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Development of Cheddar Cheese Whey based Growth Medium for *Lactobacillus helveticus* MTCC 5463

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Abstract

Whey has been used as a growth medium for lactic acid bacteria (LAB). This is the cheapest medium for production of biomass in larger amount as the whey is a by-product of dairy industry. The pure strains of *Lactobacillus helveticus* MTCC 5463 was inoculated in Cheddar cheese whey. Proteolysis was done by treating with papain (0.5%) at 50°C for 4h. Among several nutrient supplements used to promote the growth of bacteria; WPC (@ 0.5%) and inorganic salt $MnSO_4$ (@0.01%) were found most promising for boosting the growth of lactic culture. Such standardized whey based medium was compared for growth characteristics of *Lb. helveticus* MTCC 5463 to that of only whey and commercially available media (MRS) and found to have no significant difference in growth of bacteria. The viability of *Lactobacillus* in MRS as well as standardized whey based medium was also non-significant. To assess industrial feasibility for the commercialization of medium, yield of biomass of the strain was determined at laboratory scale and found to be 2.41 g from 500 ml whey. When this optimized process was scaled up to 5L capacity using fermenter for 12 h, dry yield of *Lb. helveticus* MTCC 5463 was found 5.51 g/L while total viable counts were 10.11log cfu/g. Thus, use of whey as culture media for the production of biomass of lactic acid cultures offers a low cost alternative for commercial media.

Keywords: Lactic acid bacteria (LAB), proteolysis, biomass, nutrient supplements, viable counts

Whey is serum obtained after removal of fat and casein from milk (Agustriyanto and Fatmawati, 2009). Practically it is a by-product of the cheese and casein manufacturing industry, with an annual worldwide production of about 115 million. Therefore, production of whey in very large amount and its utilization has been a continuing challenge for dairy industry. BOD and COD values of whey are very high, e.g. acid whey has BOD value of 35000 to 45000 mg per liter and COD values of ranging from 55000 to 70000 mg per liter (Mawson, 1994). Because of this high BOD value, the whey disrupts the biological operation of sewage disposal plants (Cavit Atkin *et al*. 1967). The safe disposal of whey results in increased operating costs of the effluent treatment plants due to high consumption of electrical energy. The way of whey disposal as a waste will lead to the loss of valuable milk nutrients. This in turn affects the profitability of dairy plant. Hence, utilization of this valuable by-product leads to the financial advantage in dairying, as well as, it reduces the organic load and treatment costs on the effluent treatment plant by reducing the consumption of electrical energy (Mallik and Kulkarni, 2010).

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the organic load and treatment costs on the effluent treatment plant by reducing the consumption of electrical energy (Mallik and Kulkarni, 2010). Therefore, there is continuing interest in utilizing this by-product as a fermentation substrate for the production of value-added products. The high nutritional value of whey makes it an interesting substrate for the development of fermented foods (Pescuma *et al.* 2012). This by-product is a rich substrate that has been suggested for many applications including solid enrichment in cheese manufacture, bacterial or yeast growth medium to produce biomass, animal nutrients supplement, source of added value proteins (Aguirre-Ezkauriatza *et al.* 2010).

Lactic acid bacteria are commonly important in many branches of industry, e.g., food industry and pharmaceuticals as probiotic products and dietary supplements. There are many products in market containing viable Lactic acid bacteria (LAB) cells. The primary objective of these products is to achieve persistent colonization of the bacteria in the gut during the treatment of variety of conditions such as gastro intestinal disorders (e.g., post antibiotic therapy, adjustment of microbial imbalance in the gut, liver diseases). Their positive impact on human health explains the great interest of scientists in their growth and physiology. (Polak Barecka *et al.* 2010) Most lactic acid bacteria (LAB) are facultative anaerobic, catalase-negative, non-motile and non-spore forming. Expensive culture media, which contain natural complex organic nitrogen sources such as yeast extract, malt extract and/or polypeptone, are necessary for the cultivation of lactic bacteria because nutritional requirement of lactic bacteria is very complicate (Tanaka *et al*. 1995).

