscript{2} (16-50 mM) on the catalase activity (Zotta et al. 2014) was evaluated (Figs. 3a, 3b, 3c, 3d, 3e, 3f).
  - The maximum enzymatic activity (100%) was detected at pH 7.0, even if the purified protein had a significant residual activity (35%) also at pH 4 and 10 (Fig. 3a).
  - The optimal temperature for catalase functionality was 25°C, but noticeable activity was found within 20-60°C (80-40% of residual activity; Fig. 3b).
  - Potassium cyanide (≥15 μM), sodium azide (≥0.75 μM) and hydroxylamine (≥0.75 μM) significantly impaired the catalase activity (<20% of residual activity), confirming that the purified enzyme harboured a heme prosthetic group (Figs. 3d, 3e, 3f).
  - High concentrations (50 mM) of H\textsubscript{2}O\textsubscript{2} also reduced (<10%) the activity of heme-catalase (Fig. 3c).
  - NADH-peroxidase activity (estimated MW of 50 kDa, theoretical pI ~5.0) was not detected in the purified extract.

**CONCLUSIONS**

- This is the first study that optimized a purification protocol for a non-heterologous heme-catalase in LAB.
- Heme-catalase of \textit{L. casei} N87, having a broad stability, may be exploited for several biotechnological applications.
- However, further structural characterization studies are needed to understand and exploit the enzyme functionality.

References


