Soy whey based medium for optimized phytase activity in *Saccharomyces cerevisiae* MTCC 5421 and α -D-galactosidase and antibacterial activities in *Lactobacillus plantarum* MTCC 5422 by response surface methodology

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Abstract Soy whey based growth medium for yeast enabled the native isolate of *Saccharomyces cerevisiae* MTCC 5421 to produce 198 U/ml of phytase activity against that of 135 U/ml in potato dextrose broth. In the same medium for lactic acid bacteria, the isolate of *Lactobacillus plantarum* MTCC 5422 was able to elaborate α -D-galactosidase activity of 10.6 U/ml in comparison to 6 U/ml in Lactobacillus MRS broth. Using this formulated medium, a central composite experimental design based on 5 variables of 3 factors namely incubation temperature, pH level and incubation period showed that *S. cerevisiae* could produce 200 U/ml of phytase in 36 h at 30 °C and pH 3.5. Similarly, *L. plantarum* in 33 h at 37 °C and pH 6.6 exhibited 14.2 U/ml of α -D-galactosidase activity, whereas the antibacterial activity of 14.4 AU/ml against *Bacillus cereus* was evident in 42 h at 42 °C and pH 6.0. The phytase and antibacterial activity units visualized in response surface plots were more or less close to those obtained with the experimental design treatments.

Keywords *Lactobacillus plantarum* · *Saccharomyces cerevisiae* · α -D-galactosidase · Phytase · Antibacterial · Soy whey based medium

Introduction

Among the desirable microbial cultures involved in traditional food fermentations, the important ones are those

of lactic acid bacteria (LAB) and yeasts, which constitute the more familiar group of probiotics (Asa and Torkel 2006). These microbial cultures are known to elaborate beneficial attributes, which may include enzymes hydrolyzing anti-nutrient and anti-flatulence factors as well as antibacterial against foodborne pathogenic bacteria. An earlier study included biochemical and molecular characterization of desirable attributes in native isolates of *Lactobacillus plantarum*, *Bifidobacterium adolescentis* and *Saccharomyces cerevisiae*, wherein these cultures are known to be involved in the preparation of quality consistent traditional fermented foods (Roopashri and Varadaraj 2009).

In traditional food fermentations, the enzymes of importance are phytase and α -D-galactosidase acting on phytate and raffinose family of oligosaccharides occurring in most of the cereals and legumes. Microbial phytases have been reported to be more promising and are easily isolated when produced extracellularly in a culture medium as well as the culture could be used as microbial starter, if desired, in food fermentation (Chadha et al. 2004). Similar to phytase, the absence of α -D-galactosidase in human system, results in hindered digestion of raffinose family of oligosaccharides, often causing flatulence. Species of *Lactobacillus* are known to produce α -D-galactosidase and help in the breakdown of the complex carbohydrates and enhance the nutrition level of many such foods (Kaplan and Hutkins 2000; Tzortzis et al. 2003). As a means to achieve higher performance of microbial associated activities, there have been developments in the selection of chemically prepared culture medium. Response surface methodology (RSM) has increasingly been in use to optimize growth related factors during different phases of fermentative and biotechnological processes as well as in

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the preparation of traditional foods (Vohra and Satyanarayana 2002; Tellez-Luis et al. 2003; Tsen et al. 2009; Chakraborty et al. 2011; Jain et al. 2011; Modha and Pal 2011).

In general, *Lactobacillus* MRS broth and like are in use for the elaboration of desirable attributes associated with selected beneficial microbial cultures. Considering the potential of desirable microbial cultures to utilize oligosaccharides in the substrate, in the present study, an attempt was made to evaluate a laboratory formulated soy whey based medium for the elaboration of optimized phytase activity in *Saccharomyces cerevisiae* and α -D-galactosidase and antibacterial activities in *Lactobacillus plantarum*.

Materials and methods

All dehydrated media, chemicals and reagents used in this study were procured from HiMedia Laboratories, Mumbai, India.