Lactic acid bacteria have complex growth factor requirements including B vitamins, several amino acids, and purine and pyrimidine bases. Whey protein hydrolysate (WPH) is a potential nutrient supplement which is readily useable by microbes. It can be readily produced by dairy processors on-site, either by direct hydrolysis of whey, or by the hydrolysis of WPC (Fitzpatrick *et al*. 2001).

Probiotic bacteria grow slowly in milk because of a lack of proteolytic activity, and the usual practice is to add yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) to reduce the fermentation time. *Lactobacillus delbrueckii* ssp. *bulgaricus* produces essential amino acids owing to its proteolytic nature, and the symbiotic relationship of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* is well established; the former organism produces amino nitrogen for the latter organism. Such starter cultures may necessitate the incorporation of micronutrients (peptides and amino acids) through whey powder (WP), whey protein concentrate (WPC), acid casein hydrolysate (ACH), or tryptone for reducing the fermentation time and for improving the viability of probiotic bacteria (Dave *et al.* 1998b).

Bhuvaneshwari and Sivasubramanian (2011) investigated the treatment of the organic wastes using microbiological process for effective usage of waste and to develop value added products from it. The organic wastes used in this processes were domestic wastes, vegetable wastes, fruit wastes, bakery wastes and whey. They used *Lactococcus lactis subsp lactis for* synthesis of lactic acid. The optimal production of lactic acid and bacterial growth were 35.45 g/l and 1.34 g/l respectively from whey by *Lactobacillus rhamnosus*.

Fermentation of whey by Lactic Acid Bacteria (LAB) usually focuses on the production of lactic acid. Alternatively, whey or whey permeate has the potential as a culture medium for the propagation of dairy cultures. Whey or UF whey permeate are cheap and readily available sources for use as fermentation media (Parente and Zottola, 1991).

Whey has mainly been used to grow LAB for purposes of lactic acid production, its potential as a growth medium for biomass production has not been explored. Therefore, looking into the nutritional value of whey proteins, the hydrolysed whey proteins can be a good ready to serve nutrient to enhance biomass production of the beneficial LAB. Further enhancement can be done by nutrient supplementation.

MATERIALS AND METHODS

Unsalted cheddar cheese whey was collected from Amul Dairy, Anand. Whey was hydrolysed by method described by Macwan *et al.* 2016.

Inoculum preparation

The pure strains of *Lactobacillus helveticus* MTCC 5463were acquired from the culture collection of Dairy Microbiology Department, Anand Agricultural University, Anand, Gujarat. The strains were activated from its frozen form (stored in 10% glycerol at -80 °C) by giving one transfer in respective broth. This was followed by two successive transfers into sterile respective broths under incubation conditions of 37 ºC for 12 h.

Nutrient supplementation

As source of nitrogen (Yeast extract and Whey Protein Concentrate) and ammonium salts (Ammonium Sulphate, Magnesium Sulphate and Manganese Sulphate) were tested as a supplement at yeast extract (0-3%) (Aeschlimann *et al.* 1990), whey protein concentrate (0-2%) (Bury *et al*. 1998), ammonium sulphate (0-1%) (Arasaratnam *et al.* 1996), manganese sulfate (0.003-0.02 % w/v) and magnesium sulfate (0.005-0.01% w/v) (Eldeleklioğlu *et al*. 2013) rate. Yeast Extract and Whey Protein Concentrate were added in cheddar cheese whey before papain treatment (Macwan *et al.* 2016). Whereas ammonium sulphate, $MgSO_4$ and $MnSO_4$ were added after papain treatment. Effect of each supplement was checked individually.

Samples collection and analyses

Cultures (were inoculated at the rate of 2% (v/v), incubated at 37°C and samples were withdrawn after 0, 6, 12 and 24 h of incubation. All the samples were analysed for changes in pH, titratable acidity and viable count.

Determination of biomass yield

The cells were harvested from each media by centrifugation at 6000xg for 20 min at 4 °C (REMI C30, India). Cell pellet was washed twice with saline water (0.85% w/v) and the wet yield was determined gravimetrically.

Measurement of total viable counts

Serial dilutions of the samples were prepared by aseptically transferring 1 g of the pellet to 9 ml phosphate buffer dilution blank to obtain 1:10 dilution. Subsequently 1 ml of above dilution was used for making further dilutions in 9 ml phosphate buffer tubes. Suitable dilutions were prepared and poured in a set of sterile MRS agar plate in duplicates. After setting of the agar, another layer of the same medium (5-7ml) was poured. The plates were then incubated at 37±2 ºC for 48 h. After incubation, plates were removed for counting the colonies. The bacterial count was expressed as log cfu/ml.

