# The Adhesion of *Lactobacillus salivarius* REN to a Human Intestinal Epithelial Cell Line Requires S-layer Proteins

**Ran Wang [1#], Lun Jiang [1#], Ming Zhang[2], Liang Zhao[1], Yanling Hao[1], Huiyuan Guo[1], Yue Sang[3], Hao Zhang[1] & Fazheng Ren[1*]**

**[1]The Innovation Centre of Food Nutrition and Human Health (Beijing), College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, P. R. China**

**[2]School of Food and Chemical Engineering, Beijing Technology and Business University, Beijing 100048, P. R. China**

**[3]Beijing Higher Institution Engineering Research Center of Animal Product, Beijing 100083, P. R. China**

**# R Wang and L Jiang contributed equally to this work**

**[*]To whom correspondence should be addressed.**

**Fazheng Ren, China Agricultural University, Beijing 100083, P. R. China.**

**Tel.: 86-010-62736344; fax: 86-010-62736344.**

**Email: renfazheng@263.net**

**Supplemental Data**

**This article contains Supplemental Fig. S1 and Table S1, S2 and S3.**

**Supplemental Figure S1. The electrostatic potential map of CbpA and ENO1.**

**Supplemental Figure S2. The sequence analysis of SlpA, SlpB and RlpA.**

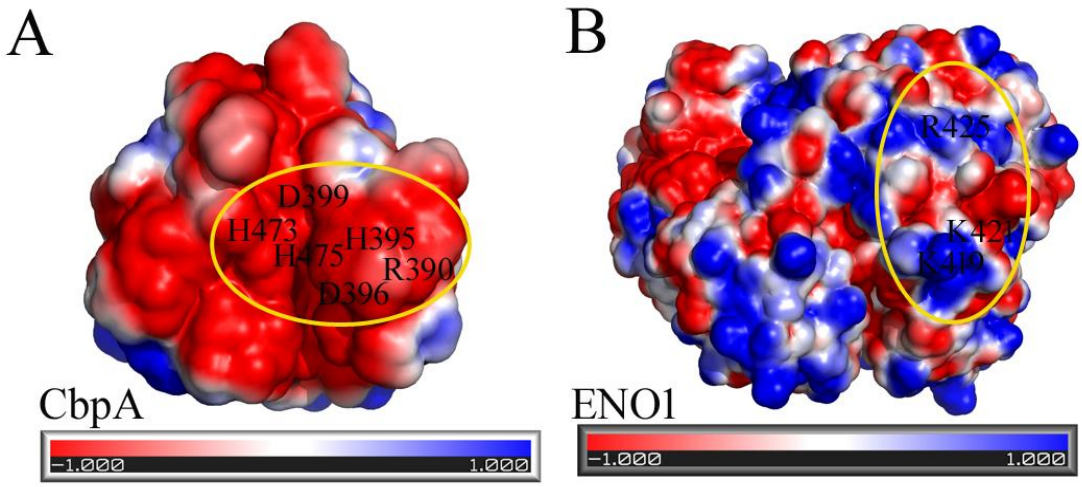
**Supplemental Figure S3. Enolase (ENO1), the putative CbpA receptor of HT-29 cells.**

