

**Aim of the Experiment:****DETERMINATION OF TRANSIT TOLERANCE TO BILE SALTS****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 higher concentrations of bile. Bile is a physiological aqueous solution produced and secreted by the liver. It consists mainly of bile salts, phospholipids, cholesterol, conjugated bilirubin, electrolytes, and water. Bile travels through the liver in a series of ducts, eventually exiting through the common hepatic duct. Probiotic viability is generally determined by quantifying its resistance to bile salts in *in vitro* tests, which measures microbial survival after given periods of contact, i.e.,  $[\log \text{ colony forming units (cfu) of surviving viable cells}] / [\log \text{ cfu of initial viable cells}]$ . Bile tolerance is one of the most crucial properties for probiotic bacteria, as it determines its ability to survive in the small intestine, and consequently its capacity to play its functional role as a probiotic. The ability of the examined strains to reduce the pH in the presence of different percentages of bile salts or Oxgall was considered as the bile tolerance

**Materials**

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

**Procedure**

1. MRS broth was supplemented with different concentrations (1.0, 1.5, and 2.0%) of oxgall to simulate the bile conditions as prevalent in human gut.
2. Another set of MRS broth, without bile salt was adjusted to pH 7.0, and was used as control.
3. Afterwards, to obtain bile survival a similar procedure was followed as described

earlier for acid resistance in section

4. 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.
5. 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.
6. Further, vortexed and re-suspended in equal volumes of bile added MRS and control MRS broth.
7. One milliliter culture was taken at (0, 1, 2 and 3 h) and further 10- folds were prepared in 5 mL of 0.1% peptone water.
8. 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) Bile 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				
1.0%				
1.5%				
2.0%				

## Interpretation