Microbial cultures These included (i) native isolates of *Saccharomyces cerevisiae* MTCC 5421 and *Lactobacillus plantarum* MTCC 5422 (Roopashri and Varadaraj 2009) and (ii) a strain of *Bacillus cereus* F 4810 (for antibacterial assay) obtained through courtesy from Dr. J.H. Kramer, Central Public Health Laboratory, United Kingdom. All these microbial cultures were maintained in the culture stock of the Department of Human Resource Development, CFTRI (India). The culture of *L. plantarum* was maintained at 6 °C in MRS broth; that of *S. cerevisiae* on potato dextrose agar (PDA) agar slant and *B. cereus* on nutrient agar slant. The cultures were propagated successively twice in the respective broths, prior to their use in experimental trials.

Broth media for growth of microbial cultures The potato dextrose broth (PDB) and *Lactobacillus* MRS broth used in the experimental trials were those of Hi-Media. The formulated basal culture medium namely soy whey broth was prepared by soaking aliquots of 50 g quantities of well dried soy bean seeds in Milli-Q water (A10 Elix 3, Millipore Corporation, Billerica, USA) for 12 h at ambient temperature (18–26 °C), after which the hydrated beans were blended with 200 ml water. The resultant slurry was filtered through double-folded muslin cloth. The soy milk so obtained was adjusted to pH 4.5–4.7 using 6 M citric acid and boiled for 5 min till precipitation occurred. This was then placed at 6 °C for overnight and the resultant clear supernatant was collected and filtered through sterile 0.22 μ m membrane filters (Millipore, Bengaluru, India). The clear supernatant was stored at 6 °C till further use.

For the culture of *S. cerevisiae*, the medium formulated was soy whey based for yeast (SWBY). The basal medium was added with 1% yeast extract, 2% glucose and 0.1%

ammonium sulphate and pH adjusted to 5.2 ± 0.2 . The prepared medium was sterilized at 121 °C for 15 min. Prior to use in the experimental trials, 6 ml of membrane filtered soy whey was added to the pre-sterilized basal medium. In the case of *L. plantarum*, the formulated medium was known as soy whey based for *Lactobacillus* (SWBL), wherein the basal medium was added with 1% each of peptone and yeast extract, 2% galactose, 0.2% each of tri ammonium citrate and dipotassium phosphate, 0.5% sodium acetate, 0.1% polysorbate 80, 0.01% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.005% $\text{MnSO}_4 \cdot \text{H}_2\text{O}$. The pH of prepared medium was adjusted to 6.5 ± 0.2 and sterilized at 121 °C for 15 min. Prior to use in the experiment, the basal medium was added with 6 ml of membrane filtered soy whey.

Preparation of cell suspension Cell suspensions of the individual microbial cultures were prepared for use in the experimental trials. Isolate of *L. plantarum* was grown in *Lactobacillus* MRS broth under static aerobic condition for 18 h at 37 °C and that of isolate of *S. cerevisiae* was grown in PDB for 24 h at 30 °C at 110 rpm in an orbital shaker incubator (Alpha Scientific Co., Bengaluru, India). After incubation period, the respective culture broths were centrifuged at 8000 rpm at 10 °C for 20 min (Superspin R-V/F_M, Plasto Crafts, Mumbai, India), washed with sterile 0.85% saline and the pellets were resuspended in 10 ml of sterile saline and stored at 6 °C until further use. This suspension was serially diluted and appropriate aliquots of the dilutions were used to get an initial level of $2.3 \log_{10}$ CFU/ml.

Evaluation of broth media for desirable attributes elaborated by microbial cultures The broth media were evaluated for optimized phytase, α -D-galactosidase and antibacterial activities specific to the selected microbial cultures in the present study. The ability of *S. cerevisiae* MTCC 5421 to elaborate extracellular phytase in the formulated SWBY and PDB was evaluated. Aliquot of individual medium was inoculated with a viable population of $2.3 \log_{10}$ CFU/ml and incubated in a shaker (110 rpm) incubator at 30 °C for 12, 24, 36 and 48 h, respectively. Aliquot of culture broth from each of the specified incubation period was centrifuged at 8000 rpm for 15 min at 4 °C and phytase activity in the resultant supernatant was determined by the method of Heinonen and Lahti (1981). One unit of the enzyme activity was defined as the amount of phytase required to liberate 1 μ mol of inorganic phosphate per min under defined assay conditions.

