

	Description	Scientific Name	Max. Score	Mean Score	Query Cover	Value	Pct Ident	Acc. Len	Accession
✓	brothodent ardens (Lutarna monodentipes)	Lutarna monodentipes	352	352	100	16.122	100.00%	177	WP_045645464.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	339	339	100%	20.117	106.61%	177	WP_138324938.1
✓	23k_brothodent ardens (Lutarna monodentipes)	Lutarna monodentipes	298	298	100%	74.86	99.24%	132	WP_045645464.1
✓	brothodent ardens (Lutarna costaeensis)	Lutarna costaeensis	162	162	100%	14.77	59.64%	108	WP_070922433.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	111	112	100%	36.28	41.27%	157	WP_138324938.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	111	111	100%	36.28	54.50%	157	WP_138324938.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	111	111	100%	46.27	54.50%	149	WP_181272769.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	111	111	100%	46.27	54.50%	149	WP_181272769.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	111	111	100%	46.27	54.50%	151	MG01770062.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	110	110	100%	26.27	54.50%	145	WP_181272769.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	110	110	100%	26.27	54.50%	141	WP_181272769.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	110	110	100%	26.27	54.50%	157	WP_181272769.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	110	110	100%	26.27	54.50%	157	WP_181272769.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	108	108	100%	26.27	54.50%	170	MG01311143.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	108	108	100%	26.26	54.50%	157	WP_181272769.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	92	92	100%	37.07	41.17%	157	WP_181272769.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	91	91	100%	36.27	50.20%	133	WP_030000000.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	91	91	100%	36.27	50.20%	133	WP_030000000.1
✓	MAL730792C.2 brothodent ardens (undetermined)	undetermined Lutarna	90	90	83%	36.19	43.57%	156	WP_030000000.1
✓	brothodent ardens (Euboscopus albertensis)	Euboscopus albertensis	72	72	100%	39.12	32.73%	149	WP_112812511.1
✓	brothodent ardens (Lutarna seelae)	Lutarna seelae	71	71	100%	36.12	32.73%	161	WP_030000000.1

**Distribution of the top 90 Blast Hits on 90 subject sequences**

The chart displays the coverage (Cov) of the top 90 Blast Hits across 90 subject sequences. The x-axis represents coverage from 0 to 150. The y-axis lists the subject sequences. The bars are colored in alternating green and pink. The top 10 sequences have the highest coverage, with the first sequence reaching approximately 140.

Sequence

SignalP-4.1 prediction (gram+networis): Sequence

Score

Position

C-score  
S-score  
Y-score

MKSDIKKRYILVIGALNMDSEFVDTVMNDSVGGSSNLPDQGMNDDPFLVDITLDESQNVIN

#	Measure	Position	Value	Cutoff	signal peptide?
1	max. C	33	0.650		
2	max. Y	33	0.601		
3	max. S	33	0.678		
4	mean D	1-32	0.575		
5	D	1-32	0.591	0.450	YES

