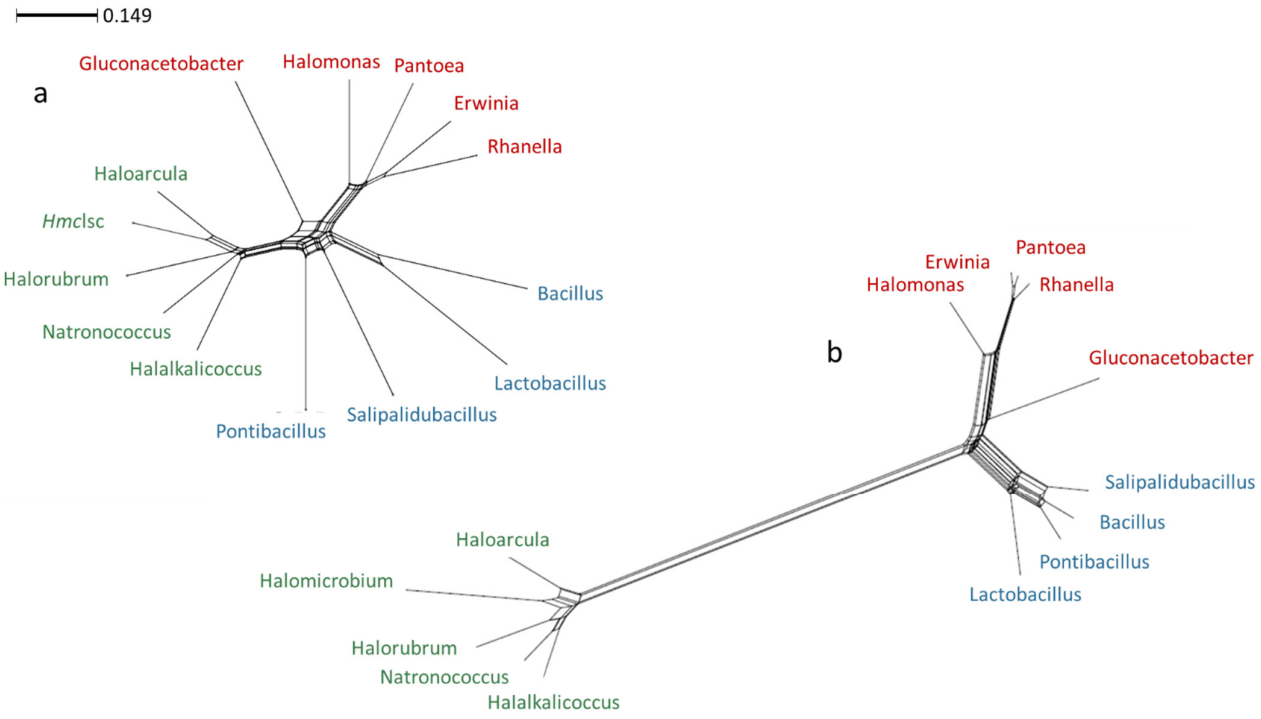

Figure S1. Phylogenetic networks generated in SplitsTree. NeighborNet splits analysis based on selected GH68 sequences (a) from Fig. 1a and 16S sequences (b) from Fig. 1b. See Fig. 1 legend for species details. The scale bar indicates 0.00149 substitutions/site.

