

Title	Cold induced expression of a novel levansucrase gene sacB1 enhances exopolysaccharide production and stress resilience in <i>Leuconostoc mesenteroides</i>
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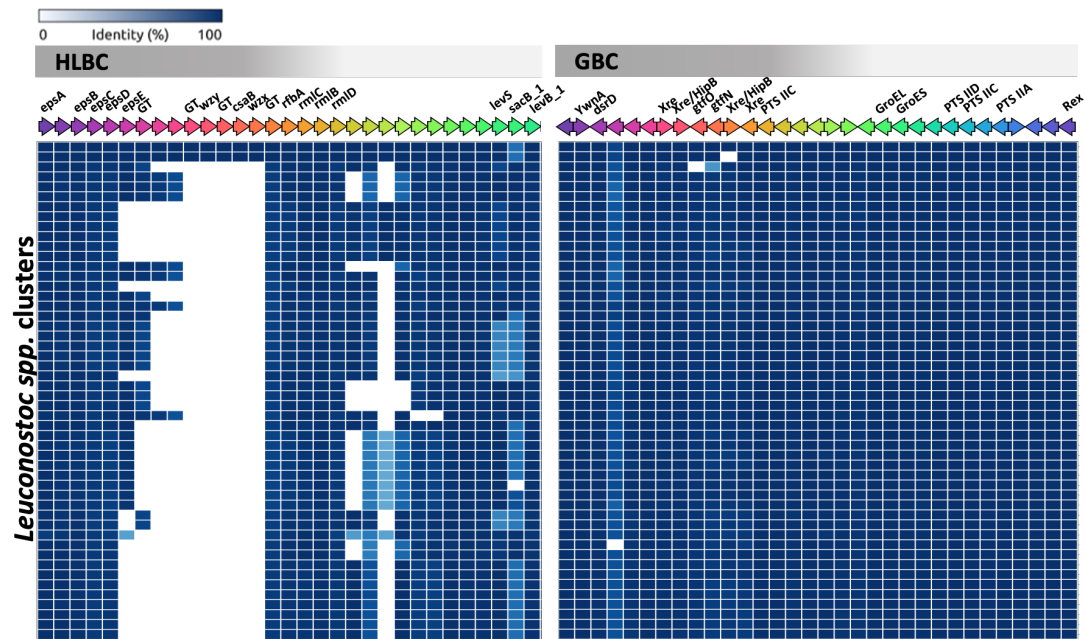
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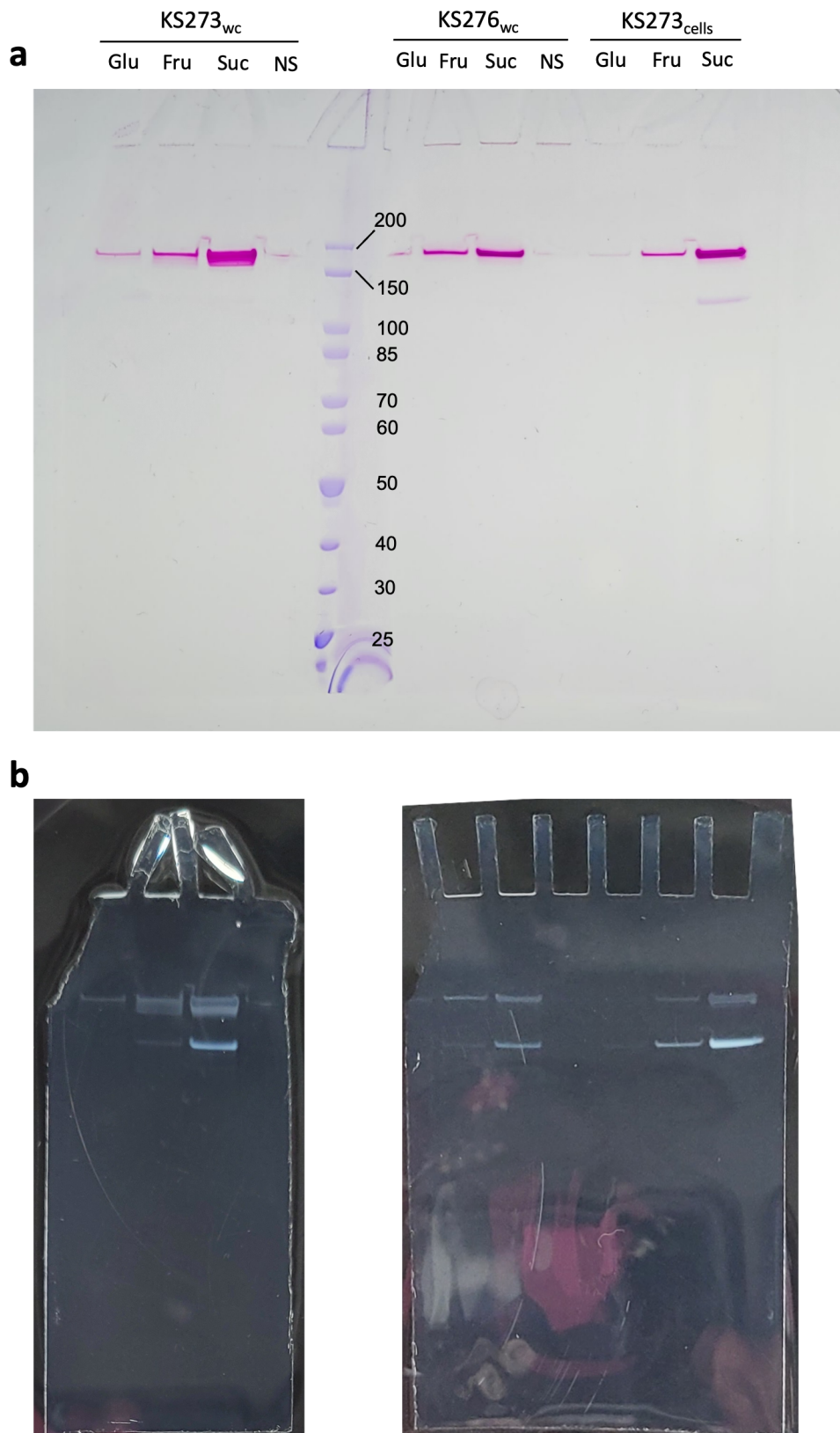
Supplementary information

