# BIOCOMPATIBILITY STUDY OF *LACTOBACILLUS CASEI* ISOLATED FROM CUCUMBER AND EVALUATION OF PROBIOTIC EFFECTS IN THE HUMAN GUT

*[1]Nannu Shafakatullah*
*Dept. of Bio-Sciences,*
*Mangalore University,*
*Mangalagangothri-574199,*
*Mangalore, Karnataka, INDIA.*
*Email: shafakhath@yahoo.com*

*[2] M. Chandra*
*Dept. of Bio-Sciences,*
*Mangalore University,*
*Mangalagangothri-574199,*
*Mangalore, Karnataka, INDIA.*
*Email: drchandram1@gmail.com*

**ABSTRACT:** Probiotics are live microorganisms introduced orally in the gastrointestinal tract (GIT) that are able to contribute positively to the activity of intestinal microflora and therefore, to the health of its host. A variety of probiotic supplements are currently available in the market which target towards improving the balance and activity of the intestinal microflora. Probiotics must have robust survival properties in the gut in order to exert any beneficial health promoting properties. Many in vitro properties, such as adhesion, co-aggregation, aggregation, hydrophobicity, resistance to pH, bile, etc., are usually investigated to determine if a specific selected strain would be suitable as a probiotic. *Lactobacillus casei* has been isolated from raw cucumber and identified based on phenotypic, physiological and biochemical characteristics. The isolate was studied for its survival at acidic pH, bile salt, intestinal juice, gastric juice, different NaCl concentrations, their action against pathogens, and resistance to antibiotics, and their aggregation and co-aggregation properties. The organism has shown well resistance to gastric acids, bile digestion antibiotics and pathogenic microbes and also exhibited good aggregation and co-aggregation properties.

**Key words:** Biocompatibility, cucumber, gastrointestinal tract, *Lactobacillus casei*, probiotics

## 1. INTRODUCTION

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1]. The use of probiotic bacteria for the health of human has been increased from the last decade due to the increased research on benefits of the probiotics for human. Knowledge of gut health and awareness of general health consciousness in human leads to the search of new probiotic bacteria [2-7]. It has been proved that irritable bowel syndrome, inflammatory bowel disease, and antibiotic-induced diarrhea that occur due to the imbalance in the intestinal microflora can be reversed by the intake of probiotics. Lactobacillus species are "Generally Recognized as Safe" (GRAS) microorganisms and they are the most commonly used microorganisms as probiotics and are the most desired intestinal microflora. It is important to study the biocompatibility of the lactobacillus species before using them as probiotics. Acidic pH, bile salts, and gastric and intestinal juice in the gastrointestinal tract (GIT) are the major stress factors that the probiotics should overcome in order to survive in GIT. Other than their survival capabilities, the probiotic microbes consumed should possess the capacity to adhere and colonize in the gastro intestinal tract. More the adherence capacity of the probiotics more is their chance to retain themselves in the GIT and provide positive effect to the consumer. In accordance with the FAO/WHO (2002) guidelines [8] in order to prevent the transmission of antibiotic resistance genes from the probiotics to the intestinal pathogens it is recommended that the antibiotic resistance/susceptibility pattern of every probiotic strain (including bacteria with GRAS status) is to be determined.

Due to the development of antibiotic resistant pathogens there is increased interest in the alternative antimicrobial strategies for treatment and prevention of infections by using probiotics and their antimicrobial metabolites. Hence, antimicrobial activity against pathogens is a desirable property of a potential probiotic strain. The present study was aimed at isolation, identification, characterization and biocompatibility study of the Lactobacillus strain isolated from cucumber. The biocompatibility properties were investigated through in vitro assays.

