IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 10, Issue 10 Ver. II (October 2017), PP 45-51
www.iosrjournals.org

Studies on optimization of cost effective medium for production of Probiotic *Lactobacillus plantarum* RSLP003, and enhanced stability even at simulated harsh conditions by Co-Microencapsulation

G.Babul Reddy[1*], Karuganti Sukumar[1], G.Swarnabala[2], K. Ravi Kumar[2], B.Rajasekhar[2], K.Venkatanand Reddy[2], V.Muneel[2]
RADOSOM, R&D center, Som Phytopharma (India) Ltd, Hyderabad, India
Corresponding author: babulreddy@agrilife.in

Abstract: *Lactobacillus plantarum RSLP003 was isolated from Fruit juice wastes and identified by phenotypic studies as well as biochemical characterization. A cost effective growth medium for Lactobacillus plantarum RSLP003 was optimized. In the present study, Modified MRS (MMRS) medium was formulated by using Cabbage juice medium (CJM) and half strength MRS medium, which showed highest yield in broth (5.2X10^9/ml)and cell pellet (4.5X10^{11}/g).Co-Microencapsulation was done employing Calcium alginate, Pregelatinized starch, Chitosan, Flax seed oil and Inulin. Inulin was used as a Prebiotic. Survivability of co-microencapsulated cells were checked in low pH condition (pH 1.5), different bile concentrations (1%, 1.5%&2%) and different temperatures (70^{0}C, 80^{0}C, 90^{0}C) i.e. simulated gastric environment. In all the parameters co-microencapsulated cells showed good survivability when compared to the free cells. These results establish Lactobacillus plantarum RSLP003 as a potential human and animal gut probiotic.*

Keywords: *Cabbage juice medium (CJM), Co-Microencapsulation, Lactobacillus plantarum, Prebiotic, Simulated gastric environment.*

Date of Submission: 09-10-2017 Date of acceptance: 27-10-2017

I. Introduction

In recent days, usage of Antibiotics is revisited, due to misusage and increasing antibiotic resistance of disease causative microorganisms and further side effects on host. Therefore, to replace the Antibiotics an alternative, safe and long term solution for treating the diseases is required. Probiotics have been proved to be safe, natural and eco – friendly alternative.

Probiotics are living microbes when administered in required amounts, show beneficial effects on host [1]. Among the probiotics, *Bifidobacteria* and Lactic acid bacteria (LAB) are the most common microbial groups [2]. In Lactic Acid Bacteria, species of *Lactobacillus*, *Enterococcus, Pediococcus* and *Bifidobacterium* are most prevalent [3]. *Lactobacillus* genus includes various species that shows high degree of diversity. Among all the species, *Lactobacillus plantarum* is flexible and versatile species which can be found in various environmental niches. *L.plantarum* is usually found as a natural habitat in human gastro intestinal tract [4].

Lactobacillus plantarum is a Gram positive, non-spore forming and homofermentative, rod shaped bacterium [5]. *Lactobacillus plantarum* differs from other species by showing unique features like large genome which shows the ability to adapt at various conditions and fermentation of different carbohydrates [4]. *L.plantarum* requires high Manganese and it can build up large quantities of intracellular manganese [6].Manganese protects the microbe against the toxicity of oxygen by reducing them to Hydrogen peroxide and oxygen radicals[7].By Manganese co-factor Pseudocatalase, Hydrogen peroxide is converted to oxygen and water [8].

In order to show useful effects of probiotics, it must survive in severe acidic conditions of gastrointestinal tract and high load of microbes should reach the large intestine which helps them to colonize and proliferate. Literature shows recommendation of ingestion of probiotic at a range of Colony Forming Units is 10^7-10^9 per day per adult. This is the optimum microbial load, so after reaching the colon at least 10^6-10^7 Colony Forming Units must be colonized to show commensalism and colonization. Most of the probiotic species cannot withstand low pH in gastric tract and also exposure of oxygen may limit their effectiveness [1].

