

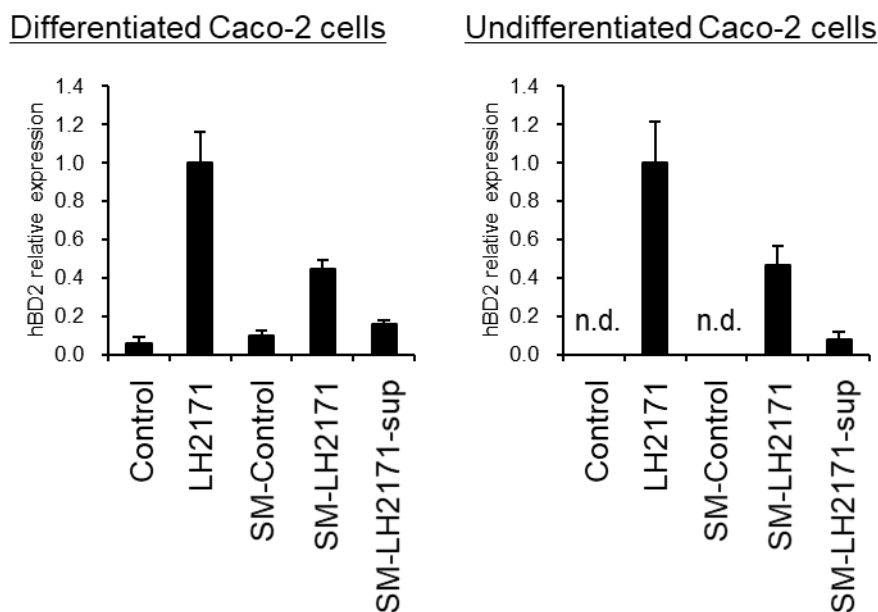
Supplementary Material

S-layer protein of *Lactobacillus helveticus* SBT2171 promotes human β -defensin 2 expression via TLR2–JNK signaling

