

Table S1. List of primers used in the study

Primer name	Sequence (5' – 3')	Restriction site	Purpose
PRO 6722	AT <u>AGATCT</u> CAAGGCCGATGGTGCGGTCG	<i>Bgl</i> III	To amplify the 5' HR1 of <i>srtA</i> gene
PRO 6723	ATGAGCTCCTGGCCCTCAGACGGGCATC	<i>Ecl</i> II3611	To amplify the 3' HR1 of <i>srtA</i> gene
PRO 6724	AT <u>ATTTAAAT</u> CGCAGGAAGCGTGGAGCCAA	<i>Swa</i> I	To amplify the 5' HR2 of <i>srtA</i> gene
PRO 6725	AT <u>CTCGAGC</u> GCCCAACAAAAGGCGTTC	<i>Xho</i> I	To amplify the 3' HR2 of <i>srtA</i> gene
PRO 7080	GATGCCCGTCTGAGGGCCAG		5' flanking region of <i>srtA</i> gene to check gene replacement
PRO 7081	TTGGCTCCACGCTTCCTGCG		3' flanking region of <i>srtA</i> gene to check gene replacement 3' <i>lab129_01548</i> to check <i>srtA</i> gene organization
cat96F	TCAAATACAGCTTTTAGAACTGG		5' cat cassette pNZ5319 (36)
cat96R	ACCATCAAAAATTGTATAAAGTGGC		3' cat cassette pNZ5319 (36)
EryintF	CGATACCGTTTACGAAATTGG		5' ery cassette pNZ5319 (36)
EryintR	CTTGCTCATAAGTAACGGTAC		3' ery cassette pNZ5319 (36)
PRO 7838	GGAAGGCACGTTAGGAATCATTACC		5' pNZ5319 to check gene replacement
PRO 7839	GAGATCTTCTTCACGTTACTAAAGGG		3' pNZ5319 to check gene replacement
PRO 8177	ATGAGCTCGTTGCATTAGTTGATTGAGATCG	<i>Sac</i> I	To amplify the 5' of <i>srtA</i> gene for complementation
PRO 8150	ATGAGCTCGAAGATATCGATACCCCTAGAATGTAATAAT	<i>Sac</i> I	To amplify the 3' of <i>srtA</i> gene for complementation
pseu 383	CCCCTGACGAGCATCACAA		3' pLAB1301 to check insertion in the MCS
pseu 384	GCGTCAGACCCCGTAGAAAA		5' pLAB1301 to check insertion in the MCS
S&P-00012	AGGCCGAAGAACGCGATA		To amplify the 5' of <i>lab129_01546</i> for qRT-PCR
S&P-00013	ACGTACTGCGCCACTTGTTG		To amplify the 3' of <i>lab129_01546</i> for qRT-PCR
S&P-00014	GCGCTGCCAGCTATAACATG		To amplify the 5' of <i>lab129_01548</i> for qRT-PCR
S&P-00015	TGATGAATGCCAGCAATAATATTTG		To amplify the 3' of <i>lab129_01548</i> for qRT-PCR
S&P-00016	CCGATGCCACGTGCTTTATT		To amplify the 5' of <i>lab129_01549</i> for qRT-PCR

