

Choice for host-specific high-adhesive *Lactobacillus* strains

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ABSTRACT

Adhesive ability was tested in seven *Lactobacillus* isolated from the chicken digestive tract after cultivation with CaCo-2 cells (intestinal epithelial cells), MDCK (dog kidney) cells and CEF (chicken embryo fiber) cells. We noted the following important observations regarding the adhesive ability between different *Lactobacillus* strains and three cell types: the adhesive interaction between the SDnB₇, SDnE₁ and SDnA₃ *Lactobacillus* strains and CaCo-2 cells was greater compared to controls, the adhesive effect between SDnB₁ and CEF cells was also greater than controls and *Lactobacillus* showed only minimal adherence to MDCK cells. Incubation time also affected *Lactobacillus* adherence to CaCo-2 cells: adhesive ability was optimal at 37°C when incubated for 2 days and this was confirmed by a local increase in the concentration of *Lactobacillus* around CaCo-2 cells when incubated for 24 h as opposed to 3 h. Adherence ability in *Lactobacillus* was also tested at various concentrations (10⁸, 10⁷, 10⁶, 10⁵ and 10⁴). The number of *Lactobacillus* that adhered around the cells was significantly increased in the treatment with 10⁸ bacterial cells. Transmission electron microscopy revealed that the cellularity of the junction between CaCo-2 cells and *Lactobacillus* was not compromised. Transmission electron microscopy also revealed that the thalli fabric structure remained intact

Keywords: Host Specific; CaCo-2 Cells; MDCK Cells; CEF Cells; High Adhesion *Lactobacillus*

1. INTRODUCTION

The definition for probiotics has gradually changed with an increasing understanding of how it influences human and animal health. A potentially successful probiotic strain is expected to have several desirable properties in order to show benefit. These properties include the ability to adhere to cells while remaining non-pathogenic. Adherence to the intestinal mucosa is considered selec-

tion criteria for probiotic microorganisms [1-11].

Lactobacillus bacteria have the capacity to adhere to intestinal epithelial cells. Different species of these bacterium vary in their adherence ability. Rinkinen (2003) and Gusils (2002) suggested that the adhesive ability of probiotic strains is species specific but not host-specific [12,13]. We, on the other hand, aimed to demonstrate that strains were not only species-specific but also host-specific.

We isolated *Lactobacillus* bacteria from the chicken digestive tract and tested their adherence to CaCo-2 cells, CEF cells and MDCK cells. The choice of high adhesive strains based on host-specificity will serve to advance research in adhesive mechanisms and production.

2. MATERIALS AND METHODS

2.1. CaCo-2 Cells, MDCK Cells and CEF Cells

CaCo-2 cells (intestinal epithelial cells) were obtained from the cell bank, China science college, Shanghai. MDCK cells were obtained from the medical science college of Beijing. CEF cells were retrieved after 10 days from an SPF chicken embryo.

CaCo-2 cell culture: 10% serum of new cattle DMEM culture liquids (penicillin, 100 ten thousands unit, streptomycin, 100 µg/l) (pH 7.0 to 7.2); 0.1 M phosphate buffer (pH 7.4); cell digestive liquids (0.02% EDTA: D-Hanks liquids = 10:1) (not included in Ca²⁺, Mg²⁺) When cells grew to a particular confluency we passaged them.

CEF cell culture: 5% serum of new cattle DMEM culture liquids (penicillin, 100 ten thousands unit, streptomycin, 100 µg/l) (pH 7.0 to 7.2); 0.1 M phosphate buffer (pH 7.4); cell digestive liquids (0.02% EDTA: D-Hanks liquids = 10:1) (not included in Ca²⁺, Mg²⁺) When cells grew to a particular confluency we passaged them.

MDCK cell culture: 10% serum of new cattle DMEM culture liquids (penicillin, 100 ten thousands unit, streptomycin, 100 µg/l) (pH 7.0 to 7.2); 0.1 M phosphate buffer (pH 7.4); cell digestive liquids (0.02% EDTA: D-Hanks liquids = 10:1) (not included in Ca²⁺, Mg²⁺) When cells grew to a particular confluency we passaged

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them.

