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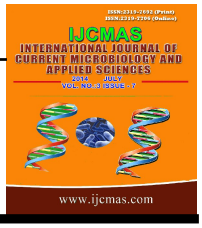


Original Research Article

Investigation of tolerance of *Lactobacillus casei* to the presence of acids, bile salts and Deconjugation of bile salts

K.G.Deshpande*, C.B.Dolas, and N.S.Chavan

MGM College of Agricultural Biotechnology, Aurangabad, India
**Corresponding author*

Keywords

Lactobacillus casei Deconjugation of bile salts, MRS medium

A B S T R A C T

A lab experiment was carried out during summer session of 2012-13 at Dept. of Post Harvest and Food Biotechnology, MGM College of Agricultural Biotechnology, Aurangabad to study the "Investigation of tolerance of *Lactobacillus casei* to the presence of acids, bile salts and deconjugated bile salts*"*.The experiment was laid out in completely randomized design with 3 replications. *Lactobacillus casei* strains were isolated from serial dilution of curd and grown on MRS medium unaerobically at 37°C for 48 hrs. Catalase test and gram staining were performed to check the characteristics of *Lactobacillus casei*. The isolated *Lactobacilli* were exposed to artificial gastric juice and artificial intestinal juice. *Lactobacillus* were exposed to gastric juice of pH 2, 3 and 4 for 0min, 30min 60min and 90min.resp. The interaction time of lactobacilli with gastric juice of different pH was gradually increased to see the period of viability of *Lactobacillus* in the acidic environment of human gut. Among three pH of gastric juice used the lactobacilli which were exposed to gastric juice of pH4 for 90min showed significant results in terms of no. of colonies. Similarly *Lactobacilli* were exposed to intestinal juice of pH8 with different concentration i,e 0.5%, 1.0% and 1.5% and incubated at 0min, 30min, 60min, 90min and 180min respectively. Among all this treatments *Lactobacillus casei* which was exposed to intestinal juice of 1% and incubated for 180min showed significant viability in terms of no. of colonies. In case of other treatments also *Lactobacillus* showed considerable viability when incubated with intestinal juice.

Introduction

Lactobacillus strains are a major part of the probiotics micro flora of the intestine and of fermented dairy products, and are found in a variety of environments. The aim of the study was to find out the ability of bile and acid tolerance and antibacterial properties of the twenty eight isolates of three group *lactobacilli* namely *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus delbruki*. For this purpose twenty eight different *Lactobacillus* strains that isolated from Koozeh cheese as a traditional cheese were screened. The acid tolerance test was 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 De Man, Rogosa,

and Sharpe (MRS) agar to be incubated at 37°C for 24 hours. All cells were selected for bile tolerance test in MRS broth containing bile concentrations of 0% as control and 0.3% as test. Then cell counts were enumerated after 24 hours of incubation on MRS agar. Results showed twenty seven isolates did not have ability to tolerate acid and bile salts and antimicrobial activity against four indicator bacteria included *Eshirichia coli, Listeria monocytogenesis, bacillus cereus, Salmonella entritidis*. Only one Isolate namely *Lactobacillus casei* could tolerate acid and bile salt and had antibacterial activity against of *L. monocytogenesis*. (Hassan Hassanzadazar *et.al* 2012).

The lactic acid bacteria are second only to yeast in importance in their service to human beings. They have been used worldwide in the generation of safe, storable and organoleptically pleasing foodstuffs for centuries. These foods include fermented milk products, bread and cereals, beverages, vegetables and, particularly in the Far East, preserved and ensiled fish products. In recent research, lactic acid bacteria are considered as one of the probiotic bacteria because of their beneficial effects for humans. In the correct quantity, the bacteria can ensure the balance of flora in vivo and advance the health of the host .As probiotic bacteria, the lactic acid bacteria are confronted with several challenges, such as high acid concentration and high bile salt concentration. Acid tolerance is the principal characteristic of every strain that can survive and function in the alimentary canal. The depressant effect of bile salts on the growth of bacteria has some relationship with the bile salts concentration and bacteria characteristics. The bacteria that can survive and metabolize in normal physiobile salt concentrations may have the ability to survive in the transport process of the intestinal tract . It is essential to separate the bacteria that have high bile salt tolerance Hence, whether they can survive in the acid and bile salt conditions in the human gastrointestinal tract is accepted as one of the desirable properties used to select potential probiotic strains .(Xia Chen *et.al* 2009).