S&P-00017	TTCCTGTCGCAAACGCATT		To amplify the 3' of <i>lab129_01549</i> for qRT-PCR
S&P-00026	CTGGTGCTGCTAAGGCTCTTG		To amplify the 5' of housekeeping gene for qRT-PCR
S&P-00027	GTTGTGCATGGCCTTGTAATTTAC		To amplify the 3' of housekeeping gene for qRT-PCR
S&P-00028	TTGGTCTCGTTAGTCGGTGTTG		To amplify the 5' of <i>lab129_01550</i> for qRT-PCR
S&P-00029	G TTCAGACCCGTGTGAGCATT		To amplify the 3' of <i>lab129_01550</i> for qRT-PCR
S&P-00030	GCCGTTCAAGCGGTCAAT		To amplify the 5' of <i>lab129_01551</i> for qRT-PCR
S&P-00031	GCCACACCCGTTACATTAACATC		To amplify the 3' of <i>lab129_01551</i> for qRT-PCR
S&P-00038	ACGTGATTGGCTCGATTGC		To amplify the 5' of <i>lab129_01547 (srtA)</i> for qRT-PCR
S&P-00039	GCTGACACCATTACCGATTGG		To amplify the 3' of <i>lab129_01547 (srtA)</i> for qRT-PCR
PRO 7809	ATGAGCTCGGCTAAGACGGTTAAGCAG		5' <i>lab129_01547</i> to check <i>srtA</i> gene organization by PCR on cDNA of CMPG5300
PRO 8917	ATCTCGAGCGCATTGGGGCTCTCATCATC		3' <i>lab129_01549</i> to check <i>srtA</i> gene organization by PCR on cDNA of CMPG5300
S&P-00042	GCGCTTGATACCATGCACAAAGTC		3' <i>lab129_01550</i> to check <i>srtA</i> gene organization by PCR on cDNA of CMPG5300
S&P-00043	GGCATTGACCGCTTGAACGGCCTTAGC		3' <i>lab129_01551</i> to check <i>srtA</i> gene organization by PCR on cDNA of CMPG5300

Table S2. Protocols used for optimization of electroporation.

S. No	Buffer set used for washing	Culture conditions	Voltage	Electroporation efficiency (number of transformants/ μ g DNA)
1	Set I	Anaerobic	200 V	3
2		Anaerobic	400 V	0
3		Aerobic w/o shaking	200 V	1
4		Aerobic w/o shaking	400 V	0
5		Aerobic with shaking	200 V	4
6		Aerobic with shaking	400 V	0
7	Set II	Anaerobic	200 V	4
8		Anaerobic	400 V	16
9		Aerobic w/o shaking	200 V	24
10		Aerobic w/o shaking	400 V	48
11		Aerobic with shaking	200 V	21
12		Aerobic with shaking	400 V	13

Table S3. Putative sortase-dependent proteins (SDPs) of *L. plantarum* CMPG5300

Protein ID	product	AA	LPXTG motif	signal peptide [#]
lab129_00260	cell surface protein	2489	LPQTGE	MNRFITSKQHYKMYKKGRFWVFAGITVATFTLNPLISRA [#]
lab129_00360	cell surface protein	834	LPQTDE	MNKLLYTSITTAALFVGTQLGVNNAQA
lab129_00362	cell surface protein, SEC10/PgrA surface exclusion domain	903	LPQTNE	MKKSRIALMSTALVAAGATAIGTATTAQA
lab129_00533	cell surface protein, alpha/beta hydrolase family	901	LPQTDE	MKRNSQQSTTVVDHYKMFKDGKHWVYAGITIAGLGLSTLMLTTNALA [#]
lab129_00830	mucus-binding protein, LPXTG-motif cell wall anchor	845	LPQTDE	MSKDNQKMTGDSVYRVKMYKDGKRWVYAGATTLALAAGLVFANVNASA [#]
lab129_00902	cell surface adherence protein, collagen-binding domain	727	LPQTSE	MRRKLVGYMLSMMLTVILALFMLGSTAHA
lab129_00908	cell surface adherence protein, collagen-binding domain	506	LPQTNE	MRKKWRWLLALTGIFFLMFGPPLVSQA
lab129_01009	cell surface protein precursor, CscD family ^s	123	LPQTNE	MAKFRRLVLLSLSLGLALAGGCRSPDALA
lab129_01040	cell surface adherence protein, collagen-binding domain	990	LPQTSE	MRLIDFKTWIMGTAAMLTLIVTNQTVSA
lab129_01118	adherence-associated mucus-binding protein	1345	LPQTDE	MRYTRGKWRVTNPKVWLFSSVLILGWRIVPTVAQA
lab129_01174	cell surface protein precursor	463	LPQTGE	no (pseudogene)
lab129_01196	cell surface protein precursor, CscD family ^s	125	LPQTGV	MMRWLLIRGLLGLLIGLPSIGGAAQTVGTSSTQIQFYTSRSA
lab129_01448	cell surface protein precursor	1231	LPKTNS	MYTENTGKHHRNLPLVWLLPLLVVISFWGVSQNIMVVDA
lab129_01662	cell surface protein precursor	834	LPQTDE	MMERKRTNFKMYKIGRRWAFACAVILTMGTTTLVARA [#]
lab129_01674	cell surface protein precursor	419	LPQTGE	MSKALKIVMGITMLTGGIMAQKMTVHA
lab129_01688	cell surface protein precursor	617	LPQTNE	MKLSKRGLFWLLGLVSFTILLFFNQLPGAQA
lab129_01986	cell surface protein precursor, CscD family ^s	113	LPQTDE	MVQRFSVCVATILISLIMLTITGVA
lab129_02128	mucus-binding protein	1192	LPQTDE	MKPNNVNNQNKRHQSRWVITSATAMILTTLIASQAAAA