In another set of experimental trials, intracellular α -D-galactosidase activity of *L. plantarum* MTCC 5422 was evaluated in the formulated SWBL and *Lactobacillus* MRS broth with an initial inoculum of $2.3 \log_{10}$ CFU/ml, followed by incubation at 37 °C under static aerobic

condition. Culture broth aliquot at 12, 24, 36 and 48 h of incubation was subjected to centrifugation at 8000 rpm for 15 min at 4 °C. The resultant cells were washed twice in 50 mM McIlvaine buffer of pH 5.8, followed by 2 times sonication in the same buffer each of 15 min at 100 W. The sonicated buffer was centrifuged at 8000 rpm for 15 min at 4 °C to obtain a cell-free extract, which was stored at 6 °C till further use. The α -D-galactosidase activity was assayed in McIlvaine buffer by measuring spectrophotometrically the *p*-nitrophenol (PNP) released by the action of the enzyme upon its specific substrate, *P*-nitrophenyl- α -D-galactopyranoside (PNPG) at 400 nm in a UV-VIS Spectrophotometer (Genesys 5, Milton Roy Co., USA). One unit of enzyme was defined as the amount of enzyme that released 1 μ M of PNP from the substrate PNPG per ml per min under the assay conditions (Church et al. 1980).

The antibacterial activity elaborated by the isolate of *L. plantarum* MTCC 5422 in SWBL was assayed in extracellular culture supernatant of the culture broth incubated at 37 °C for 12, 24, 36 and 48 h, respectively. Antibacterial activity was performed against the indicator bacterial species of *B. cereus* F 4810 by agar well diffusion assay as described earlier (Schillinger and Lucke 1989; Varadaraj et al. 1993). Antibacterial activity in the culture supernatants was defined as the reciprocal of the highest dilution showing definite inhibition of indicator bacterial strains and expressed as activity units per millimetre (AU/ml).

The formulated SWBY and SWBL media devoid of any microbial cultures were assayed for activities of phytase, α -D-galactosidase and antibacterial. All experiments were performed in duplicates and average values with standard errors have been presented at requisite and appropriate places.

Experimental design for desirable attributes in microbial cultures The experimental design was a 3 factor and 5 level central composite design, wherein the factors were incubation temperature, pH level and incubation period. For phytase activity in *S. cerevisiae*, the broth medium was SWBY and the 5 levels were in the range of 25–35 °C for incubation temperature, 3.5–5.5 of pH levels and 24–48 h of incubation period. In respect of α -D-galactosidase and antibacterial activities with *L. plantarum*, the medium was SWBL and the 5 levels were in the range of 30–45 °C for incubation temperature, 5.8–6.6 of pH levels and 18–48 h of incubation period. The selected broth media in requisite quantities of 20 ml each were inoculated with respective microbial species at an initial level of 2.3 log₁₀ CFU/ml, mixed well and subjected to the experimental runs as per the design. Sample aliquots from individual experimental treatments were drawn and assessed for phytase, α -D-galactosidase and antibacterial activities, respectively, as described previously. All experiments were performed in

duplicates and average values with standard errors have been presented at requisite and appropriate places.

For each of the desirable attributes specific to the microbial species used in the study, average values in terms of activity units were used to perform multiple regression analysis using Microsoft Excel Software Programme 2007 (Microsoft Corporation, Redmond, WA, USA) with the following equation:

$$\text{Prd} = x_1 + x_2T + x_3p + x_4t + x_5T^2 + x_6T^*p + x_7p^2 + x_8p^*t + x_9t^2 + x_{10}t^*T$$

Where x_1 to x_{10} are the coefficients; T is the incubation temperature (°C), p is the pH level and t is the incubation period (h). Based on the derived coefficients, response surface plots were generated to describe the parameters towards optimized activity of the desirable attributes in the microbial species grown in individual selected broth media.