Bifidobacteria are Gram-positive, non-gas producing, and anaerobes with bifid morphology. Today, this genus, which belongs to the Actinomycetaceae group, includes 30 species. Bifidobacteria represent up to 91% of the total gut bacterial population during the early stages of life.The mechanisms allowing intestinal bacteria to resist physiological bile concentrations remain poorly understood and have been mainly related to bile salt modifying enzymes or to the membrane proteins that either take up or extrude these compounds. These adaptive mechanisms are very important to ensure the adaptation of bifidobacteria to the intestinal environment. Some Bifidobacterium strains because of their health-promoting effects in the human and animal intestinal tract are being included as probiotic active ingredients in functional foods. Some health-promoting effects, such as treatment of diarrhoea and balancing of the intestinal microbiota, have been established for some strains of bifidobacteria. Moreover, several anti-mutagenic and anti-carcinogenic activity which increased the immune response and reduced serum cholesterol levels, have been proposed. One commonly found species of bifidobacteria in breast-fed infants is *B.Pseudocatenulatum*, which is largely unexplored as probiotic.(Babak Rasti *et.al* 2011).

Probiotic such as Lactobacillus acidophilus were found to excrete bile salt hydrolase (BSH) (cholylglycine hydrolase;

EC3.5.1.24), the enzyme that catalyzes the hydrolysis of glycine- and taurine-conjugated bile salts into amino acid residues and free bile salts (bile acids). BSH was found to be present in several bacterial species of the gastrointestinal tract, such as *Lactobacillus sp*., *Bifido-bacterium* longum, Clostridium perfringens and *Bacter-oides fragilis ssp. fragilis* . He found precipitated halo and opaque granular white material on agar plugs around L. acidophilus colonies, which were confirmed to be cholate, chenodeoxycholate and deoxycholate, produced by the deconjugation of taurocholic acid and taurodeoxycholic acid. The major route of cholesterol excretion from humans and other mammals is through faeces. Cholesterol is the precursor of primary bile salts that are formed in the liver and are stored as conjugated bile salts in the gall bladder for secretion in the gastrointestinal tract . Conjugated bile salts are secreted into the small intestine for absorption of dietary fat, hydro-phobic vitamins and other fat-soluble compounds. A small fraction of bile salts that are not absorbed is lost as free bile salts in faeces. Free bile salts were less soluble than conjugated bile salts, resulting in lower absorption in the intestinal lumen . At the physiological pH of the intestinal lumen, deconjugated bile salts can be transported through the epithelium and into the blood stream of the host, or precipitated. Thus, in a steady-state situation, deconjugation of bile acids can reduce serum cholesterol levels by increasing the formation of new bile acids that are needed to replace those that have escaped the enterohepatic circulation (M.T.Liong *et.al* 2005).

Materials and Methods

The experiment was conducted in the Department of Post Harvest and Food Biotechnology of MGM College of Agricultural Biotechnology, Aurangabad.

Test Organism: *Lactobacillus casei*

Isolation of *Lactobacilli*

Lactobacillus Casei was isolated from the milk samples of MGM Dairy. These were subcultured 3 times by growing in MRS broth at 37°C for 24 hrs.

Gram Staining

Gram staining was performed to check grams nature of organism. Overnight suspension of *Lactobacillus* was prepared.

d) Catalase Test

Catalase test was performed to check the anaerobic nature of the isolated lactobacillus.

e) Tolerance *of Lactobacillus casei* to gastric juice

1. After isolation and identification by using biochemical reaction the culture was subjected for its acid tolerance by subjecting it to the artificial gastric juice. One aliquat (0.2 ml) from suspension of bacteria was transfered in a 2ml sterile eppendroff tube and was mixed in 0.3ml of 0.5% sterile Nacl solution and 1ml stimulated gastric juice of pH 2, 3, and 4 respectively. This mixture was vertexed for 10 sec and was incubated at 37 ^{o}c. (Emese Both *et.al* 2010)

Viability of strains was analysed by determination of CFU/ml after different periods of incubation. (0min, 30min, 60min, 90min) in the stimulated gastric juice by inoculation on MRS solid media after an incubation time of 48hrs at 37^{o}C.

f)Tolerance of *Lactobacillus casei* to bile salts

Bacterial cultures were obtained on MRS medium, after incubation at 37^{o}C, for 48 hrs,

the colonies were suspended in 0.5% Nacl solution

One aliquat (0.2ml) from suspension of bacteria was transferred in 2ml sterile eppendroff tube and mix with 0.3ml of 0.5% sterile Nacl solution and 1ml stimulated intestinal juice (0.5%, 1% and 1.5%). This mixture was vertexed for 10sec followed by incubation at 37ºC for 48hrs (Seun Jang *et.al*).

