P-02-12

Protease production characteristics of *Pediococcus pentosaceus* isolated from Harbin dry sausages (#200)

Fangda Sun, Huan Zhang, Baohua Kong

Northeast Agricultural University, College of Food Science, Harbin, China

Introduction

Harbin dry sausage is a traditional Chinese natural fermented meat product. *P. pentosaceus* showed a clear effect on improving the flavour of Harbin dry sausage. The objective of the present study was to investigate the production, purification and biochemical characteristics of the microbial protease from *P. pentosaceus* R1 isolated from Harbin dry sausages. Enzyme activity, culture condition, enzyme kinetic parame ters, were analysed.

Methods

1. Optimizing culture conditions

The basic fermentation conditions were incubation time 24 h, initial pH 6, and fermentation temperature 37 °C. The fermentation conditions in MRS basic medium were sequentially optimized as follows: incubation time (0-96 h), initial pH of the medium (2-9), and incubation temperature (17-52 °C).

2. Protease preparation and purification

Fermented broth (500 mL) was added to a clean centrifuge tube, and then centrifuged at 10000 ×*g* for 10 min at 4 °C. The obtained supernatants were stored it at 4 °C for further research. We used 80% saturated ammonium sulphate to precipitate crude protease, then loaded on DEAE-Sepharose FF column and gel filtration column, each tube was collected for 10 min. The active protease collections were quickly freeze-dried.

3. Estimation of K_m and V_{max} values

The maximum velocity of the reaction (V_{max}) and the Michaelis constant (K_m) of *P. pentosaceus* R1 protease were determined by using casein as the substrate at various concentrations (2.5-40 mg/mL) to evaluate the enzyme kinetics. The initial rate measurements were used to for fitting of the curve to the Michaelis-Menten equation.

Results

1. Effect of fermentation time, initial pH, and fermentation temperature on crude protease production

Fig. 1A shows the the crude protease activity first increased (0-36 h) and then decreased (36-96 h) with increasing fermentation time. The protease activity reached the maximum value of 47.60 U/mL at 36 h and was significantly higher than the protease activity of other fermentation times (P<0.05); then, the activity decreased rapidly to approximately 4.6 U/mL after 96 h.

As shown in Fig. 1B, with increasing pH, the optimum initial pH of *P. pentosaceus* R1 protease production was 4, and the corresponding protease activity reached 48.70 U/mL; the enzyme activity then decreased monotonically to 11.66 U/mL at pH 9. The different initial medium pH (acidic or alkaline medium) has a significant effect on the protease production by *P. pentosaceus* R1.

Fermentation temperature is another critical parameter that affects the microbial cell growth and enzyme production . The protease production was relatively low at 17 °C (20.30 U/mL) and achieved maximum activity of 50.25 U/mL at 42 °C; protease production then decreased rapidly when the fermentation temperature was above 42 °C (Fig. 1C).

According to the above results, the relative high protease activity was obtained in the sample with incubation 36 h, initial pH 4, and incubation temperature 42 °C.

2. Protease purification

Fig. 2 shows the purification curve of ion exchange chromatography and gel filtration. Two protease activity peaks were observed in ion exchange chromatography and the highest protease activity peak was found for fractions 4-8. The gel filtration curve (Fig. 2B) has two peaks in fractions 12-22 and 29-37, but the protease activity was obtained only at the first peak (12-22), so we combined these fractions and used this sample as a purified protease preparation. A 29.6 kDa extracellular protease was purified using ammonium sulphate precipitation, ion exchange layer and gel filtration (Fig. 2C).

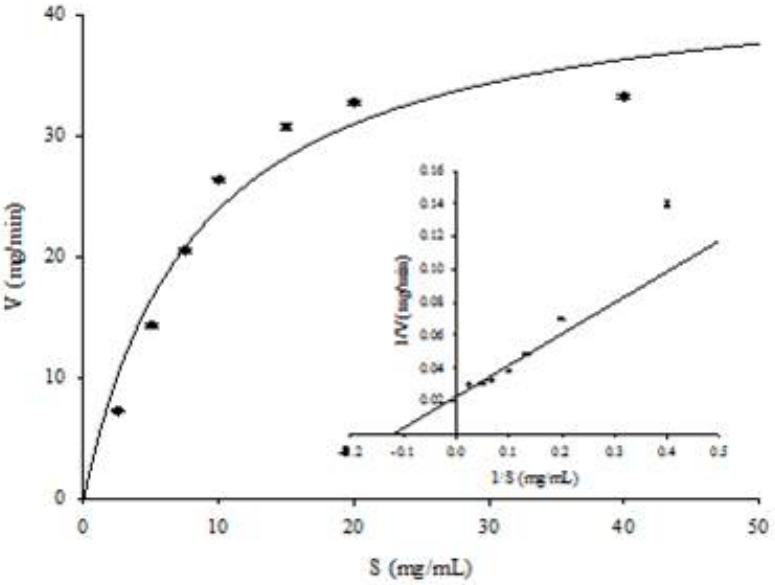
3. Determination of protease kinetic parameters

The kinetic parameters of *P. pentosaceus* R1protease was shown in Fig. 3. Michaelis-Menten plot of the protease reveals maximum velocity and Michaelis constant of the protease. The solid curve is the non-linear fit of the Michaelis-Menten data. The Lineweaver-Burk plot transformation of the previous plot was shown as the inset of the picture. From these plots, V_{max} and K_m were measured as 43.9 mg/min and 8.3 mg/mL, respectively, which is promising for application in fermented meat products due to the reaction speed and substrate affinity. The above conclusion indicated that the K_m value of *P. pentosaceus* R1protease was higher than that of the partial microbial protease; this observation shows that the protease was only weakly able to bind to the substrate, possibly due to the differences in the strain and the enzyme structure.

