Supplementary material

Screening for new surface anchoring domains for *Lactococcus lactis*

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**Table S1.** Strains, primers, plasmids and synthetic genes used in this study.

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| --- | --- | --- |
| **Strain, primer plasmid, or gene** | **Relevant features or sequence** | **Reference** |
| **Strains** |  |  |
| *E. coli* DH5α | endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR F- Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17(rK- mK+), λ– | Invitrogen |
| *L. lactis* NZ9000 | MG1363 *nisRK* Δ*pepN* | NIZO |
| **Primers** |  |  |
| 2xLysM-F-Eco | 5’- GAATTCTCCGTTCATGATGTGGAAAC-3’ | This work |
| 2xLysM-R-TAA-Xba | 5’- TCTAGATTAATAATTTAAAGTTTGACCAGCATAAATC-3’ | This work |
| 3xLysM-F-Eco | 5’-GAATTCTCTAGTGCGAGCGCTGTTAC-3’ | This work |
| 3xLysM-R-TAA-Xba | 5’- TCTAGATTAAATTTTAATGGTTTGGCCTGG-3’ | This work |
| CW\_1-F-Eco | 5’- GAATTCTCAACGATTAAAGTGGTTAATAAATC-3’ | This work |
| CW\_1-R-Spe | 5’- TTAATAACTAGTCATGACACCCGTTGATGAATC-3’ | This work |
| CW\_1-R-TAA-Xba | 5’- TCTAGATTACATGACACCCGTTGATGAATC-3’ | This work |
| Cpl-7-F-Eco | 5’- GAATTCGGCGGAATCGCTCATGCG-3’ | This work |
| Cpl-7-R-Spe | 5’- TTAATTACTAGTTGTCAGAATGTTATTAACTTCTGTTTG-3’ | This work |
| Cpl-7-R-TAA-Xba | 5’-TCTAGATTATGTCAGAATGTTATTAACTTCTGTTTG-3’ | This work |
| WxL1-F-Eco | 5’-GAATTCGCGACAGTAGGAAATACATCAAG-3’ | This work |
| WxL1-R-TAA-Xba | 5’-TCTAGATTAACTATAAAGATTCCAGTTTAAAGTTGTAG-3’ | This work |
| WxL3-F-Eco | 5’-GAATTCACTGGGGGAGATTATACATC-3’ | This work |
| WxL3-R-TAA-Xba | 5’-TCTAGATTAATTCGTTAAGGACCAAATTAATTGTG-3’ | This work |
| AM7-F-Eco | 5’-AGAATTCAGTTGGGCTCTTCATGGAC-3’ | This work |
| AM7-R-Xba | 5’-TTCTAGATTATGGTTTAGTAGCCGTTACTCC-3’ | This work |
| AM12-F-Eco | 5’-TGAATTCTATTTAGCTCGCATTGGG-3’ | This work |
| AM12-R-Xba | 5’-TTCTAGATTAAAATGGATTTGTATCACTAATTGC-3’ | This work |
| SK1-F-Eco | 5’-TGAATTCTATTTCATCACGGAGCTTAATAAAC-3’ | This work |
| SK1-R-Xba | 5’-TTCTAGATTATTTTTTAGCAATGATAGGCTTATC-3’ | This work |
| 1358-F-Eco | 5’-AGAATTCATGGTTACTGGTAAAGGAC-3’ | This work |
| 1358-R-Xba | 5’-TTCTAGATTAATAACAAAGACCTGTTCGG-3’ | This work |
| Clu1072-F-Eco | 5’-AGAATTCGGAAATAAATCATTGATGACAAATG-3’ | This work |
| Clu813-F-Eco | 5’-AGAATTCGTCCCTCCAAAAGTAGTG-3’ | This work |
| Clu-R-Xba | 5’-TTCTAGATTATTTACGAATCTTACGAACAG-3’ | This work |
| **Plasmids** |  |  |
| pGEM-T Easy | Apr, cloning vector for PCR products | Promega |
| pNZ8148 | pSH71 derivative, P*nisA,* Cmr, nisin-controlled expression | (Kuipers et al., 1993; de Ruyter et al., 1996; Mierau and Kleerebezem, 2005) |
| pSDBA3b | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *acmA3b* | (Skrlec et al., 2017) |
| pSD-2LysM | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *2lysm* | This work |
| pSD-3LysM | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *3lysm* | This work |
| pSD-CW | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *cw* | This work |
| pSD-Cpl | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *cpl* | This work |
| pSD-2CW | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *2cw* | This work |
| pSD-2Cpl | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *2cpl* | This work |
| pSD-WxL1 | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *wxl1* | This work |
| pSD-WxL3 | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *wxl3* | This work |
| pSD-AM7 | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *am7* | This work |