Determination of biomass yield on dry basis (Yx)

The wet biomass of lactic cultures obtained as per the description given in 3.3.2.1. The total solids of the pellet was determined according to the procedure described in IS: 1479 (II) (1961). In a dry and clean previously weighed stainless steel dish, 2 g of the sample was taken and the weight of sample was recorded. The duplicate dishes were placed on hot plate till the moisture got evaporated (indicated by a slight brown colour). The dishes were then transferred to the oven maintained at a temperature of 102 ± 2 °C for 1h or till the constant weight between two consecutive readings was recorded. The dishes were then transferred to desiccator for 5 min. Then final weight was recorded. Total solid was calculated by using the following formula. To obtain the percentage total solids content of the sample, the % moisture was subtracted from 100.

$$\text{Moisture (\% by weight)} = 100\ (W_1\text{-}W_2) / (W_1\text{-}W)$$

Where,

W_1 = weight in g of the dish with material before heat to constant weight;

W_2 = weight in g of the dish with material after heating to constant weight;

W = weight in g of the empty dry dish

The biomass was calculated on dry matter basis and expressed as g/L.

Upscaling of Optimized Process for Production of Biomass at Pilot Scale

Batch experiments were conducted in a 5 lit fully automatic fermenter (Shree Biocare, India). Optimized whey based medium was inoculated with 2% (w/v) culture 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 rpm of agitator was fixed at 80 rpm and the dissolved oxygen content was kept below 20%. After 12 h of fermentation Samples were collected from 1lit of thoroughly mixed fermented media from bioreactor to determined viability and total biomass yield of bacteria.

RESULTS AND DISCUSSION

Effect of nutrient supplementation

The supplements were nitrogen sources (yeast extract, whey protein concentrate and ammonium sulphate) and inorganic salts (magnesium sulphate and manganese sulphate). To evaluate effect of these nutrient supplements, all were added at the optimized rate as reported in the literature.

The effects of nutrients supplements on the cell growth of the *L. helveticus* during the batch fermentation of cheese whey is illustrated in Table 1. It appeared from the results that treatment (supplementation of nitrogen source) has no significant effect on changes in pH during growth of cultures. After 24 h of incubation growth of *L. helveticus* resulted in highest drop in whey containing yeast extract, closely followed by the whey containing WPC. Smaller drop in pH of whey supplemented with WPC may have been due to increased buffering action of proteins. The maximum cell number obtained without the addition of nutrient supplements was 8.92 log cfu/ml. WPC addition resulted in growth of 9.28 log cfu/ml of *L. helveticus* and which did not differ significantly with that of addition of yeast extract. Addition of ammonium sulphate did not improve growth characteristics.

Table showed that addition of inorganic salts had significant effect on drop in pH. Drop in pH was highest in $MnSO_4$ containing wheyafter 24 h of incubation, i.e., 3.48. As fermentation time increased pH decreased significantly for all the treatments. However, interaction between treatment and period was again statistically non-significant. Data showed that total viable count increased by addition of inorganic salts. Between the treatments the difference was statistically significant. 9.24 log cfu/ml was

Table 1: Changes in pH after nutrient supplementation during growth of lactic cultures

Incubation period (h)	*Lactobacillus helveticus* MTCC 5463						
	Nitrogen source				Inorganic Salts		
	Control	Yeast extract (0.5%)	WPC (1%)	$(NH_4)_2SO_4$ (0.25%)	Control	$MgSO_4$ (0.01%)	$MnSO_4$ (0.01%)
0	5.82	5.99	6.02	6.05	5.82	5.96	5.96
6	5.01	5.24	5.30	5.44	5.01	5.07	4.85
12	4.25	4.22	4.35	4.32	4.25	4.21	3.88
24	3.79	3.57	3.65	3.94	3.79	3.73	3.48
Source of Variation	**Treatment (T)**	**Incubation period (P)**	**Interaction (T × P)**		**Treatment (T)**	**Incubation period (P)**	**Interaction (T × P)**
SEm	0.09	0.09	0.17		0.05	0.06	0.11
Test (P < 0.05)	NS	*	NS		*	*	NS
CD	NS	0.25	NS		0.16	0.18	NS
CV%	6.21				4.01		