Viability of srains was determined by CFU/ml after different periods of incubation (0min, 30min, 90min, 180min) in stimulated intestinal juice by inoculation on MRS media after an incubation time of 48 hrs at37ºC.

g) Qualitative test for deconjugation of bile salts by bacterial culture

-To MRS agar containing 0.5g/lit cysteine, 1mM of sodium taurocholate was prepared after autoclaving and solidifying, the plates were incubated anerobically for 48hrs before use. The plates were inoculated with active culture (20µlit) and incubated for 71hrs at 37ºC.The plates were observed for precipited cholic acid around colonies

h) Deconjucation of bile salts

Deconjucation of sodium glucocholate and sodium taurocholate.

10ml volume of MRS was supplemented with 6mM sodium taurocholate (102mM) respectively.-Each strain was inoculated at 1% level and incubated aerobically at 37ºC for 20hrs.-Bile salts deconjucation ability was based on release of deconjugated bile and amount of free cholic acid released by organism was measured.

i) Determination of choline released from deconjugated bile

-10ml culture of organism was adjusted to pH 7.0(1N NaoH).Cells were centrifuged at 10,000g at 4ºC for 10min.-Supernatent obtained was adjusted to pH 1.0 (10 N Hcl) i,e 1ml of Supernatent+ 1ml of ethyl acetate. This mixture was vortexed for 1min.2ml of ethyl acetate was transfered into a glass tube and evaporated under nitrogen at 60ºC.-Then residue was immediately dissolved in 1ml NaoH (0.01N) and mixed properly.-Then 1ml of furfuraldehude (1%) and 1ml of H2SO4 (16N) was added and vertexed for 1min.-Then it was heated in water bath at 65ºC for 10min.After cooling 2ml of glacial acetic acid was added and mixed and vertexed for 2min.-Then absorbance at 660nm was taken.-The amount of cholic acid released was determined by using standard cholic acid**.**

Results and Discussion

Acid tolerance of *Lactobacilli*

The culture of *Lactobacilli* was isolated, identified and confirmed by performing catalase test. It was subjected for acid tolerance by subjecting it to the artificial gastric juice. *Lactobacillus* cells were found viable after exposure to the gastric juice for 0min, 30min, 60min, and 90min. They were determined as no. of colonies. The results were interpreted in terms of no. of colonies of *Lactobacilli* exposed to gastric juice of pH 2, 3 and 4 which were summarized in Table no.4.3.1. Table.no.4.3.1 (*Lactobacillus* grown on *gastric* juice of different pH).

The results were statistically analysed by using CRD with 3 replication. According to statistical analysis the mean value were found greater than CD indicating that all values were highly significant.

Bile tolerance of *Lactobacilli*

The *Lactobacilli* were exposed to intestinal juice of pH-8 in various concentration such as 0.5%, 1% and 1.5%. The *Lactobacilli* were found to be grown on the MRS medium containing intestinal juice. `The viability of strains was determined by exposing cells to intestinal juice for 0min, 30min, 60min, 90min and 180min.The results were interpreted in terms of no. of colonies which were summarised in Table no.4.4.1

The results were statistically analysed by using CRD with 3 replication. According to statistical analysis the mean value were found greater than CD indicating that all values were highly significant.

Deconjugation of bile salts

The isolated *Lactobacilli* were checked for their ability of deconjugation of bile salts which was based on release of deconjugated bile and amount of free cholic acid released by organism around them. It was found that isolated *Lactobacilli* cells were able to deconjugate the bile salts which was observed on the surface of colonies in the form of white precipitate (free cholic acid).

The differences in the no. of colonies of different treatments were found significant. In case of gastric juice,*Lactobacillus* grown on *gastric* juice pH3 at 0 min was significantly superior over rest of others in terms of no. of colonies. This confirms that *Lactobacilli* can grow and remain viable in highly acidic pH even though its normal pH range is 6 to 6.8.