Name=Sequence SP=YES! Cleavage site between pos. 32 and 33: YMA-DG D=0.591 C=cutoff=0.450 Networks=SignalP-4.1

```

# WEBSSEQUENCE Length: 137
# WEBSSEQUENCE Number of predicted TMMs: 1
# WEBSSEQUENCE Exp number of Aba in TMMs: 25,5466
# WEBSSEQUENCE Exp number, first 60 Aba: 20,90174
# WEBSSEQUENCE Total prob of B-in: 0.8843
# WEBSSEQUENCE POSSIBLE B-term signal sequence
# WEBSSEQUENCE TMMHMM2: inside 8
# WEBSSEQUENCE TMMHMM2: TMMHMM 9 31
# WEBSSEQUENCE TMMHMM2: outside 32 177

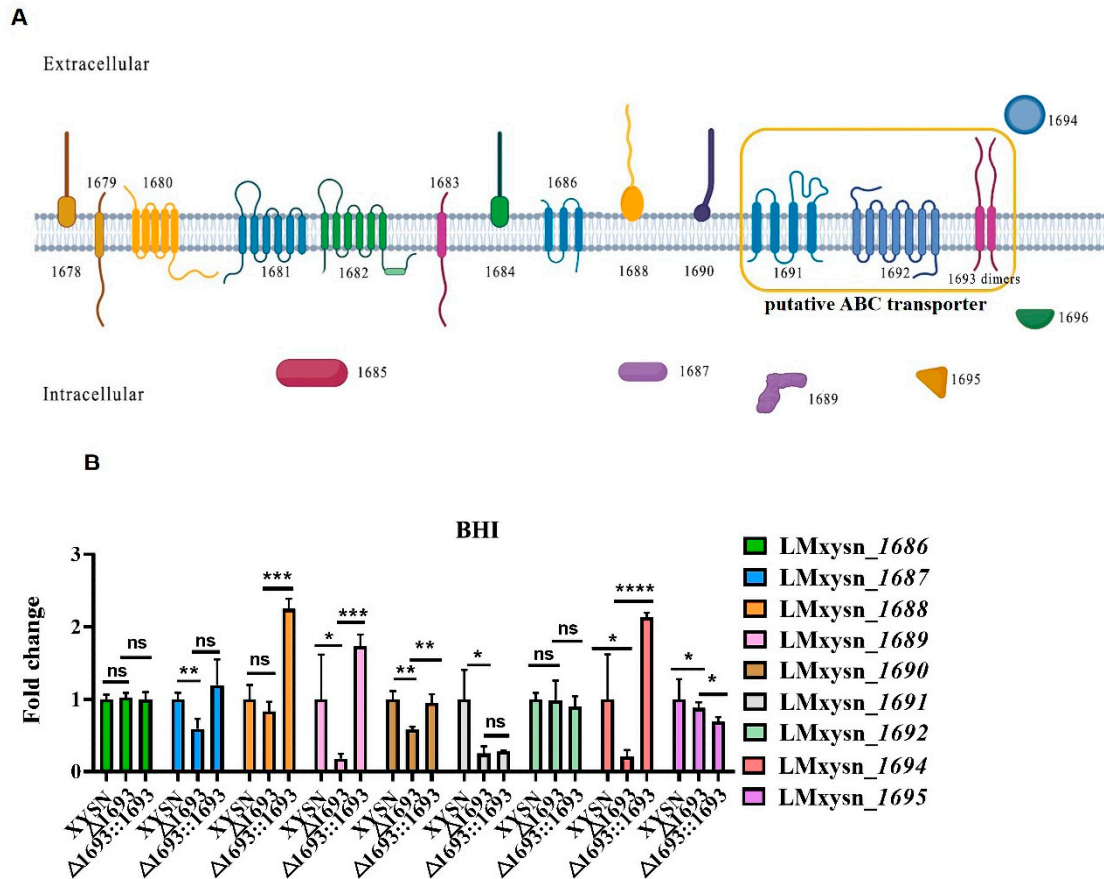
```