## 2. MATERIAL AND METHODS

### 2.1 Isolation and Identification of Bacteria

Fresh cucumber juice was prepared and 1ml of this was serially diluted to $10^{-5}$ to $10^{-6}$ and inoculated 0.1 ml on to lactobacillus MRS agar plates and incubated at 37°C for 24-48 hours anaerobically. Gram's staining, catalase activity, gas production from glucose, MRVP test, gelatine hydrolysis, growth in different NaCl was determined according to methods for lactic acid

bacteria [9-10]. The identification work was done according to the methods described in Bergey's Manual [11] and the Prokaryotes. All the strains were maintained by weekly sub culturing from 48hrs MRS agar cultures. Growth characteristics at different temperature were monitored for 7 days period. Production of ammonia from arginine was done according to the method described by Abdel-Malek and Gibson, 1948 [12], Nitrate reduction was done as described by Gerhardt et al., 1981 [13]. The isolates were maintained in MRS broth, stock cultures were stored on agar slants in refrigerator and also freeze dried and stored for longer period.

**2.2 Growth at acidic pH**

The growth behavior of culture isolate was observed at acidic pH to find the acid tolerance capacity of organisms. The Isolate was inoculated in MRS broth with pH 2 and 3 and incubation was done at 37˚C for 48-72 hrs. During these incubation time cells growth was observed and results were recorded.

**2.3 Transit Tolerance**

**2.3.1 Simulated Gastric Juice**

The simulated gastric juice was prepared freshly by suspending pepsin 1:10000 (3g/L) (SRL) in sterile NaCl (0.5%) and the pH was adjusted to 2.0 and 3.0 respectively. This was filter sterilized using 0.45µm filter. The L. casei was grown in de Man, Rogosa and Sharpe (MRS) broth at 37°C for 24 h and centrifuged at 2,500 × g at 4°C for 10 min. The collected cells were resuspended in sterile saline (0.5% NaCl) and inoculated into the simulated gastric juice (pH 2.0 and 3.0) at 108 cfu/ ml. The test was done in triplicates. Because the pH in the human stomach ranges from 1 (during fasting) to 4.5 (after a meal) and food ingestion can take up to 3 h, tolerance was assayed by determining the total viable count at 0, 1.5 and 3-h incubation in simulated gastric juice (pH 2.0 and 3.0).

**2.3.2 Simulated Intestinal Juice**

The simulated intestinal juice was prepared freshly by suspending pancreatin (1g/L) in sterile NaCl (0.5%) and adjusted the pH to 8.0. This was again filter sterilized by using 0.45µm filter. 1ml of the suspension of the *L. casei* was inoculated into 9ml of simulated intestinal juice (pH 8.0) and incubated at 37°C. The test was done in triplicates. The survival rate was assessed by determining the total viable count at 0, 2, 4, 6 and 8hrs of incubation.

**2.3.3 Bile Tolerance**

Bile plays an important role in the survival of bacteria in the small intestine. Food remains in the small intestine for around 4-6 hours [14] till it gets absorbed. The *L. casei* was screened for its survival at different bile concentrations. The organism was inoculated into 10 ml MRS broth in test tubes and incubated at 37ºC overnight in anaerobic condition. 100µl of active culture was inoculated into fresh MRS broth tubes with pH 6.5 containing 0.3%, 0.5% and 1.0% bile (CDH India). The bacterial survival was measured by MRS agar colony count by taking 100µl culture for 0, 30, 60, 90 and 180 min and aliquots spread onto MRS agar plates to calculate the CFU/ml. The experiment was determined in triplicate to calculate intra-assay variation. CFU/ml was recorded.

**2.4 Aggregation**

Aggregation [15] assay was performed by growing the isolates in MRS broth for 24 hours anaerobically at 37°C. The cells were harvested by centrifugation at 5000 rpm for 15 min, at 4˚C. The cells were washed twice and re-suspended in phosphate buffered saline (PBS) to give viable counts of approximately 108 CFU/ ml. Four ml of the cell suspension was mixed for 10 seconds in a sterile tube to determine auto aggregation during 5h of incubation, at room temperature. The upper suspension was used in each hour by transferring 0.1ml to another 3.9ml of phosphate buffer solution, and the optical density at 660nm was measured. Tests were carried out in triplicate and the results were averaged.

