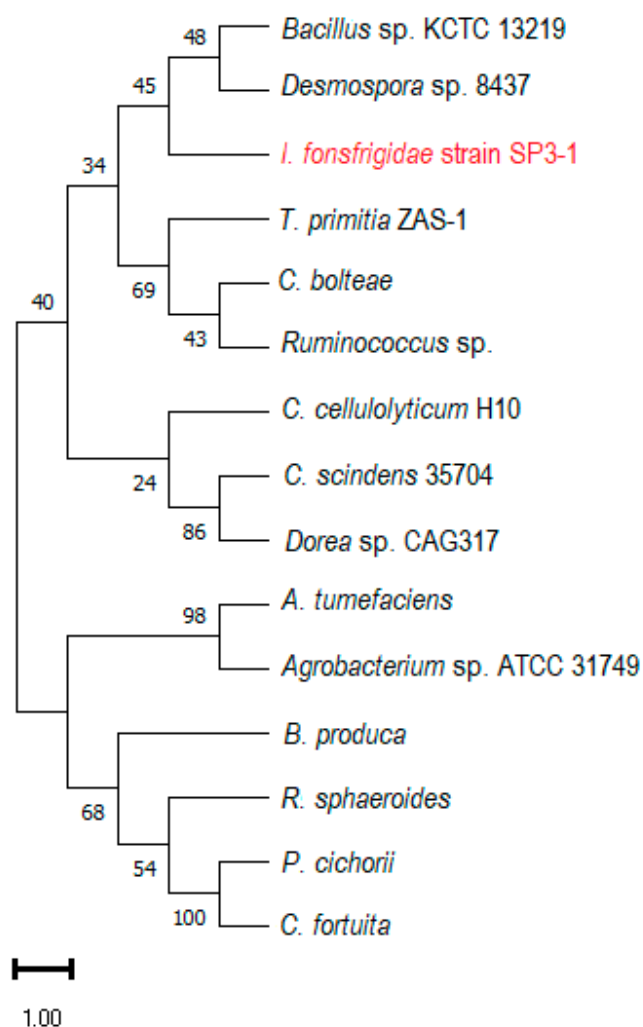
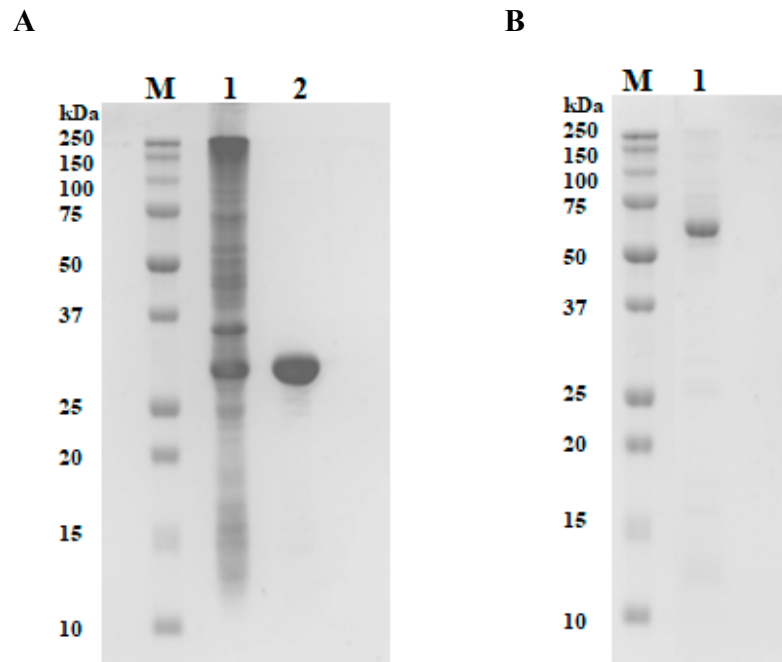


## Supplementary Materials



**Figure S1.** Phylogenetic tree analysis of IfDPEase with other characterized related members was constructed using protein sequences in MEGA 11 Program [26] by following Neighbor-Joining method with Jones-Taylor-Thornton (JTT) model + G (1000 Bootstrap). The numbers at the branching points indicated the bootstrap values. The scale for branch length is shown below the tree. Red letter is DPEase from *I. fonsfrigidiae* strain SP3-1 that has high relationship with DPEases from *Bacillus* sp. KCTC 13219, *Desmospora* sp. 8437, *Treponema primitia* ZAS-1, *Clostridium bolteae* 35704, *Ruminococcus* sp., *Clostridium cellulolyticum* H10, *Clostridium scindens* 35704, *Dorea* sp. CAG317, *Agrobacterium tumefaciens*, *Agrobacterium* sp. ATCC 31749, *Blautia produca*; DTEases from *Pseudomonas cichorii* and *Caballeronia fortuita*; and DFEase from *Rhodobacter sphaeroides*.



