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Deproteinated Cheese Whey Medium for Biomass Production of Probiotic *Lactobacillus helveticus* MTCC 5463

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A B S T R A C T

Deproteinated cheese whey was used for biomass production of probiotic strain *Lactobacillus helveticus* MTCC 5463 in a biofermenter. Optimization of growth parameters such as temperature, pH and time of incubation as well as nutrient supplementation of cheese whey was carried out using response surface methodology (RSM). Cheese whey supplemented with 0.95% yeast extract and 0.95% proteose peptone, inoculated with 6% (v/v) active culture of *L. helveticus* MTCC 5463 and fermented for 24 h at optimized temperature of 40ºC and pH 6.25 yielded 3.25 g/L dry cell biomass and 14.82 log cfu/g total viable count. The optimization of growth parameters and nutrient supplementation resulted in an increase of biomass yield from 1.997 to 3.25 g DCW/L, an enhancement 62.74 %.

Introduction

Owing to positive modulation of intestinal microbiota by probiotic, consumer's interest has sharply grown towards various probiotic foods. Probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO, 2002). The major group of probiotic bacteria belongs to the species of *Lactobacilli* and *Bifidobacteria*. There has also been growing interest in the use of probiotic lactic acid bacteria for a wide range of applications in food, pharmaceuticals and health products. The large scale production of the cell biomass of the probiotic organism is therefore necessitated to cater the industry to meet the growing demand. It is therefore important to standardize the process (upstream and downstream processing) and optimize each processing parameters (temperature, pH and time) of fermentation for yielding maximum cell biomass. Also designing of alternative low cost cultivation medium for biomass production could be useful for the large scale production of probiotic strain for its commercial application.Several workers have reported effects of growth parameters and media supplementation on growth of lactic acid bacteria and its subsequent viability (Liu *et al.*, 2010; Aguirre-Ezkauriatza *et al.*, 2010; Mondragón-parada *et al.*, 2006). Attempts have been made to optimize the fermentation

parameters and media composition in order to obtain highest microbial mass from LAB using RSM (Bevilacqua *et al.,* 2008; Polak-Berecka *et al.,* 2010; Lechiancole *et al.,* 2002).

Whey is the major by-product obtained during the preparation of dairy products such as cheese, channa, paneer, and shrikhand. It is a rich source of whey proteins, lactose, enzymes, vitamins, bioactive compounds and minerals (Agrawal *et al.,* 1989). Availability of lactose in whey and presence of essential nutrients for the growth of microorganisms makes whey one of the most potent raw materials for the production of different by-products through different biotechnological applications (Panesar *et al.,* 2007). Also, many small-size cheese plants do not have proper treatment systems for the disposal of whey and the dumping of whey constitutes a significant loss of potential food as whey retains about 40-45% of total milk solids (Panesar *et al.,* 2006).

Whey disposal poses serious pollution problems for the dairy industry to the surrounding environment because of quite high biological oxygen demand i.e. approx. 30000-50000 ppm (Gupte and Nair, 2010). In this context, fermentation of whey using LAB to produce the biomass is one of the novel ways to utilize this dairy by-product that further broadens the market potentiality of whey (Ghanadzadeh *et al.,* 2012).Whey has been used to culture lactic bacteria, but mainly for lactic acid production rather than biomass generation (Lund *et al.,* 1992; Youssef and Goma, 2005; Shahbazi *et al.,* 2005; Altiok *et al.,* 2006; Panesar *et al.,* 2007; Agarwal *et al.,* 2008). Richardson *et al.,* (1977) pioneered work on the use of whey as a low-cost alternative medium for the propagation of lactic starter cultures for cheese makers. The present study is therefore planned to optimize fermentation parameters for the production of cell biomass of *Lactobacillus helveticus* MTCC 5463 in cheese whey at pilot scale.

Materials and Methods

Materials and Media

All chemicals and reagents were of at least analytical grade and supplied by Sigma-Aldrich (Mumbai, India) unless specified. All the media used for enumeration of bacteria were purchased from Himedia (Mumbai, India). Unsalted cheddar cheese whey (CW) was procured from VidyaDairy (Anand, India). Skimmilk powder (Sagar) was purchased from local super market (Anand, India).