**Cold induced expression of a novel levansucrase gene *sacB1* enhances
exopolysaccharide production and stress resilience in *Leuconostoc mesenteroides***

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Supplementary Figure S1. Comparative pangenomic analysis of the HLBC and GBC from *Leuc. mesenteroides* KS273 with other *Leuconostoc spp.* strains from NCBI Database. Homologous gene clusters were identified using cblaster tool in CAGECAT platform with a 70% sequence identity threshold.



Supplementary Figure S2. Full length zymograms displaying polymers synthesized in situ by DsrD (173 kDa), LevS (111 kDa) and SacB_1 (115 kDa) revealed by a) Periodic acid-Schiff (PAS) gel staining and b) ethanol polysaccharide precipitation.

Supplementary Table S1. Ropy test analysis on a panel of *Leuc. mesenteroides* strains grown in different carbon sources at 8 °C for 120 h.

Species	Strain	Description	Reference/Sourc e	Ropy Test 8 °C		
				Gl u	Fr u	Su c
<i>Leuc. mesenteroides</i> subsp. <i>dextranicum</i>	KS273	Food borne; Low temp. strong slime former	This study	2+	2+	4+
<i>Leuc. mesenteroides</i> subsp. <i>dextranicum</i>	KS276	Food borne; Low temp. weak slime former	This study	1+	1+	3+
<i>Leuc. mesenteroides</i> subsp. <i>dextranicum</i>	KS277	Food borne; Low temp. weak slime former	This study	1+	1+	3+
<i>Leuc. mesenteroides</i> subsp. <i>dextranicum</i>	KS279	Food borne; Low temp. strong slime former	This study	2+	2+	4+
<i>Leuc. mesenteroides</i>	KS12	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	KS50	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	KS52	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	P1A1	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	CURRENT2 901	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	CURRENT2 902	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	CURRENT2 903	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	KS580-1	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	M120202	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	P2-1714	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	P3-1715	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	P3-1711	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	CC1-0601	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	B103003	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	B103005	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	PB1602	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	PB-A1	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	PC-D1	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	VBEM2	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	VBET2	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	VBPEM1	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	VBPEM2	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	VBPET2	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	VSEM2	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	VSET1	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	VSET2	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	VSET3	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	VSET6	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	VSPET1	Food borne strains	This study	-	-	-

