Purification and functional characterization of heme-dependent catalase in respirative strain *Lactobacillus casei* N87

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Introduction

Heme-dependent catalases are the largest group of H_2O_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 respiration-competent strain *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 *L. casei* N87 was optimised and the functional properties of the purified enzyme were evaluated.

Protein sequence of heme-catalase of *L. casei* N87

The **amino acid sequence** (486 aa length) of heme-catalase was retrieved from the genome of *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

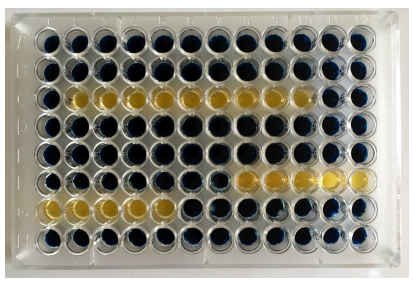


Fig. 1: Qualitative assay for heme-catalase detection in chromatographic fractions. Greenish-blue, catalase-negative fractions; yellow, catalase-positive fractions.

For all catalase concentrations the **optimal incubation parameters** were: 60 min at 25 °C in presence of 32 mM of H_2O_2, using a 0.25% (v/v) final concentration of potassium ferricyanide-ferrichloride solution (1:1).

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

Briefly, different concentrations (from 0 to 100 Units) of horseradish catalase were mixed with 32 mM of H_2O_2 and incubated at 25°C for different times (from 15 to 120 min). At the end of incubation, different concentrations (from 0.1% to 2%) of potassium ferricyanide-ferrichloride solution (1:1) were added to the reaction mix, and the change of colour from greenish blue (-) to yellow (+) indicated presence of catalase activity.

Protocol for heme-catalase purification

Total intracellular proteins were extracted from the respirative strain *L. casei* N87, grown in hemin-supplemented chemically defined medium (Ricciardi et al. 2015). Protein extracts were loaded on a DEAE Sepharose fast flow ion exchange column and eluted with a linear 0-1.0 M NaCl gradient (**step 1**). Fractions with catalase activity (potassium ferricyanide-ferrichloride assay) and MW of ~55 kDa (SDS-PAGE) were pooled (**step 2**) and further separated with a Blue-Sepharose CL-6B chromatography (**step 3**), exploiting the affinity of heme-catalase for the redox cofactor NADPH. SDS-PAGE and native-PAGE confirmed the purity and activity of purified enzyme.