2.2. *Lactobacillus* Strains

Strains were provided by the microbiology teaching and researching room of Shandong agriculture university. For SDnB₁, SDnB₇, SDnC₁, SDnE₁, SDnE₃, SDnA₃ and SDnA₄ (A, B, C and E, representative of crop, glandular stomach, small intestine and rectum, respectively) we used a 37°C incubation temperature (see 2d and 4d).

2.3. *Lactobacillus* Adherence Assay

Briefly, cell monolayers were prepared on glass coverslips and then placed in 24-well corning tissue culture plates. For the various strains, we added liquids at a volume of 200 µl and 400 µl to the 24-well plates (concentration of the strains was 1×10^8 bacteria per ml culture supernatants), incubated at 37°C in 5% CO₂ - 95% air and after 3 h or 24 h of incubation, the glass was removed and the monolayers were washed six times with sterile PBS. After drying, fixation in methanol, and staining with a Gram stain, samples were examined microscopically. For each separate strain adherence assay, the number of adherent bacteria were evaluated in 20 random microscopic sections. Adhesion assays were evaluated by two different technicians to eliminate bias.

2.4. Transmission Electron Microscopy

We mixed CaCo-2 cell suspensions (at a concentration of 10^5 /ml) with *Lactobacillus* suspensions (at a concentration of 10^7 /ml), then incubated at 37°C in 5% CO₂ - 95% air for 3 h. Liquids were then collected, washed six times with sterile PBS and centrifuged (1000 rpm/min, 5 min) to remove non-adherent bacteria. Cell were fixed with 3.5% glutaraldehyde and post-fixed with agar. Supernatant liquids were washed with 0.1 M phosphate buffer for 1 h, post-fixed with 1% OsO₄, washed with 0.1 M phosphate buffer and dehydrated in an alcohol gradient (30, 50, 70, 80, 90 and 100% ethanol) suppr resin sock wrap up; LKB super film slicer have on film slice acetate shaddock—lemmon acid lead dye; the specimens were then examined with a JEOL-1200EX transmission electron microscope.

3. RESULTS

Lactobacillus strains are host-specific and the adhesion number between cell types is variable. The *Lactobacillus* strains that successfully adhered to CaCo-2 cells is shown in **Figure 1** and **Table 1**. The effect of different *Lactobacillus* strains adherence to CaCo-2 cells is vaiable between SDnB₁, SDnB₇ and SDnE₁, while SDnA₃ had a significantly increased adhesive effect. *Lactobacillus* was shown to only slightly adhere to MDCK cells (**Fig-**

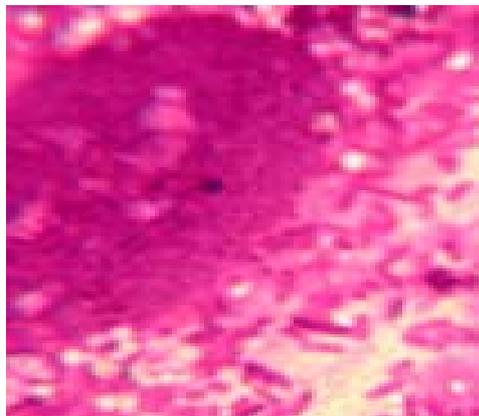


Figure 1. *Lactobacillus* adhere to CaCo-2 cells.

Table 1. Various *Lactobacillus* individual plants adhere to CaCo-2 cells.

| Individual plants | Source | Level of <i>Lactobacillus</i> adhere to CaCo-2 cell |
|-------------------|-----------------------------|---|
| SDnB ₁ | SPF chick Glandular stomach | 11.10 ± 0.78 ^a |
| SDnB ₇ | SPF chick Glandular stomach | 20.00 ± 1.46 ^b |
| SDnC ₁ | SPF chick Small intestine | 9.00 ± 1.04 ^c |
| SDnE ₁ | Chick rectum | 25.40 ± 1.78 ^d |
| SDnA ₃ | SPF chick crop | 19.20 ± 1.26 ^b |

^{a,b,c,d} values within column with different superscripts differ significantly ($p < 0.05$).

ure 2). A number of *Lactobacillus* strains successfully adhere to CEF cells, in particular, the SDnA₃ and SDnB₁ strains demonstrated good adhesion (**Figure 3**, **Table 2**).

