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RESEARCH ARTICLE

Process optimization for microencapsulation of probiotic yeasts

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BACKGROUND: Microencapsulation is a technique which improves the survival and viability of probiotics. We demonstrate encapsulation of five potential probiotic yeasts with alginate and gum as encapsulation matrices to improve their gastrointestinal transit.
METHODS: Gum extracted from various cereals viz. rice, oats, barley, finger millet and pearl millet along with alginate have been used to encapsulate five potential probiotic yeasts. Screening was carried out by measuring swelling index, encapsulation efficiency and nutritional value of microcapsules encapsulated with alginate and gum. The concentration of OBG, sodium alginate and inoculum dosage of probiotic yeasts was optimized using response surface methodology (RSM). Efficiency of alginate OBG microcapsules with or without coating materials viz. whey protein and chitosan also tested. The mucoadhesion ability and storage stability of alginate OBG microcapsules with coating materials were tested.
RESULTS: Highest encapsulation efficiency of probiotic yeasts was noted using oats bran gum (OBG) microcapsules along with alginate in all the five probiotic yeasts. Notably whey protein coated microcapsules showed maximum GIT tolerance (95%) and mucoadhesion (90%) for *L. starkeyi* VIT-MN03. The minimum loss of viability was observed in *L. starkeyi* VIT-MN03 microcapsules on 60th day of storage.
CONCLUSIONS: This is the first report on optimization and survival of microencapsulated probiotic yeasts under simulated GIT conditions using natural gum and alginate as encapsulation matrices and whey protein as coating material.

Keywords microencapsulation, oats bran gum (OBG), probiotic yeast, response surface methodology, simulated GIT condition, whey protein

Introduction

Probiotics are non-pathogenic microorganisms that are introduced orally in the gastrointestinal tract (GIT) and capable of contributing positively to the activity of intestinal microflora and health of the host by improving intestinal balance (Solanki and Shah, 2016). There are many pre-requisite conditions that must be satisfied for microorganisms to be considered as true probiotic. The selection of potential probiotic organisms includes gastrointestinal tract (GIT) resistance, adherence to epithelial cells and they must be able to contribute beneficial functions as colony resistance, immunomodulation or nutritional requirement of the normal GI microbiota when ingested by human and animal hosts (Jankovic et al., 2012). Most probiotic microorganisms are bacteria. Currently used commercial probiotic products include bacteria viz. *Bacillus, Bifidobacterium* and *Lactobacillus* (Obradovic et al., 2015). Few reports are available containing yeast as probiotics in its composition (Ragavan and Das, 2017). *Saccharomyces boulardii* is the only probiotic yeast commercialized in the market and used as a preventive and therapeutic agent for diarrhea and other GI disorders (Czerucka et al., 2007). Yeasts have already been accepted as Generally Recognized as Safe (GRAS) because of some important properties such as (1) being resistant to low pH and bile conditions (2) antibiotic resistant ability (3) rich in nutrition (4) cholesterol assimilation and better antioxidant properties (5) capable of showing antagonistic effects along with inhibition of bacterial toxins and (6) immune modulation (Pennacchia et al., 2008).

Probiotic food should be safe and must contain the appropriate probiotic microorganisms in sufficient numbers (10^6–10^7 CFU) at the time of consumption. Hence, the probiotic strains should survive and maintain their functional properties during production and storage under adverse conditions such as freezing or drying processes along with survival in human intestinal tract (Moreno et al., 2018).

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Encapsulation is a promising technique for the protection against harsh conditions to which probiotics are exposed. Microencapsulation is the most modern method which has remarkable effects on probiotic survival and viability according to several studies (Shah et al., 1995; Ying et al., 2012; Malmo et al., 2013). Probiotic encapsulation technology (PET) will help in neutralizing the problem of short shelf-life of the product and increased cell viability during GIT transit (Etchepare et al., 2015).

Alginate is the most commonly used biomaterial for microencapsulation. It has mucoadhesive property but the cross – linked alginates are usually fragile (Llanes et al., 2000). Therefore, to formulate various cross linked alginate microcapsules for easy gastrointestinal transit, blending with mucoadhesive polymers is one of the most popular approaches. Various mucoadhesive microcapsules containing sodium alginate and mucoadhesive polymers have been reported (Prajapati et al., 2008). Nevertheless, it is found that no attempt has been taken to formulate microcapsules of yeast probiotics using gum as a mucoadhesive polymer. There are reports on the use of gum viz. guar gum, xanthan gum, locust bean gum, and carrageenan gum as encapsulating materials for probiotic bacteria (Pedroso et al., 2013). No report is available on gum extracted from cereal brans used as mucoadhesive polymers so far.