**Supplemental Figure S4. Original gel drawing of PCR, SDS-PAGE and Western blotting.**

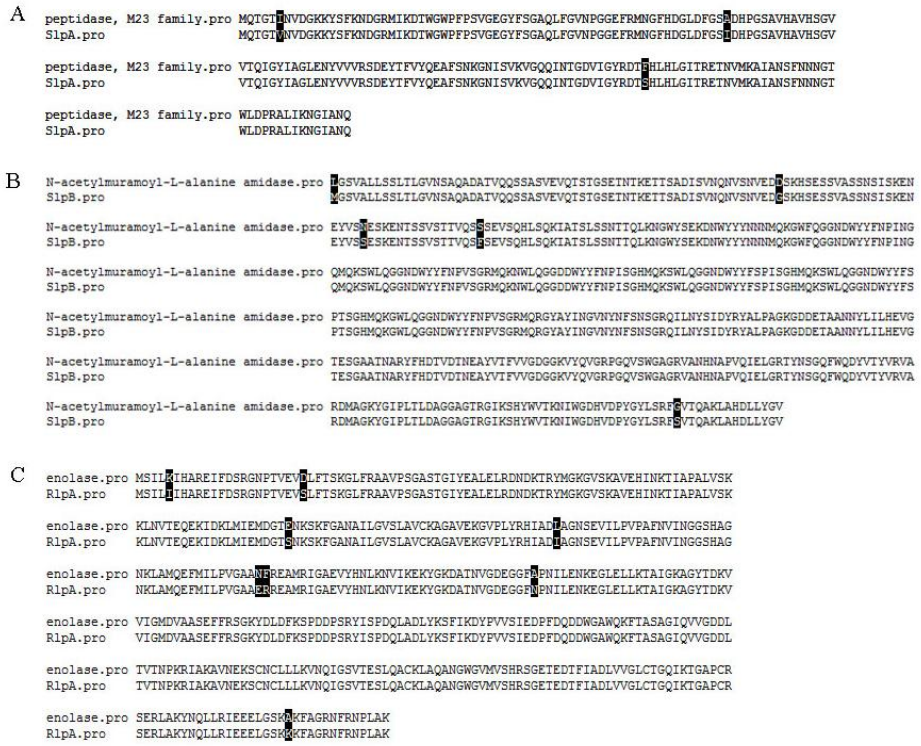
**Supplemental Table S1. Bacterial strains and plasmids used in this study.**

**Supplemental Table S2. Data collection and refinement statistics of CbpA.***

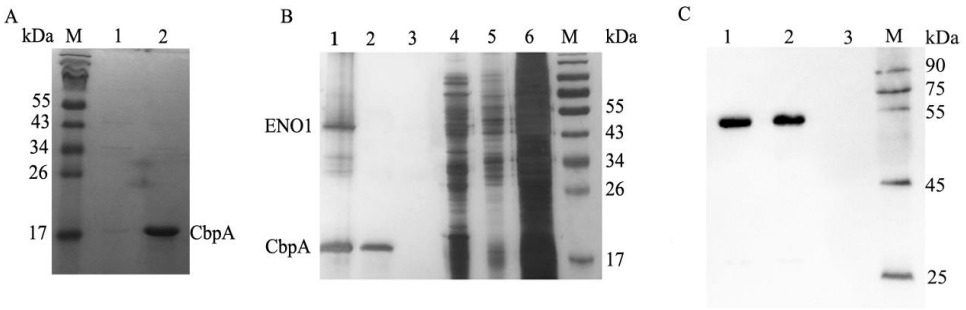
**Supplemental Table S3. The list of primers used in the study.**




**Supplemental Figure S1.** The electrostatic potential map of **A.** CbpA and **B.** ENO1 (PDB ID: 3B97). The active site of CbpA and the C-terminal of ENO1 are shown in yellow ellipse. The key residues involved in the interaction between CbpA and ENO1 are labeled.




**Supplemental Figure S2.** The sequence analysis of SlpA, SlpB and RlpA. **A.** The multiple-amino-acid sequences alignment of SlpA with M23 peptidase of *L. salivarius.* **B.** Sequences alignment of SlpB with N-acetylmuramoyl-L-alanine amidase of *L. salivarius.* **C.** Sequences alignment of RlpB with enolase. Megalign program of LaserGene was used to perform the sequences alignment.