3.1. Incubation Time Affects *Lactobacillus* Adhesion to CaCo-2 Cells

Lactobacillus SDnE₁ and SDnB₇, when incubated at 37°C for 2d, demonstrated substantial adhesion ability (**Tables 3** and **4**).

3.2. Concentration of Strains Affects *Lactobacillus* Adhesion to CaCo-2 Cells

Higher concentrations of all the *Lactobacillus* strains displayed greater adherence to CaCo-2 cells (**Table 5**).

3.3. Incubation Time Affected *Lactobacillus* Adherence to CaCo-2 Cells

A 24 h incubation time encouraged better *Lactobacillus* adherence to CaCo-2 cells compared to an incubation time of 3 h (**Table 6**).

3.4. Transmission Electron Microscopy Results

Lactobacillus adheres to CaCo-cells; the cellularity of

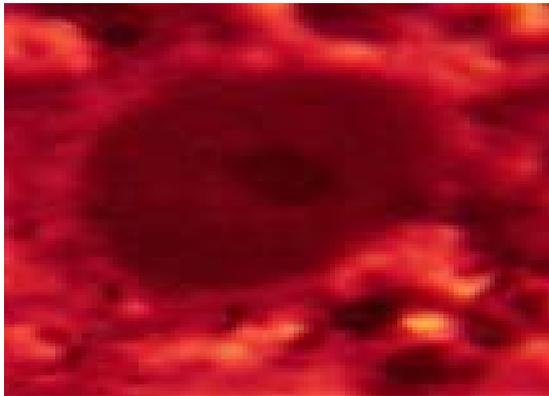


Figure 2. *Lactobacillus* can hardly adhere to MDCK cell.

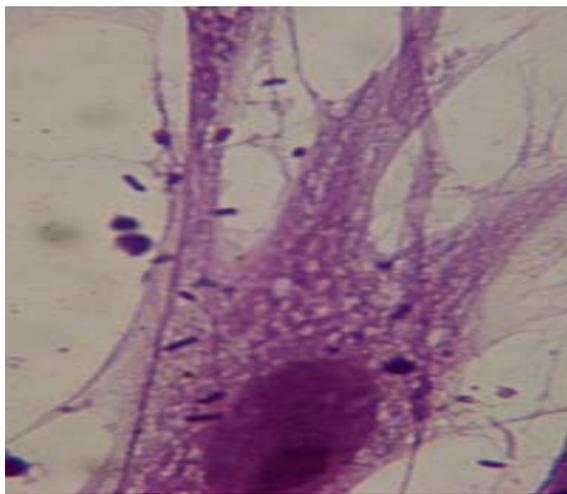


Figure 3. *Lactobacillus* can adhere to CEF cell.

Table 2. The number of different *Lactobacillus* individual plants adhere to CEF cell.

| Individual plants | Source | Level of <i>Lactobacillus</i> adhere to CEF cell |
|-------------------|-----------------------------|--|
| SDnB ₁ | SPF chick Glandular stomach | 7.20 ± 0.53 ^a |
| SDnA ₃ | SPF chick crop | 8.40 ± 0.67 ^a |
| SDnC ₁ | SPF chick Small intestine | 4.80 ± 0.53 ^b |

^{a,b}values within column with different superscripts differ significantly (p < 0.05).

Table 3. Effect of varying incubation time on the number of *Lactobacillus* individual plants adhering to CEF cells.

| <i>Lactobacillus</i> Strains SDnE ₁ incubation time (d) | Level of <i>Lactobacillus</i> adhere to CEF cell (entries/cell) |
|--|---|
| 2 | 25.40 ± 1.78 ^a |
| 4 | 16.70 ± 1.83 ^b |

^{a,b}values within column with different superscripts differ significantly (p < 0.05).

Table 4. Effect of altering incubation time on the number of *Lactobacillus* SDnB₇ individual plants adherence to CaCo-2 cells.

| <i>Lactobacillus</i> SDnB ₇ incubation time (d) | Level of <i>Lactobacillus</i> adhere to CaCo-2 cell (entries/cell) |
|--|--|
| 2 | 20.00 ± 1.46 ^a |
| 4 | 10.70 ± 0.99 ^b |

^{a,b}values within column with different superscripts differ significantly (p < 0.05).

