Professor Dr.Prasert Na Nagara Thesis Awards, Academic Year 2025

Outstanding Thesis Awards

Biological Science Section

Miss Pimsiriya Srila

Master Degree Program

Major in Biotechnology

Faculty of Agro-Industry
Kasetsart University

Microbial Cell Factories & Metabolic Engineering Laboratory
Department of Biotechnology, Faculty of Agro-Industry

BIOTEC a member of NSTDA

Heterologous Expression of D-Psicose 3-Epimerase using Lactic Acid Bacteria Expression System

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1 ABSTRACT

D-psicose 3-epimerase (DPEase) catalyzes the conversion of D-fructose into D-psicose, a low-calorie rare sugar with potential applications in the food industry. This study investigated the heterologous expression of DPEase from *Bacillus* sp. KCTC 13219 in *Lactiplantibacillus plantarum* WCFS1 using the pSIP expression system. The optimal expression condition was 12.5 ng/mL of IP-673 at 30°C for 15 hours, yielding a DPEase activity of 2.683 ± 0.083 U/mL. The purification using HisTrap™ FF column resulted in a 16.26-fold purification and a specific activity of 19.804 U/mg. The optimal conversion conditions were 65°C and pH 6.0, maintained over 80% relative activity across pH 6.0–11.0 and temperatures of 35–55°C, and achieved a 32.13% conversion rate from 20% (w/v) D-fructose. The kinetic parameters of the purified DPEase were determined as V_{max} = 47.85 µmol/min, K_m = 326.30 g/L, and k_{cat} = 334.73 s-1. These results demonstrate the feasibility of using lactic acid bacteria as safe and efficient expression hosts for the production of functional rare-sugar enzyme and highlight their potential application in food-grade biomanufacturing.

Keywords: D-psicose 3-epimerase; *Lactiplantibacillus plantarum*; Cell factory; D-psicose; Rare sugar

2 INTRODUCTION

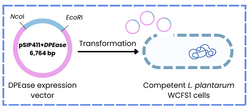
D-psicose (D-allulose) is a promising low-calorie rare sugar with approximately 70% of the sweetness of sucrose and only 0.4 kcal/g, making it attractive for food and functional food applications. Enzymatic production using D-psicose 3-epimerase (DPEase) offers an efficient and environmentally friendly approach to D-psicose synthesis. However, conventional microbial hosts such as *Escherichia coli* may present challenges related to endotoxin contamination and protein expression. Therefore, this study explored *Lactiplantibacillus plantarum* WCFS1 as a food-grade host for recombinant DPEase production using the pSIP expression system, providing a potential platform for safe and sustainable rare sugar biomanufacturing.

3 OBJECTIVES

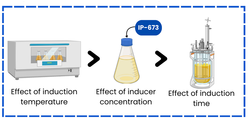
This study aimed to construct a DPEase expression vector, express DPEase in *Lactiplantibacillus plantarum* WCFS1 using the pSIP system, purify and characterize the recombinant DPEase, and investigate its potential for D-psicose production

4 METHODS

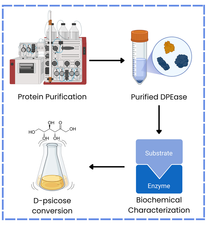
I: Construction and Transformation of DPEase Expression Vector into *L. plantarum*



II: Optimization of DPEase Expression



III: Purification, Biochemical Characterization and D-psicose Conversion



5 RESULTS

5.1 Construction and Transformation

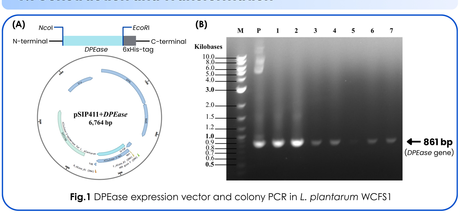
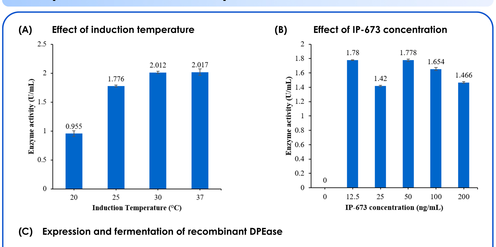
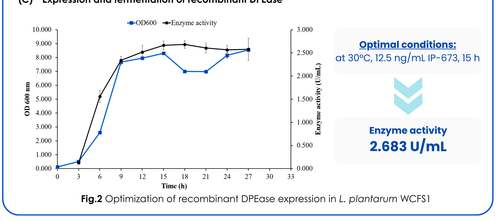