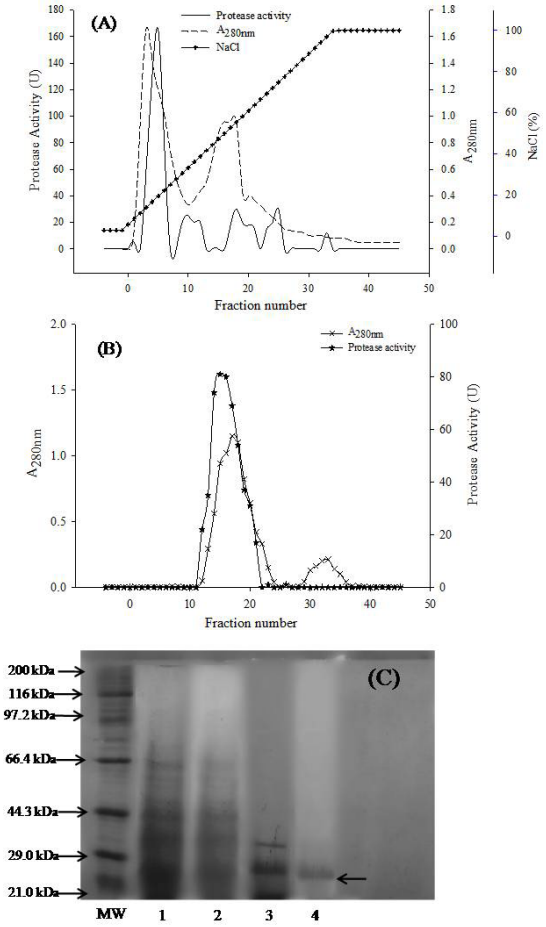
Conclusion

P. pentosaceus R1 showed higher enzyme production capacity and crude enzyme activity at 36 h fermentation time, initial pH 5 and fermentation temperature 30 °C. A 29.6 kDa extracellular protease was purified using ammonium sulphate precipitation, ion exchange layer and gel filtration. V_{max} and K_m of the protease were 43.9 mg/min and 8.3 mg/mL, respectively. In conclusion, *P. pentosaceus* can be used as a starter culture or enzyme producing strain to inoculate Harbin dry sausages.

Notes

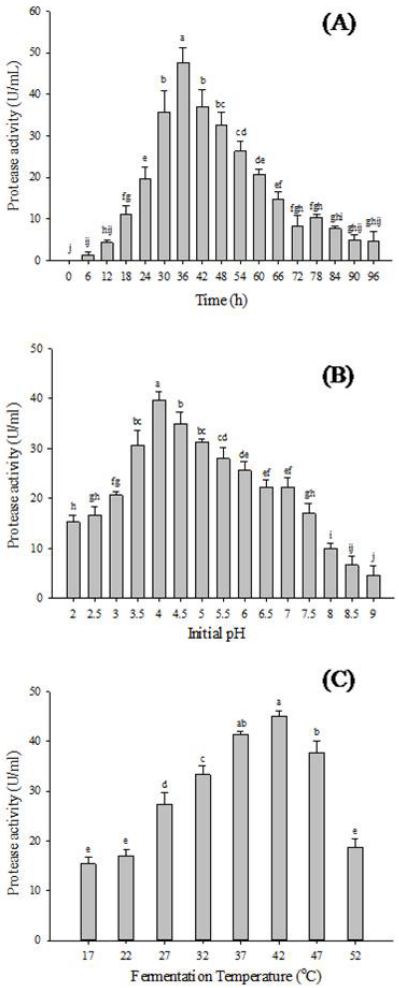


The Michaelis-Menten of protease
Fig. 3. The curve is the non-linear fit of the Michaelis-Menten data. The inset shows the Lineweaver-Burk plot. The V_{max} and K_m were 43.9 mg/min and 8.3 mg/mL, respectively.



Protease purification
Fig. 2. (A) Ion exchange pattern of the protease from *P. pentosaceus* on DEAE-Sepharose FF. (B) The separation effect of Sephadex G-75 Chroma-tograph on *P. pentosaceus* protease. Protein content was expressed as the absorbance at 280 nm. Protease activity was expressed as the absorbance at 680 nm. (C) SDS-PAGE results for the protease from *P. pentosaceus*. Lane MW, molecular weight of protein standard; Lane 1, crude protease; Lane 2, ammonium sulphate precipitation; Lane 3, DEAE-Sepharose FF; Lane 4, Sephadex G-75.

Notes



Effects of fermentation time , initial pH and temperature on the protease activity

Fig. 1. Effects of fermentation time (A), initial pH (B) and fermentation temperature (C) on the *P. pentosaceus* protease activity. Error bars refer to the standard error obtained from the analysis of triplicate samples. Different letters (a-j) indicate the significant differences of crude protease activity at different fermentation times ($P < 0.05$).

Notes