Bacterial strain

Pure strains of *L. helveticus* MTCC 5463 was provided by Department of Dairy Microbiology, AAU (Anand, India). *Lb.helveticus* MTCC 5463 (earlier known as *Lb. acidophilus* V3) strain was originally isolated from vaginal tract of a healthy adult female in India at Gujarat Agricultural University (Khedkar *et al.,* 1991). Based on the studies of its biochemical characteristics, it showed ability to grow in the presence of 0.3% sodium taurocholate, deconjugate bile acids, and reduce cholesterol *in vitro* (Ashar and Prajapati, 1998). A hypocholesterolemic effect of *L. helveticus* MTCC 5463 was reported in human subjects with different cholesterol levels (Ashar and Prajapati, 2000). A maximum reduction of 21 % was observed in volunteers having cholesterol level of 200-220 mg / dl suggesting the potential of the strain in preventing the risk of coronary heart diseases. The strain exhibited significant antimicrobial activity against *Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella enteric* serovar *typhi*, and *Escherichia coli* (Khedkar *et al*., 1990).

The strain produced extracellular polysaccharide and was able to adhere to cells of the human carcinoma cell line HT29 (Vishwanath *et al.,* 2012). Other than this a number of probiotic and synbiotic milk based products have already been developed using this strain.

The strain was activated from its frozen form (stored in 10% glycerol at -80 °C) by giving one transfer in MRS broth. This was followed by 2 successive transfers into sterile MRS broth, followed by 2 transfers into sterile whey, under incubation conditions of 37 ºC for 12 h.

Optimization of the inoculum rate

Pure strain of *L. helveticus* MTCC 5463 was grown overnight for 24 h in MRS broth. Subsequently it was inoculated at 2%, 4%, 6%, 8% and 10% (v/v) rate in to MRS broth. All the flasks were incubated at 37° C for 24 h. After incubation, samples were taken from each flask to analyze the biomass. The cells were harvested from fermented media by centrifugation at 6000xg for 20 min at 4°C (REMI C30, India). Cell pellet of the lactobacilli were washed twice with saline (0.85% w/v) and the wet yield was determined gravimetrically and expressed as g/L. Total viable counts of lactobacilli were measured using MRS Agar and expressed as log cfu/ml (De Man *et al.*,1960).

Optimization of growth parameters and media composition

CW was subjected to indirect heating at 92°C for 20 min in order to remove whey proteins by thermo coagulation and filtration of precipitate. The deproteinized whey was autoclaved and used as base media. Batch experiments were conducted in a fully automatic fermenter (Shree Biocare, India). Base media was inoculated with 6% (w/v) pure strain of *L. helveticus* MTCC 5463 and fermented in batch fermenter in pH controlled condition. Sodium hydroxide solution (6 N) and hydrochloric acid solution (6 N) were automatically fed at 0.3 ml/min flow rate using peristaltic pump. The speed of agitator was fixed at 80 rpm and the dissolved oxygen content was kept below 20%. Optimization of incubation time, temperature and pH were done using response surface methodology (RSM). The experiments were carried out with total 20 different combinations of temperature (35 ºC-45 ºC), pH (5.5– 6.5) and incubation period (12 h–24 h) as suggested by Design – Expert (ver. 9.0.2). Similarly, optimization of nutrients (yeast extract and proteose peptone) supplementation level was also done using RSM. The experiments were carried out with total of 13 different combinations of yeast extract (0.1 to 1%, w/v) and proteose peptone (0.5-1%, w/v) as suggested by Design – Expert (ver. 9.0.2). Samples were collected from 1 L of thoroughly mixed fermented media from bioreactor to determined viability and total biomass yield of *L. helveticus* MTCC 5463.

Experimental design

In the optimization of growth parameters, independent variables were pH, temperature and time of fermentation. Whereas in nutrient supplementation, the independent variables were Yeast extract (YE) and Proteose peptone (PP). For both the experiments, central composite rotatable design (CCRD) of Response Surface Methodology (RSM) using Design – Expert (ver. 9.0.2) was used. In the optimization process the response can be related to chosen factors by linear or quadratic models. Adequacy of model was evaluated using F-ratio and coefficient of determination (R^2). Model was considered adequate when F-calculated was more than tabulated F value and R^2 was more than 80%. The analysis of variance (ANOVA) tables were generated and

the effect of variables at linear, quadratic and interactive level on the individual response was described using significance at 5% levels of significance.