Table 2: Changes in total viable count after nutrient supplementation during growth of lactic cultures

Incubation period (h)	*Lactobacillus helveticus* MTCC 5463						
	Nitrogen source				Inorganic Salts		
	Control	Yeast extract (0.5%)	WPC (1%)	$(NH_4)_2SO_4$ (0.25%)	Control	$MgSO_4$ (0.01%)	$MnSO_4$ (0.01%)
12	8.92	9.32	9.28	8.97	8.91	9.18	9.24
Source of Variation	**Treatment (T)**				**Treatment (T)**		
SEm	0.07				0.08		
Test (P < 0.05)	*				*		
CD	0.20				0.23		
CV%	1.44				1.86		

obtained in $MnSO_4$ containing wheyafter 12 h of incubation.

Data from above table depicted that difference in log cfu/ml among all 3 treatments were comparatively significant. Highest count were obtained in $MnSO_4$ added whey.

However, yeast extract supplementation was not economically attractive, it was decided to supplement with whey protein concentrate as a nitrogen source.

Therefore, optimization of WPC concentration to obtain maximum cell growth was done. Results of lactic acid produced and growth obtained after 12 h of incubation are shown in Fig. 1.

Among source of inorganic salts added $MnSO_4$ could promote growth of lactic cultures significantly. Therefore, further optimization of rate of addition was done.

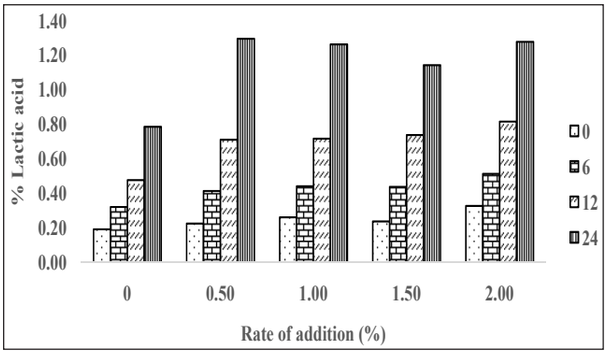

Fig. 1: Changes in titratable acidity during growth of *Lb. helveticus* MTCC 5463 in whey supplemented with WPC

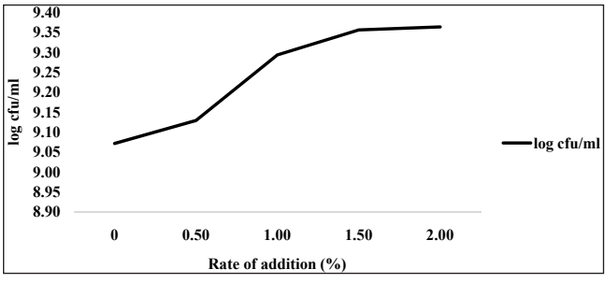

Fig. 2: Effect of WPC on change in titratable acidity of *Lb. helveticus* MTCC 5463

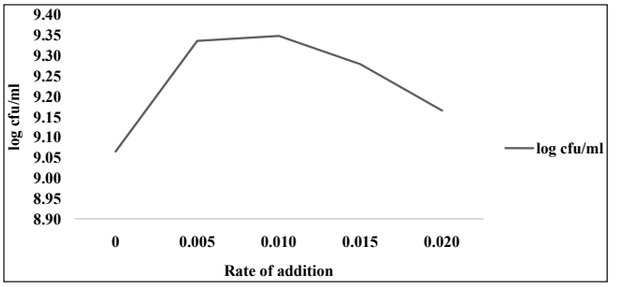

Fig. 3: Effect of WPC on change in total viable count of *Lb. helveticus* MTCC 5463