To protect the microbial cells from harsh conditions, Microencapsulation and Immobilization are mostly used [9]. In Microencapsulation process microbial cells are encapsulated by a matrix of compounds like Alginate, Soluble Starch *etc.* Microencapsulation protects the probiotic microbes form harsh conditions in

gastrointestinal tract like low pH, bile salts, oxygen exposure. In the case of ruminants, such microencapsulation will facilitate by-passing the rumen and to reach the lower GI tract. It also helps in slow release of microbes at a constant rate when favorable conditions are attained [1].

For Microencapsulation of probiotics, Polysaccharides like Carrageenan, Alginate, Chitosan and Starch are widely used for encapsulation of cells. Recent advances to increase the survivability chances of probiotics in upper gastrointestinal tract, some Oligosaccharide compounds are used as Prebiotics. Ingestion of probiotics with prebiotics increases the survivability chances of probiotic microbes as they utilize the prebiotics as instant carbohydrate source [10]. There are several prebiotic oligosaccharides used which include Fructo oligosaccharides (FOS), Inulin and Galacto oligosaccharides *etc.*[11].

In present study Inulin was used as a prebiotic which is a Fructan extracted from the roots of *Cichorium intybus*. Inulin has several health benefits on host, and yields short chain organic acids which causes the reduction of lipids and cholesterol in the host[12]. Inulin also improves the immune system and digestive health by enhancing the absorption of lactate in the colon and in the absorption of ions like Fe,Mg,Zn and Ca[13].

Mostly Alginate is used in microencapsulation technique which is a Natural polysaccharide containing D- Mannuronic acid and L- Guluronic acid residues joined by (1-4) linkages [14].Alginate is non toxic, cost effective, biocompatible and has Inotropic gelation property. But it has low physical stability in the presence of monovalent ions/chelating agents. Microencapsulation can be improved by co – encapsulation with different compounds/ polymer/ modification of alginate structure by different additives [15]. Stability of Microparticles can be increased by coating with Chitosan which is polycationic polysaccharide derived from chitin. Chitosan forms polyelectrolyte complexes with alginate. When Cationic Chitosan is added, it forms chitosan – alginate microparticles which stabilize the alginate gel [16].

Lactobacillus spp are nutritionally fastidious and culturing these microbes will be difficult as they require rich nutrient sources like carbohydrates, amino acids, peptides *etc.*, and have limited biosynthetic capabilities. De Man, Rogosa and Sharpe (MRS) broth is used mostly for culturing of Lactic acid bacteria. MRS media is complicated and very expensive for growing Lactic acid bacteria in industrial scale. So, a cost effective medium is required for culturing *Lactobacillus spp.* in large scale [17].

To decrease the cost of MRS medium Cabbage juice extract is used as substitute. Cabbage (*Brassica oleracea linne*) is herbaceous, biennial, dicotyledonous flowering, green leafy vegetable plant and which belongs to the family *Brassica* or *Cruciferae* [18].Cabbage juice was extracted from the leaves and is used for growing probiotic Lactic Acid Bacteria[19].

1.1Nutrient profile of Raw Cabbage

Nutrients	Composition per 100 g.
Carbohydrates	5.84
Sugars	3.146 g
Dietary fiber	2.47 g
Protein	1.28 g
Vitamin C	0.037%
Zinc	0.22 mg
Potassium	170 mg
Phosphorous	26 mg
Magnesium	12 mg
Calcium	40 mg
Vitamin B6	0.124 mg
Vitamine A	98 IU
Folate (Vitamin B5)	43 µg
Choline	10.67 mg
Betaine	0.45 mg
Phytosterols	11 mg
Energy	25 Kcal

In current study, *Lactobacillus plantarum* was isolated from the Fruit juice wastes and cost effective media was used for large scale production. Co-Microencapsulation was done as described below for *Lactobacillus plantarum* and its survivability was assessed in simulated gastric environment.

II. Materials and methods

2.1. Sample collection and isolation

20 samples of Fruit juice wastes were collected from the local fruit juice shops and were labeled. Samples collected were brought to laboratory in γ-irradiated sample containers and decimal dilutions were prepared and plated on De Man, Rogosa and Sharpe medium (Himedia GM 369) for all samples. After incubation at 37^0C for 48 Hrs, 6 isolates were chosen based on the colonial morphology.

2.2. Phenotypic studies and Biochemical characterization

All the 6 isolated strains were subjected to Phenotypic identification like Gram staining, Homofermentation, Catalase test and qualitative estimation of Lactic acid secretion by using standard protocol [20] & [21].