Lactobacillus grown on gastric juice of pH3 at 30min was at par with *Lactobacillus* grown on pH4 at 0min and found significantly superior over rest of other- Indicating that *Lactobacilli* grown on gastric juice pH3 at 30min and pH4 at 0min gave similar growth as compared to *Lactobacilli* grown on gastric juice pH2 (0min,30min,60min,90min), pH3 (0min, 60min, 90min) and pH4(30min, 60min, 90min).

Lactobacillus grown on gastric juice pH4 at 0min was at par with *Lactobacillus* grown on control and found significantly superior over *Lactobacilli* grown on gastric juice pH2 (0min, 30min, 60min, 90min), pH3 (60min, 90min) and pH4 (30min, 60min) - Indicating that *Lactobacilli* grown on gastric juice pH4 at 0min and control gave similar growth as compared to *Lactobacilli* grown on gastric juice pH2 (0min,30min,60min,90min), pH3 (60min,90min) and pH4 (30min,60min).

Lactobacillus grown on gastric juice pH4 at 30min was at par with *Lactobacillus* grown on gastric juice pH3 at 60min and found significantly superior over *Lactobacilli* grown on gastric juice pH2 (0min, 30min, 60min,90min), pH3 (90min) and pH4 (0min,90min) and T4-Indicating that *Lactobacilli* grown on gastric juice pH4 at 30min and gastric juice pH3 at 60min gave similar growth as compared to *Lactobacilli* grown on gastric juice pH2 (0min, 30min, 60min,90min), pH3 (90min) and pH4 (0min,90min)

Lactobacillus grown on gastric juice pH3 at 60min was at par with *Lactobacillus* grown on gastric juice pH4 at 60min and found significantly superior over *Lactobacilli* grown on gastric juice pH2 (0min, 30min, 60min, 90min), pH3 (90min) and pH4 (90min) -Indicating that *Lactobacilli* grown

on gastric juice pH3 at 60min and gastric juice pH4 at 60min gave similar growth as compared to *Lactobacilli* grown on gastric juice pH2 (0min, 30min, 60min, 90min), pH3 (90min) and pH4 (90min).

Lactobacillus grown on gastric juice pH4 at 90min was at par with *Lactobacillus* grown on gastric juice pH3 at 90min and found significantly superior over *Lactobacilli* grown on gastric juice pH2 (0min,30min,60min,90min) -Indicating that *Lactobacilli* grown on gastric juice pH4 at 90min and gastric juice pH3 at 90min gave similar growth as compared to *Lactobacilli* grown on gastric juice pH2 (0min, 30min, 60min, 90min)

Lactobacillus grown on gastric juice pH3 at 90min was at par with *Lactobacillus* grown on gastric juice pH2 at 0min and 30min and found significantly superior over *Lactobacilli* grown on gastric juice pH2 (60min,90min) -Indicating that *Lactobacilli* grown on gastric juice pH3 at 90min and gastric juice pH2 at 0min and 30min gave similar growth as compared to *Lactobacilli* grown on gastric juice pH2 (60min, 90min). *Lactobacillus* grown on gastric juice pH2 at 0min was at par with *Lactobacillus* grown on gastric juice pH2 at 30min and 60min and found significantly superior over *Lactobacilli* grown on gastric juice pH2 (90min)-Indicating that *Lactobacilli* grown on gastric juice pH2 at 0min, 30min and 60min gave similar growth as compared to *Lactobacilli* grown on gastric juice pH2 (90min)

Lactobacillus grown on gastric juice pH2 at 30min was at par with *Lactobacillus* grown on gastric juice pH2 at 60min and 90min and found significantly superior over *Lactobacilli* grown on gastric juice pH2 at 90min -Indicating that *Lactobacilli* grown on gastric juice pH2 at 30min,60min and 90min gave same growth.

Lactobacillus grown on gastric juice pH2 at 60min was at par with *Lactobacillus* grown on gastric juice pH2 at 90min -Indicating that *Lactobacilli* does not survive much when exposed to gastric juice pH2 at 60min and 90min and showed lowest no of colonies among all the treatments.

In case of intestinal juice, The differences in the no. of colonies of different treatments were found significant. *Lactobacillus* grown on 1% intestinal juice at 0min was at par with *Lactobacillus* grown on control and found significantly superior over rest of others -indicating that *Lactobacilli* grown on 1% intestinal juice at 0min and *Lactobacilli* grown on control gave similar growth as compared to *Lactobacilli* grown on 0.5% intestinal juice (0min, 30min, 60min, 90min, 180min), 1% intestinal juice (30min, 60min, 90min, 180min) and 1.5% intestinal juice (0min, 30min, 60min, 90min, 180min). This confirm that *Lactobacilli* can grow and remain viable in highly alkaline pH (8) of 1% intestinal juice even though it's normal pH i.e. 6.