In addition, to enhance the encapsulation efficiency, coating materials are added to seal the microcapsule without gaps and cracks (Ding and Shah, 2009). Though many probiotic strains are coated with chitosan, it is not mechanically resistant to gastrointestinal conditions (Desai and Park, 2005). There is report on the inefficiency of chitosan as coating material in cell survival (Suvarna et al., 2018). To overcome this problem, other coating materials viz. whey protein, poly L. lysine, sodium caseinate etc. have been used for encapsulation of probiotic bacteria (Cui et al., 2000; Gerez et al., 2012;Dash et al., 2015). Whey proteins showed potentiality in the encapsulation of lactic acid bacteria (Nag et al., 2011).

Therefore, the present research is focused on the following objectives (1) process optimization toward microencapsulation of probiotic yeasts using alginate, gum extracted from cereal brans and whey protein as coating material (2) examine the viability of microencapsulated probiotic yeasts under various storage conditions.

Materials and methods

Probiotic yeasts and growth conditions

Probiotic yeasts viz. *Yarrowialipolytica* VIT-MN01, *Kluyveromyces lactis* VIT-MN02, *Lipomycesstarkeyi* VIT-MN03, *Saccharomycopsisfibuligera* VIT-MN04 and *Brettanomycescustersianus* VIT-MN05 already reported in our previous study (Ragavan and Das, 2017) were grown in YEPD media (Himedia, India) and maintained at 37°C with 200 rpm agitation in an orbital shaker (Laboratory Tech, India) for 24-48 h. The yeast cells were then harvested by centrifugation (5000 rpm for 15 min), washed twice with distilled water and lyophilized at −80°C.

Extraction and compositional analysis of natural gum

The extraction of gum from rice bran (RB), oat bran (OB), barley bran (BB), finger millet (FM) bran and pearl millet (PM) bran was done following the standard method (Michael et al., 1996). Each material (27.5g) was blended with 250 ml of 96% ethanol for deactivation of enzyme. The supernatant was decanted and wet bran was allowed to desolventized for 2 days at 40°C . The deactivated material (6 g) was blended with 90 ml of water at 40°C , adjusted to pH 10 with 20% sodium carbonate and stirred for 30 min. The mixture was centrifuged for 10 min at 2,000 rpm (Remi, India) and the supernatant was decanted. This procedure was repeated three times. The liquid extracts of RB, BB, OB, FM and PM were cooled to 20°C and adjusted to pH 4.5 by addition of 20% (v/v) hydrochloric acid with vigorous stirring. The resulting mixture was centrifuged at 2000 rpm for 10 min and the supernatant containing gum extract was retained. The gum extract was cooled to 10°C and an equal volume of absolute ethanol was added with vigorous stirring. The mixture was centrifuged at 2000 rpm for 10 min and the supernatant was carefully decanted. The gum solids were collected and desolventized for 3 h at room temperature and dried at 60°C for 6 h. The dried gum RBG, BBG, OBG, FMG and PMG were transferred into a dialysis membrane (MW cut-off 410 kDa, Himedia, India) and dialyzed at 10°C against distilled water for 48 h. The retained gum was lyophilized at − 80°C.

The compositional analysis of gum was done for estimation of carbohydrate (Loewus, 1952), protein (Hartree, 1972), β-glucan (Claye et al., 1996) and fiber (Ajayi and Fagade, 2007). The viscosity of the gum was also tested (Amer, 2013).

Screening of natural gum

Microcapsules were formed using alginate (3%), gum (5%) extracted from cereal brans and yeast (0.1%) without optimization. Screening of natural gum was done based on swelling behavior and encapsulation efficiency (%) following the standard methods as stated below. Further experiments were carried out using the selected gum after screening.

Swelling behavior

The swelling behavior of microcapsules containing probiotic yeast was studied by measuring the percentage swelling index of the formulated microcapsules (Prajapati et al., 2008). A known amount of microcapsules (50mg) was placed in a conical flask containing 10 ml of simulated gastric fluid (pH

1.2) for a period of 60 min. The weight of the microcapsules was determined after being blotted with a piece of filter paper for the removal of excess water on the surface and transferred to fresh simulated gastro intestinal fluid (SGIF) in order to continue to swell. The weight of the microcapsules was determined after 60 min. The mass of dry microcapsules was determined at time zero and the mass of the wet microcapsules was measured at each sampling point on a weighing balance accurately. The percentage of swelling index of formulated microcapsules containing probiotic yeast was calculated using the following formula:

$$\frac{\text{Wet weight after swelling} - \text{Dry wet of beads}}{\text{Dry wet of beads}} \times 100$$

Encapsulation Efficiency (EE)

The encapsulation efficiency (EE) of yeast cells was calculated by the following equation:

$$\mathrm{EE}(\%) = (X_t/X_i) \times 100$$

Where X_t is the total amount of probiotic loaded in alginate formulated beads and X_i represents the initial amount of probiotic added in the preparation process (Solanki and Shah, 2016).