TMMHMM posterior probabilities for WEBSSEQUENCE

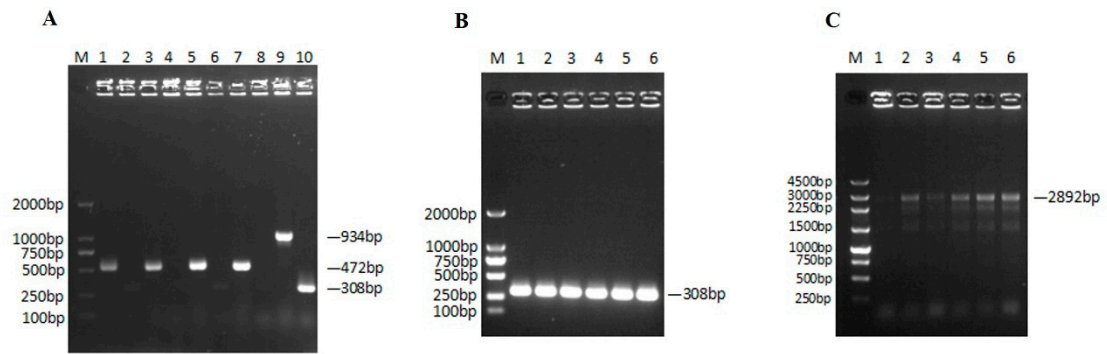
probability

transmembrane — inside — outside

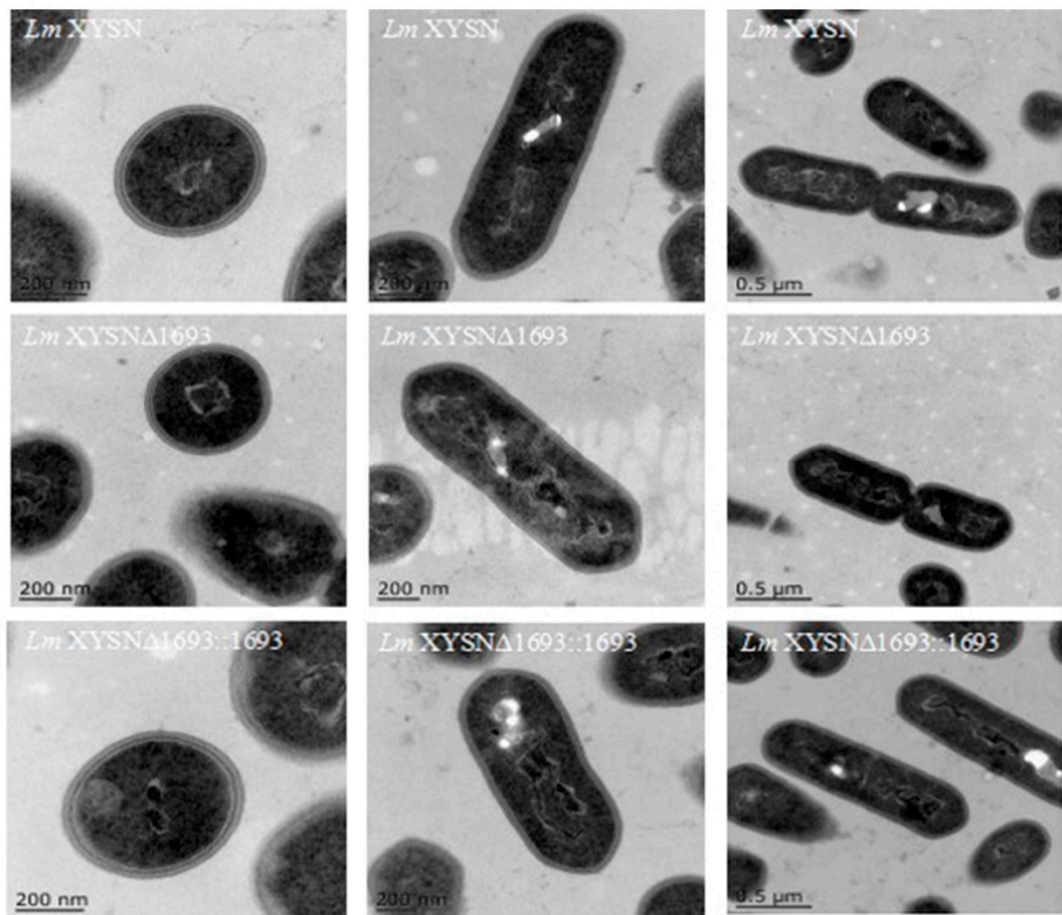
**Supplementary Figure S1. Bioinformatics prediction of LMxysn\_1693 deletion and reversion.** Homology analysis of the protein encoded by the LMxysn\_1693 using the Blast website (A and B). The signal peptide and membrane expansion structure of the protein encoded by the LMxysn\_1693 gene were analyzed using the SingnalP-6.0 website (C and D). The protein encoded by the LMxysn\_1693 gene is predicted as a hypothetical protein with a signal peptide at the N-terminus and transmembrane once.



**Supplementary Figure S2. Bioinformatics prediction and detecting expression levels of GI-7.** The cellular localization and functional predication of the proteins encoded by 20 genes distribution in the GI-7 (A). The expression levels of genes in the GI-7 were detected with LMxysn\_1693 deletion strain by qRT-PCR (B) Error bars represent SD, n = 3 independent experiments. Statistical analysis was performed using Student's t-test: ns, none significance, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .



