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Original Research Article

Measuring the Growth of *Lactobacillus fermentum* and *Bifidobacterium infantis* in the presence of N-Acetyl L-Cysteine Hydrochloric Acid

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Abstract

Aim: To study the growth of probiotic microorganisms including *Lactobacillus fermentum* and *Bifidobacterium infantis* in presence of N-Acetyl L-Cysteine Hydrochloric Acid (NACHCl)
Settings and Design*:* *Lactobacillus fermentum* and *Bifidobacterium infantis* growth in the presence of N-Acetyl L-Cysteine Hydrochloric Acid were studied by using MRS broth without any additional supplement as a control.
Material and Methods: *Lactobacillus fermentum* and *Bifidobacterium infantis* isolated from fecal samples of infants and adults were collected. Positive culture of *Lactobacillus fermentum* and *Bifidobacterium infantis* were collected from NCDC NDRI Karnal. Different concentrations of NACHCl including 0.2, 0.4, 0.6, 0.8 and 1.0 g/L were used in this study.
Results: The results were observed up to 16 hours of incubation where in 1.0 g/L i.e. the highest concentration give rise to the optical density of 1.577 for *Bifidobacterium infantis* while for *Lactobacillus fermentum*1.792 when measured at 600nm, however controls of both the cultures reached 1.136 and 1.108 respectively.
Conclusion: The application of N-Acetyl L-Cysteine Hydrochloric Acid certainly supports the growth of probiotic flora to a very good extent and can act as a potential prebiotic.

Keywords: *Lactobacillus fermentum, Bifidobacterium infantis*, N-Acetyl L-Cysteine HCl and MRS broth

1. Introduction

Isolation and identification of microbes, one of the main laboratories experimental work in the area of microbiology. Bacterial strain isolated from traditional cheese and butter etc. various sources in present study from feces and different food sample have been grown in pure cultures. This culture practice followed by various researchers over the ancient time [1]. It's very difficult task to differentiate the strain from one another based on traditional methods [2]. Various procedures employed for description or identification is not stable and hence has been modified from time to time [3]. *Bifidobacterium infantis* is a neonatal intestinal bacteria and part of human microflora which is helpful in digestion of food [4]. It can benefit the host by improving gut immunity and perform antagonistic effect to balance intestinal microbial flora [5].

Microbial flora of neonatal gut plays an important role in early life [6]. If probiotic bacterial population low in gut, stimulation of their multiplication is required [7]. Pasi *et al* 2001 showed the growth of probacteria in the presence of polyunsaturated fatty acid [8]. Zisu and Shah 2003 studied the effect of pH and temperature on the growth of *Bifidobacterium* [9]. Janner *et al* 2004 observed the growth of *Bifidobacterium lactis* supplementation of caseinomacropeptide in the MRS medium [10]. The market demand is increasing for products supplemented with probiotics for alternative and replacement foods (11). The effect of polysaccharides and peptides on the growth of *Lactobacillus* and *Bifidobacterium* has been reported by Zhang *et al* 2020 [12]. In the present investigation, measurement of growth of *Lactobacillus fermentum* and *Bifidobacterium infantis* was studied in the growth media while supplemented of with different concentrations of NACHCl.

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Materials and Methods

Lactobacillus fermentum and *Bifidobacterium infantis* were isolated from fecal samples of infants and adults. Samples were collected in clean sterilized container from volunteer mothers their children and some adults from different places. All samples were transported approximately within two hours to the laboratory and stored at 4^0C temperature until processed.

Medium and Culture Conditions

Both the probiotic species *Lactobacillus fermentum* NCDC-141 and *Bifidobacterium infantis* NCDC-271 *collected from* NCDC NDRI Karnal were cultured in MRS Broth as describe by Mahalakshmi and Murthy (2000) [13]. N-Acetyl L-Cysteine Hydrochloric Acid (NACHCl) in a concentration of 0.2, 0.4, 0.6, 0.8 and 1.0 g/L were added respectively to MRS broth. MRS broth without NACHCl was used as control for culturing of both microbes. The preparation was incubated at 37^0C temperature and spectrophotometric observations were taken on different time interval up to 16 hours.