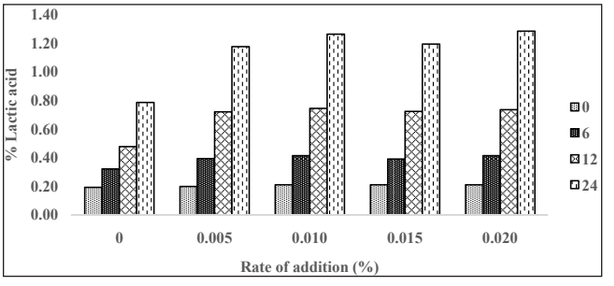

Fig. 4: Effect of MnSO4 on change in total viable count of *Lb. helveticus* MTCC 5463

It appeared from the results that treatment (supplementation of WPC) has significant effect on increase in acidity during growth of lactic cultures. After 24 h of incubation highest acidity was developed in hydrolysed whey containing 0.5% WPC for *L. helveticus.* Above figure showed that total viable count increased with increase in concentration of WPC. After 12 h of incubation 9.36 log cfu of L. helveticus/ml were counted in 1.5% WPC added whey. The growth promoting activity of WPC could be due to Caseinomacropeptides and whey protein content.

Table showed that addition of $MnSO_4$ had significant effect on viability of *Lb. helveticus* MTCC 5463. Highest count was obtained in 0.01% $MnSO_4$ containing wheyafter 12 h of incubation, i.e., 9.35 log cfu/ml followed by 0.005% $MnSO_4$ containing whey i.e., 9.34 log cfu/ml. This difference in count was statistically non-significant. No relevant data has been reported regarding effect of $MnSO_4$ and change in count during growth of *Lb. helveticus* MTCC 5463.

Table showed that addition of $MnSO_4$ had significant effect on increase in acidity. Highest acidity was developed in 0.02% $MnSO_4$ containing whey after 24 h of incubation, i.e., 1.28. Which was statistically at par with 0.01% $MnSO_4$. As fermentation time increased pH decreased significantly for all the treatments. However, interaction between treatment and period was again statistically non-significant.

Addition of 1 or 2% of a whey protein concentrate (WPC) to a whey-based medium used for fermentation with *lactobacillus delbrueckii* subsp. *bulgaricus* 11842 has produced significantly higher bacterial counts than the control whey or whey UF permeate media (Bury *et al*. 1998).

In 2013, Eldeleklioğlu and colleagues found that Manganese is an essential growth factor for *L. casei*, because of its role as a constituent of lactate dehydrogenase. Also magnesium is a critical cation and cofactor in numerous intracellular processes. Mg^{++} has an efficient role in the steps in EMP pathway. This may be the reasons of higher growth obtained in whey containing inorganic salts.

Vasala *et al.* (2005) studied pre-treatment of whey-protein-containing media by the proteolytic microbe *B. megaterium. Lactobacillus salivarius* ssp. *salicinius*, a lactic acid bacterium species that can grow at high salt concentration, was used to ferment lactic acid in cheese whey (with 3 g l^{-1} whey protein content) and lactose mother liquor (90 g l^{-1} lactose, 9 g l^{-1} proteins, 30 g l^{-1} minerals). The contribution of protease enzymes or proteolytic microbes to acid production by lactobacilli was examined. Efficient conversion of lactose to lactic acid was obtained in the presence of additional proteolytic activity.

In 1998, Gomes and co-workers found that the growth and acid production of *B. lactis* in milk were affected by the addition of proteinase-mediated hydrolyzate and, to a lesser extent, by neutrase mediated hydrolyzate; a higher degree of hydrolysis of either hydrolyzate resulted in greater biomass increase and greater acid production. The growth of *B. lactis* on un-supplemented milk was poor. When supplemented with milk hydrolyzate, growth was improved; the exponential phase of growth occurred during the first 8 to 10 h following inoculation (Gomes *et al.* 1998).

Eldeleklioğlu *et al.* (2013) investigated the effects of microorganism strain and yeast extract, $MnSO_4.H_2O$, $MgSO_4.7H_2O$ addition to cheese whey solution on the lactic acid production and enantiomeric excess of produced lactic acid. Lactic acid concentration was increased about three times according to the control experiment by adding additional nutrient supplements (yeast extract, $MnSO_4$) into the cheese whey.