Lactobacillus grown on 0.5% intestinal juice at 0min was at par with *Lactobacillus* grown on 1% intestinal juice at 90min and 0.5% intestinal juice at 30min and found significantly superior over *Lactobacilli* grown on 0.5% intestinal juice (60min,90min,180min), 1% intestinal juice (180min) and 1.5%(0min, 60min, 90min, 180min) -indicating that *Lactobacilli* grown on 0.5% intestinal juice at 0min,1% intestinal juice at 90min and 0.5% intestinal juice at 30min gave similar growth as compared to *Lactobacilli* grown on 0.5% intestinal juice (60min,90min,180min), 1% intestinal juice (180min) and 1.5%(0min, 60min, 90min, 180min)

Lactobacillus grown on 1% intestinal juice at 90min was at par with *Lactobacillus*

grown on 0.5% intestinal juice at 30min and found significantly superior over*Lactobacilli* grown on 0.5% intestinal juice (60min, 90min,180min), 1% intestinal juice (180min) and 1.5%(0min, 30min, 60min, 90min,180min) -indicating that *Lactobacilli* grown on 1% intestinal juice at 90min and 0.5% intestinal juice at 30min gave similar growth as compared to *Lactobacilli* grown on 0.5% intestinal juice (60min, 90min,180min), 1% intestinal juice (180min) and 1.5%(0min, 30min, 60min, 90min,180min).

Lactobacillus grown on 0.5% intestinal juice at 0min was at par with *Lactobacillus* grown on 0.5% intestinal juice at 30min and 90min and found significantly superior over *Lactobacilli* grown on 0.5% intestinal juice(180min) and 1.5% intestinal juice (0min, 30min, 60min, 90min, 180min) - indicating that *Lactobacilli* grown on 0.5% intestinal juice at 0min, 30min and 90min gave similar growth as compared to *Lactobacilli* grown on 0.5% intestinal juice(180min) and 1.5% intestinal juice (0min, 30min,60min,90min,180min).

Lactobacillus grown on 0.5% intestinal juice at 60min was at par with *Lactobacillus* grown on 1% intestinal juice at 180min,0.5% intestinal juice at 90min and 180min and found significantly superior over *Lactobacilli* grown on 1.5% intestinal-juice((0min, 30min, 60min, 90min, 180min) -indicating that *Lactobacilli* grown on 0.5% intestinal juice at 60min, 90min and 180min and 1.5% intestinal juice at 180min gave similar growth as compared to *Lactobacilli* grown on 1.5% intestinal-juice((0min, 30min, 60min, 90min, 180min)

Lactobacillus grown on 1% intestinal juice at 180min was at par with *Lactobacillus* grown on 0.5% intestinal juice at 90min and 180min and found significantly superior over *Lactobacilli* grown on 1.5% intestinal juice (0min, 30min, 60min, 90min,180min) - indicating that *Lactobacilli* grown on 1% intestinal juice at 180min and 0.5% intestinal juice at 90min and 180min gave similar growth as compared to *Lactobacilli* grown on 1.5% intestinal juice((0min, 30min, 60min, 90min,180min).

Lactobacillus grown on 0.5% intestinal juice at 90min was at par with *Lactobacillus* grown on 0.5% intestinal juice at 180min and found significantly superior over *Lactobacilli* grown on 1.5% intestinal juice((0min,30min,60min,90min,180min). - indicating that *Lactobacilli* grown on 0.5% intestinal juice at 90min and 180min gave similar growth as compared to *Lactobacilli* grown on 1.5% intestinal juice((0min, 30min, 60min, 90min, 180min).

Lactobacillus grown on 1.5% intestinal juice at 0min was at par with *Lactobacillus* grown on 1.5% intestinal juice at 30min,60min and 90min -indicating that lactobacilli grown on 1.5% intestinal juice at 0min,30min,60min and 90min gave similar growth. *Lactobacillus* grown on 1.5% intestinal juice at 30min was at par with *Lactobacillus* grown on 1.5% intestinal juice at 60min and 90min -indicating that *Lactobacilli* grown on 1.5% intestinal juice at 30min,60min and 90min gave similar growth.

