

EXERCISE NO. 8

Date:

Aim of the Experiment:

CELL SURFACE HYDROPHOBICITY(CSH) ASSAY

Introduction

In order to exert its beneficial effect on the host, a probiotic strain must be able to survive the gut passage of humans to reach to the action site in viable state and should be in sufficient population (normally 10^7 cells per ml). Thus, in order to survive in and colonize the gut, the bacteria should express high cell surface hydrophobicity. Cell surface hydrophobicity (CSH) is a biophysical measurement of a cell's affinity for a hydrophobic versus hydrophilic environment. Cells with higher CSH prefer a hydrophobic environment while those with lower CSH will preferentially remain in an aqueous environment. *In-vitro* CSH activity can be assayed against different hydrocarbons *viz.* *n*-hexadecane, *n*-octane and xylene.

Materials

1. Probiotic strain
2. PUM buffer (pH-6.5)
3. Microplate reader
4. Test tubes
5. Centrifuge
6. *n*-hexadecane, *n*-octane and xylene

Procedure

1. Activate the probiotic culture to be tested for overnight.
2. Activated cultures were harvested by centrifugation at 6000g for 10 min, washed pellet twice with phosphate urea magnesium buffer (PUM, pH 6.5) and finally re-suspend in the same buffer.
3. The final OD₅₈₀ was maintained near to 0.7-0.8, for each culture (200 μ L), respectively.
4. One milliliter, of cell suspension was separately mixed with 0.3 mL, each of three hydrocarbons in acid resistant tubes to fractionate two phases.
5. Following, a 10 min preincubation at 37°C, to equilibrate temperature, the samples were again vortex and allows standing in an incubator at 37°C for 60 min.
6. The aqueous phase (200 μ L) was aspirated gently and placed in a microplate reader.
7. A decrease in absorbance from the initial reading was considered as a measure of percent CSH and was calculated as:

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

Where, A_0 and A are the readings of the aqueous phase before and after the extraction at

580nm

Observations

Cell Surface Hydrophobicity (%)

Name of the Isolate	n-hexadecane	n-octane	Xylene
Control (Initial)-A₀			
After 60 min-A			
CSH% (A₀-A/A₀ X 100)			

Interpretation