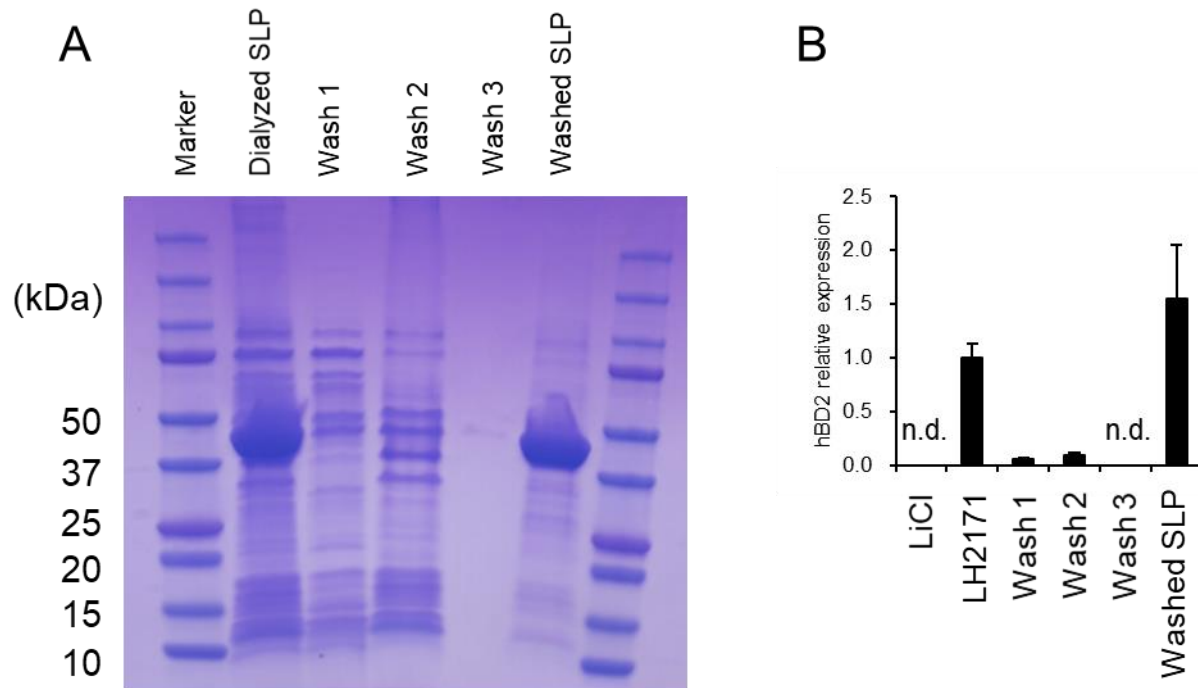
Eiji Kobatake*, Toshihide Kabuki

* Correspondence: Eiji Kobatake: eiji-kobatake@meg-snow.com

Supplementary Figures



Supplementary Figure 1. The results of hBD2 expression assays in Caco-2 cells do not change basically depending on the differentiation state. Differentiated or undifferentiated Caco-2 cells were cultured with samples shown in the following. hBD2 mRNA levels were evaluated by quantitative real time-PCR. Each experiment was performed in triplicate; data are shown as mean \pm SD. Differentiated Caco-2 cells were prepared by the culturing for 21 days. (Control: medium only, LH2171: LH2171 bacterial body, SM-Control: skim milk, SM-LH2171: skim milk fermented by LH2171, SM-LH2171-sup: supernatant of skim milk fermented by LH2171)



Supplementary Figure 2. Lithium chloride (LiCl) solution does not effect on hBD2 expression. The supernatants in SLP purification step were collected and evaluated in hBD2 expression assay. The dialyzed SLP was washed with distilled water (wash 1), 1M LiCl (wash 2), and distilled water (wash 3). SDS-PAGE profile of collected supernatants and washed SLP (**A**). Caco-2 cells were cultured with LH2171, collected supernatants, or washed SLP. hBD2 mRNA levels were evaluated by quantitative real time-PCR. Each experiment was performed in triplicate; data are shown as mean \pm SD. LiCl solution was used as Control (**B**).

CLUSTAL O(1.2.4) multiple sequence alignment

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LH2171-SLP      -ATTVTITSTTTNKPTVDLSGAGSVSESKDITVNVTPSFTL-----TSAAKGIPATLQGSI 53
CP790-prtY     -ATTVTITSTTTNKPTVDLSGAGSVSESKDITVNVTPSFTL-----TSAAKGIPATLQGSI 53
JCM1132T-slpA  AVSTVSAATTINASS---SAINTNTNAKYDVDVTPSVSAVAANT-ANNTPAIAGNLTGTI 56
JCM1120T-SLP   -----ATTI-NAD---SAINANTNAKYDVDVTPSISAIAAVAKSDTMPAIPGSLTGTI 49
CNRZ892-slpH1  -----ATTI-NAD---SAINANTNAKYDVDVTPSISAIAAVAKSDTMPAIPGSLTGTI 49
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LH2171-SLP      EASLNGTISVTADVADVAKDVTLLTDGKGVAVVSYDKNTLTNKLSDVKAGDDYMTLSGVGF 113
CP790-prtY     EASLNGTISVTADVADVAKDVTLLTDGKGVAVVSYDKNTLTNKLSDVKAGDDYMTLSGVGF 113
JCM1132T-slpA  SASYNGKTYTANLTKADTENATITAAG-----STTAVKPAELAAGVAYVTVNDVVF 107
JCM1120T-SLP   SASYNGKSYTANLPKDSGNATITDSN-----NNTVKPAELEADKAYVTVIPDVVF 99
CNRZ892-slpH1  SASYNGKSYTANLPKDSGNATITDSN-----NNTVKPAELEADKAYVTVIPDVVF 99
                . * * * . : * * : . : . * * . . : * : : : * . * * * : . *

LH2171-SLP      SFGKANAGKTLTFKLPEGV-TVEG-----ANYNKDDHKVILDQYGNVSGLKFVISKVKA 166
CP790-prtY     SFGKANAGKTLTFKLPEGV-TVEG-----ANYNKDDHKVILDQYGNVSGLKFVISKVKA 166
JCM1132T-slpA  NFGSENAGKTVTLGSANSNVKFTGTNSDNQTETNVSTLKVKLDQNGVASLTVNSIANVYA 167
JCM1120T-SLP   NFGSENAGKEITIGSANPNVTFTEKTGDQ----PASTVKVILDQDGVAKLSSVQIKNVYA 155
CNRZ892-slpH1  NFGSENAGKEITIGSANPNVTFTEKTGDQ----PASTVKVILDQDGVAKLSSVQIKNVYA 155
                . * . * * * * : * : . : . . . . . * * * * * * . . . * : *