The auto aggregation percentage was calculated by the formula: 1- ($A_t/A_0$) X 100, where, $A_t$ represents the absorbance at time t = 1, 2, 3, 4 or 5, and $A_0$ the absorbance at t = 0. Aggregation abilities of microorganisms were screened by visual observation.

**2.5 Co-aggregation**

The bacterial cells were harvested by centrifugation at 5000 rpm for 15 min after incubation at 37°C for 18h, washed twice and resuspended in phosphate buffered saline (PBS) to give viable counts of approximately 108 CFU /ml. Equal volumes (2 ml) of each cell suspension were mixed together in pairs by vortexing. Control tubes were set up at the same time, containing 4 ml of each bacterial suspension on its own. The absorbance at 660 nm of the suspensions was measured after mixing and after 5 h of incubation. The percentage of co-aggregation was calculated using the equation [16] as,

Co-aggregation (%) = [(Ax + Ay) /2) - A (x+ y)] / [(Ax + Ay)/ 2] × 100

Where x and y represent each of the two strains in the control tubes, and (x + y) the mixture of isolate tested for co-aggregation.

**2.6 Antibiotic Sensitivity test**

Antibiotic sensitivity test of the isolate was performed by standard disc diffusion method (NCCLS 1999) towards thirteen antibiotics. The pure culture of L. casei suspension was spread on the MRS agar plates to form a uniform smear. Selected antibiotic discs were aseptically transferred on to the seeded plates. The diameters of the zone of inhibition were measured using antibiotic zone scale (Himedia India) after 24 h of incubation. The experiment was repeated thrice and the average inhibitory zone diameters were compared with the standards provided by the National Committee for Clinical Laboratory Standards. Diameters of inhibition zones were measured and results were

expressed as sensitive, S (≥ 21 mm); intermediate, I (16-20 mm) and resistant, R (≤ 15 mm), respectively according to that described by Vlková et al., 2006 [17].

### 2.7 Antimicrobial Activity Test

Agar well diffusion method [18] was used to determine the inhibitory capacity of the *L. casei* against pathogenic strains such as *Pseudomonas aeruginosa, Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Bacillus subtilis*. The isolate and pathogenic strains were incubated in MRS agar medium at 37°C for 24 to 48 h.

## 3. RESULT AND DISCUSSION

### 3.1 Physiological and Biochemical Characterization

The isolate was subjected to Gram's staining and it was examined under light microscope. The strain gave blue-purple color with staining; hence it was Gram positive bacteria. Isolate was tested for catalase activity. It was catalase negative (do not show catalase activity). To test the gas production from glucose test tubes were observed for 5 days. The Isolate has shown no gas production this indicates its homofermentative nature (Table 1). Another criterion for the identification the isolate was the ability of growth at different temperatures (Table 2). From the results of 5 days observation the isolate showed growth at 15-50 °C. Growth at different NaCl concentrations was observed. The isolate has the ability to grow at 2-6% NaCl concentration. Arginine hydrolysis test was another step to follow the identification procedure. The isolate which gave the bright orange were accepted that they can produce ammonia from arginine. The yellow colour indicated negative arginine hydrolysis. The isolate has shown -ve for arginine hydrolysis. Hydrolysis of starch was negative by isolate. The isolate was non motile, non spore forming. The most useful test for the determination of strain differences is carbohydrate fermentation. Twenty one (other than glucose) different carbohydrates were used for identification. They gave different fermentation patterns when they were compared. The patterns are showed in Table 3.