Optimization for microcapsule production

Response surface methodology (RSM) used for optimization of various parameters on probiotic microcapsules production was done using Box Behnken design (BBD). The software Design Expert (Version 11) was used to reveal the interactions of different factors viz. sodium alginate (%), oats bran gum (%) and yeast inoculum (%) on probiotic microcapsule production and their evaluation. Encapsulation efficiency and GIT tolerance were used as the dependent variables (response) and the 3D contour plots were prepared to know the interactions of different factors and to evaluate the optimized conditions which influence the responses (Maran et al., 2013).

A set of 17 experiments were carried out to evaluate the effects of the three variables viz. sodium alginate (%), oats bran gum (%) and yeast inoculum (%) each with three different concentration levels of low (–1), medium (0) and high (+ 1) on responses as encapsulation efficiency (%) and GIT tolerance (%). The ranges and levels of the two variables were selected and the evaluation of microcapsules was taken as response.

Microencapsulation of probiotic yeasts

The microencapsulation of freeze-dried probiotic yeasts was done using optimized parameters. The encapsulation mixture containing 2% (w/v) sodium alginate and 10% (w/v) of OBG were mixed with 1% yeast culture (10^8 CFU ml^{-1}) was put in vortex and dropped into 0.1 M calcium chloride solution. This procedure was repeated for all the five probiotic yeast strains. Beads were formed and coated with 2% whey protein and chitosan. The coated beads were freeze-dried. The uncoated beads and free cells were served as control. Gum acacia (commercial gum) were used as positive control. Microcapsules were characterized by SEM analysis (FEI Sirion, Eindhoven, Netherlands).

Temperature and pH resistance of microencapsulated probiotic yeasts

Free cells of probiotic yeasts (in powdered form) and cell-loaded microcapsules of predetermined weight were subjected to two different heat treatments at 75°C and 90°C for 30 and 10 s, respectively and subsequently immersed in chilled water to cool down the tubes. To estimate the pH tolerance, microcapsules were kept in 10 ml of YEPD media at pH 2 and pH 3 and incubated for 24-48 h at 37°C (Fareez et al., 2017). Experiments were repeated thrice and viability of yeast cells were determined and expressed as mean ± SD.

Survival of microencapsulated probiotic yeasts in GIT simulation

GIT tolerance of encapsulated probiotic yeasts was tested following the method of Ayama et al. (2014) with minor modifications. The probiotic yeast (microencapsulated and free cells) were placed separately in a tube containing simulated gastric juice (phosphate buffer saline (g/l): sodium chloride-7.650; disodium phosphate anhydrous-0.724; dipotassium hydrogen phosphate-0.210; pH 2.0) containing 3 mg/ml pepsin (Sigma, USA) at 25°C . Incubation was done at 37°C with a shaking speed of 100 rpm for 3 h. The cells were removed after incubation for counting surviving cells and then placed in sterile simulated intestinal juice (PBS pH 8.0 containing 3mg/ml pancreatic and 1% bile salt (Sigma, USA). The tubes were incubated at 37°C for 4 h. One ml of yeast isolate was removed after incubation and the survival rate was counted by plating the contents of the microcapsules on YEPD agar medium using pour plate method.

Viability of microencapsulated probiotic yeasts during storage

The microencapsulated yeast cells were stored at 4°C for 60 days. The encapsulated cell mixture (0.1 g) was spread in YEPD agar. Colonies were counted at 10, 20, 30, 40, 50 and 60 days. Each treatment was carried out in duplicate (Jobanputra et al., 2011).

Mucoadhesion ability of microcapsules

To evaluate the mucoadhesive property of probiotic micro-

capsule, freshly excised porcine small intestine (5.5 cm × 2.5 cm) washed with 0.9% saline and placed in the slide. The microcapsules were dispersed in 100 µl saline and transferred to the intestine. Then the microscopic slide was placed vertically in a plastic cylinder containing 40 ml of 0.9% saline under shaking condition (50 rpm) at 37°C for 1 h. The microcapsules remained on the mucosa were scrapped off and incubated in simulated gastric juice at 37°C under shaking (Prajapati et al., 2008) The mucoadhesion ability of viable cells were calculated after incubation using the following formula:

$$\text{Mucoadhesion}(\%) = \frac{\text{No. of microbeads applied} - \text{No. of leached out beads}}{\text{No. of microbeads applied}} \times 100$$

Statistical analysis

The results are expressed as the mean ± standard deviation of triplicate experiments. Analysis of variance is used to compare the results using Design Expert 11 (Stat-Ease Inc., Minneapolis, MN, USA) statistical software.

Results

Compositional analysis and screening of natural gum for encapsulation

Among the five gum materials (OBG, BBG, RBG, FMG, PMG and GA), OBG was found to show maximum encapsulation efficiency and minimum swelling behavior (Fig. S1). OBG showed maximum encapsulation efficiency (91%) and least swelling index (28 µm) after 60 min which was quite comparable with the commercially available gum (Gum Acacia). Alginate OBG microcapsules were found to be stable up to 90°C under low acidic conditions (pH-2).

