Chapter 2
Lactobacillus Model Moiety a New Era Dosage Form as Nutraceuticals and Therapeutic Mediator

Abhinandan R. Patil, Sunita S. Shinde, Pratik S. Kakade and John I. D'souza

Abstract In this era Bacteria are mostly considered as pathogenic. Bacteria though are pathogenic but still a few are friendly and essential for human growth and immunity; called as Probiotics. *Lactobacillus* model moiety is one of such probiotic bacterium which when introduced in sufficient colony serves as new prophylaxis and curing agent. This will increase the innate immunity of humans. Indian market lacks the quality uni-strain product of the probiotics as Nutraceuticals and as Health enhancing drug product. Global market is full of multi-strain microbes dosage form. The best mode to utilize Nutraceutical aspect of probiotics is to convert in dry form. These purified colony; later converted into the solid dry form by spray dry (JISL mini-spray drier) technique. Starch, lactose were used as thermo-protective agent while heat drying technique. Anti-microbial screenings carried out along with In vitro cytotoxicity studies. In vitro cytotoxicity screening evaluated that *Lactobacillus* powder showings anti-cancer activity nearly same as standard drug with no side effects. Dry powder increased the shelf life of the microbes that resulted in maintenance of viability and activity.

Keywords *Lactobacillus* model moiety · Nutraceuticals · Probiotics · Pathogenic · Uni-strain

2.1 Introduction

There are more bacteria in the world today than all the humans ever born. Most of them considered as pathogen. As every coin had, two sides besides mostly bacteria are pathogen but still few are friendly essential for human growth and immunity.

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Such friendly bacteria are called probiotics. The following are microorganisms considered to be human probiotics: *Lactobacillus species:* ***Lactobacillus model moiety:*** *L. amylovorus* etc., *Bifidobacterium* species: *B. adolescentis*, etc., other lactic acid bacteria: *Enterococcus faecium, Lactococcus lactis, Leuconstoc mesenteroides, Pediococcus acidilactici, Streptococcus thermophilus* and Nonlactic acid bacteria: *Bacillus subtilis* etc.

Lactobacillus organisms are normal inhabitants of the human intestine and vagina. *Lactobacilli* are gram-positive facultative anaerobes; non-spore forming; and non-flagellated, rod or coccobacilli. To date, some 56 species of *Lactobacillus* have been identified.

- *Lactobacillus model moiety* is the most commonly known probiotic bacterium. It is found primarily in the small intestine where it produces natural antibiotics called "lactocidin" and "acidophilin". These increase immune resistance against such harmful bacteria and fungi as *Candida albicans, Salmonella, E. coli*, and *Staphylococcus aureus*.
- *Lactobacillus model moiety* implants itself the intestinal walls, as well as on the lining of the vagina, cervix, and urethra, thereby preventing other organisms from multiplying to the extent that they can cause infections. For years, it was assumed that it was the most beneficial form of the "good" bacteria; but recent research has revealed that *L. rhamnosus* may be of more importance.
- *Lactobacillus model moiety* helps control intestinal infections, thus reducing the potential of diarrhea and other infections or diseases. It also inhibits some types of cancer and helps control serum cholesterol levels. However, reaching the intestines is the problem because the *Lactobacillus model moiety* found in most commercial yogurts cannot live with stomach acids and bile.

Mostly present recently in dairy product but very rarely used in form of dosage form for treatment and as prophylaxis of diseases. To sub-culture the friendly microbes of *lactobacilli* in MRS (de Man, Rogosa and Sharpe) media is unique feature in form of Colony forming unit (CFU). To produce agent alternative to Antibiotics as Probiotics produces natural antibiotics called "lactocidin" and "acidophilin" is basic theme of formulation generation that will contribute to generate Innate immunity. Thus this enhances friendly micro flora in Gastrointestinal tract (GI) to maintain proper bowl movement. Spray drying is one of the oldest methods of encapsulation used initially for flavour capture. It is a single step continuous processing operation. The process can produce purest and finest powders with high sterility reducing the post unit operation like grinding and conditioning. Spray dried powder particles are relatively small and uniform in size and shape. Spray drying allows a uniform dispersion of powder particle by diluting the bioactive core when a low amount is required. Sulphorhodamine (SRB) assay give general idea to screen anti-cancer activity.