**Table 1: Morphological, cultural and physiological characteristics of the isolates.**

| Sl. No. | Isolate No. | Catalase test | Size(mm) | Shape | Margin | Gram's staining | Shape | Motility | Gas from glucose | Spore formation | Arginine utilization | Starch Hydrolysis | Growth in broth | Growth on slants | MR Test | VP Test | Nacl-2% | NaCl-4% | NaCl-6% | NaCl-8% | Nacl-10% | Indole test |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CU1 | -ve | >1 | Circular | Entire | +ve | rods pairs/ chains | -ve | -ve | -ve | -ve | -ve | Sediment | Smooth / flat | +ve | -ve | +++ | +++ | + | - | - | -ve |

(+++) Luxurious growth, (++) Moderate growth, (+) less growth, (-) No growth

**Table 2: Physiological characteristics of the isolates**

| Sl.No. | Isolate No. | Growth at different temperature ($^0$C) | | | | | | | Growth at different pH | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 15 | 30 | 37 | 45 | 50 | 55 | 60 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1 | CU1 | + | ++ | +++ | ++ | + | - | - | + | + | + | +++ | +++ | +++ | +++ | ++ |

(+++) Luxurious growth, (++) Moderate growth, (+) less growth, (-) No growth

**Table 3: Biochemical characteristics of the isolates by utilization of carbon sources**

| Sl. No. | Isolate No. | Fructose | Galactose | Cellobiose | Esculin | Inulin | Rhamnose | Melibiose | Mannitol | Maltose | Mannose | Ribose | Trehalose | Arabinose | Lactose | Sucrose | Xylose | Salicin | Cystein | Sorbitol | Raffinose | Glycerol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CU1 | + | + | - | + | - | - | - | + | + | + | - | + | - | + | + | - | + | - | - | + | - |

Positive reaction (+), negative reaction (-)

### 3.2 Resistance to acidic pH

Being resistant to low pH is one of the major selection criteria for probiotic strains [19-20]. Since, to reach the small intestine they have to pass through from the stressful conditions of stomach [21]. Although in the stomach, pH can be as low as 1.0, in most in vitro assays pH 3.0 has been preferred. Due to the fact that a significant decrease in the viability of strains is often observed at pH 2.0 and below [22]. Sudden decrease in the survival rate of the isolate has been observed at pH 2. At the third hour the survival rate reduced below 5%. At pH3 more than 10% survival has been observed after 3 hours of incubation.

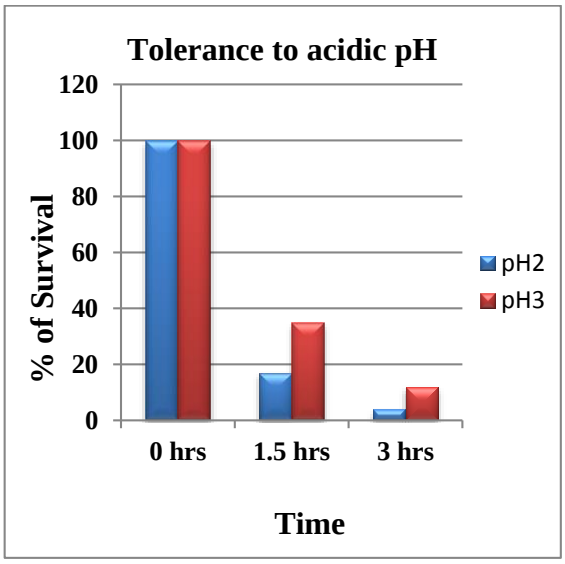


**Fig.1. Acidic pH tolerance by *L. casei***

### 3.3 Tolerance against Bile

The isolate was screened for its ability to tolerate the bile salt. Although the bile concentration of the human gastrointestinal tract varies, the mean intestinal bile concentration is believed to be 0.3% w/v and the staying time is suggested to be 4 h. Strain was screened for 3 hours in 0.3%, 0.5% and 1.0% of bile salt for its survival. The cfu values were observed. According to the results the isolate was resistant to 0.3% and 0.5% bile salt, whereas sudden fall in the number of survival organisms has been observed at 1.00% bile. The survival rate reached to 5% at the end of 3 hours of incubation at 1.00% of bile.