**Supplementary Figure S3. The construction of LMxysn\_1693 deletion and recovery strains.** PCR identification of *XYSN $\Delta$ 1693* using primers outside the homology arms of the LMxysn\_1693 gene and inside primers inside the gene (A). Lane M is Marker DL2000, lanes 1, 3, 5, and 7 are PCR amplification results of outer primers, and the band size is about 472 bp; lanes 2, 4, 6, and 8 are PCR amplification results of inner primers, and no band is amplified. Obtained, lane 9 is the identification result of *XYSN* using the outer primer, and lane 10 is the identification result of *XYSN* using the inner primer. PCR identification of *XYSNXYSN $\Delta$ 1693::1693* using primers outside the homology arms of the LMxysn\_1693 gene and inside primers inside the gene (B and C). Lane M is Marker DL2000 and Marker DL5000 respectively, the size of the amplified band of inner primers is 308bp (B), and the size of the amplified band of outer primers is 2892bp (C).



**Supplementary Figure S4. Transmission electron microscopic images depicting intact cell wall structures of XYSN, XYSN $\Delta$ 1693 and XYSN $\Delta$ 1095::1095.**

**Supplementary Table S1. Primers for construction of mutant and complement strains used in this study**

**Primers for construction of mutant and complement strains**

<b>Primer Code</b>	<b>Primers Sequences (5'-3')</b>
<b>Primer Code</b>	<b>Primers Sequences (5'-3')</b>
LMxysn_1693 P1	TTGTAAAACGACGGCCAGTGAATTCATCACTTTGGATTCTGCCTTT
LMxysn_1693 P2	AAGAGGGAGACAACGATGAAGAAAGAGTATGTTGGAACCTACACCTAT
LMxysn_1693 P3	ATAGGTGTAGGTTCCAACATACTCTTTCTTCATCGTTGTCTCCCTCTT
LMxysn_1693 P4	TGCATGCCTGCAGGTCGACTCTAGATTATGCTTCTAAACCGTCTGC
LMxysn_1693 N1	CACCTTTTACGATTACTTTTCACAC
LMxysn_1693 N2	GGTGGGTATGACCTTTCCTTATGG
LMxysn_1693 W1	TTACGGGATTTGTTGTATATCGAAG
LMxysn_1693 W2	ATAATAACTAACCCACTAACCACCT
LMxysn_1693H P1	TGTAAAACGACGGCCAGTGAATTCTGTGTTTTTCGTTATCAATGTGG
LMxysn_1693H P2	ATGTAGATAGAATTCCAGTATAACAATATCAATTCAAGTGCCTTT
LMxysn_1693H P3	AAACGCACTTGAATTGATATTGTTATACTGGAATTCTATCTACAT
LMxysn_1693H P4	GCATGCCTGCAGGTCGACTCTAGATTGGTCAGATAAAGACGTAGTAGG
LMxysn_1693H N1	CACCTTTTACGATTACTTTTCACAC
LMxysn_1693H N2	GGTGGGTATGACCTTTCCTTATGG
LMxysn_1693H W1	TTCTCTACCGCACTTTTCTTCCCC