2.2.1. Catalase test

Few drops of 3% Hydrogen Peroxide was added on to the bacterial colonies and observed for gas bubble formation.

2.2.2. Homo fermentation test

Sterilized Durham tubes were kept in inverted position in test tubes containing 5 ml of sterilized glucose broth and incubated at 30^0C for 48 Hrs. The tubes were observed for gas production.

2.2.3. Qualitative estimation of Acid production:

100 µL of 48 Hrs old broth was inoculated on MRS agar plates containing 0.004% of Bromocresol Purple Indicator and 1% Calcium carbonate. The plates were incubated at 30^0C for 48 Hrs.

Biochemical tests were conducted for all the bacterial strains isolated and comparison was done by taking *Lactobacillus plantarum* MTCC 2941 as Reference strain.

2.3. Optimization of Cost effective media for mass production of *Lactobacillus plantarum* RSLP003

2.3.1. Preparation of Modified MRS medium

Cabbage juice medium (CJM) preparation was modified by taking procedure [19] as reference. 200 g. of Cabbage outer leaves were collected from vegetable market waste were washed, chopped and mixed in 1 L. of distilled water. Chopped Cabbage bits were boiled for 30 min. and cooled to room temperature. Cabbage juice was filtered by using Muslin cloth and the extract was stored in Refrigerator.

For the preparation of Modified MRS medium (MMRS), half strength of MRS composition was dissolved in 1 L. of CJM and pH was adjusted to 6.8. Calcium carbonate (2g/L) was added to the MMRS in order to maintain proper buffering system. MMRS medium was autoclaved at 121^0C for 20 min. Similarly,1L. of De Man, Rogosa and Sharpe (MRS) medium was prepared and autoclaved.

Both MRS medium and MMRS medium were inoculated with 5%, 24 Hrs Old seed culture ($2.0X10^8$/ml) of *Lactobacillus plantarum* RSLP003and incubated at 37^0C for 48 hrs under static condition. Total viable cell count was checked after 48 Hrs, centrifuged, Cell pellet was collected and preserved in Refrigerator (4^0C). Broth and cell pellet were analyzed for total viable cell count using standard plate count protocol and the results were compared [17].

2.4. Microencapsulation of *L. plantarum* RSLP003

2 g. of Pregelatinized starch was dissolved in 100 ml of distilled water and boiled till it completely solubilized. 4 g. of Sodium alginate and 1% of Inulin were added to the above Pregelatinized Maize starch solution and mixed till they are dissolved and then autoclaved. To the above sterile Sodium alginate - gelatinized starch solution 1g. *Lactobacillus plantarum* cell pellet ($4.5X10^{11}$CFU/g.) was added and kept on shaker for 30 min. The above mixture was dissolved in 500 ml of autoclaved Vegetable oil (Flax seed oil) containing 0.2% Tween 80 and kept on shaker for 20 min. 200 ml of 0.1 M $CaCl_2$ solution was prepared and autoclaved. Sodium alginate - Pregelatinized starch mixture with Vegetable oil emulsion was aseptically added in drop by drop manner by using 250 ml burette into the sterile 0.1 M Calcium chloride solution. The mixture was kept undisturbed for 30 min so that the sodium alginate beads will settle at the bottom. Beads were collected by centrifugation and coated with Chitosan [14].

0.4 g. of Low molecular weight Chitosan was mixed in 90 ml of Distilled water which was acidified by using 0.4 ml Glacial acetic acid so that the final concentration would be 0.4% (w/v). pH of above solution was adjusted by using 1 M NaOH in the range of 5.6 – 6.0. Mixture was filtered by using Whatmann No.1 filter paper and autoclaved. Microencapsulated beads were added to 100 ml of Chitosan solution and kept on shaker for 40 min. Chitosan coated beads were harvested, washed and dried in Desiccator [22].

2.4. Survivability of Co-Microencapsulated *Lactobacillus plantarum* RSLP003 at low pH level

Survivability of co-microencapsulated *Lactobacillus plantarum* RSLP003 at pH 1.5 was checked as follow.