Measurement of Growth

Population of turbidly of both the bacterial isolate at different interval of time was measured by a Spectrophotometer (Toshniwal TVS 25A Toshiba) using logistic growth and differential equation. *Lactobacillus fermentum* and *Bifidobacterium infantis* increase their numbers by mathematical progression such as doubling of bacterial population density every generation: 1, 2, 4, 8 *etc* or 2^0, 2^1, 2^2, 2^3 ……..2^n (where n = the number of generation). Generation times were calculated in the logarithmic phase of growth using the following differential equation.

The expression of the number of populations at a given time (N), the initial number of bacterial counts in the population (N_0).

Now, the relation between number of generations (n) of bacterial cells and bacterial population is:

$N = N_0 x\ 2^n$

Taking natural logarithm on both sides:

$\ln N = \ln N_0 + n \ln 2$

$n \ln (2) = \ln N - \ln N_0$

$n \ln 2 = \ln \left(\frac{N}{No}\right)$

$n = \frac{\ln (N/No)}{\ln (2)}$ ……(1)

Now, the number of generations (n) is given by ratio of time interval (t) and generation time (t_G)

$N = t/ t_G$……(2)

Using equation 1 and 2

$t/t_G = \frac{\ln (N/No)}{\ln (2)}$

$t_G = \frac{t . \ln(2)}{\ln (\frac{N}{No})}$

or $t_G = \frac{t\ (2.303 \log 10\ (2))}{(2.303\ log 10\ (\frac{N}{No})}$

$t_G = \frac{t \log 10\ 2}{\log 10\ (N) - \log 10\ (No)}$

$t_G = \frac{0.301\ (t)}{\log 10\ (N) - \log 10\ (No)}$

Observed the growth of the bacteria by measuring the optical density of the suspension at 600 nm through a Spectrophotometer.

Results and Discussion

Growth of *Lactobacillus fermentum* and *Bifidobacterium infantis* MRS broth was observed at 0 to 06 hours without altering the growth parameters optical density at 600nm. Then after 08 hours bacteria enter into log phase turbidity of culture suspension almost unchanged for both the bacteria. In exponential phase both the bacteria optical density rapidly increases. After 16 hours optical density increased very slowly and bacterial growth enter into stationary phase.

The effect of 0.2, 0.6, 0.8 and 1.0 g/L concentrations of N-Acetyl L-Cysteine Hydrochloric acid on the growth of *Lactobacillus fermentum* and *Bifidobacterium infantis* increase gradually and then next observation decreased. Optical density of both the isolates was optimized at various incubation period with variation of concentration. The highest optical density and corresponding concentration of NACHCl in each measured period of time for the growth of *Lactobacillus fermentum* was 0.049 at 0.2 g/L (6h), 0.069 at 0.4 g/L (8h), 0.141 at 0.6 g/L (10h), 0.343 at 0.8 g/L (12 h) and 1.792 at 1.0 g/L (16 h) respectively, although the highest optical density of without incorporation of NACHCl was 1.108 at (16 h) (Table 1A & B; Figure 1 A & B).

The optimum concentration of NACHCl in MRS broth of *Lactobacillus fermentum* was 1.0 g/L incubation period for 16 h. Similarly optical density of *Bifidobacterium infantis* increased rapidly and then decreased with the increased of NACHCl in every measured period was 0.044 at 0.2 g/L (6h), 0.058 at 0.4 g/L (8h), 0.120 at 0.6 g/L (10h), 0.420 at (12 h) and 1.577 at 1.0 g/L (16 h) respectively. While the optical density of without NACHCl was 1.136 at 16 h. The optimum concentration of NACHCl in MRS broth of *Bifidobacterium infantis* was 1.0 g/L incubation period for 16 h. (Table 2A &2 B; Figure 2 A & B). The comparative data on optical density and cfu10 /mL is also depicted on the table 3 (A&B) and figures 3 (A&B). Similar results reported the effect of lactose on the growth of *Lactobacillus acidophillus* and *Bifidobacterium bifidum* [14] and measurement of optimum growth of *Lactobacillus fermentum*in MRS medium [15].