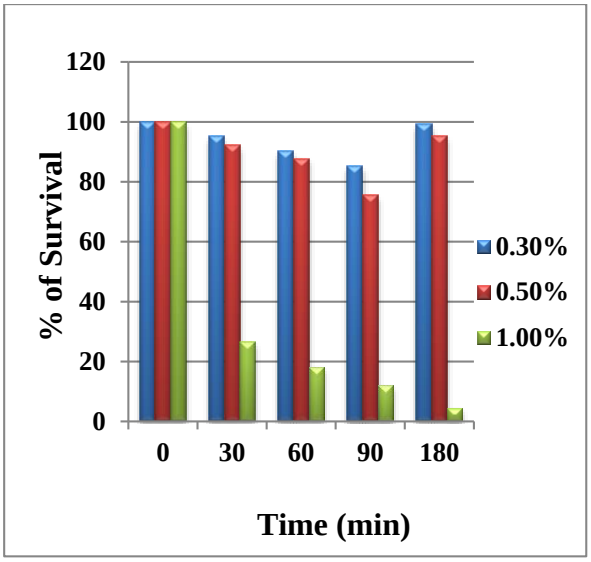


**Fig.2. Bile salt tolerance to by *L. casei***

### 3.4 Tolerance to Gastric Juice

The degree of gastric juice resistance exhibited by isolate was determined and results (Figure 3) showed that >75% of survival has been observed in gastric juice at pH 3 for 1.5 hours of incubation, whereas at pH2 the survival rate was >30% for 1.5 hours of incubation. But at 3 hours of incubation the survival rate at pH 3 reached to <40% and at pH 2 it reached to <10%.

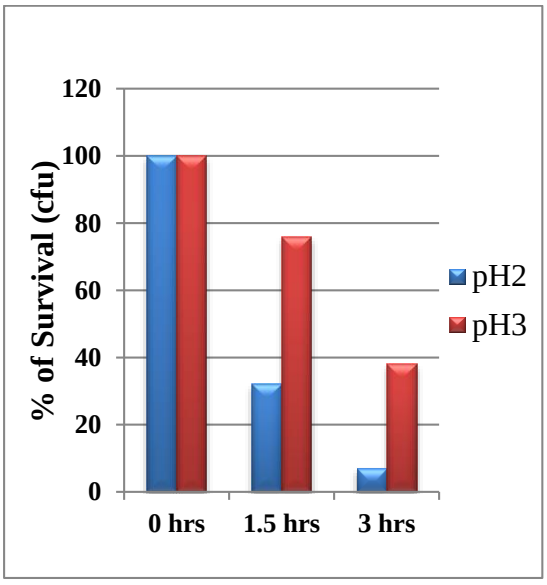


**Fig.3.Gastric juice tolerance by *L. casei***

### 3.5 Tolerance to Intestinal Juice

The isolate was tested for its ability to grow in intestinal juice. It appears that the strain exhibited good resistance to intestinal juice at pH 8 for four hours of growth (Figure 4). Good multiplication of all the isolates has been found at 6th hour of incubation.