By adding 20 ml of 0.08 M HCl containing 0.2% (w/v) of NaCl pH was adjusted to 1.5. After pre incubating the aliquot for 10 min at 37^0C, 1g. beads were transferred to each flask. Flasks were incubated at 37^0C for 2 Hrs and beads were harvested, washed and immediately used for enumeration after Depolymerization [23].

Depolymerization of co-microencapsulated beads was done by using 10 ml of filter sterilized 0.1 M Phosphate buffer with pH 7.1. Similarly, free cell suspension was subjected to pH 1.5, and enumerated after 2 Hrs of incubation. Survivability of free cells and co-microencapsulated cells were compared [24].

2.5. Survivability of Co-Microencapsulated *Lactobacillus plantarum* RSLP003 at different Bile concentrations

Survivability of *Lactobacillus plantarum* RSLP003 was checked by exposing free cells and the co-microencapsulated beads to different Bile concentrations *i.e.* 1.0%, 1.5% and 2.0%. Different concentrations of bile acids were taken and autoclaved. 1 g. of beads were transferred to the sterile bile solutions (1.0%, 1.5% and 2.0%) and incubated at 37^0C for 3 Hrs. Assay was carried out as per the similar procedure used in 2.4. Free cells were also subjected to different bile salts concentrations and enumerated [25].

2.6. Survivability of Co-Microencapsulated *Lactobacillus plantarum* RSLP003 at different temperature ranges

Free cells and Co-microencapsulated *Lactobacillus plantarum* RSLP003 were exposed to different temperature *i.e.* 70^0C, 80^0C and 90^0C for 20 min. 1 g. of Co-microencapsulated beads were taken in sterile distilled water and exposed to different temperature ranges for 20 min. Free cells were also subjected to different temperatures and enumerated. Enumeration of viable cells was carried out as per the procedure used in 2.4[24].

III. Results and discussion

Among 20 samples of Fruit juice wastes collected, 6 strains were isolated based on the colony morphology and labeled as RSLP001 to RSLP006. Phenotypic studies and Biochemical tests were done and the results were compared with reference strain *i.e. Lactobacillus plantarum* MTCC 2941.Table 1.

Table 1: Phenotypic studies and Biochemical tests

Biochemical tests	**RSLP001**	**RSLP002**	**RSLP003**	**RSLP004**	**RSLP005**	**RSLP006**	**Ref. strain**
Starch hydrolysis	+	-	-	+	-	+	-
Arginine hydrolysis	-	+	-		-	-	-
Cellobiose	+	+	+	+	+	+	+
Arabinose	+	-	+	-	-	+	+
Mannitol	-	-	+	+	-	+	+
Sorbitol	-	-	+	+	-	+	+
Maltose	+	+	+	-	+	+	+
Ribose	-	+	+	+	+	-	+
Sucrose	+	+	+	+	+	+	+
Xylose	-	-	+	-	+	-	+
Phenotypic studies							
Gram staining	+	+	+	+	+	+	+
Cell shape	Spherical	Rod	Rod	Rod	Rod	Rod	Rod
Catalase	-	-	-	-	-	+	-
Homofermentation	-	+	+	+	+	+	+
Lactic acid production	-	-	+	-	-	-	+

'+' Positive reaction, '-' Negative reaction, Ref. strain – *Lactobacillus plantarum* MTCC 2941

Among all the bacterial strains isolated, RSLP003 showed 100% similarity in all the biochemical tests and phenotypic studies when compared to the reference strain *Lactobacillus plantarum* MTCC 2941. Further studies were carried out by using *Lactobacillus plantarum* RSLP003.

Mostly for cultivation of Lactic Acid Bacteria, MRS broth is commonly used and is economically not suitable for industrial scale production [18]. In order to develop a cost effective medium for growing probiotic *Lactobacillus plantarum* RSLP003 a modified MRS medium was designed. In the present study, Cabbage juice extract was used as Natural nutritional source and was added to half strength of MRS medium composition. The nutrient materials in the MRS medium enhance the Muco-adhesive properties of probiotic *Lactobacillus spp.* in the intestine. Cabbage juice extract contains extremely high quantities of Reducing sugars, Proteins, Vitamins, Minerals, Choline and Betaine which favor the growth of probiotic intestinal microbiota. As per the reference [19] Cabbage juice medium was used for growing Probiotic Yeast cultures which has been yielded high cell density of biomass. Similarly, in current study Cabbage juice medium with half strength MRS medium showed high yields when compared to the MRS medium (Table 2).