Table 1 A: Optical Density based growth observations of *Lactobacillus fermentum* under different concentrations of NACHCl

	Optical Density					
Time in Hours	**Control (MRS)**	**MRS+ Supplement 0.2g/l**	**MRS+ Supplement 0.4g/l**	**MRS+ Supplement 0.6g/l**	**MRS+ Supplement 0.8g/l**	**MRS+ Supplement 1.0 g/l**
0 Hour	0.000	0.000	0.000	0.000	0.000	0.000
1 Hour	0.002	0.018	0.018	0.016	0.016	0.018
2 Hour	0.005	0.021	0.022	0.019	0.019	0.022
3 Hour	0.005	0.025	0.026	0.023	0.023	0.026
4 Hour	0.008	0.030	0.031	0.027	0.027	0.030
5 Hour	0.012	0.045	0.046	0.040	0.041	0.045
6 Hour	0.017	0.049	0.050	0.044	0.045	0.050
7 Hour	0.023	0.050	0.050	0.044	0.045	0.050
8 Hour	0.029	0.068	0.069	0.060	0.061	0.068
9 Hour	0.049	0.102	0.103	0.090	0.092	0.103
10 Hour	0.071	0.158	0.160	0.141	0.142	0.159
11 Hour	0.127	0.239	0.242	0.213	0.215	0.241
12 Hour	0.201	0.381	0.386	0.339	0.343	0.384
13 Hour	0.320	0.590	0.597	0.525	0.532	0.596
14 Hour	0.480	0.902	0.913	0.803	0.813	0.911
15 Hour	0.732	1.345	1.361	1.397	1.414	1.584
16 Hour	1.108	1.530	1.549	1.581	1.600	1.792

Table 1 B: Colony Forming Unit based growth observations of *Lactobacillus fermentum* under different concentrations of NACHCl

	CFU Log_{10}/mL					
Time in Hours	**Control (MRS)**	**MRS+ Supplement 0.2g/l**	**MRS+ Supplement 0.4g/l**	**MRS+ Supplement 0.6g/l**	**MRS+ Supplement 0.8g/l**	**MRS+ Supplement 1.0 g/l**
0 Hour	2.90	2.90	2.90	2.90	4.11	2.90
1 Hour	3.51	3.51	3.51	3.51	4.40	3.51
2 Hour	4.11	4.11	4.11	4.11	5.05	4.11
3 Hour	5.04	5.46	5.46	5.46	5.46	5.51
4 Hour	5.32	5.83	5.84	5.85	5.85	5.89
5 Hour	5.58	6.18	6.18	6.19	6.19	6.24
6 Hour	5.88	6.57	6.57	6.57	6.57	6.61
7 Hour	6.08	6.86	6.87	6.88	6.88	6.92
8 Hour	6.28	7.15	7.15	7.15	7.15	7.18
9 Hour	6.56	7.52	7.52	7.52	7.52	7.56
10 Hour	6.81	7.94	7.94	7.95	7.95	8.00
11 Hour	7.08	8.38	8.38	8.38	8.38	8.41
12 Hour	7.28	8.79	8.79	8.80	8.81	8.85
13 Hour	7.53	9.23	9.24	9.23	9.23	9.28
14 Hour	7.79	9.78	9.78	9.79	9.79	9.58
15 Hour	8.08	9.36	9.37	9.36	9.36	9.81
16 Hour	8.36	9.93	9.94	9.94	9.95	9.99

