

Aim of the Experiment:**DETERMINATION OF CELL ADHERENCE ABILITY BY PROBIOTIC BACTERIAL CELLS****Introduction**

Adhesion ability to the host is a classical selection criterion for potential probiotic bacteria that could result in a transient colonization that would help to promote immunomodulatory effects, as well as stimulate gut barrier and metabolic functions. Bacterial adhesion to intestinal surfaces could be driven initially by non-specific physical binding as hydrophobic interactions followed by a second stage of adhesion by specific cell wall components. A correlation between hydrophobicity and adhesion is observed in many research experiments. In this regard, the presence of some surface proteins such as cell wall–anchored proteinases have been shown to enhance hydrophobicity and adhesion in some lactic acid bacteria. The presence of adhesins in the bacterial cell wall has also an important role in the adhesion of bacteria to the intestinal lumen. Although *in vitro* experiments are key to understand the mechanisms of adhesion and select probiotic candidates with potential to adhere *in vivo*, it is difficult to extrapolate these results to the human intestinal tract where other factors such as peristaltic movements, host defence system or competition with resident microbiota could interfere in the attachment.

Materials

1. Probiotic strains
2. Caco-2 cell line
3. PBS buffer
4. DMEM with L-glutamine
5. Fetal bovine serum,
6. Gentamycin sulphate
7. Nonessential amino acids
8. Trypsin-EDTA solution
9. Giemsa stain
10. Methanol
11. 2.5% glutaraldehyde
12. Osmium tetroxide
13. Ion coater

Procedure

A. Cell line activation

Caco-2 cell culturing

1. Adherence ability of lactobacilli strains can be investigated using enterocyte-Caco-2 (non-mucus type) as epithelial cells model.
2. Briefly, Caco-2 cells were cultured routinely in a 25 cm² culture flask to monolayer in DMEM with L-glutamine and supplemented with, 10% fetal bovine serum, gentamycin sulphate (50 mg/mL; 1 µl/mL) and 1.0% nonessential amino acids.
3. To make cells confluent, these were incubated under 95% air and 5.0% CO₂ at 37°C.
4. The culture medium was replaced every 48 h to maintain the cells.
5. The adherent confluent cells were harvested by adding 3 mL of 0.25% (w/w) trypsin-EDTA solution and incubated for 20 min at 37°C.
6. When 80% of the cells were detached, fresh DMEM (7 mL) was added and mixed by doing repeated pipetting to make a single cell suspension.
7. Further, the homogenate was centrifuged at, 1500g for 5 min at room temperature.
8. Before adding cells to six wells plate, the cell concentration was calculated by hemocytometer.

B. Procedure for adhesion of bacteria to Caco-2 cells

1. The cells were seeded at a density of 4×10^4 into a six-well plate containing microscopic cover slip.
2. Overnight activated (18 h) strains of probiotic lactobacilli were washed thrice with PBS, re-suspended and diluted to 1×10^9 in DMEM.
3. One milliliter of this suspension was incubated with monolayer for 2h at 37°C (5.0% CO₂, 95% air) and non-adherent bacterial cells were removed by three successive washings with PBS (pH 7.2).
4. For cell fixation, 3 mL of methanol was added and incubated for 5-10 min, at room temperature.
5. After discarding methanol, cells were stained with 3 mL of Giemsa stain for 30 min and washed with methanol, until no colour comes in it.
6. Further, dried overnight, in an incubator at 37°C and examined microscopically at 100X magnification.
7. For each lactobacilli strain, the assay was carried out in duplicate and Caco-2 cells used in study were maintained for more than months, for differentiation and confluency.
8. For adhesion ability, bacteria were counted in cover slip per cell for 100 cells at 20 random microscopic fields. The ability to adhere was expressed in terms of bacterial cells per cell.

C. Scanning electron microscopy of adherent lactobacilli to Caco-2 cells

1. For scanning electron microscopy images, the cells were fixed with 2.5% glutaraldehyde, instead of methanol for 45 min and washed thrice with PBS at 5 min interval.
2. The cells were again refixed in 2 mL of 1.0% osmium tetroxide for 1 h and dehydrated in series of 30, 50, 70, 80% and absolute ethanol prepared in PBS (pH 7.4).
3. Wells were dried overnight; coverslips were removed carefully and gold coated in an ion coater before examined in SEM.

Observations

Name of the Isolate	Adhesion (No of Bacteria per cell)
Isolate A	
Isolate B	
Isolate C	

Interpretation