Lactobacillus grown on 1.5% intestinal juice at 60min was at par with *Lactobacillus* grown on 1.5% intestinal juice at 90min - indicating that *Lactobacilli* grown on 1.5% intestinal juice at 60min and 90min showed lowest no of colonies among all the the treatments and does not survive for long time.

Table.3.1.2 *Lactobacillus* exposed to gastric juice of different pH at different inoculation time

Treatments	pH of gastric juice	Inoculation time
T1	2	0 min
T2	2	30 min
T3	2	60 min
T4	2	90 min
T5	3	0 min
T6	3	30 min
T7	3	60 min
T8	3	90 min
T9	4	0 min
T10	4	30 min
T11	4	60 min
T12	4	90 min
T13 (CONTROL)	6.8	24 hrs

Table.3.1.3 *Lactobacillus* exposed to Intestinal juice of (pH 8) of different concentrations at different inoculation time

Treatments	Different concentration of intestinal juice (pH-8)	Inoculation time
T1	0.5	0 min
T2	0.5	30 min
T3	0.5	60 min
T4	0.5	90 min
T5	0.5	180min
T6	1.0	0 min
T7	1.0	30 min
T8	1.0	60 min
T9	1.0	90 min
T10	1.0	180min
T11	1.5	0 min
T12	1.5	30 min
T13	1.5	60 min
T14	1.5	90 min
T15	1.5	180 min
T16(CONTROL)	Growing lactobacillus at normal conditions .	

Different pH of Gastric juice	Treatments	Mean (no.of colonies)
pH-2	T1	35.33
	T2	26.33
	T3	22.66
	T4	17.66
pH-3	T5	152.33
	T6	130.33
	T7	84.00
	T8	41.33
pH-4	T9	126.33
	T10	94.33
	T11	76.33
	T12	57.00
Control	T13	114

SE=5.990 CD=17.41

Fig.1 *Lactobacillus* growth on gastric juice pH 3 at 0min.

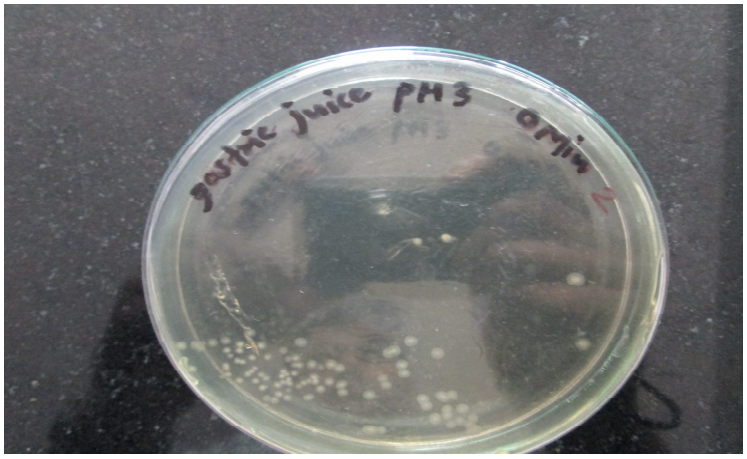


Fig.2 *Lactobacillus* growth on gastric juice pH 2 at 60min

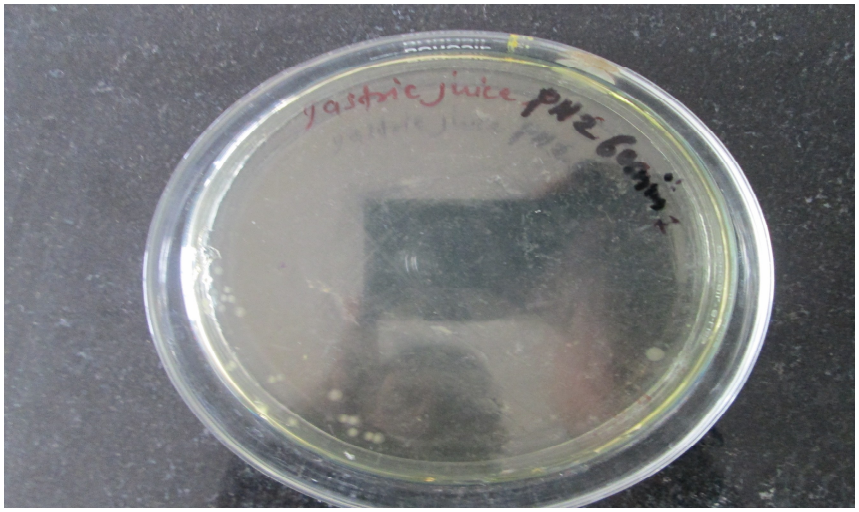


Table.4.4.1 (*Lactobacillus* grown on intestinal juice(pH8) of different concentration)