Optimization of encapsulation materials using response surface methodology

The statistical design of Box Behnken model (BBD) was applied to optimize potential microcapsule production by varying the parameters sodium alginate (A), OBG (B) and yeast inoculum (C) at different concentration range. Based on the results, response 1 (encapsulation efficiency %) and response 2 (GIT tolerance %) were obtained. The analysis of variance (ANOVA) for the obtained model was tabulated (Tables 1 and 2). The three-dimensional (3D) contour plots for the optimal levels of each variable for maximum encapsulation efficiency and GIT tolerance were illustrated in Figs. 1A and 1B. The actual and predicted responses of potential microcapsule production were highly comparable. The predicted values were calculated using regression analysis and related with experimental data which were well agreed with the predicted response values (Fig. 1C). The factors were optimized by BBD with three central points, the responses were studied and the second-order polynomial equation was given below:

$$\text{Response 1 : Encapsulation Efficiency (Y1)}$$

$$(Y1) = 82.20 - 6.12 * \text{A} - 0.5000 * \text{B} + 0.3750 * \text{C} + 1.000 * \text{AB} + 0.2500 * \text{AC} + 1.50 * \text{BC} + 1.77 * \text{A}^2 + 0.0250 * \text{B}^2 + 1.28 * \text{C}^2$$

Y1, was the response 1 representing the encapsulation efficiency (%) and A, B, and C were coded terms for the three test variables. The 3D plots showed significant influence on OBG either independently or in interaction with each other. In this case A, AB, BC, A^2, B^2 are significant model terms. All the variables had positive significance on encapsulation efficiency. Interactive effect of variables, AB (sodium alginate vs OBG) and BC (OBG vs yeast inoculum) showed most significant positive impact on encapsulation efficiency as compared to AC (sodium alginate vs yeast inoculum) and other interactions (Fig. 2A). The "Lack of Fit F-value" of 0.20 implies that Lack of Fit is not significant relative to the pure error. Non-significant lack of fit is good and considered that the model is fit. The R-Squared value was found to be 0.9882, indicating a realistic fit of the model to the experimental data. This also indicates that 98.8% variation of response can be elucidated effectively and approves that 1.2% of the variations occur while performing the experiments. The adjusted determination coefficient value was 0.9729, also confirmed that the model was highly significant with the coefficient of the variation of 2.73%. The "Pred R-Squared" value of 0.9287 is in reasonable agreement with the "Adj R-Squared" value of 0.9729. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 24.42 indicates an adequate signal, thus this model can be used to navigate the design space (Table 1). The actual encapsulation efficiency (89 ± 1%) was close to the predicted value (91 ± 0.2%) indicating the validity of the model.

Similarly, the factors were optimized by BBD with six central points, the response 2 of GIT tolerance (%) was studied and the second-order polynomial equation was given below:

$$\text{Response 2 : GIT tolerance (Y2)}$$

$$(Y2) = 81.80 - 6.25 * \text{A} + 2.00 * \text{B} - 0.7500 * \text{C} + 1.50 * \text{AB} + 0.5000 * \text{AC} + 1.50 * \text{BC} + 2.35 * \text{A}^2 + 0.3500 * \text{B}^2 + 1.35 * \text{C}^2$$

Y2, was the response 2 representing the GIT tolerance (%)