Statistical analysis

Data were subjected to statistical analysis using completely randomized design (CRD). The significance was tested at 5 % level of significance using mean value, co-efficient of variance (C.V) and critical difference (C.D).Values with $P<0.05$ were considered statistically significant. Whereas for the optimization study, Central Composite Rotatable Design (CCRD) of response surface methodology were performed using Designed expert 9.0.2 software package.

Results and Discussion

To measure the growth of *L. helveticus* MTCC 5463in three different media (MRS, Skim milk and Cheese whey), total viable counts and changes in pH were monitored after every 6 hours of incubation at 37 °C for initial 24 h and then at an interval of 12 h up to 120 h. The initial pH of the media was adjusted to 6.5 and the inoculation rate was kept constant at 6% (v/v) as optimized. The growth pattern and changes in pH are shown in figure 1 and 2 respectively.

Viable cell count clearly indicated that *L. helveticus* MTCC 5463 could efficiently grow in all the three media. Changes in cell concentration (log cfu/ml) in milk, whey and MRS media was found to be significantly ($P<0.05$) different irrespective of time. Among them the mean viable cell count was highest in MRS broth (9.12 log cfu/ml) followed by Skim milk (9.03 log cfu/ml) and cheese whey (8.72 log cfu/ml).The mean viable count, irrespective of the media, reached at the peak after 12 h of incubation (9.3 log cfu/ml) which remained statistically unchanged ($P>0.05$) till 84 h of incubation. This indicated that the average stationary phase in all the three media ranged from 12h-84 h. However there was significant decline at 96 h of incubation. The interaction effect of period of incubation and the media was non-significant but there was not much difference in the viable cell count at 12 h in all the three media. The rate of increase was very slow after 24 h of incubation and hence it is not recommended to incubate the culture for more than 24 h for harvesting the cells.

During fermentation the pH declined continuously with the increase in incubation period in all the three media as depicted in figure 2. The average change in pH was comparable in milk and whey which was significantly ($P<0.05$) lower than MRS broth. The average decline in pH drop was significant at every period of estimation up to 18 h. However further decline was comparable between 24 h to 36 h, 36 h to 60 h, 48 h to 72 h, 60 h to 96 h and 72 h to 120 h. The interaction effect of period and media was also significant. At the end of 24 h, the pH of whey and MRS medium was comparable but that of milk was significantly lower. The decline of pH in first two hours was maximum in whey followed by MRS and milk which indicated that culture enters into log phase in whey more rapidly as compared to other media.

Optimization of growth parameters

An RSM experiment was framed on the Central Composite Rotatable Design (CCRD) with three factors viz. pH, time and temperature of fermentation. The 20 experiments so generated by the Design Expert 9.0.2 software as shown in table 1 were run and the corresponding response in terms of total viable count (TVC) log cfu/g and Dry Biomass yield g/L was obtained after running the trials in biofermenter.

Effect on TVC

The average TVC of *L. helveticus* MTCC 5463 varied from 9.73 to 13.29 log cfu/g. Viability of cells was minimum when the strain was grown in cheese whey at 45 ºC for 12 h with constant maintenance of pH at 6.0 whereas maximum TVC were observed when the strain was grown in cheese whey at 40 ºC for 18 h with constant maintenance of pH at 6.5as presented in table 1. Surface response for effect of different growth parameters on TVC of *L. helveticus* MTCC 5463 grown in cheese whey is shown in figure 3. Better fit of quadratic model for TVC of the *L. helveticus* MTCC 5463 was explained on the basis of regression analysis of the data presented in table 1. Coefficient of determination (R^2) of 0.8734is in close agreement with adjusted R^2 of 0.7594. This validates experimental and predicted levels of total viable counts. Higher model F value (7.66) than tabulated F value supported the significance of model for predicting the effect of variables on TVC of *L. helveticus* MTCC 5463. Furthermore, higher adequate precision value (APV) (7.290) than required value (4.00) indicated the high and adequate prediction ability of the model.