Roy *et al*. (1986) reported 2.7 g l^{-1} h^{-1} lactic acid for a whey permeate medium supplemented with 1.5% yeast extract (YE), while Aeschlimann and von Stockar (1989) observed a higher maximum lactic acid volumetric productivity (3.8 g l^{-1} h^{-1}) in whey ultra-filtrate medium supplemented with 0.4% YE, 0.02% peptone, 1% skim milk powder, 0.02% $MgSO_4$ and 0.005% $MnSO_4$. Considering supplements other than yeast extract, Roy *et al.* (1986) reported 1.43 g 1^{-1} h^{-1} lactic acid for a whey permeate medium supplemented with 0.5% corn steep liquor, and

Vahvaselka and Linko (1987) found a maximum lactic acid volumetric productivity of 1.74 g l^{-1} h^{-1} in whey ultrafilterate supplemented with 0.5% casein hydrolysate (Chiarini *et al.* 1991).

Thus, above literature is in corroboration with the results obtained in this study. Therefore, above literature suggested that whey can be used as a fermentation medium.

Comparison of Standardized Whey Based Medium With Commercial Media

Among the nutrient supplementation done final standardized medium consisted of 0.5% WPC and 0.01% $MnSO_4$ for the growth of *Lb. helveticus* MTCC 5463. Prepared standardized whey based medium was compared for growth characteristics of *Lb. helveticus* MTCC 5463 with that of whey and commercially available media. Whey was heated at 75ºC for 10 min and after addition of 0.5% WPC it was hydrolysed with 50 mg/10 ml papain at 50ºC for 4 h described by Macwan *et al*. 2016. After cooling down to room temperature 0.01% $MnSO_4$ supplementation was done to check growth characteristics of *Lb. helveticus* MTCC 5463. Comparison was made with cheddar cheese whey and MRS medium. Cultures were inoculated at the rate of 2% in all three medium incubated at 37ºC and monitored for changes in pH, titratable acidity and viable counts. Since, colour of commercially available medium was dark it was difficult to judge color change during titratable acidity measurement. So titratable acidity was measured only for optimized whey based medium and whey.

Effect of media on growth of *Lb. helveticus* MTCC 5463

During fermentation acidity increased continuously with the increase in incubation period in all the three media as depicted in Fig. 5. The interaction effect of period and media was also significant. However, measurement of acidity of culture grown in MRS could not be possible due to colour of the medium itself. Change in colour could not be judged properly. Therefore, titratable acidity was measured for remaining two medium only. At the end of 24 h, highest increase was obtained in standardized whey medium, followed by whey. The increase in acidity in six hours was maximum in standardized whey medium followed by whey which was in concordance with that of drop in pH as indicated that culture enters into log phase in standardized whey medium more rapidly as compared to other media.

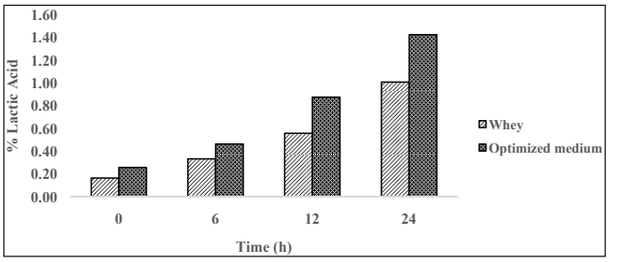

Fig. 5: Effect of medium on change in titratable acidity of *Lb. helveticus* MTCC 5463

Table 3: Total viable count of *Lb. helveticus* MTCC 5463 in different media

Incubation period (h)	Media		
	Whey	Standardized Medium	MRS
12	8.85	9.11	9.13
Source of Variation	**Treatment (T)**		
SEm	0.11		
Test (P < 0.05)	NS		
CD	0.34		
CV%	2.34		

Table showed that different media has no significant effect on growth of *Lb. helveticus* MTCC 5463. After 12 h of incubation, highest count was obtained in MRS media. However, difference in count between MRS and standardized whey medium was statistically non-significant.

Khan (2014) compared growth of *Lb. helveticus* MTCC 5463 in skim milk. Results showed that the culture enters in log phase within the first two hours and the increase in acidity reaches to more than 2% lactic acid within 24 h and then it enters into plateau. Also studied of growth curve of *Lb. helveticus* MTCC 5463 in MRS, skim milk and cheese whey. The growth pattern of viable cell count clearly indicated that *Lb. helveticus* MTCC 5463 could efficiently grow in all the

three media. It entered in log phase within 2 h and reached to peak of log phase at 24 h (10.41 ± 0.62 log cfu/ml) in MRS medium, 36 h (9.77 ± 0.35 log cfu/ml) in cheese whey and 48 h (10.27 ± 0.50 log cfu/ml) in skim milk. These results are concomitant with the results obtained in present study.