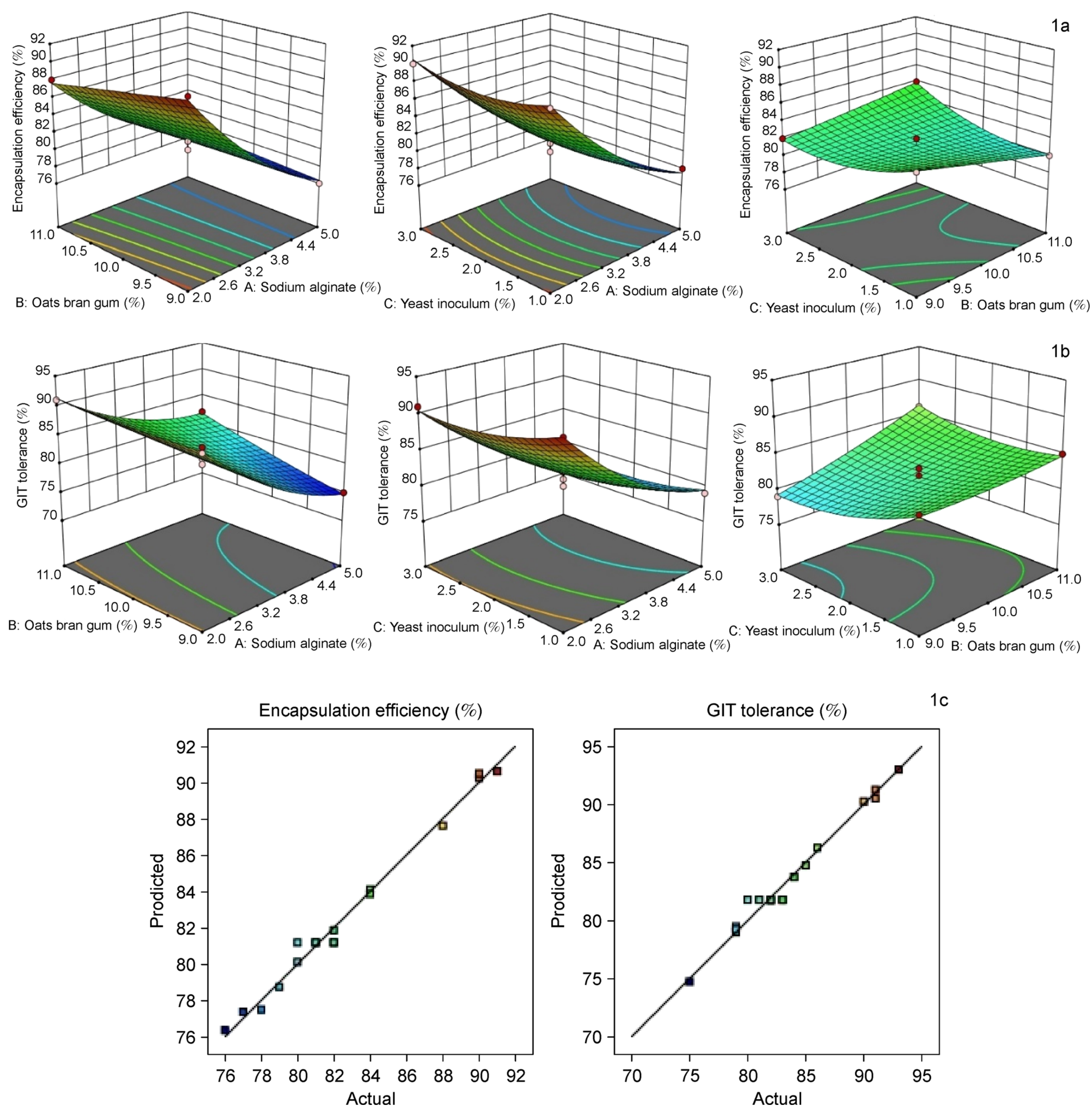

Figure 1 Response surface plots showing the effects of variables (A: Sodium alginate; B: oats bran gum; C: yeast inoculum) on potential microcapsule production for easy GIT transit. 2a: encapsulation efficiency (3D plots); 2b: GIT tolerance (3D plots); 2c: predicted vs actual values.

and A, B, and C were coded terms for the three test variables. The 3D plots showed significant influence on OBG either independently or in interaction with each other. In this case A, B, AB, BC, A^2, B^2 are significant model terms. All the variables had positive significance on encapsulation efficiency. Interactive effect of variables, AB (sodium alginate vs OBG) and BC (OBG vs yeast inoculum), had a most significant positive impact on GIT tolerance as compared to AC (sodium alginate vs yeast inoculum) and other interactions (Fig. 2B). The "Lack of Fit F-value" of 0.20 implies the Lack of Fit is not significant relative to the pure error. Non-significant lack of fit is good and considered that the model is fit. The total determination coefficient R-Squared value was found to be 0.9810, indicating a realistic fit of the model to the experimental data. This also indicates that 98% variation of response can be elucidated effectively and approves that 2% of the variations occur while performing the experiments. The adjusted determination coefficient value was 0.9565, also confirmed that the model was highly significant with the coefficient of the variation of 4.35%. The "Pred R-Squared"