Multiple regression equation generated to predict the TVC as affected by different factors in terms of coded factor is given below:

Total viable counts = +13.32+0.19* A-0.059* B-0.37* C+0.11* AB-0.18* AC+0.61* BC-0.44* A^2-0.88* B^2-0.85* C^2

Effect on biomass

Biomass yield of *L. helveticus* MTCC 5463is shown in table 1. Cheese whey inoculated (18 h fermentation time) with *L. helveticus* MTCC 5463 and incubated at 32 ºC and 6.5 pH showed minimal dry biomass yield of 0.10 g/L whereas maximum dry biomass yield of 2.63 g/L was found at 40 ºC for 28 hours and at pH 6.5. The regression analysis of the data presented in table 2 indicated that the coefficient of determination (R^2) was 0.8808 and that the model was significant. The ANOVA of quadratic model showed that model F value of 8.21 was more than the tabulated value. Adequate precision value (APV) was 10.435, which was significantly higher than minimum desirable (4.00) for high prediction value. All these parameters showed that the model can be used to describe the effect of variables on biomass production of *L. helveticus* MTCC 5463 grown in cheese whey.

Multiple regression equation generated to predict the biomass yield as affected by different factors in terms of coded factor is given below:

Biomass yield = 1.73+0.24* A+0.16* B-0.33* C-0.15* AB5.27E-03* AC-0.16* BC+0.06* A^2-0.49* B^2-0.12* C^2

Positive coefficient estimate of fermentation time (Table 2) indicates that it had significant ($P<0.01$) positive effect on biomass yield at linear level. It means with increase in the fermentation period, biomass yield from *L. helveticus* MTCC 5463 increases. Also pH has significant ($P<0.05$) effect on biomass yield. The interaction effect of all the variables in cheese whey fermentation showed non-significant effect on biomass yield. However, at quadratic level, effects of all variables were non-significant except for temperature. The surface responses for interactive effect of variables on biomass yield are shown in figure 4(a, b, c).

Current study results have shown that the physical growth parameters such as time, temperature of incubation and pH of fermentation medium has definite significant effect on production of biomass and survivability of the strain.

Table.1 Combinations generated by RSM for optimization of growth parameters

	Factor 1	Factor 2	Factor 3	Response 1	Response 2
Run	**A:Time H**	**B:Temp °C**	**C:pH**	**Dry Biomass Yield g/L**	**TVC log cfu/g**
1	28	40	6.5	2.63	13.05
2	18	40	6.5	2.15	13.29
3	24	35	6	1.64	11.85
4	18	40	7.3	1.28	10.77
5	18	40	6.5	1.63	13.29
6	18	40	6.5	1.61	13.22
7	18	48	6.5	0.85	11.47
8	24	45	6	1.75	10.93
9	12	45	6	1.86	9.73
10	24	35	7	0.92	9.95
11	18	40	6.5	1.63	13.28
12	8	40	6.5	1.45	12.28
13	18	40	6.5	1.64	13.19
14	24	45	7	0.65	10.84
15	18	40	6.5	1.62	13.27
16	12	45	7	0.49	10.96
17	12	35	7	0.40	9.91
18	12	35	6	0.89	11.72
19	18	40	5.7	1.81	12.27
20	18	32	6.5	0.10	11.37

Table.2 Regression coefficients and ANOVA of fitted quadratic model for total viable count and dry biomass yield of *L. helveticus* MTCC 5463 grown in cheese whey

Partial Coefficients	**Dry biomass yield**	**TVC**
Intercepts	1.73	13.32
A- Time of fermentation	0.24^{*}	0.19^{NS}
B-Temperature of fermentation	0.16^{NS}	-0.059^{NS}
C- pH of fermentation	-0.33^{*}	-0.37^{NS}
AB	-0.15^{NS}	0.11^{NS}
AC	$5.27E-03^{NS}$	-0.18^{NS}
BC	-0.16^{NS}	0.61^{*}
A^2	0.06^{NS}	-0.44^{*}
B^2	-0.49^{*}	-0.88^{*}
C^2	-0.12^{NS}	-0.85^{*}
Model F	8.21	7.66^{*}
R^2	0.8808	0.8734
APV	10.432	7.29

** Significant at 1% level (P<0.01), * Significant at 5% level (P<0.05), NS: Non-significant

Table.3 Combinations generated by RSM for optimization of nutrient supplementation

Run	Factor 1 A:YE %	Factor 2 B:PP %	Response 1 Yield g/L	Response 2 TVC Log cfu/g
1	0.03	0.75	2.186	13.21
2	0.6	0.75	3.244	15.11
3	0.6	0.75	3.103	15.24
4	1	0.5	2.997	14.23
5	0.2	1	2.374	14.02
6	0.6	1.1	2.978	15.26
7	0.2	0.5	2.685	14.47
8	0.6	0.4	2.828	15.12
9	0.6	0.75	3.018	15.48
10	0.6	0.75	3.244	15.68
11	0.6	0.75	3.243	15.98
12	1	1	3.622	14.85
13	1.17	0.75	3.310	15.53