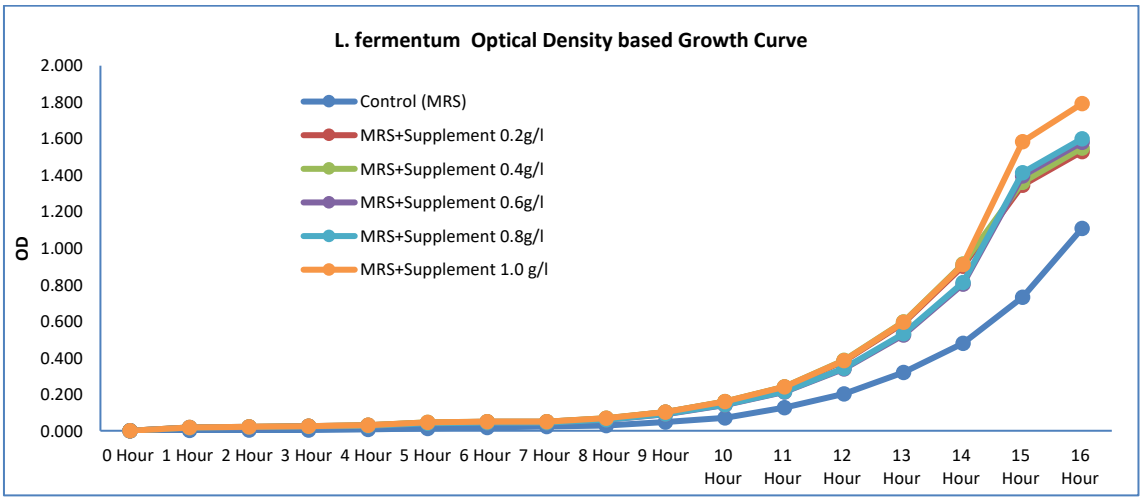

Figure 1 A: Growth curve of L. fermentum: Optical Density basis

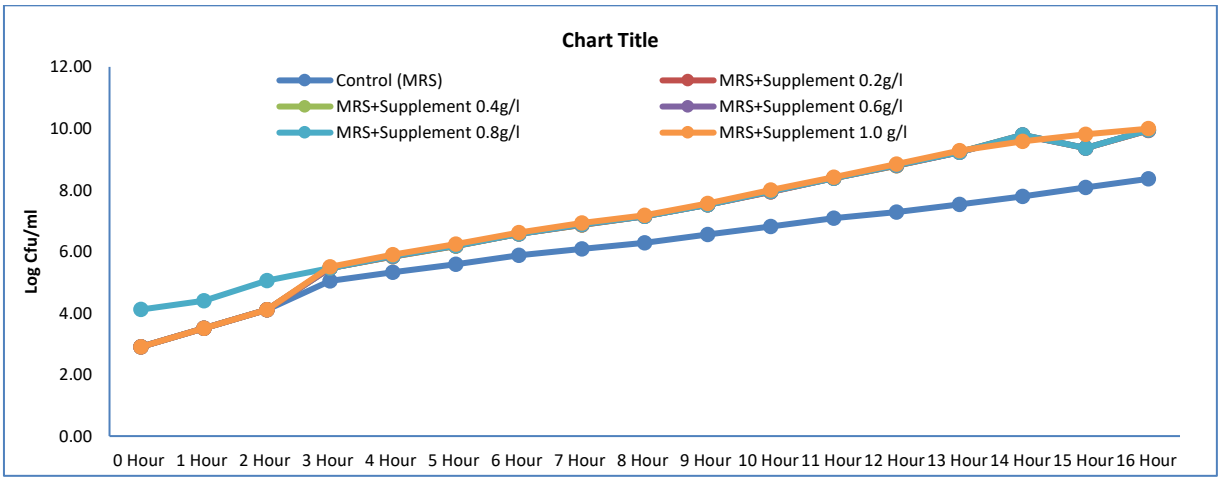

Figure 1 B: Growth Curve of L. fermentum: Log 10 CFU / mL basis

Table 2 A: Optical Density based growth observations of Bifidobacterium infantis under different concentrations of NACHCl

	Optical Density					
Time in Hours	**Control (MRS)**	**MRS+ Supplement 0.2g/l**	**MRS+ Supplement 0.4g/l**	**MRS+ Supplement 0.6g/l**	**MRS+ Supplement 0.8g/l**	**MRS+ Supplement 1.0 g/l**
0 Hour	0.000	0.000	0.000	0.000	0.000	0.000
1 Hour	0.014	0.016	0.018	0.018	0.018	0.018
2 Hour	0.017	0.019	0.021	0.022	0.022	0.022
3 Hour	0.020	0.023	0.025	0.026	0.026	0.026
4 Hour	0.023	0.027	0.030	0.031	0.031	0.031
5 Hour	0.027	0.040	0.045	0.045	0.046	0.047
6 Hour	0.032	0.044	0.049	0.050	0.051	0.051
7 Hour	0.038	0.044	0.058	0.058	0.059	0.060
8 Hour	0.044	0.060	0.058	0.059	0.059	0.060
9 Hour	0.067	0.090	0.079	0.080	0.081	0.082
10 Hour	0.100	0.141	0.119	0.120	0.121	0.123
11 Hour	0.150	0.213	0.185	0.186	0.189	0.191
12 Hour	0.224	0.339	0.411	0.415	0.420	0.425
13 Hour	0.337	0.525	0.637	0.643	0.650	0.658
14 Hour	0.505	0.803	0.974	0.983	0.995	1.007
15 Hour	0.758	0.988	1.198	1.210	1.224	1.238
16 Hour	1.136	1.258	1.525	1.541	1.558	1.577