Results and discussion

Phytase activity in *S. cerevisiae* The culture of *S. cerevisiae* was able to produce 198 U/ml of phytase in 36 h of growth in SWBY medium, as against that of 135 U/ml in PDB. Based on the suitability of SWBY, CCD based experimental data for phytase activity in *S. cerevisiae* is presented in Table 1. The activity ranged from 152 to 200 U/ml, with 200 U/ml being produced in 36 h at 30 °C and pH 3.5 and that of 152 U/ml in 43 h at 33 °C and pH 5.1. The derived regression coefficients, their standard errors and P values are shown in Table 2. The R^2 value of 0.61 did indicate an optimum degree of correlation between the experimental and predicted values. The linear coefficient and co-products of incubation temperature was significant with a lower P value ($P < 0.05$), which did indicate that incubation temperature was the primary influencing factor, followed by incubation period to a certain extent. The coefficients derived by multivariate analysis were utilized to generate response surface plots for phytase activity in *S. cerevisiae* MTCC 5421 as a function of selected incubation temperatures and periods at 3 defined pH levels. The response surface model proposed to be was:

$$\begin{aligned} \text{Prd} = & -893.91 + 58.64*(T) + 32.41*(p) + 9.72*(t) \\ & - 1.10*(T)^2 + 0.83*(T*p) - 5.63*(p)^2 \\ & + 0.35*(p*t) - 0.13*(t)^2 + 0.05*(t*T) \end{aligned}$$

The response surface plots at representative pH levels are presented in Fig. 1. Although not much variation could be observed in the generated values at the defined pH levels, it could be visualized from the surface plots that phytase activity was higher at pH 4.5–3.5, wherein an increasing

Table 1 Experimental data based on CCD for the extracellular phytase activity in the culture of *S. cerevisiae* MTCC 5421 grown in soy whey based yeast medium

Treatment number	Incubation Temp. (°C)	pH level	Incubation period (h)	Phytase activity	
				Observed (U/ml) $\pm$ SD	Predicted (U/ml)
1	27	3.9	29	172 $\pm$ 0.7	216
2	33	3.9	29	160 $\pm$ 1.3	200
3	27	5.1	29	165 $\pm$ 0.9	210
4	33	5.1	29	158 $\pm$ 0.4	199
5	27	3.9	43	168 $\pm$ 0.2	255
6	33	3.9	43	159 $\pm$ 0.5	242
7	27	5.1	43	154 $\pm$ 1.1	244
8	33	5.1	43	152 $\pm$ 0.0	237
9	25	4.5	36	190 $\pm$ 0.6	225
10	35	4.5	36	154 $\pm$ 0.9	205
11	30	3.5	36	200 $\pm$ 0.1	242
12	30	5.5	36	188 $\pm$ 0.0	232
13	30	4.5	24	172 $\pm$ 0.7	195
14	30	4.5	48	188 $\pm$ 1.1	261
15	30	4.5	36	184 $\pm$ 0.0	242
16	30	4.5	36	182 $\pm$ 0.4	242
17	30	4.5	36	188 $\pm$ 0.7	242
18	30	4.5	36	190 $\pm$ 0.2	242
19	30	4.5	36	186 $\pm$ 0.5	242
20	30	4.5	36	192 $\pm$ 1.0	242
21	30	4.5	36	188 $\pm$ 0.3	242

trend was observed over the incubation period at 28–30 °C reaching maximum activity in 48 h (Fig. 1a–c). At temperatures above 30 °C, phytase levels showed a decreasing pattern. The activity of 200 U/ml obtained in the present

Table 2 Regression coefficients, standard error and *P* values derived by multivariate analysis for phytase activity of *S. cerevisiae* MTCC 5421 grown in soy whey based yeast medium

Source	Coefficient	SE	<i>P</i>
Intercept	−893.91	604.4	0.167185
T	58.64	26.2	0.046734
p	32.41	119.1	0.790526
t	9.72	9.3	0.320236
T ²	−1.10	0.4	0.012636
T x p	0.83	2.5	0.745942
p ²	−5.63	9.3	0.556505
p x t	0.35	1.1	0.745942
t ²	−0.13	0.1	0.068261
t x T	0.05	0.2	0.828759
R ²	0.61		

experimental treatment was lower by 40 U to that visualized in the surface plot for 36 h at 30 °C and pH 3.5 (Fig. 1a).

