Supporting information

Enhancing prebiotic production by engineering the levanbiose-binding site of *Erwinia tasmaniensis* levansucrase

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Table S1 Oligonucleotide primers for mutagenesis

Mutant	Forward (5’ → 3’)	Reverse (5’→3’)
D82A	ccagatttccaggcggaaaatggg	cccattttccgcctggaaatctgg
E83A	cagatttccaggatgcgaatgggaac	gttcccattcgcatcctggaaatctg
N84A	ccaggatgaagcggggaactacg	cgtagttccccgcttcatcctgg
G85P	caggatgaaaatccgaactacgatattaagc	gcttaatatcgtagttcggattttcatcctg
N86A	ggatgaaaatggggcgtacgatattaagc	gcttaatatcgtacgccccattttcatcc
D88A	gaaaatgggaactacgcgattaagcgtgactg	cagtcacgcttaatcgcgtagttcccattttc
W371A	gatagcgtgccggcggagggggaaaaattcc	ggaatttttccccctccgccggcacgctatc
G373P	gtgccgtgggagccggaaaaattcc	ggaatttttccggctcccacggcac
R377A	ggaaaaattcgcgattggcggtactg	cagtaccgccaatcgcgaatttttcc
G379P	ccgtattccgggtactgaagcaccg	cggtgcttcagtacccggaatacgg
G380P	ccgtattggcccgactgaagcaccg	cggtgcttcagtcgggccaatacgg

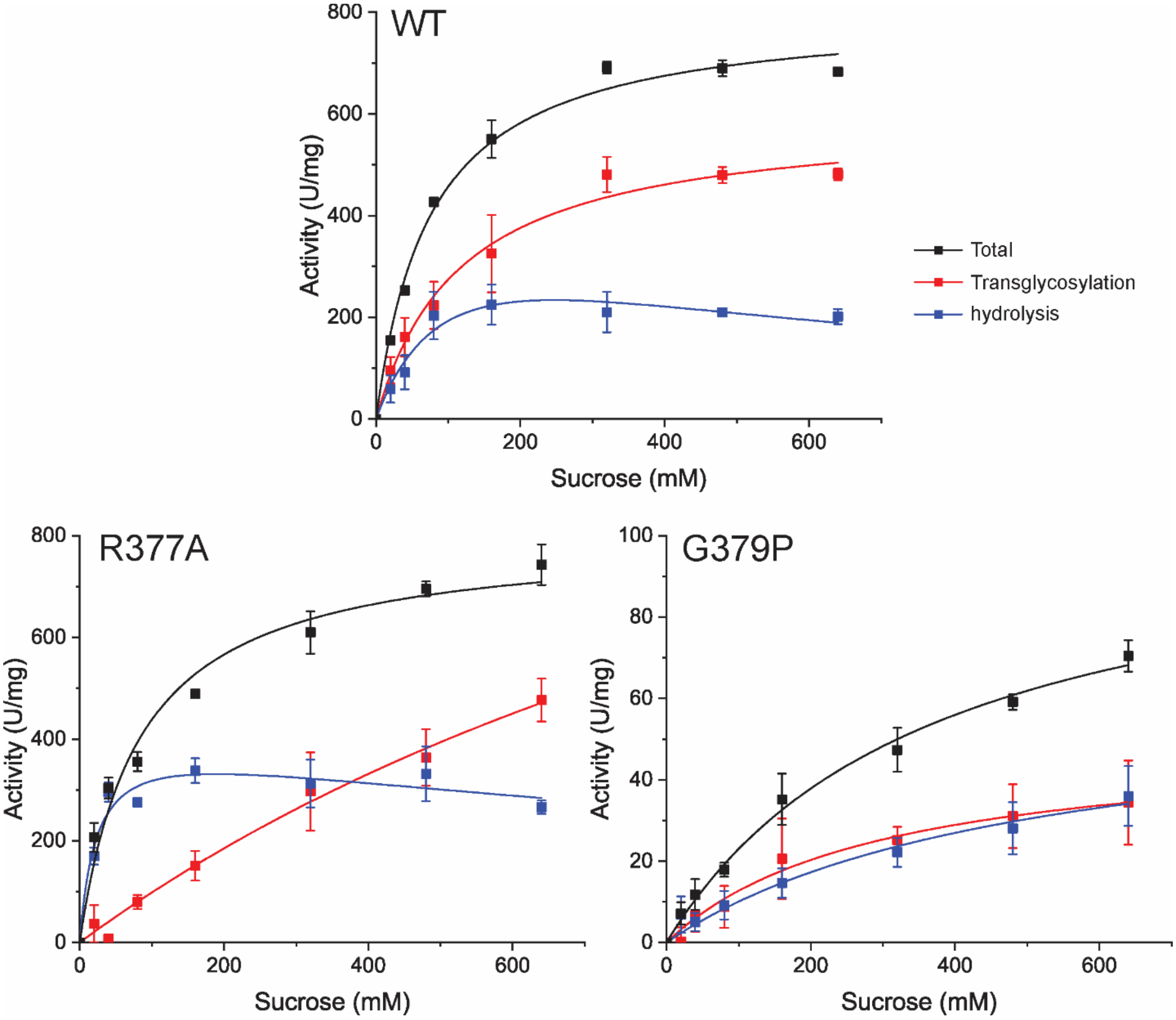

Fig. S1 Plots of total activity (V^{G}), hydrolysis (V^{F}), and transglycosylation ($V^{G\text{-}F}$) vs sucrose content for the wild-type and mutant *Et*Lscs. Kinetic parameters have been determined from the activity profiles utilizing either the Michaelis–Menten or substrate inhibition models, where applicable. Each data point signifies the mean ± standard deviation (S.D.) derived from three replicates.