Mehmood *et al.* in 2009 studied the acid production by individual LAB strain in fermented milk at 2 h interval up to 8 h. It was found that *S. thermophilus* S6, *S. thermophilus* S7 and *Lb. acidophilus* R20 produced significantly higher acidity i.e. 1.05, 1.04 and 1.02 respectively after 6 h as compared to other LAB isolates.

Prajapati *et al*. (1983) studied growth characteristics of *Lb. acidophilus* in five different media, *viz;* skim milk (SM), skim milk diluted with water (50:50), casein whey, casein whey+20% (v/v) tomato juice (CWT) and diluted skim milk+20% (v/v) tomato juice. All medium were inoculated with 1% (v/v) culture and incubated at 37 ºC for 12 days. Maximum cell count (8×10^9 cfu/ml) was produced by the strain in skim milk at the end of 48 h. They reported that diluted SMT medium was almost parallel to SM in regard to cell numbers till five days of incubation and CWT medium was closely comparable to SM medium with respect to TVC at the end of 12 days. Tomato juice was found to have protective effect on cell viability. SM showed higher acid production by the culture, amounting to 1.96% lactic acid at the end of 120 h as compared with the other media.

Khedkar *et al.* in 1989 investigated the influence of time of incubation and titratable acidity on the viable cell counts of human strains of *Lb. acidophilus* strains viz. LBKV3 and LBKI4 in skim milk. They analysed titratable acidity and viable cell count at selected time intervals of 0, 16, 20, 24, 36, 48, 72, 96 and 120 h. It was found that LBKV3 remained in exponential phase up to 20 h at 37 ºC giving 8.42×10^8 cells/ml and acidity of 0.80%. Both the cultures entered stationary phase and thereafter continued to increase progressively up to 120 h of incubation. The maximum cell count for LBKV3 was observed at 64 h of incubation and that for LBKI4 was observed at 48 h.

Polak-Berecka *et al.* (2011) optimized the media for biomass production of *Lb. rhamnosus* E/N by RSM. The study indicated a medium consisting glucose 15.44(g/L), sodium pyruvate 3.92(g/L), meat extract 8.0(g/L), potassium phosphate 1.88(g/L), sodium acetate 4.7(g/L) and ammonium citrate 1.88(g/L) were found to be optimum. Dry cell yield of 23 g/L were obtained after 18 h of fermentation of optimized media at 37°C with an inoculation rate of 2.5 (v/v) compared to 21 (g/L) of dry cell weight obtained in MRS media with same bioreactor conditions.

To summarize this stage of study in case of *Lb. helveticus* MTCC 5463, compared to MRS medium after 24 h of incubation highest pH drop as well as highest acidity developed in standardized whey based medium. As far as total viable count was concerned highest count obtained in MRS medium followed by standardized whey based medium after 12 h of incubation. However, this difference was statistically non-significant. So this optimized whey based medium can be used as a potential and viable substitute of commercially available media used to cultivate lactic acid bacteria.

Scaling Up of the Optimized Process to a Pilot Scale for Production of Biomass

The yield of biomass is an important attribute for measuring the growth of probiotic bacteria, which ultimately determines industrial feasibility of the strain to be used for commercialization. In this laboratory scale experiment after 12 h of incubation, dry yield of *Lb. helveticus* MTCC 5463, was 2.41 g from 500 ml whey. To scale up the optimized process two types of approaches were followed. In first scaling up was done at laboratory scale and then through fermenter experiment.

Scaling up of the Optimized Process at Laboratory Scale for Biomass Production

To scale up the optimized process, cultures were grown in laboratory scale. 500 ml of standardized whey based medium was prepared and cultures were added in their respective standardized whey medium. After 12 h of incubation, whole medium

was centrifuged. Pelleted biomass was checked for its dry yield by the method as described in section 3.3.2.3.