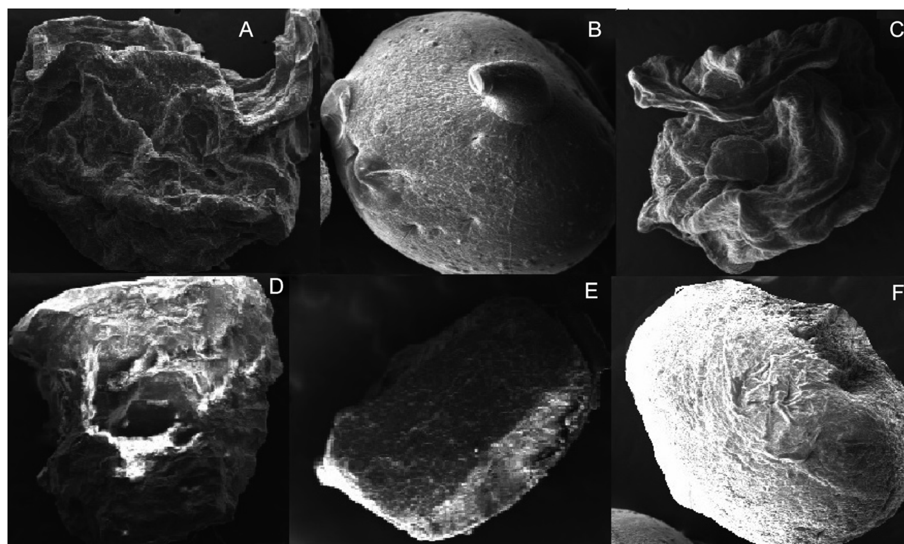

Figure 2 Morphology of alginate microcapsules with gum materials from various brans. A: rice; B: oats; C: barley; D: finger millet; E: pearl millet; F: gum acacia (commercial gum).

Table 1 ANOVA for Quadratic model for encapsulation efficiency of probiotic yeast

Source	Sum of Squares	df	Mean Square	*F*-value	*p*-value
Model	337.83	9	37.54	64.88	0.0001***
A-Sodium alginate	300.12	1	300.12	518.73	0.0001***
B-Oats bran gum	28.00	1	28.00	16.46	0.0053***
C-Probiotic yeast	1.13	1	1.13	1.94	0.2058
AB	9.00	1	9.00	14.91	0.0039***
AC	0.2500	1	0.2500	0.4321	0.5320^
BC	9.00	1	9.00	15.56	0.0056***
A^2	13.27	1	13.27	22.93	0.0020***
B^2	12.26	1	12.26	20.45	0.0081***
C^2	6.84	1	6.84	11.83	0.0108***
Residual	4.05	7	0.5786		
Lack of Fit	1.25	3	0.4167	0.5952	0.6507
Pure Error	2.80	4	0.7000		
Cor Total	341.88	16			
Std. Dev.	0.7606				
Mean	82.65				
C.V. %	0.9203				
R^2	0.9882				
Adjusted R^2	0.9729				
Predicted R^2	0.9287				
Adeq Precision	24.4265				

*** Significant; ^ non-significant

value of 0.9350 is in reasonable agreement with the "Adj R-Squared" value of 0.9565. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 22.54 indicates an adequate signal, thus this model can be used to navigate the design space (Table 2). The actual GIT tolerance (90 ± 0.1%) was close to the predicted value (90 ± 0.8%) indicating the validity of the model.

Survival of free and encapsulated yeast probiotics in simulated GIT conditions

Table 3 shows the survival of free and encapsulated yeast probiotics in simulated GIT conditions using various combinations of encapsulating materials. The maximum survival was noted in *L. starkeyi* VIT-MN03 encapsulated

Table 2 ANOVA for Quadratic model for GIT tolerance of probiotic yeast

Source	Sum of squares	*df*	Mean square	*F*-value	*p*-value
Model	401.73	9	44.64	40.06	0.0001***
A-Sodium alginate	312.50	1	312.50	280.45	0.0001***
B-Oats bran gum	32.00	1	32.00	28.72	0.0011***
C-Probiotic yeast	4.50	1	4.50	4.04	0.0844
AB	9.00	1	9.00	8.08	0.0250***
AC	1.0000	1	1.0000	0.8974	0.3750^
BC	9.00	1	9.00	8.08	0.0250***
A^2	23.25	1	23.25	20.87	0.0026***
B^2	36.51	1	31.51	27.35	0.0037***
C^2	7.67	1	7.67	6.89	0.0342***
Residual	7.80	7	1.11		
Lack of Fit	1.00	3	0.3333	0.1961	0.8941
Pure Error	6.80	4	1.70		
Cor Total	409.53	16			
Std. Dev.	1.06				
Mean	83.71				
C.V. %	1.26				
R^2	0.9810				
Adjusted R^2	0.9565				
Predicted R^2	0.9350				
Adeq Precision	22.5418				

*** Significant; ^ non-significant

with alginate and OBG coated with whey protein. Additional coating with whey protein showed remarkable improvement in the viability of all the microencapsulated yeast probiotics. In the present study, maximum survival (95%) was noted in alginate OBG microcapsules coated with whey protein whereas OBG microcapsules without coating showed 90% survival in probiotic yeast *L. starkeyi* VIT-MN03 under GIT simulation (Fig. S2). Additional coating with chitosan did not improve the viability of all the microencapsulated yeast probiotics compared to whey protein coated microcapsules (Fig. S2).

The surface morphology of the microcapsules without coating was analyzed by SEM analysis which showed significant difference in morphology of different microcapsules (Figs. 2A-2F). Alginate-OBG microcapsule exhibited smooth and uniform surface characteristics (Fig. 2B) compared to other gum materials and commercial gum (gum acacia). Table S1 shows probiotic yeast microcapsules size for each material.