A few of the earlier research investigations have focused on nutritional (nitrogen and carbon sources) and physical parameters to achieve high levels of phytase during the active growth phase of yeast cultures (Sano et al. 1999; Quan et al. 2001; Vohra and Satyanarayana 2001). Studies with other yeast cultures have revealed either lower or almost same phytase activity to that observed in the present study. Culture of *Pichia anomala* grown for 24 h at 20 °C resulted in an intracellular phytase

Fig. 1 RSM plots for extracellular phytase activity by *S. cerevisiae* MTCC 5421 in soy whey based yeast medium at pH levels of 3.5 [a], 4.0 [b] and 4.5 [c]

activity of 68 U/g, dry biomass (Vohra and Satyanarayana 2001). However, through statistical optimization of medium components by RSM, *P. anomala* showed enhanced cell bound phytase of 176 U/g, dry biomass in cane molasses medium as against 100 U/g in synthetic medium (Vohra and Satyanarayana 2002). Further, RSM based approach has been used to achieve enhanced titres of phytase activity in few of the yeasts like *P. anomala* and *Kodamea ohmeri* grown in medium based on cereals and/or legumes as substrates (Kaur and Satynarayana 2005; Li et al. 2008).

In comparison with these substrates, the formulated soy whey based medium enabled the culture of *S. cerevisiae* MTCC 5421 to produce reasonably high phytase activity of 200 U/ml, which was more so extracellular in its secretion pattern. This activity may be attributed to the inherent constituents of the basal medium, particularly the appropriate level of phytate as an inducer of phytase activity (Quan et al. 2001). The soy whey based medium devoid of any microbial growth did not exhibit any phytase activity.

Table 3 Experimental data based on CCD for the intracellular α -D-galactosidase activity in the culture of *L. plantarum* MTCC 5422 grown in soy whey based Lactobacillus medium

Treatment number	Incubation Temp. (°C)	pH level	Incubation period (h)	α -D-galactosidase activity	
				Observed (U/ml) $\pm$ SD	Predicted (U/ml)
1	32	6.0	24	4.8 $\pm$ 0.2	24.3
2	42	6.0	24	6.7 $\pm$ 0.4	30.9
3	32	6.4	24	5.2 $\pm$ 0.2	26.2
4	42	6.4	24	10.9 $\pm$ 1.1	35.2
5	32	6.0	42	6.8 $\pm$ 0.1	43.4
6	42	6.0	42	7.8 $\pm$ 0.5	48.2
7	32	6.4	42	6.9 $\pm$ 0.3	43.8
8	42	6.4	42	8.8 $\pm$ 0.4	51.1
9	30	6.2	33	2.6 $\pm$ 0.7	29.5
10	45	6.2	33	3.2 $\pm$ 0.1	40.5
11	37	5.8	33	10.6 $\pm$ 0.9	41.4
12	37	6.6	33	14.2 $\pm$ 1.3	46.2
13	37	6.2	18	2.4 $\pm$ 0.2	22.4
14	37	6.2	48	4.2 $\pm$ 0.5	51.5
15	37	6.2	33	11.0 $\pm$ 0.8	41.4
16	37	6.2	33	11.1 $\pm$ 1.1	41.4
17	37	6.2	33	10.0 $\pm$ 1.2	41.4
18	37	6.2	33	9.6 $\pm$ 0.7	41.4
19	37	6.2	33	12.0 $\pm$ 0.4	41.4
20	37	6.2	33	12.0 $\pm$ 0.9	41.4
21	37	6.2	33	9.9 $\pm$ 0.6	41.4

Activities of α -D-galactosidase and antibacterial in L. plantarum The culture of *L. plantarum* MTCC 5422 showed 10.6 U/ml of α -D-galactosidase and 13.4 AU/ml of antibacterial activities in SWBL in 36 h at 37 °C as against that of 6 U/ml and 10.2 AU/ml, respectively, in MRS broth. Based on CCD design, experimental data obtained for α -D-galactosidase and antibacterial activities are shown in Tables 3 and 4. The α -D-galactosidase activity under defined parameters ranged from 2.4 U/ml to 14.2 U/ml and that of antibacterial activity was in the range of 10.8 to 14.4 AU/ml. The derived regression coefficients, their standard errors and *P* values for the two respective activities are presented in Table 5. In view of the variations in activity values among the treatments for α -D-galactosidase, there were appreciable differences between experimental and predicted values. In the case of α -D-galactosidase activity, the linear coefficient for pH level and incubation period was significant with *P* value of <0.05 and appeared to influence this activity. However, for antibacterial activity, the linear and co-product coefficients were not statistically significant, which gives an indication

