

Aim of the Experiment:**DETERMINATION OF CELL AUTO-AGGREGATION AND COAGGREGATION ABILITY BY THE SELECTED PROBIOTIC CANDIDATE****Introduction**

Bacterial adhesion is a complex process initially based on non-specific physical interactions between two surfaces, which then enable specific interactions between adhesins (usually proteins) and complementary receptors. In order to manifest the beneficial effects, probiotic bacteria need to achieve an adequate mass through aggregation. Auto-aggregation of probiotic strains appeared to be necessary for adhesion to intestinal epithelial cells and the ability to coaggregate with other bacteria such as pathogens may form a barrier that prevents colonization by pathogenic microorganisms. Adherence of bacterial cells is usually related to cell surface characteristics. Physicochemical characteristics of the cell surface such as hydrophobicity may affect auto-aggregation and adhesion of bacteria to different surfaces. Auto-aggregation ability test together with cell-surface hydrophobicity and coaggregation could be used for preliminary screening identifying potentially adherent bacteria with properties suitable for commercial purposes.

Materials

1. Probiotic strain
2. MRS Broth
3. Spectrophotometer
4. Test tubes
5. PBS buffer
6. Centrifuge
7. Vortex mixture
8. MacFarland turbidity standard

Procedure**Cell Auto-Aggregation**

1. Probiotic lactic acid bacterial strains were grown for 18 h at 37°C with MRS liquid medium.
2. The cells were harvested by centrifugation at 5,000 g for 15 min, washed twice and resuspended in phosphate buffered saline (PBS) to give viable counts of approximately 10^8 CFU/ml.
3. The OD at 595 nm of a 200 μ L sample was adjusted to ~ 0.5 , for equilibration of bacterial cell density in each sample and validated with MacFarland standard 2.

- Thereafter, a 200 μL aliquot of sample was gently taken out after, 2, 4 and 6 h of incubation at 37°C .
- Decrease in absorbance was consider as positive sign for auto-aggregation ability and calculated by following formula:

$$\text{Auto-aggregation \%} = [(A_0 - A) / A_0] \times 100$$

Where A_0 = Initial OD and A =OD at interval hours.

Cell Coaggregation

- The method for preparing the cell suspensions for coaggregation was the same as that for autoaggregation assay.
- Two different probiotic lactic acid bacterial strains (X and Y) were grown for 18 h at 37°C with MRS liquid medium.
- The cells were harvested separately by centrifugation at 5,000 g for 15 min, washed twice and resuspended in phosphate buffered saline (PBS) to give viable counts of approximately 10^8 CFU/ml.
- Equal volumes of each cell suspension (X and Y) were mixed together in pairs by vertexing for 10 s.
- Thereafter, a 200 μL aliquot of sample was gently taken out after 2, 4 and 6 h of incubation at 37°C separately and well as in mixed samples.
- The absorbance (A) at 595 nm of the suspensions were measured after mixing and after 5 h of incubation at room temperature.
- The percentage of coaggregation was calculated using the equation

$$\text{Coaggregation}\% = [(A_x + A_y) / 2] - A(x+y) / [(A_x + A_y) / 2] \times 100$$

where, x and y represent each of the two strains in the control tubes, and (x + y) the mixture.

Observations

Autoaggregation and Coaggregation

Name of the Isolate	0 hour	2hour	4 hours	6 hours
Autoaggregation (%)				
Co-aggregation (%)				

Interpretation