CFU/ml counts of 48 H. old broth showed almost 7 folds increase in MMRS ($5.2X10^9$/ml) when compared to MRS ($8X10^8$/ml) Fig 1(a). After centrifugation Cell pellets were collected and cell pellet from MMRS showed 27% increase in the yield when compared to MRS. 1 g. of Cell pellet was taken and enumerated for viable cell count.Table 2 &Fig. 1(b).

Table 2: Optimization of cost effective medium for growing *Lactobacillus plantarum* RSLP003

Medium	Broth (Total Viable CFU/ml)	Cell pellet weight per 100 ml(in grams)	Cell pellet (Total viable CFU/g.)
MRS	$8.0X10^{8}$	0.98	$1.1X10^{11}$
MMRS	$5.2X10^{9}$	1.25	$4.5X10^{11}$

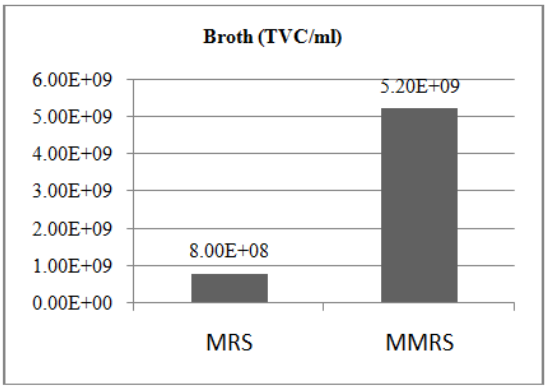

Figure 1(a)

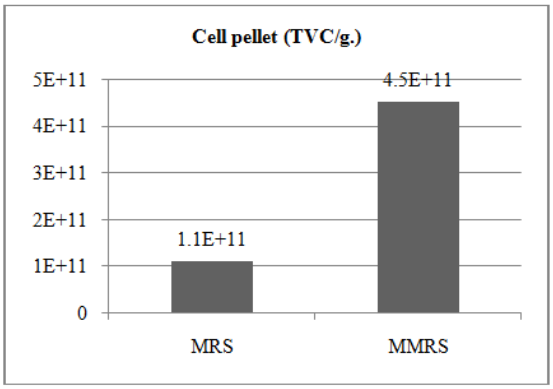

Figure 1 (b)

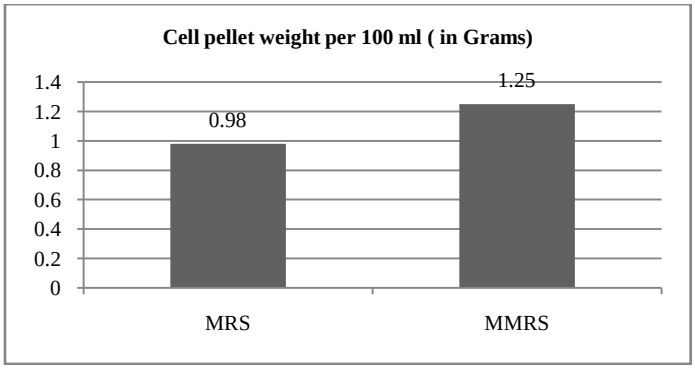

Figure 1(c)

Fig 1(a): TVC counts of 48 H broth, 1(b): TVC counts of cell pellet, 1(c): Comparison of cell pellet weight

Lactobacillus plantarum RSLP003 cells were Microencapsulated by Calcium alginate - Pregelatinized starch, Flax seed oil and using Inulin as prebiotic for the probiotic bacteria and beads were coated with Chitosan. In the present study, survivability of Co-microencapsulated and free cells were compared in simulated gastric environment.