**Supplemental Figure S3. Enolase (ENO1), the putative CbpA receptor of HT-29 cells. A.** Recombinant CbpA was purified using $Ni^{2+}$-agarose affinity chromatography. Lane M: marker. Lane 1: flow-through liquid after purification with $Ni^{2+}$-agarose affinity chromatography. Lane 2: purified CbpA. **B.** The receptor of His-CbpA on HT-29 cells was identified using a His pull-down assay. Lane 1: bait protein (His-CbpA)-prey protein complex. Lane 2: bait protein (His-CbpA). Lane 3: control without bait protein. Lane 4: flow-through bait after binding to glutathione agarose. Lane 5: flow-through prey after incubation with immobilized bait. Lane 6: lysate of HT-29 cells. **C.** Western blotting analysis with anti-ENO1 polyclonal antibodies. Extracts from HT-29 cells were co-immunoprecipitated by anti-CbpA antibody. Co-immunoprecipitated proteins were analyzed by Western blotting with anti-ENO1 polyclonal antibodies. Lane 1: the receptor of His-CbpA. Lane 2: the receptor of CbpA. Lane 3: the control agarose resin.

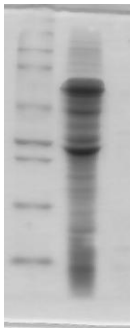


Figure. 2A

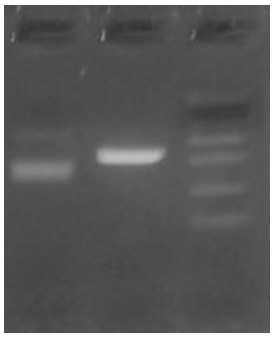


Figure. 2D

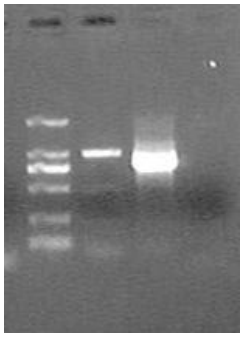


Figure. 2D

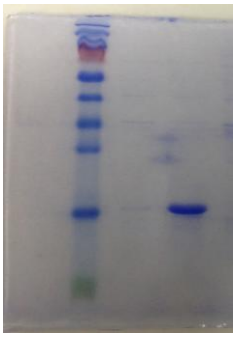


Figure. 6A

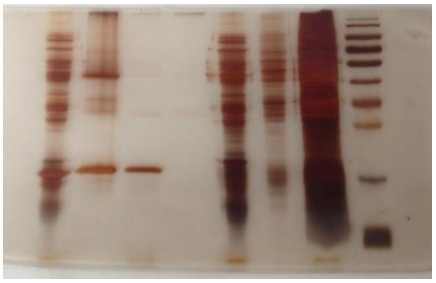


Figure. 6B

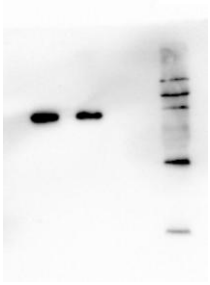


Figure. 6C

**Supplemental Figure S4. Original gel drawing of PCR, SDS-PAGE and Western blotting.**

## Table S1. Bacterial strains and plasmids used in this study.

| Bacterial strains or plasmids | Relevant properties | Reference of source |
|---|---|---|
| *L. salivarius* REN | Isolated from fecal samples of healthy centenarians | [1] |
| *L. salivarius* REN Δ *cbpA* | *L. salivarius* REN with deletion of the *cbpA* gene | This study |
| *L. salivarius* REN Δ *nam-amidase* | *L. salivarius* REN with deletion of the *nam-amidase* gene | This study |
| *E. coli* EC1000 | Host for pORI19, chromosomal *repA*+ (pWV01) | [2] |
| pORI19 | $Em^r$ $Ori^+$ *repA*$^-$ *lacZ*' derivative of pORI28 | [2] |
| pTRK669 | Temperature sensitive-helper plasmid, *repA*$^+$, $Cm^r$ | [3] |
| pORS001 | pORI19 containing flanks of *cbpA* | This study |
| pORS002 | pORI19 containing flanks of *NAM-amidase* | This study |
| pET28b+ | Expression vector | This study |