Table.4 Regression coefficients and ANOVA of fitted quadratic model for total viable count and biomass yield of *L. helveticus* MTCC 5463 grown in cheese whey

Partial Coefficients	Dry biomass yield	TVC
Intercepts	3.17	15.5
A- Level of yeast extract	0.39*	0.48*
B- Level of proteose peptone	0.066 NS	0.046 NS
AB	0.23*	0.27 NS
A^2	-0.19*	-0.65*
B^2	-0.11*	-0.26 NS
Model F	40.14*	4.49*
R^2	0.9663	0.7624
APV	20.726	6.029

** Significant at 1% level (P<0.01), * Significant at 5% level (P<0.05), NS: Non-significant

Figure.1 Growth pattern of Lb. helveticus MTCC 5463 in MRS, Skim milk and Cheese whey

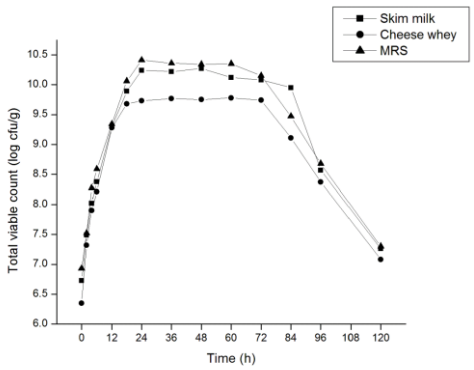


Figure.2 Changes in pH during fermentation of different media (MRS, Skim milk and Cheese whey)

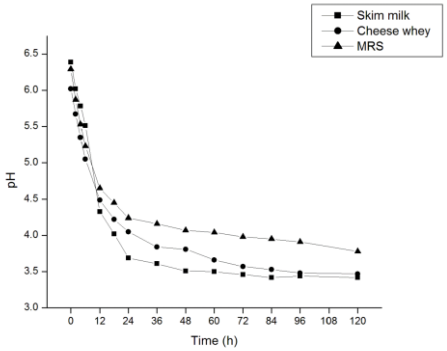
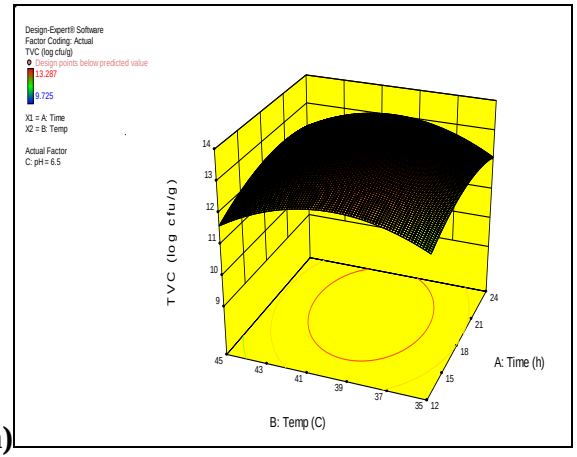
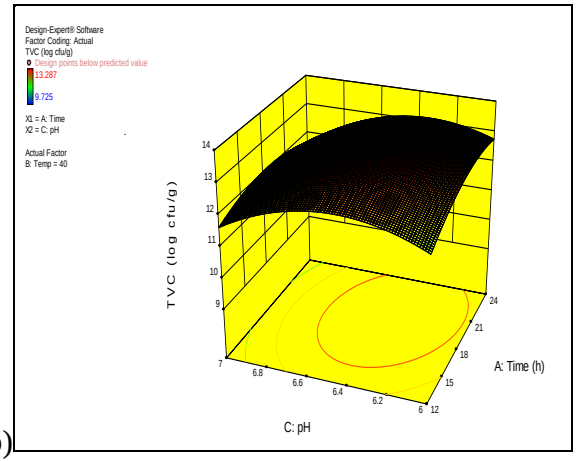


Figure.3 Surface response for effect of different growth parameters on TVC of *L. helveticus* MTCC 5463 grown in cheese whey; (a) Time and temperature (b) Time and pH (c) Temperature and pH



a)



b)

c)