Table 5. Effect of different concentrations on the number of *Lactobacillus* individual plants adhering to CaCo-2 cells.

| Different concentration (10 ⁸ bacteria/ml) | Level of <i>Lactobacillus</i> adhere to CaCo-2 cell (entries/cell) |
|---|--|
| 1 | 20.20 ± 1.70 ^a |
| 0.1 | 13.70 ± 0.86 ^b |
| 0.01 | 10.00 ± 0.97 ^c |
| 0.001 | 6.90 ± 0.74 ^d |
| 0.0001 | 3.30 ± 0.60 ^e |

^{a,b,c,d,e}values within column with different superscripts differ significantly (p < 0.05).

Table 6. Effect of different incubation time on the number of *Lactobacillus* individual plants SDn E₁ adherence to CaCo-2 cells.

| Action time (h) | Level of <i>Lactobacillus</i> adhering to CaCo-2 cells (entries/cell) |
|-----------------|---|
| 3 | 15.10 ± 1.49 ^a |
| 24 | 25.40 ± 1.78 ^b |

^{a,b}values within column with different superscripts differ significantly (p < 0.05).

the junction between CaCo-2 cells and *Lactobacillus* was preserved (**Figure 4**).

4. DISCUSSION

The human gastrointestinal tract is colonized by a variety of gram-positive and gram-negative organisms including bacteria belonging to the *Lactobacillus* genus. The human CaCo-2 cell line has proven to be one of the best model systems for studying interactions between bacterial cells and intestinal epithelial cells [14].

We investigated the adherence of *Lactobacillus* species to CaCo-2 cells quantitatively. The two methods we used to calculate the number of *Lactobacillus* adhering to intestinal epithelial cells included: counting and see. fore is directive use numerical value and post by subjective imagination connect them can precise to response adhesion effect.

We found that *Lactobacillus* have the ability to adhere to intestinal epithelial cells and CEF cells but adhesion to MDCK cells was minimal. This observation is a clear example of host-specificity that until now, had not been

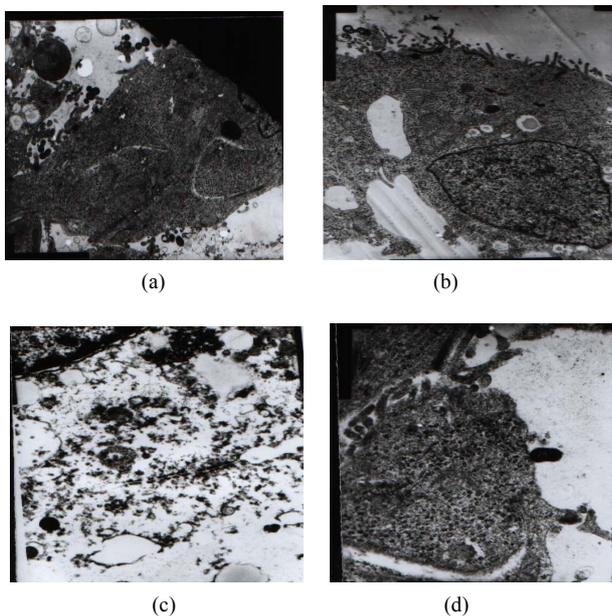


Figure 4. Results of transmission electron microscopy reveal that (respective magnifications: 7500 \times , 7500 \times , 15K \times and 20 K \times). (a), (d): Cellularity of the junction between CaCo-2 cells and *Lactobacillus* is preserved; (b): The thalli fabric remains intact; (c): Two-cell connection, cell structure is unaffected.

reported. Intestinal epithelial cells are tumor derived and for this reason, we also incubated primary intestinal epithelial cells from 10 SPF chicken embryos. The adhesion effect was very similar to that seen in the tumor-derived cell line.

Incubation time and concentration have a significant effect on *Lactobacillus* adhesion. We demonstrated that incubation for 2d at 37 $^{\circ}$ C and a 10 8 cells/mL bacterial concentration enhanced adhesion ability. This culture conditions will be useful for future adhesion experiments.

5. ACKNOWLEDGEMENTS

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