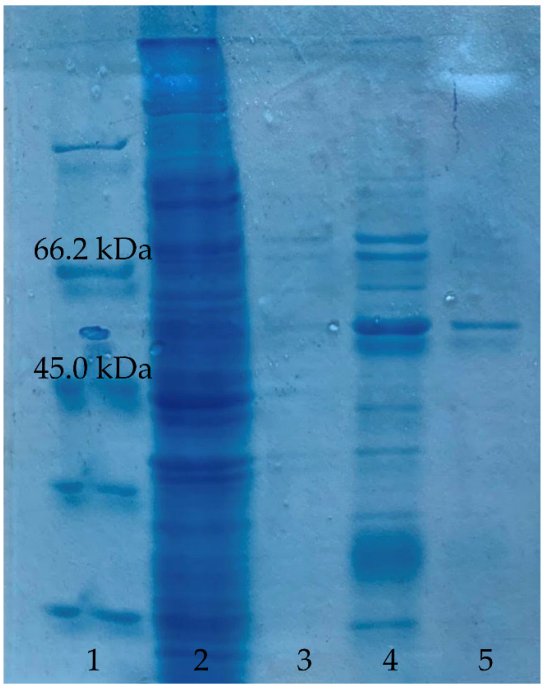

Figure S2. SDS-PAGE analysis of *Hmc*Isc fractions. Lanes: **(1)** reference protein ladder, **(2)** induced crude extract, **(3,4,5)** 1st, 2nd (peak fraction) and 3rd fractions of 1 ml each, containing *Hmc*lsc, collected from the IMAC via one-step non-gradient elution with 300 mM imidazole, respectively.