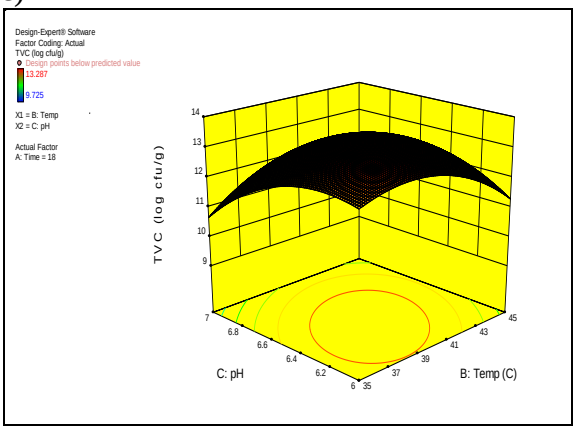
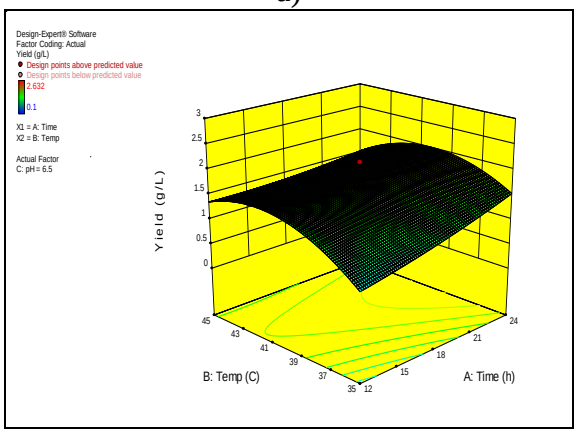
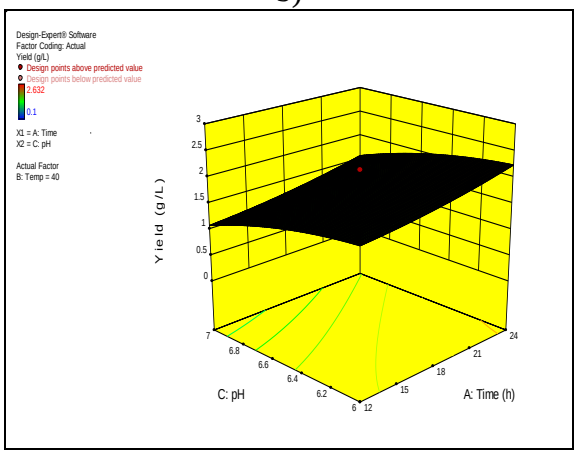


Figure.4 Surface response for effect of different growth parameters on biomass yield of *L. helveticus* MTCC 5463 grown in cheese whey; (a) Time and temperature (b) Time and pH (c) Temperature and pH

a)



b)



c)

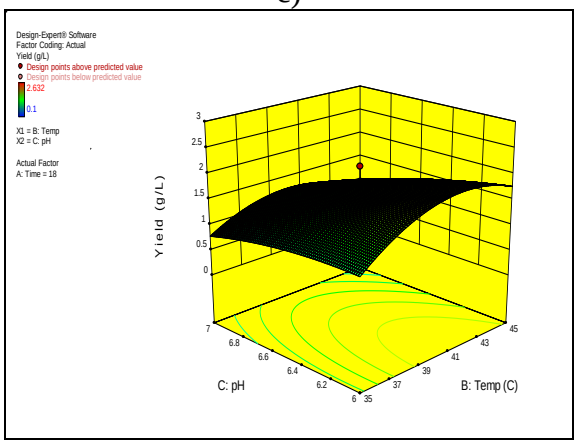


Figure.5 Surface response effects of different nutrient supplementation (yeast extract and proteose peptone) on TVC of *L. helveticus* MTCC 5463 grown in cheese whey