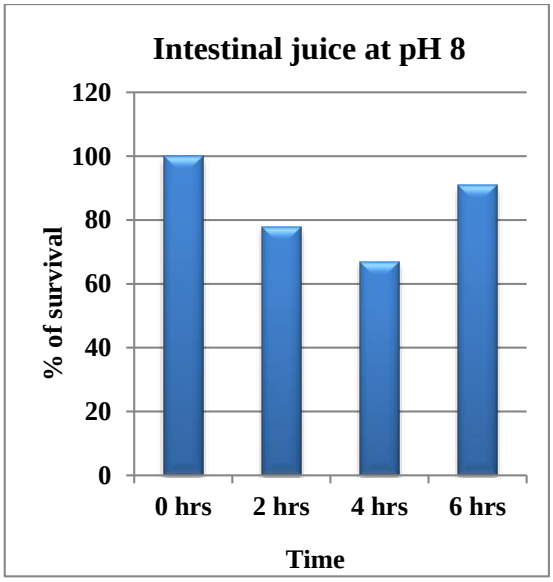


**Fig.4. Intestinal juice tolerance by *L. casei***

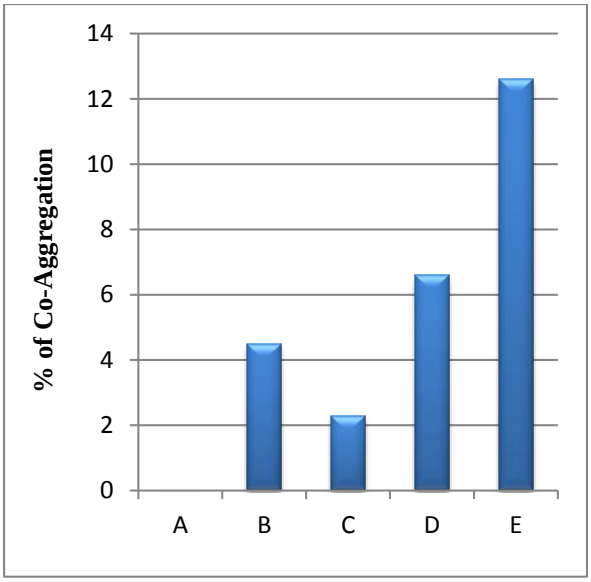


**Fig.6. Co-aggregation by *L. casei*, A**- *B. subtilis.* **B**- *E.coli*, **C**- *K. pneumonia*, **D**- *P. aeruginosa,* **E**- *S.aureus*

### 3.6 Aggregation

On the basis of sedimentation characteristics aggregation capability of the isolate was tested. L. casei has exhibited good amount of aggregation during the test time of 5 hours (Figure 5).

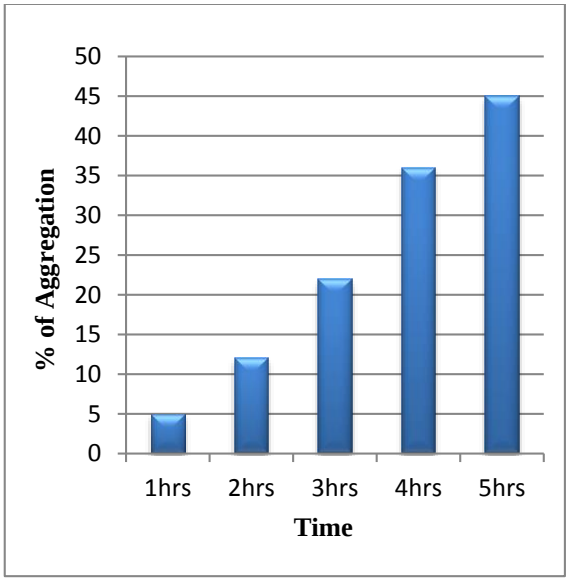


**Fig.5. Aggregation by *L. casei***

### 3.7 Co-aggregation

The co-aggregations of L. casei with five pathogenic bacteria were examined. Results were expressed as the percentage reduction after 5 h in the absorbance of a mixed suspension compared with the individual suspension. Good co-aggregation of L. casei with S. aureus has been seen. There was no co-aggregation between L. casei and B. subtilis. 2-6% of co-aggregation has been seen with E. coli, K. pneumonia and P. aeruginosa (Figure 6).

### 3.8 Antibiotic Sensitivity test

The determination of antibiotic sensitivity of the isolate is an important prerequisite prior to considering it safe for human and animal consumption. The isolate was subjected to antibiotic susceptibility test. The results are given in Table 4. The isolate was resistant to most of the antibiotics used. According to earlier reports, specific antibiotic resistance traits among probiotic strains may be desirable [23]. It has been said by many authors that probiotics should be resistant to certain antibiotics when used along with antibiotics to prevent gastrointestinal disorders. Whereas others claim that antibiotics resistant probiotics used may serve as host of antibiotic resistance genes, which can be transferred to pathogenic bacteria.