| pSD-AM12 | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *am12* | This work |
| pSD-SK1 | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *sk1* | This work |
| pSD-1358 | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *1358* | This work |
| pSD\_I07 | pNZ8148 containing gene fusion of *sp*Usp45, *darpin* and *acmA3b* | This work |
| pDARP-2LysM | pNZ8148 containing gene fusion of *sp*Usp45, *darpin* and *2lysm* | This work |
| pDARP-3LysM | pNZ8148 containing gene fusion of *sp*Usp45, *darpin* and *3lysm* | This work |
| pDARP-2CW | pNZ8148 containing gene fusion of *sp*Usp45, *darpin* and *2cw* | This work |
| pDARP-WxL3 | pNZ8148 containing gene fusion of *sp*Usp45, *darpin* and *wxl3* | This work |
| pDARP-AM7 | pNZ8148 containing gene fusion of *sp*Usp45, *darpin* and *am7* | This work |
| pDARP-AM12 | pNZ8148 containing gene fusion of *sp*Usp45, *darpin* and *am12* | This work |
| pDARP-1358 | pNZ8148 containing gene fusion of *sp*Usp45, *darpin* and *1358* | This work |
| pSD-sLPXTG | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *slpxtg* | This work |
| pSD-lLPXTG | pNZ8148 containing gene fusion of *sp*Usp45, *b-dom* and *llpxtg* | This work |
| pEva3-cAcmA | pNZ8148 containing gene fusion of *sp*Usp45, *eva3* and *acmA3b* | (Skrlec et al., 2017) |
| pEva3-AM12 | pNZ8148 containing gene fusion of *sp*Usp45, *eva3* and *am12* | This work |
| **Synthetic genes** |  |  |
| *am7* | AGTTGGGCTCTTCATGGACAATCAGTTCAGGCAGTTCAAGACTATTTTATTGCTGGTATCCAACGATACATGGGTGGGGCATCTACTTCAACGGGTAATGATTATACATCTCAAAATACAACATATACGTTGAATAAGGAATTACTTGATATGTATCTTATATTTACAGTTGACACGAAACGTTGGTATATTTCAAATGGCGTTGGTGTTCGTTATGTCCGTACAACTAGAATGCTTGCAAATTATCAAGATAATTTCGGTAAACTTCAATTACCTACAGACAAAATGTATCAAGTTGAATTAGATAAAGAATTTGGAGTAACGGCTACTAAACCATAA | This work |
| *am12* | TATTTAGCTCGCATTGGGATTTCTAAAAATCAATTGGCAAATGACCTTGCGCATGGAGTAGGCGGTACAGTTCCAGCCCCAACATCAAAGCCAGCACCAGCTCCAGCTTCAAAACCTAGTAAATCAGCTCCAAAGGTACAAACAAATGTTGTTTATGGTTTACATCAAAAAGGGGGAGGGTGGTTAGGTGAAATTACGAATTTCAATAATAGTAATAGTTCAGGATTTGCTGGTTTGCCAAGTAATTCACATGATTTGTTATATATGCGTGTTACACACGGAGCTTTAAAATACCGTGTTCATACAATTGAAGATGGTTGGTTGGGTTGGGTTACTAACGGAAATAAAAACGATACCGTAAATGGCTGCGCAGGTATTTCTGGTCATACTATTGATGGGGTACAAGCATATTTTTACACTCCAAGTGGCGAAGTCTATCAACAGGCCCACTATCGTTCACAAACGGCCAAACGTGCTGGATGGTTAGCTCCAGTTGTAGATGATTCTGATTTTGCTGGCATTTTTGGTGAACCATTAGACCGTTTGCAAGTAGCAATTAGTGATACAAATCCATTTTAA | This work |
| *sk1* | TATTTCATCACGGAGCTTAATAAACGCCTTACAGGACAAAACAACACACAAACTAATACTGAGTTGGAAGATGATGATTTAATGAAGTTTACATATACCAACGGAGATAAAACCACATATTATTTTAACGGCGAGAAAGTTATTGCTTTGTCTCATCCAGATCAATTGGCTATTGTGCGTAAAACATATAAAGAGACTACAGGAAAGGATTTGAAGAATTTTGATTGGAAAGGTTCTCCAATTGATATAAGATTCATGCAAGCAAACGGTATTGATAAGCCTATCATTGCTAAAAAATAA | This work |
| *1358* | ATGGTTACTGGTAAAGGACAAGCAGCAGATAAGGCAGCCATTAACACTCGTCCTTATCCAAGTGAATTTTGGGGAGTTGTACGCCCACCTATTACATCAGGAGGGAATATTACTCCTCCTGACCAAAACGGCACTGGAGGGAAACTTACAGCTCAAAGAGGTACATTTAAAGCCAACTCTGCTGTAAATATAAGACGTGCTCCTAATACCAAAACAGGCACAGTGGCTGGAGTACTTAAAGCTGGTCAAACAGTCAACTATGATAATATCATTGACGCAGATGGATGGCGTTGGGTTTCTTGGGTTGGCGCTTCTGGTAATCGAAATTATTCTGCTGTACGACGCTTATCTGATAATTTCCGAACAGGTCTTTGTTATTAA | This work |



**Figure S1.** Clustal W alignment of the conserved ChW sequence (HMM logo from Pfam; upper row) with part of the cAM12 sequence (middle row). \*, conserved residues; colon (:), similar residues; dot (.), residues with weak similarity.

**References**

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