2.2 Materials and Methods

2.2.1 Preparation of Bacterial Suspension

Lactobacilli Species (*Lactobacillus model moiety*) were obtained from the "Warana Diary" (Warananagar) M.S; for long-term maintenance, this organism was stored as glass bead cultures in freezer at −20 °C. Man, Rogosa, Sharpe—MRS broth Obtained from the "Siffin Pharma" (Germany) (Fig. 2.1).

Latter these microbes were harvested into the natural media like pasteurised milk of cow and buffalos.

2.3 Methodology

The exact four procedure applied to generate the formulation as follows.

2.3.1 Part A

The milk of cow/buffalo were pasteurised before use to nil the other microbes if present in the milk. The pasteurization and tyndallisation gave the platform to the new inoculated microbes to get flourish into the milk after cooling of this milk. Tyndallisation was carried out in beaker for 3 days and then microbes were inoculated to get semi-solids beads (Fig. 2.2).

2.3.2 Part B: Homogenization and Spray Dry

This semi-solid mass obtained by 24 h incubation in oven were broken down/converted into liquid state by Homogenization. 2,300 rpm (rotation per min) used to break the bead/semi solid mass formation. The spray-drying process of *Lactobacilli* Species in the various media was undertaken in a laboratory scale spray dryer (Jisl mini-spray dry). The excipients used for the spray dry were lactose and starch solution. This combination of lactose and starch (2:1) acts as the thermo-protective agent to prevent the mortality of the microbes or decrease in the cell count while at the spray dry procedure due to the heavy inlet temperature. The viability at different combination were carried out for lactose and starch solution as 1:1, 1:2, 2:1 etc. to check for good results (Fig. 2.3).

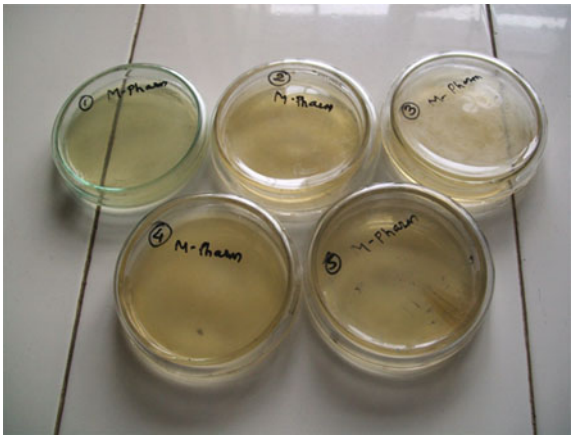

Fig. 2.1 Multiple plates

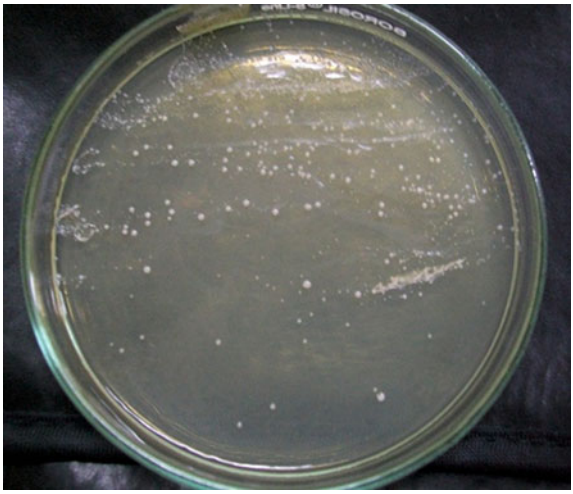

Fig. 2.2 Plate of MRS

2.3.3 Part C: In Vitro Cytotoxicity Studies: SRB Assay

Cell line: Colon cancer HT-29 procured from (N.C.C.S) National Centre for Cell Science, Pune. Standard anticancer drug Capacitabin obtained as gift sample from the Atmatara foundation and research unit (Kolhapur). Stock solution of *Lactobacillus model moiety* powder of 1 mM in 0.25 % Dimethyl sulphoxide (DMSO) was prepared and further dilution done in 10, 50 and 100 μM with phosphate buffer saline (PBS). Stock solution of Capecitabine of 1 mM in distilled water was prepared and further dilution done in 10, 50 and 100 μM with phosphate buffer saline (PBS).