<i>Leuc. mesenteroides</i>	VCEM3	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	VCET2	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	CRMFB(A)	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	1M	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	2M	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	CRMFB(B)	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	1M	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	APC 2294	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	APC 2143	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	APC 2137	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i>	5.4(APC)	Food borne strains	This study	-	-	-
<i>Leuc. mesenteroides</i> subsp. <i>dextranicum</i>	DSM 20240	Type strain; EPS producer	DSMZ culture collection	-	-	-
<i>Leuc. mesenteroides</i> subsp. <i>mesenteroides</i>	DSM 20343	Type strain; EPS producer	DSMZ culture collection	-	-	-

Supplementary Table S2. Primers used in this study

Primer	Sequence	Template	Tann [°C]	Product	Purpose	Amplification size
NcoI-Lev2-for	cggccatggaggagg aaaaATGAGAA	KS273 DNA	61	<i>sacB_1</i>	Cloning into pNZ44	3116
XhoI-Lev2-rev	gccctcgag CTGCTTTAAATCAGG					
NcoI-Prom_Lev2-for	cggccatgg CTGTAATCTATGGCTAC					
XhoI-Lev2-rev	gccctcgag CTGCTTTAAATCAGG	KS273 DNA	61	<i>P_sacB_1</i>	Cloning <i>P_sacB_1</i> into pNZ	3430
XhoI-pNZ-for	gccctcgag TGCATATTTTCGGCAAT					
NcoI-pNZ-rev	cggccatgg TCCCGTCTCTGGATG	pNZ44	61	pNZ vector (promoterless)		2517
pNZ44-for	AG	pNZ44-based vectors	61	Region of MCS	Selection of positive clones	variable
pNZ44-rev	G					

Sequence in bold indicates restriction site. Underline sequence indicates the Shine-Dalgarno sequence.

Supplementary Table S3. Comparative phylogenetic analysis of the *sacB_1* gene occurrence

in other *Leuc. mesenteroides* strains in NCBI database.

Strain	Query Cover	% Ident	Accession	Source
<i>Leuc. mesenteroides</i> Lm10	100%	99.4	CP147495.1	Birch sap
<i>Leuc. mesenteroides</i> SRCM217106	100%	99.27	CP128496.1	Kimchi
<i>Leuc. mesenteroides</i> SRCM103356	100%	99.27	CP035139.1	Kimchi
<i>Leuc. mesenteroides</i> CBA7131	100%	98.88	CP021966.1	Kimchi
<i>Leuc. mesenteroides</i> subsp. <i>mesenteroides</i> ATCC 8293	100%	98.88	CP000414.1	Fermented olives

<i>Leuc. mesenteroides</i> FDAARGOS_1033	100%	98.88	CP065995.1	Clinical
<i>Leuc. mesenteroides</i> PLO3	100%	98.79	CP103383.1	Kimchi
<i>Leuc. mesenteroides</i> SRCM103460	100%	98.79	CP035746.1	Kimchi
<i>Leuc. mesenteroides</i> MSL129	100%	98.79	CP128560.1	Kimchi
<i>Leuc. mesenteroides</i> subsp. <i>mesenteroides</i> CBA3650	100%	98.73	CP159465.1	Kimchi
<i>Leuc. mesenteroides</i> subsp. <i>mesenteroides</i> MSJK0064	100%	98.73	CP136668.1	Winter pickled vegetables
<i>Leuc. mesenteroides</i> WiKim0121	100%	98.37	CP098784.1	Kimchi
<i>Leuc. mesenteroides</i> WiKim32	100%	98.28	CP037752.1	Kimchi
<i>Leuc. mesenteroides</i> subsp. <i>mesenteroides</i> J18	97%	99.25	CP003101.3	Kimchi
<i>Leuc. mesenteroides</i> subsp. <i>mesenteroides</i> DRC0211	97%	99.25	CP013016.1	Kimchi
<i>Leuc. mesenteroides</i> SRCM102735	100%	98.09	CP028255.1	Soybean paste
<i>Leuc. mesenteroides</i> ML2	100%	98.09	CP143438.1	Fermented soybean
<i>Leuc. mesenteroides</i> WiKim33	100%	98.09	CP021491.1	Kimchi
<i>Leuc. mesenteroides</i> SRCM103453	100%	98.09	CP035271.1	Kimchi