Viability of the encapsulated yeast cells

Fig. S3 shows the loss of cell viability after 2 months of storage at 4°C . The results indicated that microcapsule coated with whey protein showed the highest viability (98%) on 60th d in case of probiotic yeast *L. starkeyi* VIT-MN03. Alginate-OBG microcapsule with whey protein coating acted as a good barrier to prevent the cell release.

The surface morphology of alginate-OBG microcapsules with coating materials before and after GIT simulation was studied (Fig. S4). No changes were noted in the surface of alginate-OBG-WP (whey protein coated) microcapsule whereas little changes were observed in case of alginate-OBG-C (Chitosan coated) microcapsule after GIT simulation.

Mucoadhesion ability of microcapsules

Mucoadhesion ability of microcapsules for five probiotic yeasts have been tested on porcine intestine. Alginate OBG-WP microcapsule of *L. starkeyi* VIT-MN03 showed maximum mucoadhesion capacity (90%) followed by *S. fibuligera* VIT-MN04 (80%) as shown in Fig. 3.

Nutritional value of gum

Among all the gums extracted from various cereal brans, OBG was found to contain maximum carbohydrate (30%), protein (22%), fiber (48%) and β-glucan (66%) which revealed the nutritional quality of gum. Maximum viscosity of OBG was found to be (1.88 mPa/s) which may be responsible for enhancing the mucoadhesion ability of the microcapsules (Table S2). This result indicates that OBG served as nutritionally rich mucoadhesive polymer for the preparation of microcapsules.

Table 3 Survival of microencapsulated probiotic yeasts under simulated gastrointestinal conditions

Yeast strains	Type of encapsulation	Untreated microcapsule (CFU/ml)	Treated microcapsule (CFU/ml)	Cell survival (%)
Yarrowia lipolytica VIT-MN01	Free cells	2.26×10^6	1.59×10^6	70×0.1
	Oats gum + Alginate	2.30×10^6	1.68×10^6	75×0.8
	Oats gum + Alginate + Chitosan	2.32×10^6	1.77×10^6	79×0.2
	Oats gum + Alginate + Whey protein	2.35×10^6	1.98×10^6	84×0.3
	Oats gum + Chitosan	2.33×10^6	1.82×10^6	78×0.8
	Oats gum + Whey protein	2.44×10^6	1.96×10^6	80×0.2
Kluyveromyces lactis VIT-MN02	Free cells	2.18×10^6	1.53×10^6	72×0.1
	Oats gum + Alginate	2.60×10^6	1.98×10^6	76×0.4
	Oats gum + Alginate + Chitosan	2.11×10^6	1.70×10^6	80×0.2
	Oats gum + Alginate + Whey protein	2.10×10^6	1.82×10^6	86×0.1
	Oats gum + Chitosan	2.33×10^6	1.90×10^6	81×0.3
	Oats gum + Whey protein	2.10×10^6	1.76×10^6	83×0.2
Lipomyces starkeyi VIT-MN03	Free cells	2.12×10^6	1.71×10^6	80×0.2
	Oats gum + Alginate	3.06×10^6	2.76×10^6	90×0.1
	Oats gum + Alginate + Chitosan	3.10×10^6	2.83×10^6	91×0.1
	Oats gum + Alginate + Whey protein	3.24×10^6	3.18×10^6	95×0.2
	Oats gum + Chitosan	3.20×10^6	2.76×10^6	86×0.3
	Oats gum + Whey protein	3.12×10^6	2.94×10^6	93×0.2
Saccharomycopsis fibuligera VIT-MN04	Free cells	2.06×10^6	1.61×10^6	78×0.4
	Oats gum + Alginate	2.90×10^6	2.32×10^6	82×0.2
	Oats gum + Alginate + Chitosan	3.10×10^6	2.61×10^6	84×0.1
	Oats gum + Alginate + Whey protein	2.95×10^6	2.73×10^6	92×0.1
	Oats gum + Chitosan	3.01×10^6	2.50×10^6	83×0.2
	Oats gum + Whey protein	2.80×10^6	2.54×10^6	90×0.1
Brettanomyces custersianus VIT-MN05	Free cells	2.12×10^6	1.49×10^6	72×0.2
	Oats gum + Alginate	2.37×10^6	1.79×10^6	80×0.1
	Oats gum + Alginate + Chitosan	2.23×10^6	1.97×10^6	81×0.3
	Oats gum + Alginate + Whey protein	2.30×10^6	2.03×10^6	88×0.1
	Oats gum + Chitosan	2.20×10^6	1.86×10^6	84×0.4
	Oats gum + Whey protein	2.25×10^6	1.90×10^6	85×0.2

Average values (SD±) from three independent repetitions are presented

Discussion

There is report on the use of various gums viz. guar gum, xanthan gum, locust bean gum, and carrageenan gum as encapsulating materials which showed improved survival of probiotic bacteria such as *Lactobacillus rhamnosus and Bifidobacterium longum* (Ding and Shah, 2009).