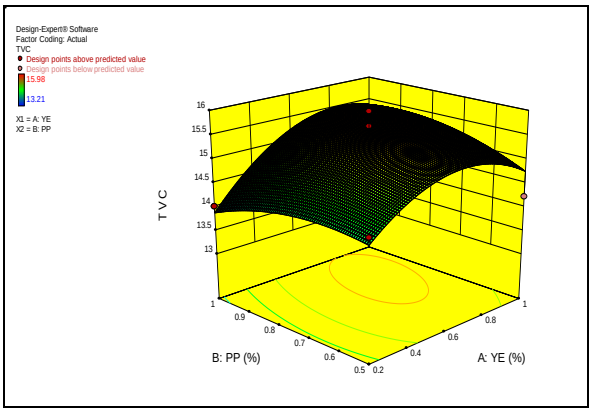
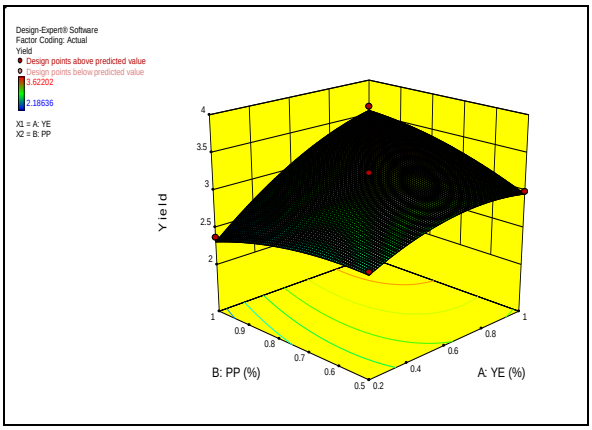


Figure.6 Surface response effects of different nutrient supplementation (yeast extract and proteose peptone) on biomass yield of *L. helveticus* MTCC 5463 grown in cheese whey



The major influence of pH over biomass production in fermentation processes is due to the fact that the catalytic activity of the enzymes and metabolic activity of the microorganism depend on extracellular pH. According to Hofvendahl and Hahn-Hägerdal (2000) and Wood and Holzapfel (1995), optimal pH for lactic acid production by lactic acid bacteria varies between 5.0 and 7.0 and is dependent on the strain which is in accordance with the results obtained in this study (pH 6.25) for *L. helveticus* MTCC 5463. Also it is indicated in Bergey's manual (Bergey *et al.*, 1974) that the optimum temperature for lactobacilli ranges from 30 ºC– 40 ºC and pH from 5.5 -6.2. Similarly, the optimum temperature for highest viable biomass production was found to be 40 °C. This result is in agreement with observation reported by Tango and Ghaly (1999) mentioning that maximum biomass was obtained at 42 ºC. However, slightly higher temperature (45 °C) was also reported by other researchers for *L. helveticus* (Kulozik and Wilde, 1999). This may be attributed to strain to strain to variation. On the basis of the results, further trials for the biomass production of *L. helveticus* MTCC 5463 were taken keeping temperature at 40 ºC, pH at 6.25 and incubation period of 24 hours.

Effect of nutrient supplementation on biomass production

An RSM experiment was framed on the Central Composite Rotatable Design (CCRD) with two factors *viz*., Yeast extract and Proteose peptone. The 13 experiments so generated by the Design Expert 9.0.2 software were run and the corresponding response in terms of TVC log cfu/g and Dry Biomass yield g/L was obtained after running the trials in biofermenter (Table 3).

Effect on TVC

The results obtained are shown in table 3. The average TVC of *L. helveticus* MTCC 5463 varied from 13.21 to 15.98 log cfu/g. Total viable count was minimum (13.21 log cfu/g) when the strain was fermented in cheese whey supplemented with 0.03 % YE and 0.75% PP at optimized growth parameters whereas maximum TVC of 15.98 log cfu/g was obtained when cheese whey was supplemented with 0.6% YE and 0.75% PP.

Better fit of quadratic model for TVC of the *L. helveticus* MTCC 5463 was explained on the basis of regression analysis of the data presented in table 4. Coefficient of determination (R^2), 0.7624is in close agreement with adjusted R^2 of 0.5926. This validates experimental and predicted levels of total viable counts. Higher model F value 4.49 than tabulated F value supported the significance of model for predicting the effect of variables on TVC of *L. helveticus* MTCC 5463. Furthermore, higher adequate precision value (APV) (6.029) than required value (4.00) indicated the high and adequate prediction ability of the model. Surface response effects are shown in figure 5. Multiple regression equation generated to predict the TVC as affected by different factors in terms of coded factor is given below:

Total Viable counts = +15.5+0.48* A+0.046* B+0.27* AB-0.65* A^2-0.26* B^2

Effect on biomass

Cheese whey supplemented with 0.03 %YE and 0.75% PP showed minimal dry yield of 2.186 g/L whereas maximum dry yield of 3.622 g/L was found in medium supplemented with 1 %YE and 1 % PP and fermented at 40ºC for 24 hours.