Fig. 2.3 Spray dry JISL

2.3.4 Part D: Anti-microbial Screening (Well Assay)

MRS agar plates were prepared; that surface of the MRS agar plates was inoculated with the swab containing 24 h culture *V. cholera* and for *S. dysentriae*. Wells were punched with the gel puncher 80 mg *Lactobacillus model moiety* powder (L.A.P.) were added to the wells punched in the centre of the plates. Plates were incubated for 24 h at 37 °C. *S. dysentriae* were also incubated on the MRS agar plates, separately and wells were punched. Wells, with a diameter of 5 mm, then cut in the agar using sterile gel puncher.

2.4 Results and Discussion

The results for the **Part A** work were in form of the CFU (colony forming units) count as,

Fig. 2.4 CFU count in semi solids mass form in beaker

Table 2.1 Batch dilution at 10^6 CFU/ml by plate count for the cow milk

Sr. no.	1:1	1:2	2:1
1	12	15	35
2	12	14	34
3	12	14	34

- Count in Milch animals

  (a) Cow = 10^6 CFU/ml (colony forming units).
  (b) Buffalos = 10^7 CFU/ml (colony forming units).

This difference in the cell count was due to the difference in the fat, nutrients avail in both animals milk (Fig. 2.4).

The results for the **Part B and Part C** work were obtained in form liquid generated at the rotation per min (rpm) via Homogenization carried out from 1,000 to 2,300 rpm. The results seen in case at 2,300 rpm for 20 min were optimal. This solution obtained by this above procedural was acceptable for the next spray dry mythology. The large size particle generally clogged the spray dry at the gun nozzle but size reduction at 2,300 rpm for 20 min prevented the above discussed problem.

The spray drying carried out by taking the combination of the Lactose and starch in different ratios and the cell count obtained by serial dilution for the cow milk is given in Table 2.1.

The results were optimal for the cow milk as by spray dry. The 2:1 ratio of (lactose and starch) gave good cell count as 35×10^6 CFU/ml (average).

Similarly, the spray drying carried out by taking the combination of the Lactose and starch in different ratios and the cell count obtained by serial dilution for the Buffalo milk is given in Table 2.2.

Table 2.2 Batch dilution at 10^6 CFU/ml by plate count for the buffalos milk

Sr. no.	1:1	1:2	2:1
1	12	14	21
2	12	15	21
3	12	14	22

Table 2.3 Other thermo protective agent used and there effects

Nature/excipients	Lactose	Starch	Lactose + Starch
Powder nature	Amorphous/nonhygroscopic	Hygroscopic	Amorphous/hygroscopic
Cell count	Less	Less	Good

The results were optimal for the Buffalos milk as by spray dry. The 2:1 ratio of (lactose and starch) gave good cell count as 21×10^6 CFU/ml (average).

2.4.1 Other Thermo Protective Agent Used and There Effects Observed by Spray Dry

The spray dry results obtained in case of the Lactose alone or starch alone is not so good than lactose and starch used in combination at (2:1) ratios is given in Table 2.3.

2.4.2 Survival Rate During Spray Drying

The powder were collected and packed in the air tight container. The mortality rate is less by the utilization of the lactose and the starch in the composition (2:1) kept in dark air tight container at room temperature than keeping in open containers.

The power viability or cell count has been checked by simple serial dilution technique for both cow and buffalos spray dried powder as:

For 1 g in 10 ml = 10×10^6 CFU/ml *approx in case of cow;
1 g in 10 ml = 70×10^6 CFU/ml *approx in case of buffalo spray dried powder.

2.4.3 Part D: In Vitro Cell Cytotoxicity Studies

(Table 2.4) (Fig. 2.5).

Table 2.4 SRB assay

Concentration (μM/ml)	Percentage cell cytotoxicity [a](L.A.P.)/test	Percentage cell cytotoxicity [b]std. drug
10	48.87 ± 0.9536	50.23 ± 0.5752
50	68.56 ± 0.6894	69.85 ± 0.5275
100	97.83 ± 0.3768	86.53 ± 0.3217
IC_{50} value	11	9

[a] (L.A.P.) = *Lactobacillus model moiety*
[b] Std. drug = Capacitabin/capa.