Reports are available on optimization of microencapsulated probiotic bacteria using RSM (Chen et al., 2005; Anekella and Orsat, 2013; Behboudi-Jobbehdar et al., 2013). The evaluation of probiotic bacteria *Lactobacillus sporogenes* loaded sodium alginate with carboxymethyl cellulose mucoadhesive beads using design expert software is also reported (Solanki and Shah, 2016). So far no report is

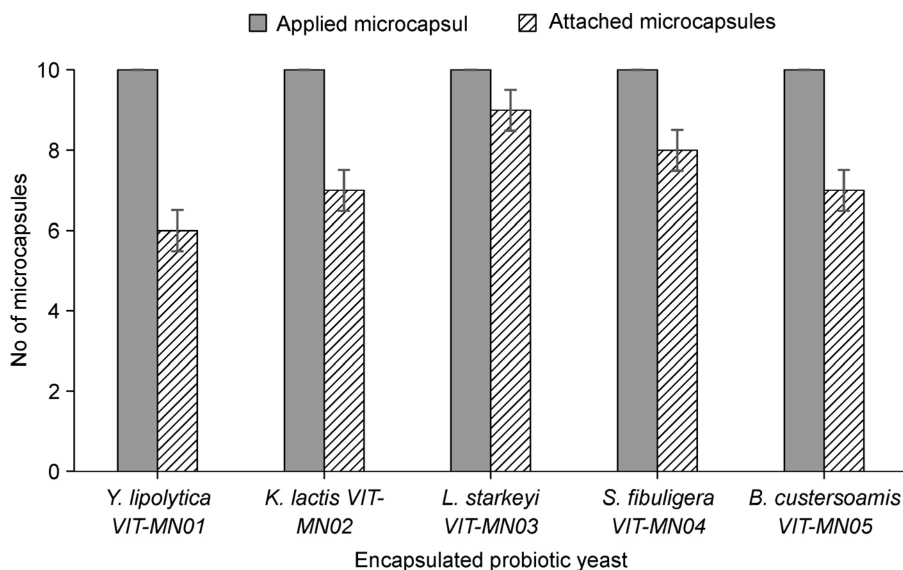

Figure 3 Mucoadhesion ability of microencapsulated probiotic yeasts on porcine intestine.

available on microencapsulation of probiotic yeasts using RSM.

Gbassi et al. (2009) reported that whey protein coating on microcapsule of alginate containing probiotic cultures of *Lactobacillus plantarum* showed effective protection in probiotic viability. *Saccharomyces boulardii* microspheres with whey protein and alginate in the ratio of 62: 38 showed 95% encapsulation efficiency (Hébrard et al., 2009). Dash et al. (2015) reported the inefficiency of chitosan as coating material in cell survival.

Kanmani et al. (2011) reported the survival of micro-encapsulated bacteria *Enterococcus faecium* MC13 with alginate and a chitosan coating at –20°C for 6 months. Stability of probiotic *Lactobacillus plantarum* microcapsules coated with chitosan was tested during storage for up to 45 days at 4°C, 30°C and 37°C (Albadran et al., 2015).

Solanki and Shah (2016) has already reported the advantageous properties of mucoadhesive beads of probiotic *Lactobacillus sporogenes* encapsulated with sodium alginate and carboxymethyl cellulose. Mucoadhesion can significantly help easy gastrointestinal transit when alginate is blended with mucoadhesive polymers. In the present work, OBG served as mucoadhesive polymer.

Conclusion

In the present study, microcapsules of yeast probiotics were successfully developed and optimized using Box Behnken design (BBD). The actual encapsulation efficiency (89 $\pm$1%) and GIT tolerance (90 $\pm$0.1%) were close to the predicted values (91 $\pm$0.2%) and (90 $\pm$0.8%) respectively which indicated the validity of the model. The optimal formulation of microcapsule contained 2% alginate, 10% OBG and 1% yeast inoculum. The optimized microcapsule coated with whey protein demonstrated good entrapment efficiency, high mucoadshesivity and extended survival for a period of 60 days. The coating with chitosan did not improve the viability of microencapsulated yeast probiotics compared to whey protein coated microcapsules. In addition, use of oat bran gum (OBG) served as an efficient and nutritionally rich mucoadhesive polymer blended with alginate helped in easy gastrointestinal transit of microencapsulated yeast probiotics under simulated GIT condition. To the best of our knowledge, this is the first report on optimization and evaluation of microencapsulated probiotic yeasts for improved survival under simulated GIT condition.

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Conflict of interest statement

Mangala Lakshmi Ragavan and Nilanjana Das declare that they have no conflict of interest.

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