lab129_02262	cell surface protein precursor, CscD family [§]	123	LPQTSE	MKRLGFTIAISTLLCWGWSAPIIGVA
lab129_02270	cell surface protein precursor, CscD family [§]	141	LPQTSE	MPNKWWRLILGVMLVLSWAIPVRA
lab129_02388	cell surface protein precursor, CscD family [§]	92	LPQMNG	no
lab129_02464	mucus-binding protein precursor	2219	LPQTND	MRNRLNRLGLESKSHYKLYKSGRRWVAASITVFSVGIGLTFSQV [#]
lab129_02519	cell surface protein precursor	1005	LPQTSE	MRLIVRSVRLFLKKWGITINYRESEVKCYKMYKSGKMWLFASASLLLLNTQLLTAHA [#]
lab129_02520	cell surface protein precursor	1039	LPQTGE	MEQVKKRYKMYKSGKVWLFAGITLVTLNMNVVTGRA [#]
lab129_02713	mucus-binding protein	1179	LPQTSE	MNKRKIITNPPKWHLITGIAATILAGILTNQDAFA
lab129_02725	mucus-binding protein	2026	LPQTGE	MQRRRLQRAQLTEKRTYKMYKKGRLWLIAGLSTFTLGASLLPMTGRA [#]
lab129_02822	mannose-specific adhesin	1204	LPQTDE	MLRNNYFGETKTHYKLYKCGKNWAVMGISLFSGLGMLVTSRPSVA [#]
lab129_03163	cell-wall proteinase, peptidase S8 family	1482	LPATGD	MRRNSMTEMKRHYKLYKSGSKWKAIAITVSAGAIVLSGNVTQSVAA [#]
lab129_03234	collagen-binding protein, C-terminus	230	LPQTGD	no
lab129_03333	mucus-binding protein, C-terminus	301	LPQTDE	no

[#] Some SDPs have a special predicted KxYKxGKxW signal peptide (TIGR03715). This model describes a novel form of signal peptide that occurs as an N-terminal domain with a recognizable motif, reminiscent of the YSIRK and PEP-CTERM forms of signal peptide. This domain tends to occur on long, low-complexity (usually Serine-rich and heavily glycosylated) proteins of the *Firmicutes*. As with YSIRK, the majority of these proteins have the LPxTG cell wall-anchoring motif at the C-terminus (Bensing *et al.*, 2007).

[§]CscD are often encoded by gene clusters involved in the biosynthesis of putative cell-surface protein complexes for carbohydrate utilization, which are conserved in specific Gram-positive bacteria. CscD proteins form a very unusual group among the LPxTG-proteins, since they are extremely short (90–140 residues) and have only 40–60 residues between the signal peptide (which is removed by signal peptidase I) and the LPxTG-anchoring motif (which is cleaved by sortase) (Siezen *et al.*, 2006).

References

Bensing BA, Siboo IR, Sullam PM. 2007. Glycine residues in the hydrophobic core of the GspB signal sequence route export toward the accessory Sec pathway. *J. Bacteriol.* **189**:3846-3854

Siezen RJ, Boekhorst J, Muscariello L, Molenaar D, Renckens B, Kleerebezem M. 2006. *Lactobacillus plantarum* gene clusters encoding putative cell-surface protein complexes for carbohydrate utilization are conserved in specific gram-positive bacteria. *BMC Genomics.* **7**:126.