**Table 4: Antibiotic resistance of the isolates**

| **Antibiotics** | ***Lactobacillus casei*** |
|---|---|
| Ampicillin | R |
| Chloramphenicol | S |
| Erythromycin | S |
| Gentamycin | R |
| Kananamycin | R |
| Lincomycin | S |
| Nalidixic acid | S |
| Neomycin | R |
| Norfloxacin | S |
| Penicillin | S |
| Streptomycin | I |
| Tetracycline | R |

| | |
|---|---|
| Vancomycin | R |

S-Sensitive, R-Resistance, I-Intermediate

### 3.9 Antimicrobial Activity Test

Antimicrobial activity helps to select the potential probiotics strains. Antimicrobial activity usually targets the intestinal pathogens. The isolate was examined for antibacterial activity. L. casei was grown with indicator microorganisms, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus and Bacillus subtilis. The antibacterial effect on the indicator microorganisms was determined by diameter of inhibition zones. Lactobacilli casei has a high ability to inhibit the growth of pathogenic microorganisms. The degree of inhibition was highest in S. aureus, whereas the inhibition was moderate in E. coli, K. pneumonia and P. aeruginosa. The isolate could not inhibit the growth of spore forming B. subtilis.

**Table 5: Antimicrobial activity of the isolates**

| **Strain** | ***Lactobacillus casei*** |
|---|---|
| ***B. subtilis*** | - |
| ***E.coli*** | ++ |
| ***Klebsiella pneumonia*** | ++ |
| ***P. aeruginosa*** | ++ |
| ***Staphylococcus aureus*** | +++ |

Degree of inhibition: + = Moderate inhibition zone (6-9 mm); ++ = Strong inhibition zone (10-14mm) +++ = Very strong inhibition zone (15-18mm); - = No inhibition zone

## 4. CONCLUSION

Lactobacillus casei has shown good survival in acidic pH, different bile concentrations, gastric juice and intestinal juice. The organism exhibited good survival in the presence of different antibiotics. The isolate is also able to inhibit the growth of different pathogenic microorganisms examined. All these characteristics of the organism will help it to survive in the stomach and proliferate in the intestine. This will help strains to reach the small intestine and colon and contributing to the balance of intestinal microflora.

**ACKNOWLEDGEMENT**

The authors are thankful to Indian Council of Medical Research (ICMR) for financial assistance. No. 3/1/2/40/Nut./2013 dated 20.11.2013.

**REFERENCES**

1. FAO/WHO, 2001 "Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria: report of a Joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria," Tech. Rep., Food and Agriculture Organization/World Health Organization, Cordoba, Argentina.
2. J. M. T. Hamilton-Miller, S. Shah, and C. T. Smith, 1996. "'Probiotic' remedies are not what they seem," The British Medical Journal, vol. 312, no. 7022, pp. 55–56.
3. J. M. T. Hamilton-Miller, S. Shah, and J. T. Winkler, 1999. "Public health issues arising from microbiological and labelling quality of foods and supplements containing probiotic microorganisms," Public Health Nutrition, vol. 2, no. 2, pp. 223–229.
4. F. Canganella, S. Paganini, M. Ovidi et al., 1997. "A microbiological investigation on probiotic pharmaceutical products used for human health," Microbiological Research, vol. 152, no. 2, pp. 171–179.
5. G. Klein, A. Pack, C. Bonaparte, and G. Reuter, 1998. "Taxonomy and physiology of probiotic lactic acid bacteria," International Journal of Food Microbiology, vol. 41, no. 2, pp. 103–125.
6. U. Schillinger, 1999. "Isolation and identification of lactobacilli from novel-type probiotic and mild yoghurts and their stability during refrigerated storage," International Journal of Food Microbiology, vol. 47, no. 1-2, pp. 79–87.
7. C. Dunne, L. O'Mahony, L. Murphy et al., 2001. "In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings," The American Journal of Clinical Nutrition, vol. 73, no. 2, pp. 386–392.
8. FAO/WHO, 2002. "Guidelines for the evaluation of probiotics in food," Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food, Food and Agriculture Organization/World Health Organization, London, UK, Ontario, Canada.
9. Hargrove, R.E. and J.A. Alford, 1978. Growth rate and feed efficiency of rats fed yoghurt and other fermented milks. J. Dairy Sci., 61: 11-19.
10. Roissart, H. and F.M. Luguet, 1994. Bacterieslactiques. Aspects fondamentauxettechnologiques. Uriage, Lorica, France. Vol.1. p. 605.