LH2171-SLP      YDSANTNAVSYFYDAKSGLVATQGSYMT-LAENGNLNV DALLKALNDKYEAMQFKDGSFQT 225
CP790-prtY     YDSANTNAVSYFYDAKSGLVATQGSYMT-LAENGNLNV DALLKALNDKYEAMQFKDRSFQT 225
JCM1132T-slpA  INTT DNSNVNFYDVTSGATVINGAVSVNADNQGQVNVANVVAAINS KYFAAQYADKKLNT 227
JCM1120T-SLP   IDTTYNSNVNFYDVTTGATVTTGAVSIDADNQGQLNITSVVAAINS KYFAAQYDKKQLTN 215
CNRZ892-slpH1  IDTTYNSNVNFYDVTTGATVTTGAVSIDADNQGQLNITSVVAAINS KYFAAQYDKKQLTN 215
                : : : . . * * * * . : * * * : : : : : : : * * * * * * : . : .

LH2171-SLP      VKVNTTADDVKALEKAGIKVDAANNFEAPDTFTVTLNAKSDVNGKTASLPVVVTVPNGK 285
CP790-prtY     VKVNTTADDVKALEKAGIKVDAANNFEAPDTFTVTLNAKSDVNGKTASLPVVVTVPNGK 285
JCM1132T-slpA  RTAN-TEDA IKAALKDQKIDVNSVGYFKAPHTFTVNVKATSNTNGK SATLPVVVTVPNVA 286
JCM1120T-SLP   VTFD-TETAVKDALKAKQKIEVSSVGYFKAPHTFTVNVKATS NKNKGSATLPVTVTPNVA 274
CNRZ892-slpH1  VTFD-TETAVKDALKAKQKIEVSSVGYFKAPHTFTVNVKATS NKNKGSATLPVTVTPNVA 274
                . : * * * : * : * . . . . * * * * * * : * * : * * * * * * *

LH2171-SLP      STVVPSQSKT IMHNAYYYDKDAKRVGTDKVTRYNAVTVAMNTTKLANGISYYEVIENGKA 345
CP790-prtY     STVVPSQSKT IMHNAYYYDKDAKRVGTDKVTRYNAVTVAMNTTKLANGISYYEVIENGKA 344
JCM1132T-slpA  EPTVASVSKRIMHNAYYYDKDAKRVGTDKVTRYNSVSVLPNTT-TINGKTYQVVENGKA 345
JCM1120T-SLP   DPVVPSQSKT IMHNAYFYDKDAKRVGTDKVTRYNTVTVAMNTTKLANGISYYEVIENGKA 334
CNRZ892-slpH1  DPVVPSQSKT IMHNAYFYDKDAKRVGTDKVTRYNTVTVAMNTTKLANGISYYEVIENGKA 334
                . . * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

LH2171-SLP      TGKYINADNIDGKRTLKHNAYVYESSKKRANKVVLKKGTEVTTYGNPYTFKNGKYYKI 405
CP790-prtY     TGKYINADNIDGKRTLKHNAYVYESSKKRANKVVLKKGTEVTTYGNPYTFKNGKYYKI 404
JCM1132T-slpA  VDKYINAA NIDGKRTLKHNAYVYASSKKRANKVVLKKGTEVTTYGAS YTFKNGKYYKI 405
JCM1120T-SLP   TGKYINADNIDGKRTLKHNAYVYKTSKKRANKVVLKKGTEVTTYGGSYKFKNGQRYKI 394
CNRZ892-slpH1  TGKYINADNIDGKRTLKHNAYVYKTSKKRANKVVLKKGTEVTTYGGSYKFKNGQRYKI 394
                . . * * * * * * * * * * * * * * * * * * * * * * * * * * * *

LH2171-SLP      GADTKKTYVRVENFD 420
CP790-prtY     GADTKKTYVRVENFD 419
JCM1132T-slpA  GDNTDKTYVKVANFR 420
JCM1120T-SLP   GANTEKTYVKVANFE 409
CNRZ892-slpH1  GANTEKTYVKVANFE 409
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Supplementary Figure 3. Multiple alignment of deduced amino acid sequences. Amino acid sequences of the predicted mature forms of LH2171 SLP, *L. helveticus* CP790 prtY (accession no. BAA86287.1), *L. acidophilus* JCM1132^T slpA (accession no. CAA50535.1), *L. helveticus* JCM1120^T SLP (accession no. CAB46985.1), and *L. helveticus* CNRZ892 slpH1 (accession no. CAA62606.1) were analyzed with Clustal Omega.