The probiotic bacteria should survive while passing through the gastrointestinal tract and after reaching the colon at least 10^{6} - 10^{7} cells must be survived and colonized. Low pH of gastric juice shows negative impact on the microbial cell viability [23]. In present study, Co-microencapsulated *Lactobacillus plantarum* RSLP003 was assessed at low pH condition (pH-1.5). Both the Co-microencapsulated cells and free cells were treated with pH 1.5 solution and total viable cell count was checked after 120 min. Before checking the viable cell count the beads were subjected to depolymerization by using 0.1 M Phosphate buffer [24].When compared to free cells ($6.1X10^{5}$/g.) the co-microencapsulated cells ($3.0X10^{7}$/g.) showed almost 2 logs high survivability and found that the constituents which were used in microencapsulation *viz.* Calcium alginate, Gelatinized starch, Inulin, Flax seed oil and Chitosan have protected the cells from the effect of low pH. Table 3& Fig.2.

Table 3: Survivability of *L.plantarum* RSLP003at Low pH level

Survivability at pH 1.5 (Values in CFU/g.)	
Free cells	$6.1X10^{5}$
Encapsulated cells	$3.0X10^{7}$

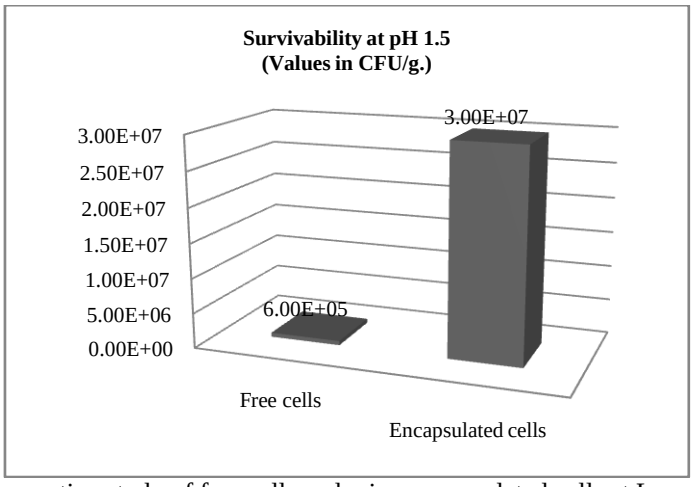

Figure 2: Comparative study of free cells and microencapsulated cells at Low pH conditions

Survivability of probiotic *Lactobacillus plantarum* RSLP003 was checked with different concentrations of Bile acids as it was proven to be showing antimicrobial activity by dissolving the bacterial cell membranes [26]. S. Mandal *et al.,* have studied the survivability of Microencapsulated *Lactobacillus casei*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in simulated bile concentrations and the results shown are encouraging [24]. In present study, Co-microencapsulated *Lactobacillus plantarum* RSLP003 was assessed with various Bile acid concentrations *i.e.,* 1%, 1.5%&2%. When microencapsulated cells and free cells of *Lactobacillus plantarum* RSLP003 were exposed to 1% and 1.5% of bile acid concentration, the entrapped cells showed almost 2 logs increase in the survivability compared to free cells. When exposed to 2% of bile acid concentration, microencapsulated cells showed 3 logs increase in survivability by comparing with free cells. Table 4

Table 4: Survivability of *Lactobacillus plantarum* RSLP003 at different Bile concentrations

Bile concentration	**Free cells (CFU/g)**	**Encapsulated cells (CFU/g)**
1%	$6.5X10^7$	$7.5X10^9$
1.5%	$1.5X10^6$	$6.0X10^8$
2%	$8.0X10^4$	$4.0X10^7$

As some probiotics will be incorporated in heat treated foods, the survivability of bacteria will reduce due to high temperatures. So, survivability of probiotic bacteria can be increased by Microencapsulation [24]. In present study, free cells and co-microencapsulated cells were exposed to 70^0C, 80^0C and 90^0C for 20 min. As per the observation the co-microencapsulated cells showed almost 5 logs more survivability compared to free cells and at 80^0C co-microencapsulated cells survived up to minimal probiotic dosage [1]. At 90^0C no survivability was observed in free cells whereas microencapsulated cells survived. Table 5

Table 5. Survivability of *Lactobacillus plantarum*RSLP003 at different temperature ranges

Temperature	**70^0C/20 min**	**80^0C/ 20 min**	**90^0C/ 20 min**
Free cells	$3.4X10^4$	$1.5X10^2$	No viability
Encapsulated cells	$3.0X10^9$	$2.0X10^7$	$1.6X10^4$