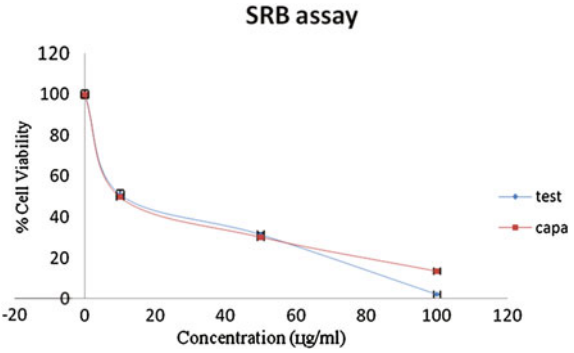

Fig. 2.5 SRB assay

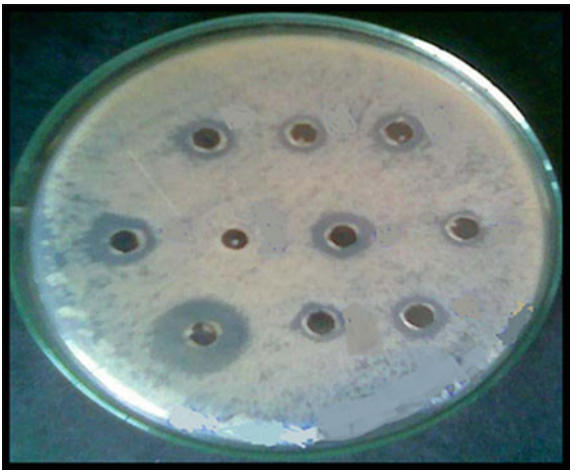

Fig. 2.6 Zone of inhibition on *Vibrio cholera*

2.4.4 Part E

The inhibitory activity against *V. cholera* and *S. dysentriae* was seen in form of clearance obtained with each organism as (Figs. 2.6, 2.7):

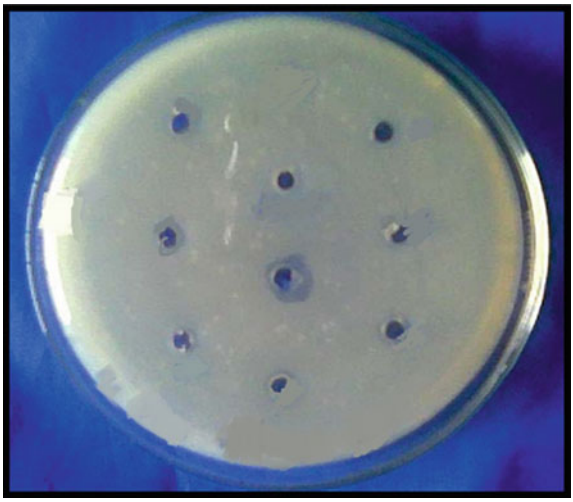

Fig. 2.7 Zone of inhibition of *Shigela dysentriae*

Table 2.5 Zone of inhibition study

Sr. no.	Name of organism	Zone of inhibition (mm)
1	*Vibrio cholera*	9
2	*Shigella dysentriae*	7

2.4.5 Organisms Zone of Clearance

See Table 2.5.

2.5 Conclusion

Dry powder increased the shelf life of the microbes and the formulation can be used any time by mere opening the pack kept at the airtight container. Lactose and starch given optimized thermo protection effect that prevented the cell death by heat in the spray dry temperature of inlet heat of 100–110 °C.

Revalidation of the powder carried out by serial dilution method in which the spray dry powder is taken in the saline water and is added in 10 ml water, and then shaken well. From that 10 ml solution 1 ml of the sample is transfer in next 10 ml test tube and shaken and diluted to 10 times. This procedure repeated till 10^6 times. Latter plates of MRS media were prepared to check cell count, which was found optimal in case of the buffalos spray dry powder than cow's powder. Best Nutraceutical agent was been accessed by antibiotic effects seen successfully in case of *V. cholera* and *S. dysentriae* zone of inhibition study. Better choice therapeutic mediators in cancer study were verified by SRB test.

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