The yield of biomass is an important attribute for measuring the growth ofprobiotic bacteria, which ultimately determines industrial feasibility of the strain to be used for commercialization. The cell pellet obtained by centrifugation (6000 rpm for 20 min at 4 ºC) of the ferment was washed once with phosphate buffer saline (0.85%). The cell pellet was dried up to constant moisture in an oven maintained at 102 ± 2 ºC. In laboratory scale experiment after 12 h of incubation, dry yield of *Lb. helveticus* MTCC 5463 2.41 g was obtained from 500 ml whey. The average protein content of the whey was 1.10± 0.06 g per 100 ml whey. Thus, for production of 2.41 g biomass 5.5 g whey proteins was present in the whey used for the study.

Scaling up of the Optimized Process at Pilot Scale for Biomass Production

To scale up the optimized process at pilot scale fermenter experiment was done. After 12 h of incubation, dry yield was calculated. Also Total viable count of both the medium and the pelleted biomass were counted.

In fermenter experiment after 12 h of incubation, dry yield of *Lb. helveticus* MTCC 5463 was 5.51 whereas total viable count obtained were 10.11log cfu/g.

Total viable count of the fermented whey not only measure the survivability of bacterial cell but also denotes the survivability against the metabolites produced by cell itself. Data regarding the total viable count after 12 h in biomass obtained after pelleting the 12 h fermented optimized whey medium.

Khan (2014) found Biomass yield of the *Lb. helveticus* MTCC 5463 in cheese whey. Cheese whey inoculated (18 h fermentation time) with *Lb. helveticus* MTCC 5463 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 in media fermented at 40ºC at pH 6.5 for 28 hours. They obtained an average TVC of *Lb. helveticus* MTCC 5463 varied from 13.21 to 15.98 log cfu/g biomass after 24 h of bioreactor fermentation. 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 parameter (39.89 ºC, 6.259 pH for 24 h) whereas maximum TVC of 15.98 log cfu/g was obtained when cheese whey was supplemented with 0.6% YE and 0.75% PP. Thus, results obtained in present study are inlined with results obtained by this author.

Mondragón-Parada and co-workers (2006) worked to isolate and characterize lactic acid bacteria (LAB) strains to be used for biomass production using a whey-based medium supplemented with an ammonium salt and with very low levels of yeast extract (0.25 g/L). The biomass yield on lactose, the percentage of lactose consumption, and the maximum increase in cell mass obtained in the bioreactor were 0.165 g of biomass/g of lactose, 100%, and 2.0 g/L, respectively, which were 1.44, 1.11, and 2.35 times higher than those found in flask cultures.

Lavari *et al.* (2014) explored double use of cheese whey (culture medium and thermos protectant for spray drying of lactobacilli) for their capacity to produce biomass of *Lb. paracasei* JP1, *Lb. rhamnosus* 64 and *Lb. gasseri* 37. All the cultures were found to ferment the media and at highest biomass production, the viability of the cultures ranged between 8-9 log cfu/g of biomass.

Above reported study suggested that biomass yield increased by about two times in bioreactor experiment to that of in laboratory experiments. Therefore, results obtained in this study are in accordance with the literature cited above. Throughout the fermentation optimum pH, temperature and the amount of dissolved oxygen were kept constant. This might be the probable reason for getting higher biomass yield in fermenter compared to flask experiment.

Therefore, attempts made to up scale the process for production of biomass at laboratory scale was turned out successfully and indicated the promise for further scaling up. Similarly, attempts made to up scale the process for production of biomass at pilot scale was also found successful.

CONCLUSION

LAB biomass could be produced from whey, which is a cheap and abundant crude feedstock. It was demonstrated that it is possible to produce LAB biomass using a whey-based medium supplemented with minimal amounts of yeast extract and to substitute most of the expensive yeast extract with the far less expensive ammonium salts without a significant decrease in the final biomass concentration and in the biomass yield.

Whey protein concentrates can stimulate the growth of lactic acid bacteria in whey or UF whey permeate broths. Supplementation with the major whey proteins (α-lactalbumin and β-lactoglobulin) was only 63% as effective as WPC. The components responsible for the increase growth are heat stable as the stimulatory effect is not lost after heating to 121ºC for 15 min. The heat stable components might be a-nucleotides, non-protein nitrogen, or some specific heat stable peptides not present in Bacto-peptone.

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