Table 2 B: Colony Forming Unit based growth observations of Bifidobacterium infantis under different concentrations of NACHCl

	CFU Log_{10}/mL					
Time in Hours	**Control (MRS)**	**MRS+ Supplement 0.2g/l**	**MRS+ Supplement 0.4g/l**	**MRS+ Supplement 0.6g/l**	**MRS+ Supplement 0.8g/l**	**MRS+ Supplement 1.0 g/l**
0 Hour	2.90	2.90	2.90	2.90	2.90	2.90
1 Hour	3.51	3.51	3.51	3.51	3.51	3.51
2 Hour	4.11	4.11	4.11	4.11	4.11	4.11
3 Hour	5.04	5.18	5.23	5.28	5.28	5.28
4 Hour	5.32	5.45	5.49	5.54	5.54	5.54
5 Hour	5.58	5.70	5.75	5.80	5.81	5.81
6 Hour	5.88	6.00	6.04	6.08	6.08	6.08
7 Hour	6.08	6.20	6.26	6.30	6.30	6.30
8 Hour	6.28	6.45	6.49	6.54	6.54	6.54
9 Hour	6.56	6.72	6.76	6.81	6.82	6.83
10 Hour	6.81	6.97	7.04	7.08	7.08	7.08
11 Hour	7.08	7.23	7.28	7.32	7.32	7.32
12 Hour	7.28	7.45	7.49	7.54	7.54	7.54
13 Hour	7.53	7.71	7.76	7.81	7.81	7.82
14 Hour	7.79	7.97	8.04	8.08	8.08	8.08
15 Hour	8.08	8.26	8.30	8.34	8.34	8.34
16 Hour	8.36	8.54	8.59	8.64	8.64	8.65