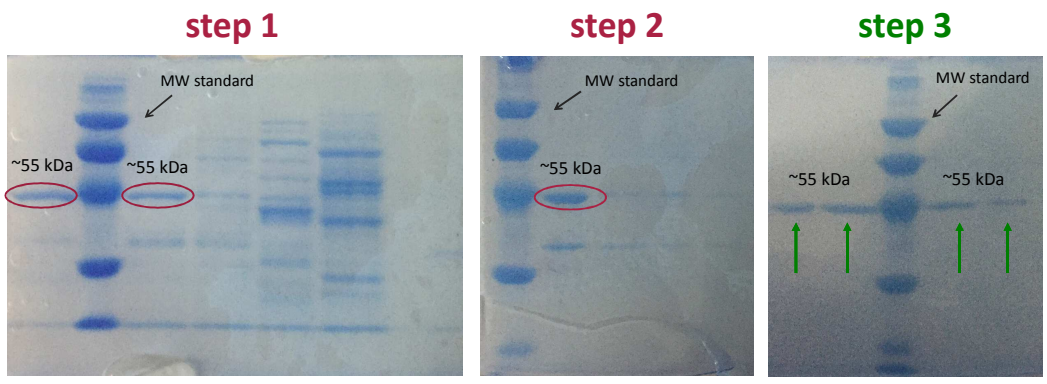
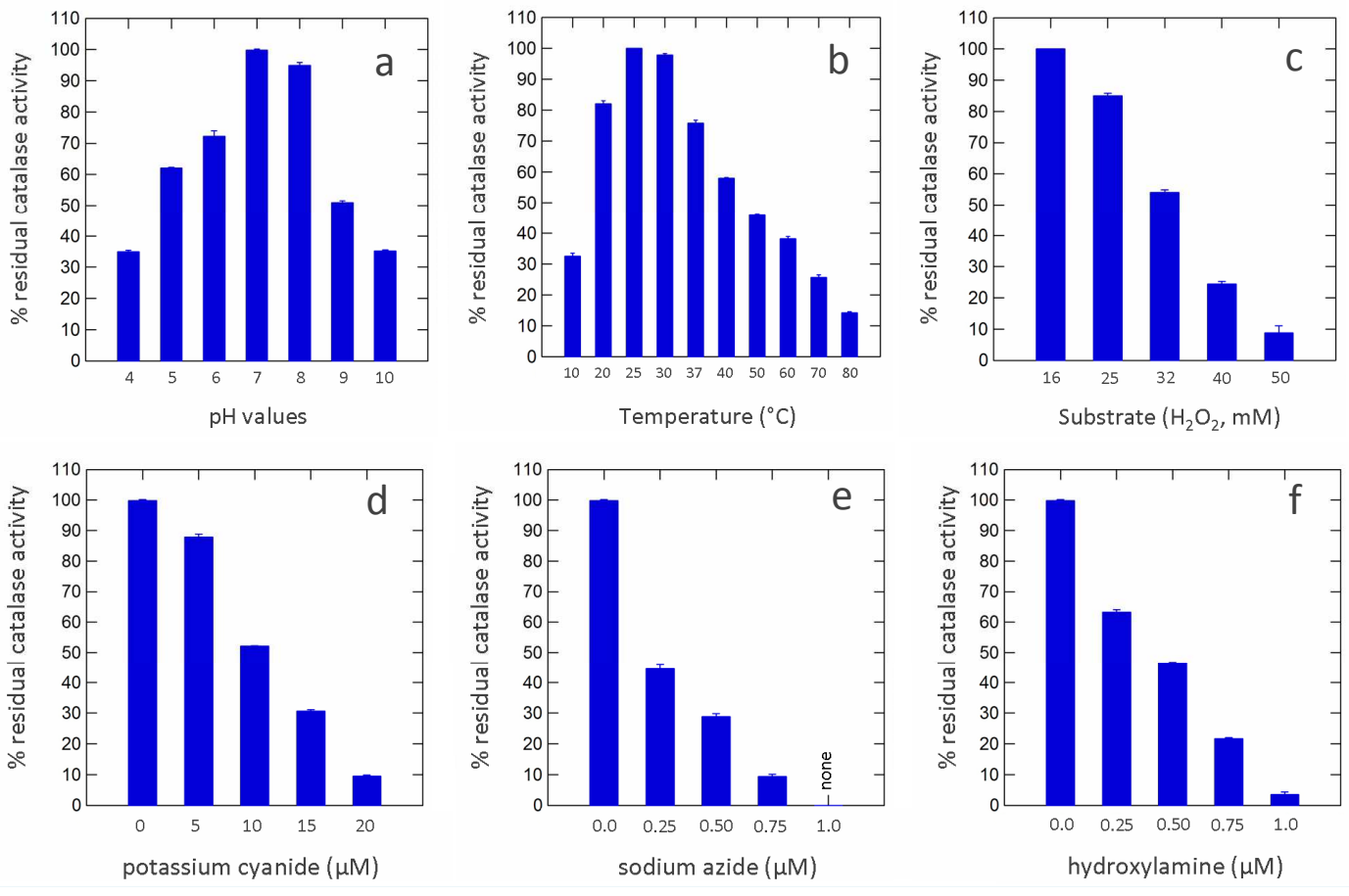


Fig. 2: Example of SDS-PAGE (12%) of chromatographic fractions recovered in the different purification steps. Step 1: fractions eluted from DEAE Sepharose column; step 2: fractions from DEAE Sepharose, with catalase activity and MW ~55 kDa, pooled and loaded on the Blue-Sepharose column; step 3: fractions eluted from the Blue-Sepharose, containing purified heme-catalase.

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_2O_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_2O_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 *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

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