Fig.1 DPEase expression vector and colony PCR in *L. plantarum* WCFS1

5.2 Optimization of DPEase expression



(C) Expression and fermentation of recombinant DPEase



Optimal conditions:
at 30°C, 12.5 ng/mL IP-673, 15 h

Enzyme activity
2.683 U/mL

Fig.2 Optimization of recombinant DPEase expression in *L. plantarum* WCFS1

5.3 Purification of DPEase in *L. plantarum*

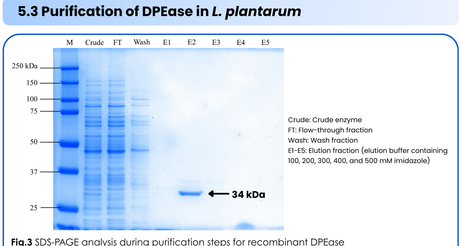


Fig.3 SDS-PAGE analysis during purification steps for recombinant DPEase

Table 1 Purification table of purified recombinant DPEase using FPLC

Sample	Total volume (mL)	Total protein (mg)	Total enzyme activity (U)	Specific activity (U/mg)	Purification factor (fold)
Crude enzyme	150	364.950 ± 0.433	444.605 ± 1.665	1.218	1
Purified DPEase	45	7.290 ± 0.052	144.372 ± 0.288	19.804	16.26

5.4 Biochemical characterization

Optimum pH and temperature

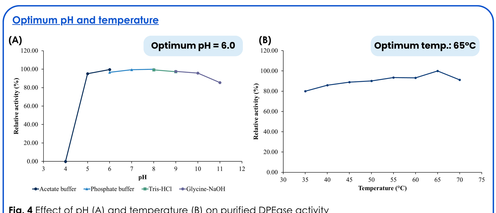


Fig. 4 Effect of pH (A) and temperature (B) on purified DPEase activity

pH and thermal stability

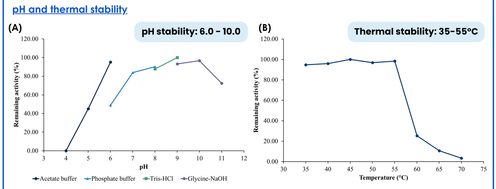
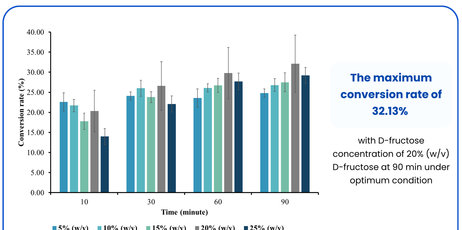


Fig. 5 Effect of pH (A) and temperature (B) stability on purified DPEase activity

5.5 D-psicose conversion



The maximum conversion rate of 32.13%

with D-fructose concentration of 20% (w/v) D-fructose at 90 min under optimum condition

Fig. 6 D-psicose conversion from D-fructose 5, 10, 15, 20, and 25% (w/v) using purified DPEase

6 CONCLUSION

- DPEase was successfully heterologously expressed in *L. plantarum* WCFS1 using the pSIP system
- Optimized production achieved 2.683 ± 0.083 U/mL at 30°C for 15 h with 12.5 ng/mL IP-673, with 16.26-fold purification
- Purified DPEase showed broad pH and temperature stability, with optimal activity at pH 6.0 and 65°C
- D-fructose was converted to D-psicose with a maximum conversion rate of 32.13%

Acknowledgment

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Thesis Book File