IV. Conclusion

In present study, optimization of cost effective medium for achieving high cell density and microencapsulation of probiotic *Lactobacillus plantarum* RSLP003 was carried out to protect the probiotic bacterial cells from different simulated harsh conditions. MMRS medium showed high cell density ($5.2X10^9$/ml) of *Lactobacillus plantarum* RSLP003 when compared to expensive MRS medium ($8.0X10^8$/ml) and MMRS can be used for industrial production of *L.plantarum*. Microencapsulation studies of *Lactobacillus plantarum* RSLP003 at various simulated harsh conditions has been proven high survivability rates when compared to the non encapsulated free cells. Survivability of microencapsulated cells is increased due to the microencapsulating agents such Calcium alginate, Pregelatinized starch, Inulin, Flax seed oil & Chitosan which were used in the process. This microencapsulation method is feasible for large scale production.

Acknowledgment

We wish to express our gratitude to Dr. Venkatesh Devanur and Dr.G. Vijaya Raghavan for helpful and critical discussions. This work was supported by Som Phytopharma (India) Ltd, Hyderabad.

References

[1]. Amal Bakr Shori, Microencapsulation Improved Probiotics Survival during Gastric Transit, HAYATI Journal of Biosciences xxx, 2017, 1-5.
[2]. Alex Galanis, YiannisKourkoutas, Chrysoula C. Tassou and Nikos Chorianopoulos, Detection and Identification of Probiotic Lactobacillus plantarum Strains by Multiplex PCR Using RAPD-Derived Primers, Int. J. Mol. Sci. 2015, 16, 25141-25153.
[3]. Neethu Maria Jose , Craig R. Bunt and Malik Altaf Hussain,Comparison of Microbiological and Probiotic Characteristics of Lactobacilli Isolates from Dairy Food Products and Animal Rumen Contents,Microorganisms 2015, 3, 198-212.
[4]. MichielKleerebezem, Jos Boekhors, Richard van Kranenburg, DouweMolenaar, Oscar P. Kuipers, Rob Leer,RenatoTarchini, Sander A. Peters, Hans M. Sandbrink, Mark W. E. J. Fiers, Willem Stiekema, Rene´ M. Klein Lankhorst, Peter A. Bron, Sally M. Hoffer, Masja N. Nierop Groot, Robert Kerkhoven, Maaike de Vries, BjournUrsing, Willem M. de Vos, and Roland J. Siezen, Complete genome sequence of Lactobacillus plantarum WCFS1,PNAS,2003.1990-1995.
[5]. Molin G., The role of Lactobacillus plantarum in foods and in human health, Handbook of fermented functional foods, (Farnworth, E.R (Ed), 2010).
[6]. Frederick S. Archibald & Irwin Fridowich, Manganese, Superoxide Dismutase, and Oxygen Tolerance inSome Lactic Acid Bacteria,Journal of Bacteriology, 1981, 928-936.
[7]. Frederick S. Archibald & Irwin Fridowich Manganese and Defenses against Oxygen Toxicity inLactobacillus plantarum,Jounal of Bacteriology, 1981, 442-451.
[8]. Yasuhisa Kono and Irwin Fridovich, Isolation and Characterization of the Pseudocatalase of Lactobacillusplantarum ,The journal of Biological chemistry,1983,6015-6019.
[9]. C. S. FaVaro-Trindade and C. R. F. Grosso, Microencapsulation of L. acidophilus (La-05) and B. lactis(Bb-12) and evaluation of their survival at the pH values ofthe stomach and in bile,J. microencapsulation, vol. 19, no. 4, 485-494.
[10]. Eun Y. Ann, Younghoon Kim, Sejong Oh, Jee-Young Imm, Dong-Jun Park, Kyoung S. Han &Sae H. Kim, Microencapsulation of Lactobacillus acidophilus ATCC 43121 with prebiotic substrates using a hybridisation system, International Journal of Food Science and Technology , 2007, 411–419.
[11]. S. Sathyabama, M. Ranjith kumar, P. Brunthadevi, R. Vijayabharathi, V. Brindhapriyadharisini, Co encapsulation of probiotics with prebiotics on alginate matrix and its effect on viability in simulated gastric environment, LWT - Food Science and Technology 57,2014, 419-425.