**Supplementary Table S2. Primers for quantitative real-time PCR detection used in this study**

**Primers for quantitative real-time PCR detection**

Primer Code	Primers Sequences (5'-3')	Primer Code	Primers Sequences (5'-3')
gyrB P1	AGACGCTATTGATGCCGATGA	gyrB P2	GTATTGCGCGTTGTCTTCGA
bsh P1	TAATGGAGACAGCGGTAAAT	bsh P2	TTATTCCGTGGATTCTTGTT
sigB P1	TCGGATGGAAGTACGATTAC	sigB P2	TCTTCTGTTCTCGCTCATCT
brtA P1	TTATTACGGCGTTCACTTAT	brtA P1	TTATTACGGCGTTCACTTAT
prfA P1	AGCCAACCGATGTTTCTGT	prfA P2	TATTAGCGAGAACGGGACC
ami P1	TGGCACCTCCGCTACTATGA	ami P2	CCGTTGCAGTCCCATCTC
actA P1	TATGCGTGCGATGATGGT	actA P2	ACCTCGCTTGACTGCTCT
hly P1	AAATGCCACTAAATCAAACG	hly P2	CACTGTAAGCCATTTCTGCA
LMxysn_1686 P1	CAGTGGCACATCAATACCCT	LMxysn_1686 P2	TTGGGTTTGTATTAAGTGGT
LMxysn_1687 P1	GTTCGGTTGCTTCATAGGTT	LMxysn_1687 P2	ACCAAACGAAGATTTACCT
LMxysn_1688 P1	TGGCGATGTTTCCTGTATTT	LMxysn_1688 P2	CCAAAAGAAGGAATCGAAAT
LMxysn_1689 P1	CCTTGTCTTAGAATGTTCC	LMxysn_1689 P2	TCACGAACGAAGAACAAAAC
LMxysn_1690 P1	CATCTGGCAAAGCATCTGTA	LMxysn_1690 P2	CATTCTACGAGCGTATTGG
LMxysn_1691 P1	TGTGATTTTACTCCCGATGT	LMxysn_1691 P2	CATTATTTGGTTTTGGAGGA
LMxysn_1692 P1	TCCAAATTGTTATCAGACGC	LMxysn_1692 P2	AGGTAATTTTCTCCCAACAG
LMxysn_1694 P1	AAAGTCCTTCAAATAAACCAT	LMxysn_1694 P2	TCTGAAAGTGTTGAAGGAGGA
LMxysn_1695 P1	ACTTTGTATTGATAAGCATCTCCA	LMxysn_1695 P2	CGTAGTAGGAACGGTAGGTAAAGG

**Supplementary Table S3. Determination the minimum inhibitory concentration of Lm**

Categories	XYSN (µg/mL)	XYSNΔ1693 (µg/mL)	XYSNΔ1693::1693 (µg/mL)	ATCC25923 (µg/mL)
PG	64	64	64	64
CL	32	32	32	32
CHL	1	2	1	1
NVA	1	1	1	2
AMP	1	1	1	1

The effect of LMxysn\_1693 gene deletion on bacterial resistance was detected by the minimum inhibitory concentration experiment using different antibiotics. The antibiotics used in this experiment were penicillin G, vancomycin and ampicillin (acting on the cell wall), polymyxin B (acting on the cell membrane), and

chloramphenicol (acting on the ribosomal 50S subunit).

**Supplementary Table S4. Physiological and biochemical characteristics of Lm**

	AMY	PIPLC	dXYL	ADH1	BGAL	AGLU	APPA	CDEX	AspA	dRAF	O129R
XYSN	+	+	-	-	-	+	-	+	-	-	+
XYSNΔ1693	+	+	-	-	-	+	-	+	-	-	+
XYSNΔ1693::1693	+	+	-	-	-	+	-	+	-	-	+
	BGAR	AMAN	PHOS	LeuA	ProA	BGURr	AGAL	PyrA	BGUR	SAL	SAC
XYSN	-	+	-	-	-	-	-	-	-	+	-
XYSNΔ1693	-	+	-	-	-	-	-	-	-	+	-
XYSNΔ1693::1693	-	+	-	-	-	-	-	-	-	+	-
	AlaA	TyrA	dSOR	URE	POLYB	dGAL	dRIB	ILATk	LAC	dTRE	ADH2s
XYSN	-	+	-	-	+	+	+	-	-	+	-
XYSNΔ1693	-	+	-	-	+	+	+	-	-	+	-
XYSNΔ1693::1693	-	+	-	-	+	+	+	-	-	+	-
	NAG	dMAL	BACI	NOVO	NC6.5	dMAN	dMNE	MBdG	PUL	OPTO	
XYSN	+	+	+	+	+	-	+	+	-	+	
XYSNΔ1693	+	+	+	+	+	-	+	+	-	+	
XYSNΔ1693::1693	+	+	+	+	+	-	+	+	-	+	

Biochemical determination was conducted with wild strains, deletion strains and restored strains, - means negative result, + means positive result, which is consistent with the description in the instructions.