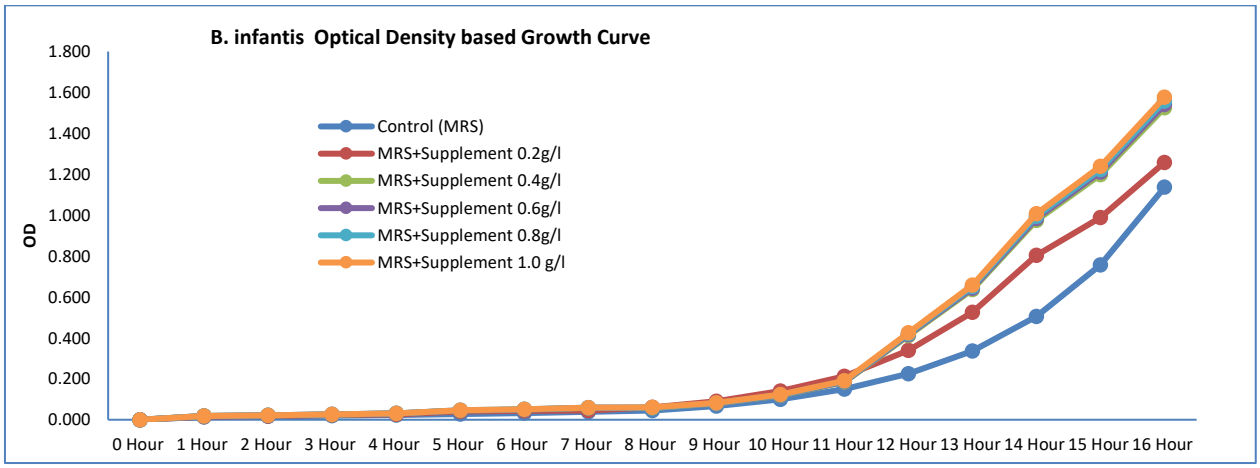

Figure 2 A: Growth curve of *B. infantis:* Optical Density basis

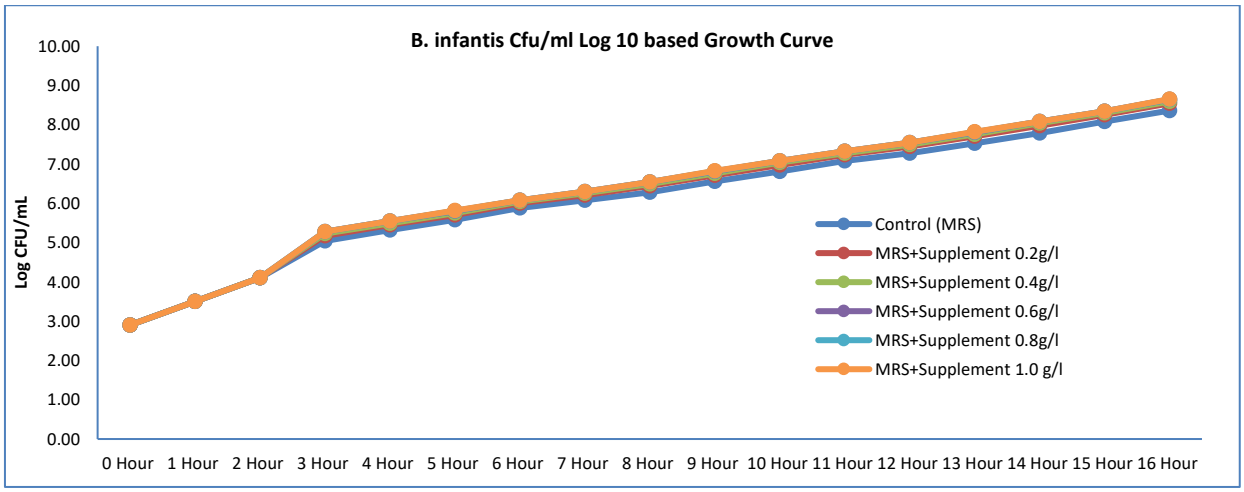

Figure 2 B: Growth Curve of *B. infantis:* Log 10 CFU / mL basis

Table 3A: Comparative data on optical density obtained at control and 1.0g/L of NACHCl for both the culture

	Bifidobacterium		***Lactobacillus***	
Time in Hours	**Control (MRS)**	**MRS+ Supplement 1.0 g/l**	**Control (MRS)**	**MRS+ Supplement 1.0 g/l**
0 Hour	0.00	0.000	0.000	0.000
1 Hour	0.01	0.018	0.002	0.018
2 Hour	0.02	0.022	0.005	0.022
3 Hour	0.02	0.026	0.005	0.026
4 Hour	0.02	0.031	0.008	0.030
5 Hour	0.03	0.047	0.012	0.045
6 Hour	0.03	0.051	0.017	0.050
7 Hour	0.04	0.060	0.023	0.050
8 Hour	0.04	0.060	0.029	0.068
9 Hour	0.07	0.082	0.049	0.103
10 Hour	0.10	0.123	0.071	0.159
11 Hour	0.15	0.191	0.127	0.241
12 Hour	0.22	0.425	0.201	0.384
13 Hour	0.34	0.658	0.320	0.596
14 Hour	0.51	1.007	0.480	0.911
15 Hour	0.76	1.238	0.732	1.584
16 Hour	1.14	1.577	1.108	1.792