Table 4 Experimental data based on CCD for the extracellular antibacterial activity in the culture of *L. plantarum* MTCC 5422 grown in soy whey based Lactobacillus medium

Treatment number	Incubation Temp. (°C)	pH level	Incubation period (h)	Antibacterial activity	
				Observed (AU/ml) $\pm$ SD	Predicted (AU/ml)
1	32	6.0	24	13.2 $\pm$ 0.4	11.0
2	42	6.0	24	13.5 $\pm$ 0.2	9.4
3	32	6.4	24	13.2 $\pm$ 0.5	11.2
4	42	6.4	24	13.2 $\pm$ 0.1	9.6
5	32	6.0	42	13.2 $\pm$ 0.3	11.0
6	42	6.0	42	14.4 $\pm$ 0.7	9.4
7	32	6.4	42	12.5 $\pm$ 0.2	10.8
8	42	6.4	42	14.0 $\pm$ 0.6	9.2
9	30	6.2	33	12.5 $\pm$ 0.1	11.8
10	45	6.2	33	13.2 $\pm$ 0.3	9.2
11	37	5.8	33	13.2 $\pm$ 0.7	10.4
12	37	6.6	33	12.8 $\pm$ 0.4	10.2
13	37	6.2	18	10.8 $\pm$ 0.2	10.0
14	37	6.2	48	12.3 $\pm$ 0.1	9.7
15	37	6.2	33	13.1 $\pm$ 0.7	9.8
16	37	6.2	33	12.5 $\pm$ 0.4	9.8
17	37	6.2	33	12.5 $\pm$ 0.9	9.8
18	37	6.2	33	13.1 $\pm$ 0.3	9.8
19	37	6.2	33	13.1 $\pm$ 0.5	9.8
20	37	6.2	33	12.5 $\pm$ 1.1	9.8
21	37	6.2	33	13.1 $\pm$ 0.3	9.8

Table 5 Regression coefficients, standard error and *P* values derived by multivariate analysis for the activities of α -D-galactosidase and antibacterial in the culture of *L. plantarum* MTCC 5422 grown in soy whey based Lactobacillus medium

Source	Coefficient α -D-galactosidase	SE	<i>P</i>	Coefficient Antibacterial	SE	<i>P</i>
Intercept	441.29	296.9	0.165323	158.01	130.8	0.25244
T	4.71	3.4	0.188728	−0.91	1.5	0.551364
p	−193.14	87.4	0.049283	−43.00	38.5	0.288042
t	3.91	1.8	0.053708	0.33	0.8	0.685863
T ²	−0.10	0.0	4.12E-05	0.01	0.0	0.176064
T x p	0.59	0.5	0.268463	0.0	0.2	1
p ²	14.80	6.8	0.053604	3.60	3.0	0.262756
p x t	−0.24	0.3	0.403968	−0.05	0.1	0.661257
t ²	−0.03	0.0	7.31E-05	−0.0	0.0	0.155947
t x T	−0.01	0.0	0.268463	0.00	0.0	0.203884
R ²	0.90			R ²	0.60	

T, Incubation temperature (°C); p, pH level; t, Incubation period (h); R², Coefficient of determination

that all the 3 factors may either exert influence or no influence.

The coefficients derived by multivariate analysis were utilized to generate response surface plots for the culture of *L. plantarum* MTCC 5422 as a function of selected incubation temperature and period at 3 selected pH levels for α -D-galactosidase activity (Fig. 2) and only 2 pH levels for antibacterial activity (Fig. 3). The response surface model proposed for α -D-galactosidase activity was:

$$\begin{aligned} \text{Prd} = & 441.29 + 4.71*(T) - 193.14*(p) + 3.91*(t) \\ & - 0.10*(T)^2 + 0.59*(T*p) + 14.80*(p)^2 - 0.24*(p*t) \\ & - 0.03*(t)^2 - 0.01*(t*T) \end{aligned}$$

and for antibacterial activity it was:

$$\begin{aligned} \text{Prd} = & 158.01 - 0.91*(T) - 43.00*(p) + 0.33*(t) \\ & + 0.01*(T)^2 + 0.0*(T*p) + 3.60*(p)^2 - 0.05*(p*t) \\ & - 0.0*(t)^2 + 0.00*(t*T) \end{aligned}$$