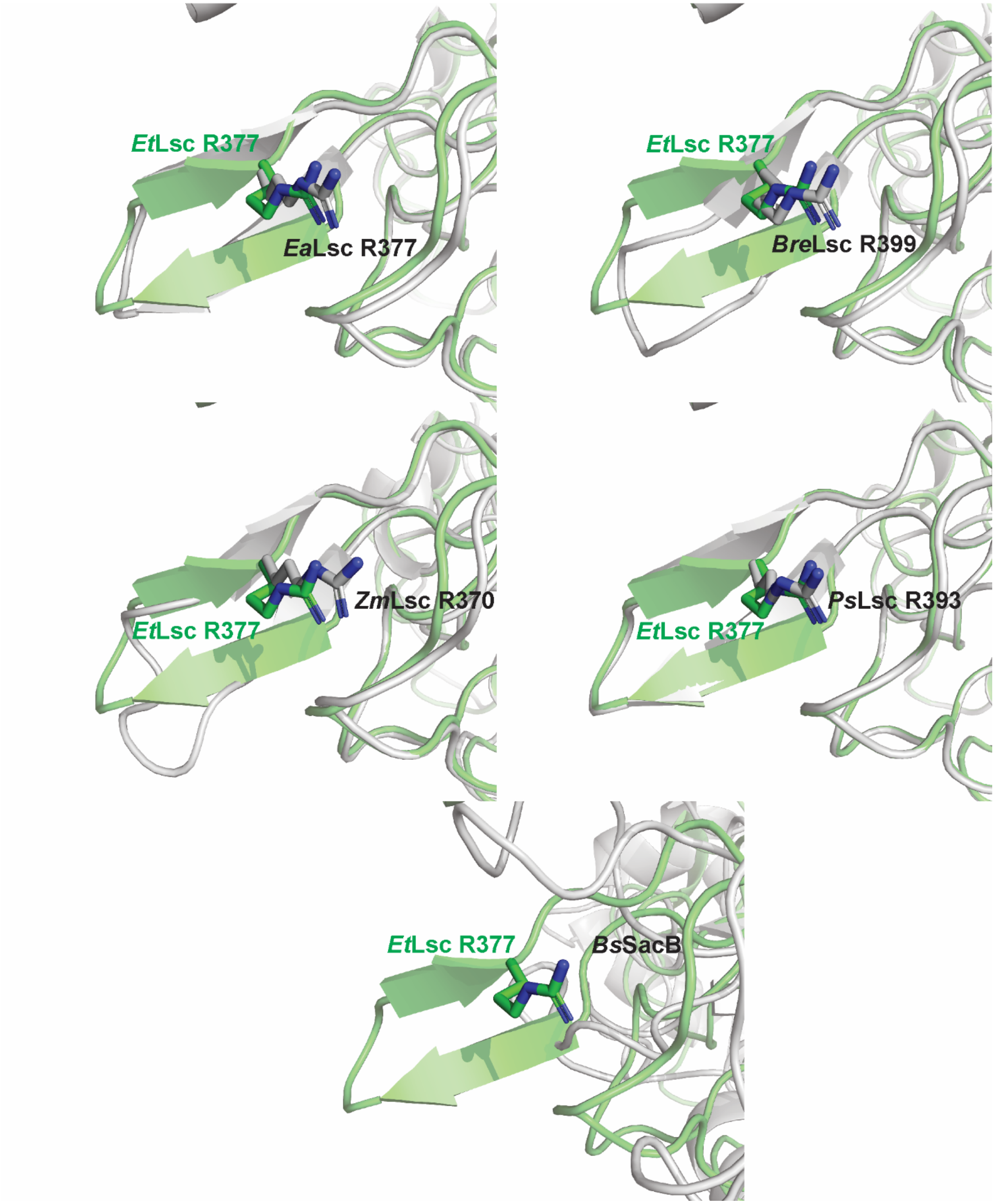

Fig. S2 Structural comparison of the R377 position in *Erwinia tasmaniensis* levansucrase (EtLsc) with corresponding residues in other levansucrases: *Erwinia amylovora* (*Ea*Lsc), *Brenneria sp.* (*Bre*Lsc R399), *Zymomonas mobilis* (*Zm*Lsc), *Pseudomonas syringae* (*Ps*Lsc), and *Bacillus subtilis* (*Bs*SacB).