[12]. Siok-Koon Yeo, Lay-GaikOoi, Ting-Jin Lim and Min-TzeLiong, Antihypertensive Properties of Plant-Based Prebiotics, Int. J. Molecular Sciences, 2009, 3517-3530.
[13]. Sherif M. Abed,Abdelmoneim H. Ali, Anwar Noman, SobiaNiazi, Al-FargaAmmar, Amr M. Bakry, Inulin as Prebiotics and its Applications in Food Industry and Human Health; A Review, International Journal of Agriculture Innovations and Research,Volume 5, Issue 1, 2016, 2319-1473.
[14]. Mohammad Ali KhosraviZanjani, Babak GhiassiTarzi, Anousheh Sharifanand NimaMohammadi, Microencapsulation of Probiotics by Calcium Alginate-gelatinized Starch with Chitosan Coating and Evaluation of Survival in Simulated Human Gastro-intestinal Condition**,** Iranian Journal of Pharmaceutical Research , 13 (3), 2014, 843-852.
[15]. Honam Chun, Cheol-Hyun Kim, and Young-Hee Cho, Microencapsulation of Lactobacillus plantarum DKL 109 using External Ionic Gelation Method, Korean J. Food Sci. An. Vol. 34, No. 5, 2014, pp. 692~699.
[16]. J. S. Lee, D. S. Cha and H. J. Park, Survival of Freeze-Dried Lactobacillus bulgaricus KFRI 673 in Chitosan-Coated Calcium Alginate Microparticles,J. Agric. Food Chem. , 52, 2004,7300-7305.
[17]. KiBeom Lee, Sang-Kee Kang and Yun JaieChoi,A low-cost Lactobacillus salivariusL29 growth medium containing molasses and corn steep liquor allows the attainment of high levels of cell mass and lactic acid production,Academic journals, 2013,2013 – 2018.
[18]. Oguwike F.N, Offor C.C, Nwadighoha A.N3, Ebede S.O, Evaluation of Efficacy of Cabbage Juice (Brassica OleraceaLinne) As Potential Antiulcer Aggent and Its Effect on the Haemostatic Mechanism of Male Albino Wistar Rats, IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e2279-0861.
[19]. Min Ho Choi, Yun Hee Park, Production of yeast biomass using waste Chinese cabbage, Biomass and Bioenergy 25,2003,221 – 226.
[20]. Aneja, K.R, Experiments in Microbiology, Plant Pathology and Biotechnology. New Delhi: New Age Publishers, 2005.
[21]. PritiKhemariya, Sudhir Singh, Namrata Jaiswal & S. N. S. Chaurasia,Isolation and Identification of Lactobacillus plantarum from Vegetable Samples,FoodBiotechnology,VOL. 30, NO. 1,2016, 49–62.
[22]. WunwisaKrasaekoopt, Bhesh Bhandari, Hilton Deeth, The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria, International Dairy Journal 14 ,2004, 737–743.
[23]. A.V. Rao, N. Shiwnarain and 1. Maharaj, Survival of Microencapsulated Bifidobacterium pseudolongumin Simulated Gastric and Intestinal Juices, Can. Inst. Food Sci. Technol. J. Vol. 22, No. 4, pp. 345-349,1989.
[24]. S. Mandal, A.K. Puniya, K. Singh, Effect of alginate concentrations on survival of microencapsulatedLactobacillus casei NCDC-298, International Dairy Journal 16 (2006) 1190–1195.
[25]. Ki-Yong Lee and Tae-RyeonHeo, Survival of Bifidobacterium longumImmobilized in Calcium Alginate Beads in Simulated Gastric Juices and Bile Salt Solution, Applied and environmental microbiology,2000,869–873.
[26]. Ma´ire Begley, Colin Hill, and Cormac G. M. Gahan, Bile Salt Hydrolase Activity in Probiotics, Applied and Environmental Microbiology,2006,1729–1738.

G.Babul Reddy. "Studies ¬¬¬¬on optimization of cost effective medium for production of Probiotic Lactobacillus plantarum RSLP003, and enhanced stability even at simulated harsh conditions by Co-Microencapsulation." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS), vol. 10, no. 10, 2017, pp. 45–51.