The response surface plot for α -D-galactosidase activity revealed that higher units could be obtained with pH 6.6 and 6.4 at 37–45 °C and the same progressively increased during 28–44 h (Fig. 2a and b). Incubation temperatures and pH levels lower than 37 °C and 6.4, respectively, resulted in reduced α -D-galactosidase activity. The experimental design treatment resulted in only 14.2 U/ml, which was much lower than the visualized units in surface plots, although the degree of coefficient of determination (0.90) was indicative of good agreement (Table 5).

The surface plots generated for antibacterial activity showed higher units at pH 6.4 and 6.6 (Fig. 3a and b). The pattern observed was of interest in that with increase in incubation temperature (33 to 45 °C) and period (28 to 44 h), there was a marginal decrease in the antibacterial

activity units. However, an activity of 14.0 AU/ml obtained in the experimental design treatment was more or less same (slightly higher) with those visualized in surface plots, despite an optimum coefficient of determination degree.

Fig. 2 RSM plots for intracellular α -D-galactosidase activity by *L. plantarum* MTCC 5422 in soy whey based Lactobacillus medium at pH levels of 6.4 [a] and 6.6 [b]

Fig. 3 RSM plots for antibacterial activity against *B. cereus* F 4810 exhibited by *L. plantarum* MTCC 5422 in soy whey based Lactobacillus medium at pH levels of 6.4 [a] and 6.6 [b]

The cultures of LAB are known more for their probiotic attributes, rather than for prebiotic characteristics. Earlier studies have shown that α -D-galactosidase present in LAB can bring about maximum hydrolysis of stachyose and raffinose, wherein selected heterofermentative species of *Lactobacillus* harbouring this enzyme were used as starter cultures in the preparation of fermented soy bean products (LeBlanc et al. 2004). In a study with *L. acidophilus* FTCC 0291, RSM enabled to derive optimized culture medium for growth and α -D-galactosidase activity. The optimized attributes did indicate relationship between growth and decreasing levels of reducing sugars and oligosaccharides (De Fatima Viana et al. 2005; Fung et al. 2008).

In contrast, the present study revealed that soy whey broth with added peptone, yeast extract, galactose and requisite salts could support good growth and result in appreciable α -D-galactosidase and antibacterial activities as visualized in the generated response surface plots (Figs. 2 and 3). The ability of *L. plantarum* to hydrolyze raffinose and stachyose could have enhanced the desired activities, as

there was the availability of raffinose family of carbohydrates in the growth medium, wherein the enzyme activity in bacterial species would get activated to a higher level, an observation documented in one of the earlier research studies (Silvestroni et al. 2002).

Conclusion

The laboratory formulated soy whey based medium enabled an appreciable phytase activity in the culture of *S. cerevisiae* MTCC 5421 and α -D-galactosidase and antibacterial activities in *L. plantarum* MTCC 5422. These activity levels did relate well with those derived in response surface plots, although an optimum correlation was derived based on the coefficient of determination degree. It is quite likely, that genotypic characteristics of the organism as well as cultural and nutritional attributes may be of significance in the performance of microbial cultures for the elaboration of such desirable characteristics.