**Figure S2.** (A) SDS-PAGE of crude enzyme (lane 1) and purified IfDPEase (lane 2). (B) Native-PAGE of the purified IfDPEase (lane 1). Lane M is standard marker proteins.

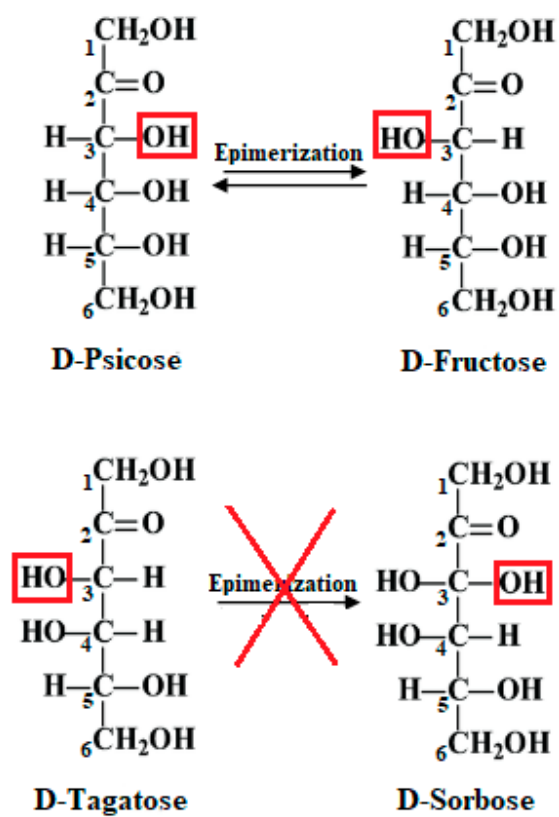
A



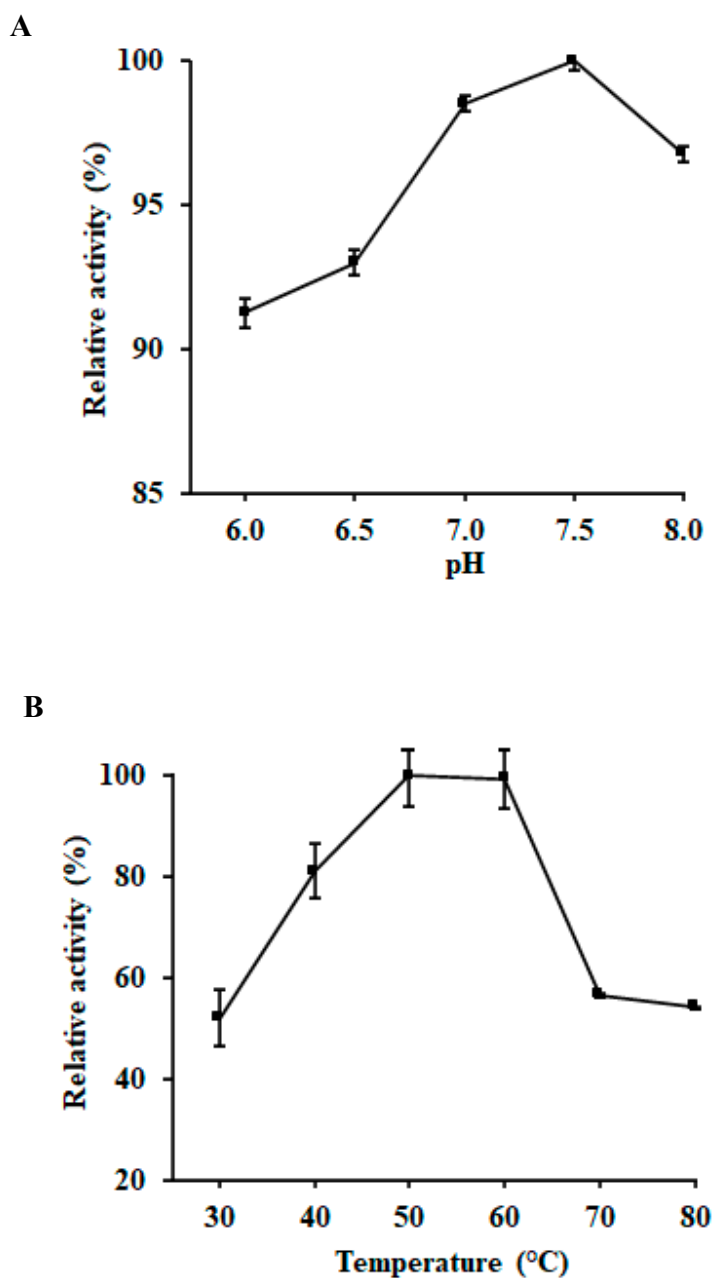
B

Name of sequence	IfdPEase
Length of Sequence	269
Preicted On	Fri Oct 14 08:52:32 2022
NON ALLERGEN	
Prediction by mapping of IgE epitope	
The protein sequence does not contain experimentally proven IgE epitope	
Name of sequence	IfdPEase
Length of Sequence	269
Preicted On	Fri Oct 14 08:55:41 2022
Blast RESULT	
BLAST Results of ARPS : No Hits found	
NON ALLERGEN	

**Figure S3.** The protein allergen for food safety was performed. (A) Using the AllerTOP v.2.0 database by searching for full IfdPEase sequence compare with database (<https://www.ddg-pharmfac.net/AllerTOP/>). (B) Using the Algpred (<http://crdd.osdd.net/raghava/algpred/>) database by comparing sequence in UniProtKB and mapping of IgE epitope. Based on these results, IfdPEase was found to be non-allergenic.

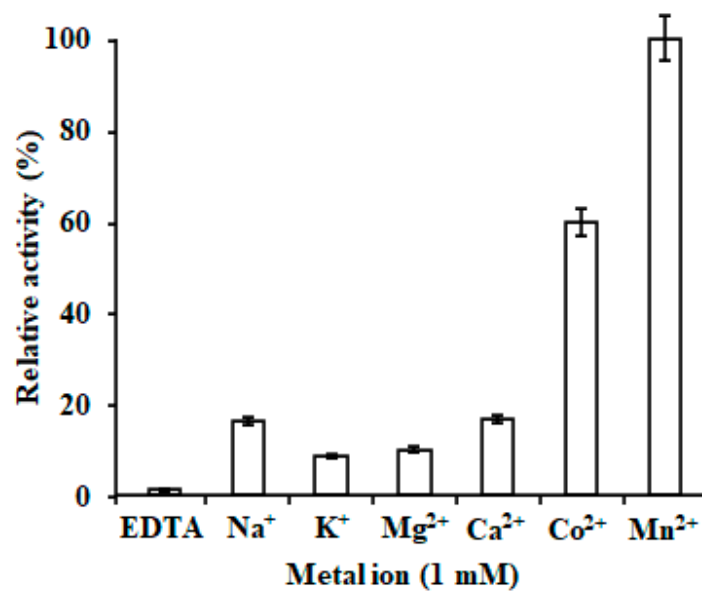


