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 (10^8, 10^7, 10^6, 10^5 and 10^4). The number of lactobacillus that adhered around the cells was significantly increased in the treatment with 10^8 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 selection 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 Ca^{2+}, Mg^{2+})

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 Ca^{2+}, Mg^{2+})

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 Ca^{2+}, Mg^{2+})

When cells grew to a particular confluency we passaged them.
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 cover-slips 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 $1 \times 10^8$ 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 Electrictron 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 and dehydrated in an alcohol gradient (30, 50, 70, 80, 90 and 100% ethanol) suprr resin sock wrap up; LKB super film slicer have on film slice acetate shaddock—lemon 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 variable between SDnB₁, SDnB₇ and SDnE₁, while SDnA₃ had a significantly increased adhesive effect. Lactobacillus was shown to only slightly adhere to MDCK cells (Figure 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 SDnB₁, 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 Electrictron 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.

<table>
<thead>
<tr>
<th>Individual plants</th>
<th>Source</th>
<th>Level of lactobacillus adhere to CEF cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDnB1</td>
<td>SPF chick Glandular stomach</td>
<td>7.20 ± 0.53a</td>
</tr>
<tr>
<td>SDnA3</td>
<td>SPF chick crop</td>
<td>8.40 ± 0.67b</td>
</tr>
<tr>
<td>SDnC1</td>
<td>SPF chick Small intestine</td>
<td>4.80 ± 0.53b</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Lactobacillus Strains SDnE1 incubation time (d)</th>
<th>Level of lactobacillus adhere to CEF cell (entries/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>25.40 ± 1.78a</td>
</tr>
<tr>
<td>4</td>
<td>16.70 ± 1.83b</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Lactobacillus SDnB7 incubation time (d)</th>
<th>Level of lactobacillus adhere to CaCo-2 cell (entries/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>20.00 ± 1.46a</td>
</tr>
<tr>
<td>4</td>
<td>10.70 ± 0.99b</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Different concentration (10^8 bacteria/ml)</th>
<th>Level of lactobacillus adhere to CaCo-2 cell (entries/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.20 ± 1.70a</td>
</tr>
<tr>
<td>0.1</td>
<td>13.70 ± 0.86b</td>
</tr>
<tr>
<td>0.01</td>
<td>10.00 ± 0.97b</td>
</tr>
<tr>
<td>0.001</td>
<td>6.90 ± 0.74b</td>
</tr>
<tr>
<td>0.0001</td>
<td>3.30 ± 0.60b</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Action time (h)</th>
<th>Level of lactobacillus adhering to CaCo-2 cells (entries/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>15.10 ± 1.49a</td>
</tr>
<tr>
<td>24</td>
<td>25.40 ± 1.78b</td>
</tr>
</tbody>
</table>

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
concentration enhanced adhesion ability. This culture provided us with MDCK cells; and D. guo (service Microscopie Elec-
tion effect was very similar to that seen in the tumor-
derived cell line.

Figure 4. Results of transmission electron microscopy reveal that (respective magnifications: 7500×, 7500×, 15K× and 20 K×). (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 adhe-
sion 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°C and a 10⁹ cells/mL bacterial concentration enhanced adhesion ability. This culture conditions will be useful for future adhesion experi-
ments.

5. ACKNOWLEDGEMENTS

This work was supported by the Shandong agriculture university microbiology teaching and researching room. We thank the cell bank of china science college Shanghai that provided us with CaCo-2 cells (intestinal epithelial cells); medical science college of Beijing that provided us with MDCK cells; and D. guo (service Microscopie Electronique, Shandong Agriculture University) for technical assistance during the electron microscopy study. This work is supported by the ministry of Agriculture of China: the earmarked fund for Modern Agro-industry Technology Research System of China.

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