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References

- Asa L, Torkel W (2006) Lactic acid bacteria as probiotics. *Curr Issues Intest Microbiol* 7:73–89
- Chadha BS, Harmeet G, Mandeep M, Saini HS, Singh N (2004) Phytase production by the thermophilic fungus *Rhizomucor pusillus*. *World J Microbiol Biotechnol* 20:105–109
- Chakraborty SK, Kumbhar BK, Chakraborty S, Yadav P (2011) Influence of processing parameters on textural characteristics and overall acceptability of millet enriched biscuits using response surface methodology. *J Food Sci Technol* 48:167–174
- Church FC, Meyers SP, Srinivasan VR (1980) Isolation and characterization of alpha-galactosidase from *Pichia guilliermondii*. In: Underkofler LA, Wulf ML (eds) *Developments in industrial microbiology*. SIM, Arlington, pp 339–348
- De Fatima Viana S, Guimaraes VM, Jose IC, Oliveira MG De Almeida e, Costa NMB, De Barros EG, Moreira MA, De Rezende ST (2005) Hydrolysis of oligosaccharides in soy bean flour by soy bean α -galactosidase. *Food Chem* 93:665–670
- Fung WY, Woo YP, Liong MT (2008) Optimization of growth of *Lactobacillus acidophilus* FTCC 0291 and evaluation of growth characteristics in soy whey medium: A response surface methodology approach. *J Agric Food Chem* 56:7910–7918
- Heinonen JK, Lahti RJ (1981) A new and convenient colorimetric determination of inorganic orthophosphate and its application to the assay of inorganic pyrophosphatase. *Anal Biochem* 113:313–317
- Jain SK, Verma RC, Murdia LK, Jain HK, Sharma GP (2011) Optimization of process parameters for osmotic dehydration of papaya cubes. *J Food Sci Technol* 48:211–217
- Kaplan H, Hutkins RW (2000) Fermentation of fructooligosaccharides by lactic acid bacteria and bifidobacteria. *Appl Environ Microbiol* 66:2682–2684

- Kaur P, Satyanarayana T (2005) Production of cell-bound phytase by *Pichia anomala* in an economical cane molasses medium: Optimization using statistical tools. *Process Biochem* 40:3095–3102
- LeBlanc JG, Garro MS, Savoy De Giori G (2004) Effect of pH on *Lactobacillus fermentum* growth, raffinose removal, α -galactosidase activity and fermentation products. *Appl Microbiol Biotechnol* 65:119–123
- Li X, Chi Z, Liu Z, Yan K, Li H (2008) Phytase production by a marine yeast *Kodamea ohmeri* BG3. *Appl Biochem Biotechnol* 149:183–193
- Modha H, Pal D (2011) Optimization of *rabadi*-like fermented milk beverage using pearl millet. *J Food Sci Technol* 48:190–196
- Quan CS, Zhang L, Wang Y, Ohta Y (2001) Production of phytase in a low phosphate medium by a novel yeast *Candida krusei*. *J Biosci Bioengg* 92:154–160
- Roopashri AN, Varadaraj MC (2009) Molecular characterization of native isolates of lactic acid bacteria, bifidobacteria and yeasts for beneficial attributes. *Appl Microbiol Biotechnol* 83:1115–1126
- Sano K, Fukuhara H, Nakamura Y (1999) Phytase of the yeast *Arxula adeninivorans*. *Biotechnol Lett* 21:33–38
- Schillinger U, Lucke FK (1989) Antibacterial activity of *Lactobacillus sake* isolated from meat. *Appl Environ Microbiol* 55:1901–1906
- Silvestroni A, Connes C, Sesma F, De Giori GS, Piard JC (2002) Characterization of the *melA* locus for α -galactosidase in *Lactobacillus plantarum*. *Appl Environ Microbiol* 68:5464–5471
- Tellez-Luis SJ, Moldes AB, Alonso JL, Vázquez M (2003) Optimization of lactic acid production by *Lactobacillus delbrueckii* through response surface methodology. *J Food Sci* 68:1454–1458
- Tsen J-H, Lin Y-P, An-Erl King V (2009) Response surface methodology optimization of immobilized *Lactobacillus acidophilus* banana puree fermentation. *Int J Food Sci Technol* 44:120–127
- Tzortzis G, Jay AJ, Baillon MLA, Gibson GR, Rastall RA (2003) Synthesis of α -galactooligosaccharides with α -galactosidase from *Lactobacillus reuteri* of canine origin. *Appl Microbiol Biotechnol* 63:286–292
- Varadaraj MC, Devi N, Keshava N, Manjrekar SP (1993) Antimicrobial activity of neutralized extracellular culture filtrates of lactic acid bacteria isolated from a cultured Indian milk product (*dahi*). *Intl J Food Microbiol* 20:259–267
- Vohra A, Satyanarayana T (2001) Phytase production by the yeast *Pichia anomala*. *Biotechnol Lett* 23:551–554
- Vohra A, Satyanarayana T (2002) Statistical optimization of the medium components by response surface methodology to enhance phytase production by *Pichia anomala*. *Proc Biochem* 37:999–1004