**Table S2.** Data collection and refinement statistics of CbpA.*

| | CbpA (PDB: 5GT1) |
|---|---|
| **Data collection** | |
| Wavelength | 1.1000 |
| Space group | *P*43212 |
| Cell dimensions | |
| a,b,c (Å) | 95.607, 95.607, 39.208 |
| α,β,γ (˚) | 90.0, 90.0, 90.0 |
| Resolution (Å)† | 50.0-1.85 (1.88-1.85) |
| *R*sym (%) | 9.5 (45.9) |
| *I*/σ | 37.0 (3.4) |
| Completeness (%) | 93.0 (57.2) |
| Total No. of reflections | 415874 |
| Unique reflections | 16222 |
| Redundancy | 15.9 (5.2) |
| **Refinement** | |
| Resolution (Å) | 50.0-1.85 (1.85-1.90) |
| No. of reflections | 14186 (598) |
| $R_{work}/R_{free}$(%) | 17.2/19.7 (23.8/29.7) |
| No. of atoms | |
| Protein | 1171 |
| Ligand/ion | 9 |
| Water | 141 |
| *B*-factors(Å$^2$) | |
| Protein | 19.41 |
| Ligand/ion | 31.20 |
| Water | 30.60 |
| rms deviations | |
| Bond lengths (Å) | 0.009 |
| Bond angles (°) | 1.19 |
| Ramachandran Plot (%)[1] | 97.4/2.6/0.0/0.0 |

*Three crystal experiments for each structure.

$R_{sym}=\Sigma_h\Sigma_i|I_{h,i}-I_h|/\Sigma_h\Sigma_I I_{h,i,}$ where $I_h$ is the mean intensity of the *i* observations of symmetry related reflections of *h*.

[1]Residues in most favored, additional allowed, generously allowed and disallowed regions of the Ramachandran plot.

**Table S3**. The list of primers used in the study. a: F or R indicates forward primer or reverse primer, b: the underlines stand for restriction enzyme cutting site, c: 5' or 3' indicates location of primer upstream or downstream of target gene.

| primers[a] | Sequence (5'-3')[b] | Restriction enzyme cutting site | Target[c] |
|---|---|---|---|
| AIE51F | CCGGAATTCCAAACAGGTACTGTCAATGTAGATG | *Eco*R Ⅰ | 5' *lspA* |
| AI52R | CCACACCACTATGAACTGCCTCATCAGAACGAACTACT | - | 5' *lspA* |
| AI33F | GCAGTTCATAGTGGTGTGGAGTAGTTCGTTCTGATGAG | - | 3' *lspA* |
| AIB34R | CGCGGATCCCTGATTAGCAATTCCAT | *Bam*H Ⅰ | 3' *lspA* |
| BIE51F | CCGGAATTCCAAAAAGGCTGGTTTCAG | *Eco*R Ⅰ | 5' *lspB* |
| BI52R | TAGTCAATGGAGTAGTTCATCACCCTTACCTGCTGGT | - | 5' *lspB* |
| BI33F | AACTACTCCATTGACTAACCAGCAGGTAAGGGTGATG | - | 3' *lspB* |
| BIB34R | CGCGGATCCCACACCATACAATAAATCAT | *Bam*H Ⅰ | 3' *lspB* |
| ORI19DF | GGAAATTATCGTGATCAACAAGTTTA | - | pORI19 |
| ANF | CCCATGGGCCAAACAGGTACT | *Nco* Ⅰ | *lspA* |

## Reference


1. Zhang, M., Qiao, X. W., Zhao, L., Jiang, L. & Ren, F. Z. *Lactobacillus salivarius* REN counteracted unfavorable 4-nitroquinoline-1-oxide-induced changes in colonic microflora of rats. *J Microbiol* **49**, 877-883 (2011).

2. Law, J. *et al.* A system to generate chromosomal mutations in *Lactococcus lactis* which allows fast analysis of targeted genes. *J Bacteriol* **177**, 7011-7018 (1995).

3. Russell, W. M. & Klaenhammer, T. R. Efficient system for directed integration into the *Lactobacillus acidophilus* and *Lactobacillus gasseri* chromosomes via homologous recombination. *Applied and environmental microbiology* **67**, 4361-4364 (2001).