**Figure S4.** Reaction scheme for the catalysis of keto-sugars by IfDPEase



**Figure S5.** (A) Effect of pH on IfDPEase activity was determined by incubating 50  $\mu$ M of purified enzyme with D-fructose (10 mg/mL) containing 1 mM of  $Mn^{2+}$  for 5 min in buffers with a pH range of 6.0-8.0 (sodium phosphate buffer, pH 6.0-7.0; Tris-HCl buffer, pH 7.0-8.0). The reaction was done at 50  $^{\circ}$ C for 5 min. The maximum pH activity of IfDPEase at pH 7.5 was considered as 100% relative activity. (B) Effect of temperature on IfDPEase activity was determined under the conditions described above. The reaction was done with a temperature range of 30 to 80  $^{\circ}$ C, pH

7.5 for 5 min. The maximum temperature activity of IfDPEase at 50 °C was considered as 100% relative activity. Values are mean of three replications  $\pm$  standard deviation.



**Figure S6.** Effect of metal ions on IfDPEase activity. The reactions were done with D-fructose (10 mg/mL) and purified enzyme (5  $\mu$ M) at 50 °C, pH 7.5 for 5 min with different metal ions. Metal ions used are Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Co<sup>2+</sup> and Mn<sup>2+</sup>. The values are shown as percentages of the optimal activity with Mn<sup>2+</sup>, taken as 100% relative activity. Values are the mean of three replications  $\pm$  standard deviation.