**11.** Holzapfel, H.W. and U. Schillinger, 1992. The genus Leuconostoc in: The prokaryotes (2nd Edn.). Eds. Balows. A Truper H.G. DwinkinM.Harder W and Schleifer K. Springer-Verlag, New York, USA, pp: 1508-1534.

**12.** Abdel-Malik, Y. and T. Gibsoren, 1948. Studies on the bacteriology of milk. The Streptococci of milk. J. Dairy Res., 15: 233.

**13.** Gerhardt, P., R.G.E. Murray, R.N. Costilow, E.W. Nester, W.A. Wood, N. R. Kneg and G.B. Philips, 1981. Manual of Methods for General Bacteriology. eds. Gerhardt P. Murray R.G.E. CostiloSw R.N. Nester E.W. Wood, W.A. Krieg N.R. Philips G.B. Am. Soc. Microbiol., Washington USA, pp: 419.

**14.** Prasad, J., Gill, H., Smart, J., and Gopal, P.K. 1998. Selection and Characterization of Lactobacillus and Bifidobacterium strains for use as probiotic. International Dairy Journal 8:993-1002.

**15.** Del Re, B., Sgorbati, B., Miglioli, M. and Palenzona, D. (2000) Adhesion, autoaggregation and hydrophobicity of 13 strains of Bifidobacteriumlongum. Letters in Applied Microbiology 31, 438–442

**16.** Handley PS, Harty DWS, Wyatt JE, Brown CR, Doran JP, Gibbs ACC. 1987. A comparison of the adhesion, coaggregation and cell-surface hydrophobicity propertiesoffibrillar and fimbriate strains of Streptococcus salivarius. J Gen Microbiol.; 133: 3207-3217.

**17.** Vlkova´ E., Rada V., Popela´rˇova´ P., Trojanova´ I., Killer J. 2006. Antimicrobial susceptibility of bifidobacteria isolated from gastrointestinal tract of calves. Livestock Science, 105, 253–259.

**18.** Liasi S.A., Azmi T.I., Hassan,M.D., Shuhaimi M., Rosfarizan M. and Ariff A.B., 2009. Antimicrobial activity and antibiotic sensitivity of three isolates of lactic acid bacteria from fermented fish product, Budu. Malaysian Journal of Microbiology, 5(1), 33-37

**19.** Ouwehand, A., Salminen, S., and Isolauri, E. 2002. Probiotics: an overview of beneficial effects. Antonie van Leeuwenhoek, 82: 279-289.

**20.** Çakır, İ. (2003). Determination of some probiotic properties on Lactobacilli and Bifidobacteria. Ankara University Thesis of Ph.D.

**21.** Chou, L.S and Weimer, B. (1999). Isolation and characterization of acid and bile tolerant isolates from strains of Lactobacillus acidophilus. Journal of Dairy Science 82:23-31.

**22.** Prasad R., Sankhyan S.K. and Karim S.A., 1998. Growth performance of broiler rabbits fed on diets containing various types of protein supplements, Indian J. Anim. Prod.Manage., 14 (4), 227-230

**23.** Charteris WP, Kelly PM, Morelli L, Collins JK (1998). Antibiotic susceptibility of potentially probiotic Lactobacillus species.Journal of Food Protection 61(12): 1636-1643.