The regression analysis of the data presented in table 4 indicated that the coefficient of determination (R^2) was 0.9663 and that the model was significant. The ANOVA of quadratic model showed that model F value of 40.14 was more than the tabulated value. Adequate precision value (APV) was 20.726, which was significantly higher than minimum desirable (4.00) for high prediction value. All these parameters showed that model can be used to describe the effect of variables on biomass production of *L. helveticus* MTCC 5463 grown in cheese whey.

The coefficient estimates of dry biomass model (Table 4) showed that supplementation of cheese whey with YE and PP showed significant ($P<0.05$) positive effect on yield at linear level. However, at quadratic level, both YE and PP did impart significant ($P<0.05$) negative effect on yield of *L. helveticus* MTCC 5463. Interaction of all variables with each other also imparted positive significant effect on yield ($P<0.05$). Surface responses for interactive effect of variables on yield are shown in figure 6.

Multiple regression equation generated to predict the biomass yield as affected by different factors in terms of coded factor is given below:

Biomass yield = +3.17+0.39* A+0.066* B+0.23* AB-0.19* A^2-0.11* B^2

The optimized value for level of YE and PP i.e. 0.95% (w/v) for each, were slightly higher than the level of supplementation reported by Masuda *et al.,* (2006). On the other hand, the level optimized for different media constituents in the present study are found to be less than the data reported by other researchers (Liew *et al.,* 2005; Bevilacqua *et al.,* 2008; Chang and Liew, 2012). The difference found in the studies regarding level of YE and PP to obtain maximum biomass production from lactobacilli may be mainly due to addition of other ingredients such as glucose, meat extract, tryptone, casein or other protein hydrolysates and salts along with YE and PP which may provide growth nutrients to lactobacilli and thereby reduces the requirement of YE and PP. Nevertheless, from commercialization point of view, cost may be considered as one of the important factor for selection of different nitrogen and carbon source to be used as media supplementation. Also the quantity of growth nutrients available in base media (non-supplemented) may also be considered as important factor for selecting a range of nutrients at different level of supplementation.

The stimulating effect of yeast extract on the growth of lactic acid bacteria is well known as yeast extract is a rich source of amino acids, peptides, nucleotides and group B vitamins, most of which are essential for stimulating the growth of lactobacilli (Møretrø *et al.,* 1998). Our findings that PP, when used in combination with YE has a positive effect on growth of *L. helveticus* MTCC 5463 may have a number of explanations. Both the composition and average peptide length of complex nitrogen sources for bacteriological media differ widely (Oxoid Manual, 1990). However, since the proteolytic system of lactobacilli is similar to that of lactococci (Kunji *et al.,* 1996) it is conceivable that, because of competition of di, tri, and oligopeptide for the available carriers, increase of peptide concentration may not result automatically in increased growth. In fact, increased concentration of peptides whose amino acid composition is different from that required for balanced growth may limit the uptake of essential peptides and amino acids, thus resulting in growth limitation. Based on the results obtained from the experiment it was decided to supplement cheese whey by addition of yeast extract and proteose peptone at 0.95% (w/v) each to achieve the highest cell biomass.

The present study concluded that cheese whey supplemented with yeast extract and proteose peptone for the growth of probiotic *L. helveticus* MTCC 5463 showed the medium based on this low-cost by-product was suitable for producing high amount of cell biomass. In general, fermentation in this economical medium under optimized growth condition could be used as an alternative to the standard media (MRS broth), which appear to be too expensive for growing LAB on an industrial scale.

Cell biomass of *L. helveticus* MTCC 5463, an important microorganism recognized as an indigenous probiotic strain with maximum number of healthy living cells of the order of 14 log cfu/g was obtained by optimizing the process and parameters of fermentation. The whole experiment has helped in standardizing the conditions for de proteinated cheddar cheese whey fermentation for maximum biomass yield. Based on the results obtained in various phases, it could be concluded that cheddar cheese whey supplemented with 0.95% yeast extract and 0.95% proteose peptone, inoculated with 6% (v/v) active culture of *L. helveticus* MTCC 5463 and fermented for 24 h at temperature of 40 ºC and pH 6.25 could yield 3.25 g/L dry cell biomass and 14.82 log cfu/g total viable count.

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