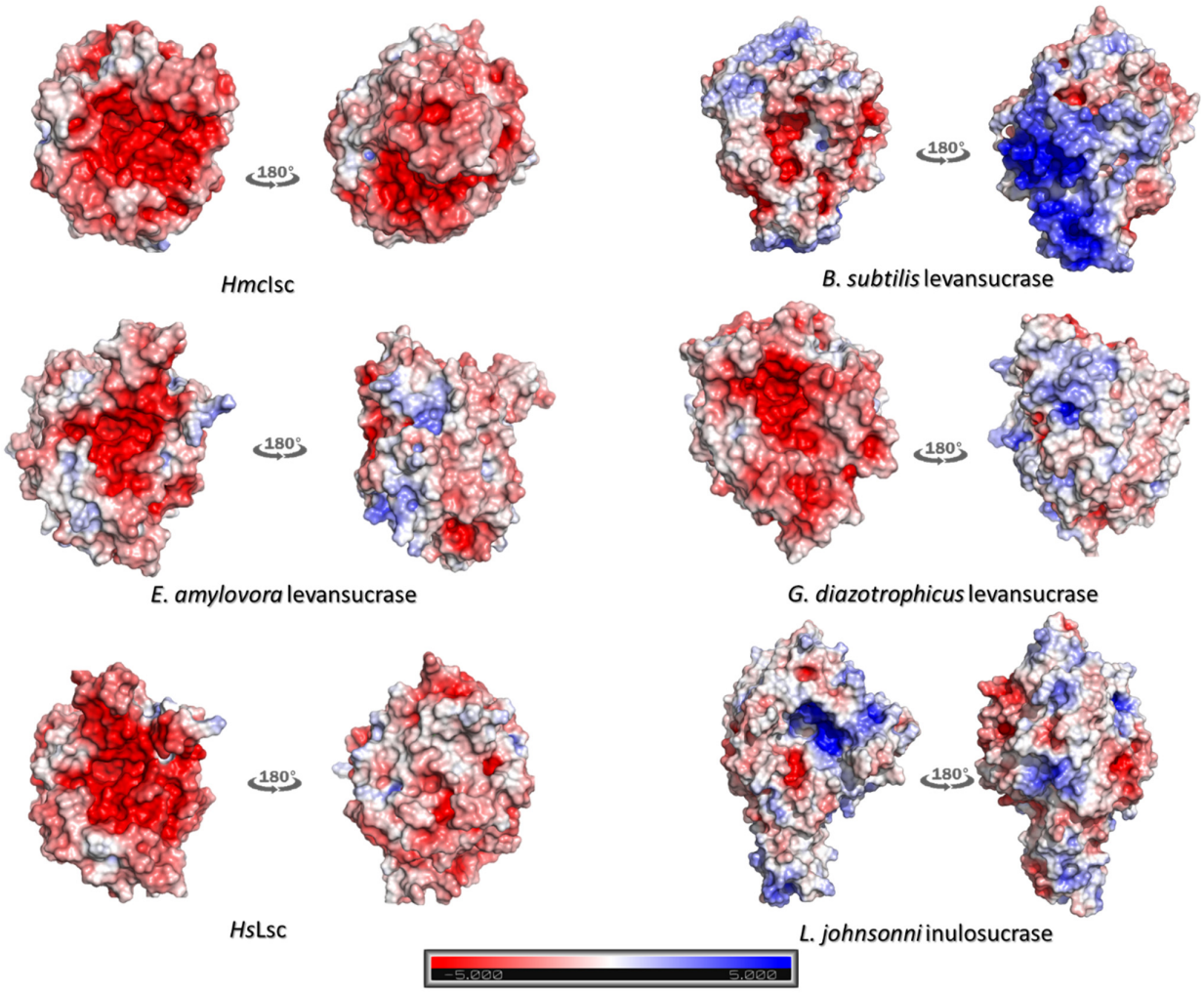

Figure S3. Surface analysis of GH68 sucrases. Surface analysis of the *Hmc*Isc inulosucrase model compared to the 3D structures of levansucrases from *B. subtilis* (1OYG), *E. amylovora* (4D47), *G. diazotrophicus* (*1w18*), a model for *H. smyrnensis* levansucrase (*Hs*Lsc) and the inulosucrase from *L. johnsonii* (2YFR). Positively (blue) and negatively charged regions (red) are indicated at pH 7.0.

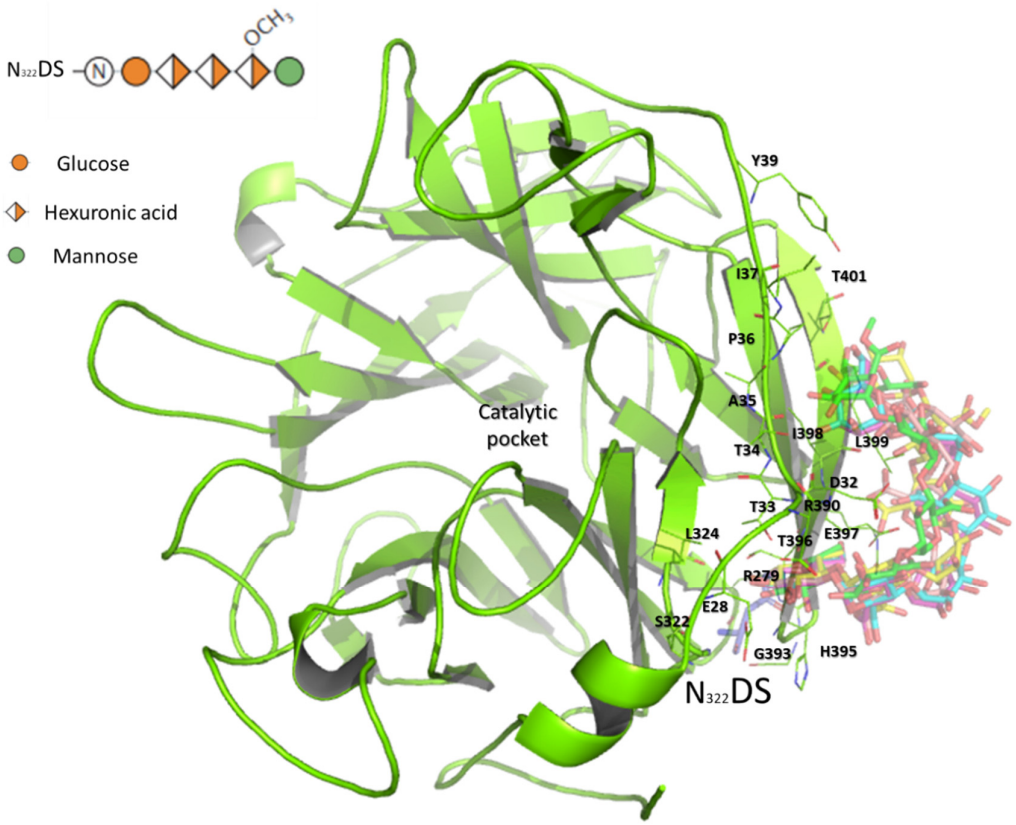

Figure S4. Glycosylation model of *Hmc*Isc. Model of the glycosylation on N322 of *Hmc*Isc showing the top 5 conformations of the glycosylation chain as revealed through covalent docking analysis and interaction with neighboring amino acids.

