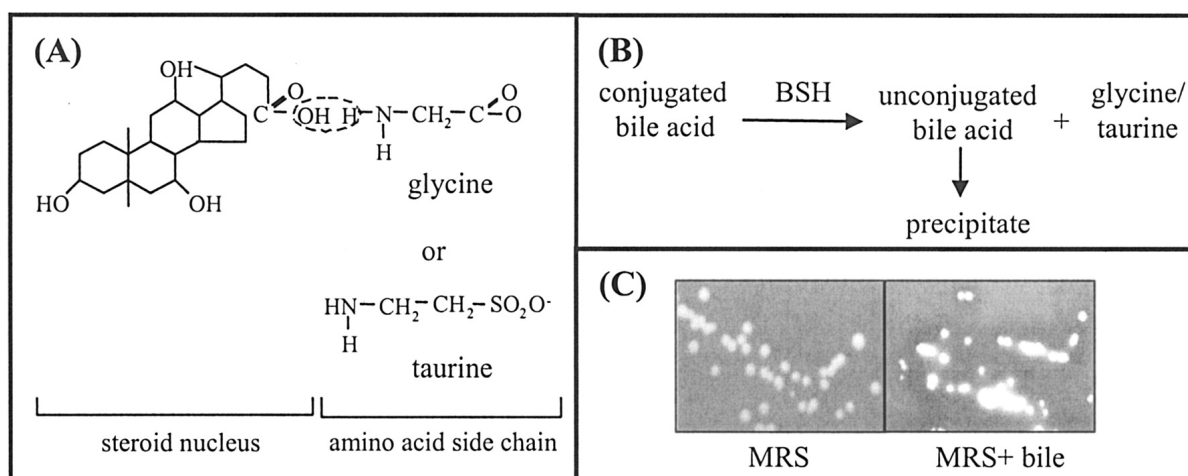


Aim of the Experiment:**BILE SALT HYDROLASE (BSH) ACTIVITY ASSAY****Introduction**

Bile is a yellow-green aqueous solution whose major constituents include bile acids, cholesterol, phospholipids, and the pigment biliverdin. It is synthesized in the pericentral hepatocytes of the liver, stored and concentrated in the gallbladder interdigestively, and released into the duodenum after food intake. Bile functions as a biological detergent that emulsifies and solubilizes lipids, thereby playing an essential role in fat digestion. This detergent property of bile also confers potent antimicrobial activity, primarily through the dissolution of bacterial membranes.

The primary bile acids, cholic and chenodeoxycholic acid, are synthesized *de novo* in the liver from cholesterol. The solubility of the hydrophobic steroid nucleus is increased by conjugation as an N-acyl amidate with either glycine (glycoconjugated) or taurine (tauroconjugated) prior to secretion. The resulting molecules are therefore amphipathic and can solubilize lipids to form mixed micelles.

Bile acids are efficiently conserved under normal conditions by a process termed enterohepatic recirculation. Conjugated and unconjugated bile acids are absorbed by passive diffusion along the entire gut and by active transport in the terminal ileum. Reabsorbed bile acids enter the portal bloodstream and are taken up by hepatocytes, reconstituted, and resecreted into bile. Approximately 5% of the total bile acid pool (0.3 to 0.6 g) per day eludes epithelial absorption and may be extensively modified by the indigenous intestinal bacteria. One important transformation is deconjugation, a reaction that must occur before further modifications are possible. Deconjugation is catalyzed by bile salt hydrolase (BSH) enzymes (EC 3.5.1.24), which hydrolyze the amide bond and liberate the glycine/taurine moiety from the steroid core. The resulting acids are termed unconjugated or deconjugated bile acids.



(A) Chemical structure of bile acids. Primary bile acids are synthesized in the liver from

cholesterol and are conjugated with either glycine or taurine prior to secretion. The carboxyl group of the bile acid and the amino group of the amino acid are linked by an amide bond.

(B) Reaction catalyzed by BSH enzymes. BSHs cleave the peptide linkage of bile acids, which results in removal of the amino acid group from the steroid core. The resulting unconjugated bile acids precipitate at low pH.

(C) Detection of BSH activity: *L. plantarum*, which was grown overnight in MRS broth, was streaked onto MRS (Difco) agar (A) or MRS agar supplemented with 0.2% (wt/vol) glycodeoxycholic acid (GDCA; Sigma) (B) and incubated anaerobically for 48 h. The white precipitates around colonies and the clearing of the medium are indicative of BSH activity.

Materials

1. Probiotic strain
2. MRS agar and Broth
3. Anaerobic Gas pack jar
4. Test tubes
5. PBS buffer
6. Centrifuge
7. Sodium taurocholate hydrate (TCA), Sodium deoxycholate (DC), Sodium taurodeoxycholate (TDC) and sodium tauroglycocholate (TGC),

Procedure

1. Bile salt deconjugation activity among the *Lactobacillus* strains was tested according to the direct plate assay.
2. Bile salt-MRS agar plates were prepared by adding a common 0.037 g/L of calcium chloride and individual bile salt from four used one-0.5% (w/v) sodium taurocholate hydrate (TCA), sodium deoxycholate (DC), sodium taurodeoxycholate (TDC) and sodium tauroglycocholate (TGC), to prepare different bile salt plates.
3. Before use, the plates were anaerobically incubated at 37°C in an anaerobic Gaspack jar system, with anaerobic pouches, subsequently for three days.
4. For induction of *bsh* gene and acclimatization of cultures to bile salts, they were initially grown in 0.3% (w/v) bile containing MRS broth for subsequent generations.
5. After that, they were streaked on the respective bile salt plate, for deconjugation and incubated for 72 h under anaerobic conditions at 37°C.
6. BSH positive cultures were attributed by two different means - White precipitated halos around colonies due to liberated Cholic or deoxycholic acid and opaque white colonies without formation of halos.

Observations

Bile Salt hydrolase assay

Name of the Isolate	Sodium taurocholate hydrate (TCA)	Sodium deoxycholate (DC)	Sodium taurodeoxycholate (TDC)	Sodium tauroglycocholate (TGC)
Control				
Isolate A				
Isolate B				
Isolate C				

Interpretation