Intestinal juice	T/R	Mean
0.5%	T1	58.00
	T2	47.66
	T3	41.00
	T4	37.33
	T5	31.33
1.0%	T6	147.00
	T7	101.00
	T8	75.33
	T9	54.66
	T10	39.00
1.5%	T11	13.33
	T12	9.33
	T13	6.33
	T14	4.00
	T15	-
Control	T16	136.66
	Total	

SE=3.982 CD=11.499

Fig.3 *Lactobacillus* growth on 1% intestinal juice (pH8) incubated at 0 min.

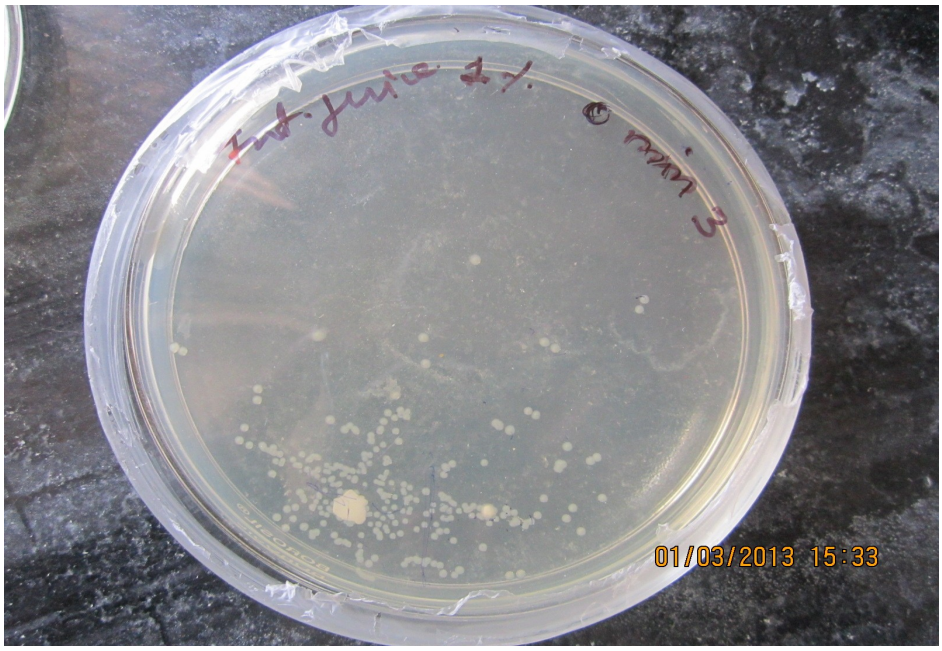


Fig.4 *Lactobacillus* growth on 1.5% intestinal juice (pH8) incubated at 60 min.