Table S1. Development of primers for cloning. Primers designed to clone the *Hmc*Isc gene in frame with the N-terminal and C-terminal His tag. Restriction site consensus sequences are bold and underlined.

Primer	Sequence (5′ to 3′)
C-terminal 6xHis-Tag forward primer	GAT**CCATGG**GCATGAGCAAGGACAGTGCTGG (*Nco*I)
C-terminal 6xHis-Tag reverse primer	GAT**CTCGAG**GCGGCGGTACGATCCG (*Xho*I)
N-terminal 6xHis-Tag forward primer	GAT**CATATG**AGCAAGGACAGTGCTGGG (*Nde*I)
N-terminal 6xHis-Tag reverse primer	GAT**CTCGAG**CTAGCGGCGGTACGATC (*Xho*I)
GH68 forward primer	ATGAGCAAGGACAGTGCTGG
GH68 reverse primer	GCGGCGGTACGATCCG

Table S2. Protein purification table for *Hmc*Isc. Comparison of induced and uninduced cell extracts and the purified IMAC peak fraction.

	Total protein (mg)	Volume (ml)	Total activity (mU)	Specific activity (U/g protein)	Purification (fold)
Crude cell extract	50.10	5.5	56.0±1.3	1.1**	1
IMAC (peak fraction*)	0.54	1.0	30.5±1.0	56.5±2.0	51.4
Uninduced cell extract (control)	23.59	5.5	2.3±0.3	0.1**	-

*Peak fraction refers to the protein fraction on lane 4 on SDS-PAGE (Fig. S2).
**Standard error values for specific activities of crude cell extracts are below 0.02.

Table S3. Sodium ion interactions in the *Hmc*Isc model. Interactions of sodium ions at 4 M concentration with amino acid side chains and backbones of *Hmc*Isc protonated at pH 7.0. Each interaction represents one different Na^+ atom interacting with the respective amino acids. The interactions were measured and calculated using MOE. The total energy of all the interactions is -399.62 Kcal/mol.

Interactions with amino acid side chains			
Interaction	**Amino acid**	**Energy Kcal/mol**	**Distance A°**
1	D53	-31.88	2.34
	E272	-13.15	2.33
	Total	**-45.03**	
2	D302	-18.11	2.22
	D291	-15.51	2.35
	P333	-3.48	2.34
	Total	**-37.1**	
3	E186	-28.18	2.37
	N239	-1.53	2.45
	Total	**-29.71**	
4	D127	-27.11	2.32
	E59	-19.09	2.22
	F126	-2.35	2.3
	Total	**-48.55**	
5	E425	-26.19	2.36
	T159	-2.3	2.45
	Total	**-28.49**	
6	D365	-16.66	2.32
	H292	-4.26	2.45
	Total	**-20.92**	
7	D48	-20.2	2.62
	P47	-1.84	2.43
	D44	-1.49	3.61
	Total	**-23.53**	
8	D4	-25.39	2.41
	Q23	-3.3	2.47
	G18	-2.45	2.36
	Total	**-31.14**	
Interactions with amino acid backbones			
Interaction	**Amino acid**	**Energy Kcal/mol**	**Distance A°**
9	D291	-3.42	2.42
	L298	-3.15	2.35

	F294	-1.55	2.37
	Total	**-8.12**	
10	R434	-29.95	2.39
	V109	-3.02	2.43
	V110	-0.87	2.7
	Total	**-33.84**	
11	D360	-19.41	2.23
	E374	-3.49	2.31
	A361	-3.29	2.38
	Total	**-26.19**	
12	E113	-18.73	2.21
	F111	-2.78	2.31
	E112	-1.24	2.35
	Total	**-22.75**	
13	E211	-3.98	2.38
	F204	-3.15	2.34
	H213	-2.52	2.38
	Total	**-9.65**	
14	D413	-32.05	2.31
	L414	-2.55	2.37
	Total	**-34.6**	