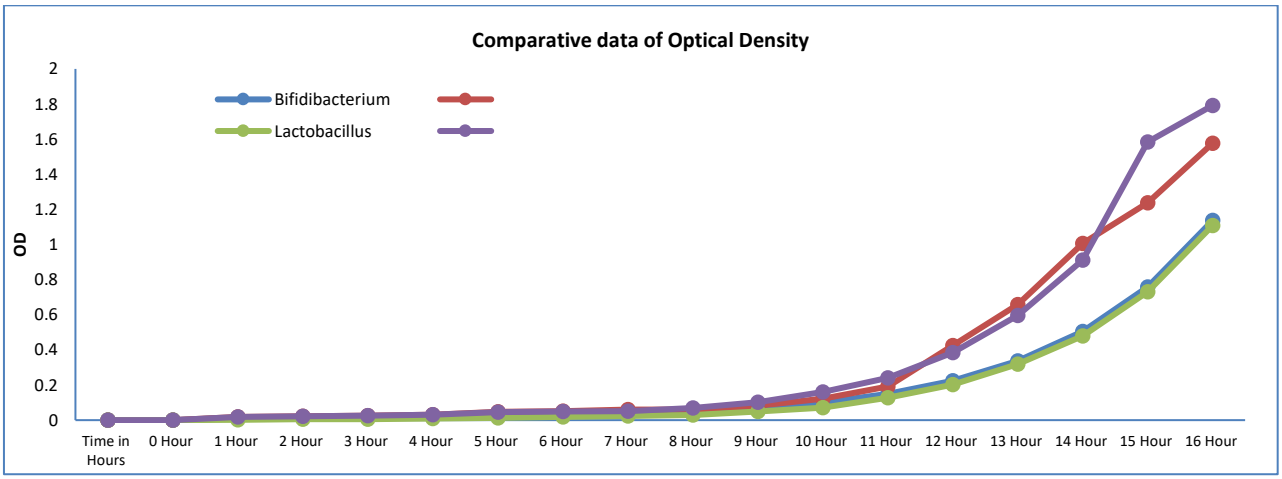

Fig 3 A Comparative data on optical density obtained at control and 1.0g/L of NACHCl for both the culture

Table 3B: Comparative data on Cfu Log 10 / mL obtained at control and 1.0g/L of NACHCl for both the culture

	Bifidibacterium		*Lactobacillus*	
Time in Hours	**Control (MRS)**	**MRS+ Supplement 1.0 g/l**	**Control (MRS)**	**MRS+ Supplement 1.0 g/l**
0 Hour	2.90	2.90	2.90	2.90
1 Hour	3.51	3.51	3.51	3.51
2 Hour	4.11	4.11	4.11	4.11
3 Hour	5.04	5.28	5.04	5.51
4 Hour	5.32	5.54	5.32	5.89
5 Hour	5.58	5.81	5.58	6.24
6 Hour	5.88	6.08	5.88	6.61
7 Hour	6.08	6.30	6.08	6.92
8 Hour	6.28	6.54	6.28	7.18
9 Hour	6.56	6.83	6.56	7.56
10 Hour	6.81	7.08	6.81	8.00
11 Hour	7.08	7.32	7.08	8.41
12 Hour	7.28	7.54	7.28	8.85
13 Hour	7.53	7.82	7.53	9.28
14 Hour	7.79	8.08	7.79	9.58
15 Hour	8.08	8.34	8.08	9.81
16 Hour	8.36	8.65	8.36	9.99

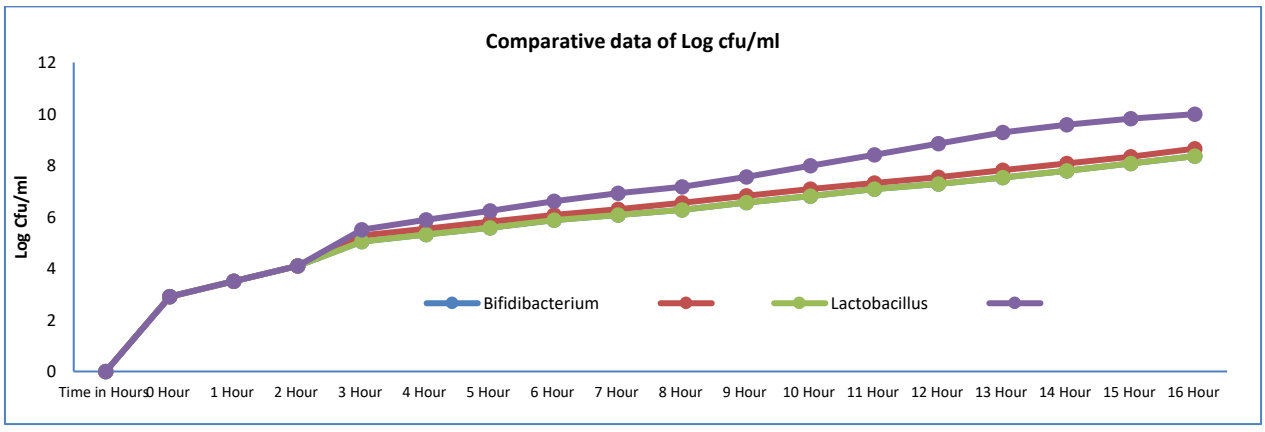

Fig 3 B Comparative data on CFU Log 10 / mL obtained at control and 1.0g/L of NACHCl for both the culture

Conclusion

Supplementation of NACHCl promote the growth of Lactobacillus fermentum and Bifidobacterium infantis. to a very good extent and can act as a potential prebiotic.