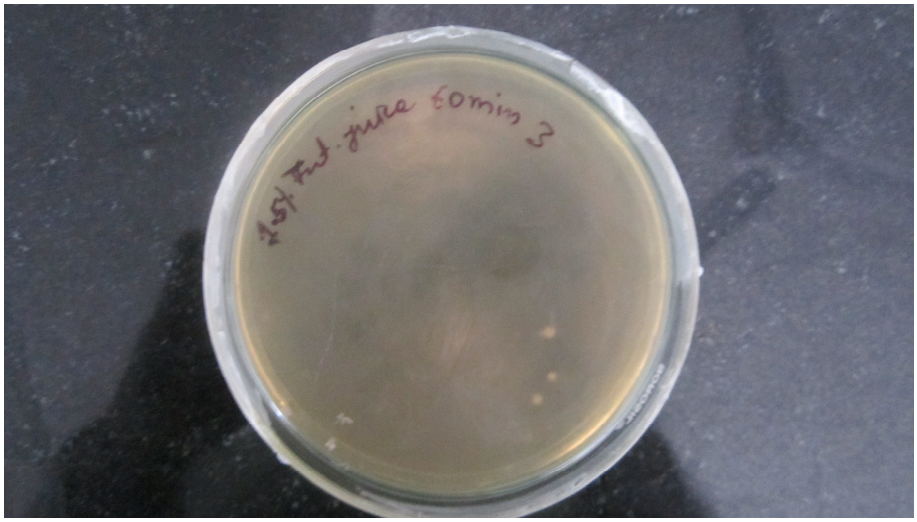
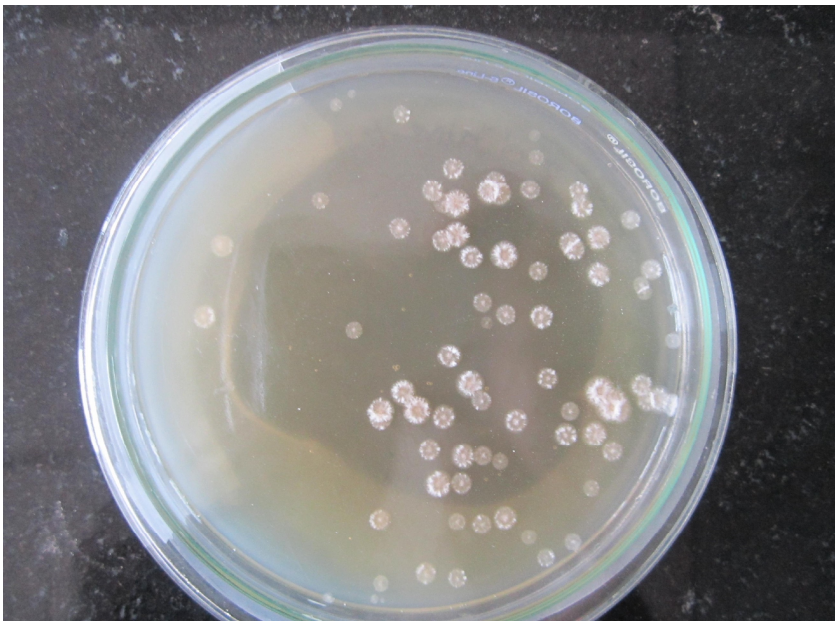


Fig.5 Cholic acid released around *Lactobacillus* colonies



In this experiment the potency of *Lactobacillus casei* against different pH values was checked. As per the objectives the isolated *Lactobacilli* were exposed to artificial gastric juice and artificial intestinal juice. *Lactobacillus* were exposed to gastric juice of pH 2,3 and 4 for 0min,30min60min and 90min. The interaction time of *Lactobacillus* with gastric juice of different pH was gradually increased to see the period of viability of *Lactobacillus* in the acidic environment of human gut(upper intestine). Among three pH of gastric juice used, the *Lactobacilli* which were exposed to gastric juice of pH4 for 90min showed significant results in terms of no. of colonies. This indicates that *Lactobacillus* can survive in upper intestine at lowest pH for at least 90min. The purpose of this assesment was to see whether they can survive in gut for 90min or not.

Similarly with respect to another objective we have checked the ability of lactobacilli to grow at high pH (pH8) which is regular environment of lower intestine. In this experiment the *Lactobacilli* were incubated with artificial intestinal juice of pH8 with various concentrations such as 0.5%, 1% and 1.5% among all this treatments the *Lactobacillus casei* which was exposed to intestinal juice of 1% and incubated for 180min showed significant viability in terms of no. of colonies. In case of other treatments also *Lactobacillus* showed considerable viability when incubated with intestinal juice.

These *Lactobacillus* were tested for deconjugation ability of bile salts. This was confirmed by the occurance of precipitate on surface of colonies. This precipitate was due to the production of cholic acid by deconjugation of bile salts. Thus we can conclude that the *Lactobacilli* were found to be potent as they successfully grown and remain viable in the presence of highly acidic environment as well as highly alkaline environment i,e in upper and lower intestine respectively. The acidity of gastric juice, which is determined by hydrochloric acid concentration, influences the viability of

probiotic strains, which manifests in different modes, depending on bacterial strains.

The earlier report suggests that such bacteria which can grow at extreme acidic and alkaline environment and can survive there for 180 min which is normal period of retention of food in intestine. They can show competent probiotic activity within this period by multiplying and inhibiting the other micro flora. In this experiment the isolated lactobacilli strain was found to be more resistant in gastric juice than in intestinal juice.

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