

**Aim of the Experiment:****DETERMINATION OF ACID TOLERANCE ACTIVITY****Introduction**

The fermented foods serve as good sources of new potential sources of lactic acid bacteria. Therefore, use of strains of such origin would be of great importance, consumers who suffer from lactose intolerance. 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 tolerance to low (acidic) pH and high tolerance to higher concentrations of bile. They are supplemented as probiotics that are live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance. Probiotic viability is generally determined by quantifying its resistance to simulated gastric juice or to simulated intestinal fluid in *in vitro* tests, which measures microbial survival after given periods of contact, i.e., [log colony forming units (cfu) of surviving viable cells]/ [log cfu of initial viable cells. The acid tolerance test can be studied under pH 2.0 and 3.0 with 7.5 as control. The cell count for the acid tolerance test was obtained at an interval of 0, 1, 2 and 3 hours respectively and was pour plated on deMan, Rogosa, and Sharpe (MRS) agar to be incubated at 37 °C for 24 hours.

**Materials**

1. Probiotic strain
2. Peptone water (0.1%)
3. MRS agar and Broth
4. HCl
5. Spectrophotometer
6. Test tubes
7. PBS buffer
8. Centrifuge

**Procedure**

1. Selected strains of lactobacilli were evaluated at different pH values-1.5, 2.0, 2.5 and 6.5 (control) (1N HCL) in MRS broth, as prevalent in human stomach.
2. The OD of each culture was adjusted near to 0.6-0.7 at 595 nm and compared with McFarland standards that corresponds to  $\log^{8-9}$  cfu/mL.
3. Overnight grown cells at 37°C were harvested by centrifugation at 5000g at 4°C for 10 min and washed twice with sterile PBS (pH 7.4) to remove debris.

4. Further, vortexed and re-suspended in equal volumes of acidified-MRS and control MRS broth.
5. One millilitre culture was taken at (0, 1, 2 and 3 h) and further 10- folds were prepared in 5 mL of 0.1% peptone water.
6. To evaluate the acid tolerant an aliquot of 100  $\mu$ L was pour plate on MRS agar plates, incubated at 37°C for 24-48 h and viable counts were enumerated in terms of log cfu/mL

## Observations

### A) Acid Tolerance

Name of the Isolate	0 hour (log cfu/mL)	1 hour (log cfu/mL)	2 hours (log cfu/mL)	3 hours (log cfu/mL)
Control				
pH 1.5				
pH 2.0				
pH 6.5				

## Interpretation