References

[1]. Bulut C, Gunes H, Okuklu B, Harsa S. Killic S. Coban HS and Yenidinya AF. Homofermentative lactic acid bacteria of a traditional cheese, Comlekpeyniri from cypadocia region. J. Diary Res, 2005 ; 72:19-24

[2]. Mohania D. Nagpal R. Kumar M. Bhardwaj A Yadav M. Jain S. Marotta F. Singh V. Parkash O and Yadav H.Molecular approaches for identification and characterization of Lactic acid bacteria. J. Dig Dis, 2008; 9:190-198.

[3]. Niu H, Zhou XH and Zhang XG. Breast milk contains probiotic with anti-infantile diarrhea effects that may protect infants as they change to solid foods. Env. Microb. 2021;23(3):1750–1764.

[4]. Sakib KM, Rahman MM and Abul KA. Isolation of Bifidobacteriumfrom infant feces and their antibacterial activity against pathogenic bacteria. Int. J. Pl. Sci. 2015;7(1):67–77

[5]. Michalkiewicz J. Krotkiewski M, Gackowska L, Wyszomirska- Golda M, Helmin-Basa A, Dzierzanowska D. and Madalinski K. Immunomodulatory effects of lactic acid bacteria on human peripheral blood mononuclear cells. Microb. Ecol Health Dis.2003 ;15, 185–192

[6]. Thomson P, Medina DA and Garrido D. Human milk oligosaccharides and infant gut Bifidobacteria: molecular strategies for their utilization. Food Microb. 2018;75:37–46.

[7]. Jianlong H, Kouta S and Tohru S. Determination of the ribosome-binding sequence and spacer length between binding site and initiation codon for efficient protein expression in Bifidobacterium longum 105-A. J. Biosci. Bioeng. 2012 ;113(4): 442-444.

[8]. Pasi E, Kankaanpa SJ and Salminen EI. The influence of polyunsaturated fatty acids on probiotic growth and adhesion. FEMS Microb. Lett. 2001;194:149–153.

[9]. Zisu B. and Shah NP. Effects of pH, temperature, supplementation with whey protein concentrate, and adjunct cultures on the production of exopolysaccharides by Bifidobacterium L80, 1275. J. Dairy Sci. 2003;86(11):3405.

[10]. Janer C, Pelaez C and Requena T. Caseinomacropeptide and whey protein concentrate enhance Bifidobacterium lactis growth in milk. Food Chem.2004;86(2) : 263–267.

[11]. Singh B, Ram C. Singh D, Pal N, Singh A, Singh R et al. Alternative and Replacement Foods, Volume 17, Handbook of Food Bioengineering (I-XX) Multi-Volume SET, entitled "Potential of novel bioactive peptides as functional food ingredients in preventing cardiovascular disease", ELSEVIER, Academic Press 2018**:**411-431,

[12]. Zhang C, Zhang Y and Li H. The potential of proteins, hydrolysates and peptides as growth factors for Lactobacillus and Bifidobacterium: current research and future perspectives. Food Funct. 2020;11(3):1946–1957.

[13]. Mahalakshmi, R., and VVPS. Murthy. "Growth of Bifidobacterium bifidum in whey-based media." Journal of Industrial Microbiology and Biotechnology. 2000; 25, (4):177-179.

[14]. Hartemink R, Kok BJ, Weenk GH and Rombouts FM. Raffinose-Bifidobacterium (RB) agar, a new selective medium for Bifidobacteria. J. Microb. Meth. 1996; 27: 33-43.

[15]. Charteris WP. Kelly PM, Morelli L and Collins JK. Effect of conjugated bile salts on antibiotic susceptibility of bile salt-tolerant Lactobacillus and Bifidobacterium isolates. J. Food Prot. 2000 ;63: 1369–1376.