RESEARCH ARTICLE

Evaluation of bile tolerance in dairy and human origin *Lactobacillus* fermentum strains

Aiswarya S. Panicker and Pradip V. Behare

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Abstract Eleven *Lactobacillus fermentum* strains including seven dairy and four faecal origin were studied for in vitro bile tolerance at 0, 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 % bile concentration to assess their probiotic potential. At 0.3 and 0.6% bile concentration except NCDC-400, all dairy origin strains shown 3 to 5 log reduction in count, while faecal origin strains did not show much reduction. Almost similar trend was observed for dairy strains at 1.2 to 1.5 % bile concentration whereas BIF-18, BIF, 19 and BIF 20 from faecal strains shown better growth. *Lactobacillus fermentum* BIF-19 from faecal and *Lactobacillus fermentum* NCDC-400 from dairy origin were most bile tolerant. Out of all strains BIF-19 exhibited highest survival in all concentration of bile.

Keywords : Probiotic, *Lactobacillus, L.fermentum, bile* tolerance, NCDC 400, BIF 19

Introduction

Probiotics are defined as "live microorganisms which, when administered in adequate number, confers health benefit to the host" (FAO/WHO, 2002). Species of *Lactobacillus* and *Bifidobacterium* are the most commonly used probiotics. Particularly, *Lactobacillus fermentum* is a part of a number of ethnic as well as commercial probiotic preparations. Based on proven health promoting effects, certain strains like ME-3 and PCC are commercially marketed as probiotics. For a probiotic strain to exert beneficial effect on the host, it must be able to survive and retain its activity in the host's digestive tract. They encounter various stresses during the passage through the gastro-intestinal tract, which may affect the functionality of these strains. Among the various hurdles present, bile salt is more detrimental for the surviving organism. The human liver secretes as much as a litre of bile into the small intestine each day, and thus exposure to bile is a serious challenge to probiotics. Therefore, tolerance to bile salt has often been used as the most important selection criteria for potential probiotic strains. The concentration of bile acids in human GI tract varies between 0.2 and 2% following food ingestion (Hofman, 1998). The main function of bile is to facilitate the digestion of fat but it also acts like antimicrobial detergent. It can disorganize the structure of the cell membrane and cause dissociation of integral membrane proteins that leads to leakage of cell content followed by cell death (Bergeley *et al*., 2005). Tolerance to bile salt greatly varies from strain to strain and may be dependent on the type of sources from which the potential organisms have been isolated. It has been reported that human originated strains can give better probiotic results since they are obtained from the similar environment that may reduce adaptability issues in the GI tract conditions. Nevertheless, certain dairy and non-dairy based isolates have proven well as probiotic and are also available in the market for commercial use. In this project, we aim to evaluate bile tolerance among the dairy and human based *Lactobacillus fermentum* strains which could be used as indigenous probiotic cultures in near future.

Materials and Methods

The *Lactobacillus fermentum* strains used in the study was isolated from dairy and human samples (Table 1). They were always maintained in MRS broth and glycerol stocks at 4°C. Tolerance for bile acids was tested as per the method of Gilliland *et al*. (1984). All the cultures were grown in MRS broth overnight at 37°C. The actively grown cells were harvested by centrifugation and resuspended in MRS

Aiswarya S. Panicker and Pradip V. Behare (✉)
Dairy Microbiology Division, National Dairy Research Institute, Karnal-132001, Haryana, India

Pradip V. Behare
Scientist, NCDC, Dairy Microbiology Division, National Dairy Research Institute, Karnal 132001, Haryana, India
E-mail : pradip_behare@yahoo.com

broth supplemented with 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8% w/v ox bile (Himedia Laboratories Pvt. Ltd, Mumbai, India) and without supplement (as a control). Survival was evaluated by plate count on MRS agar, after 0, 3 and 6 hrs of incubation in MRS broth containing bile salts reflecting the time spent by food in the small intestine and subsequently the plates were incubated at 37°C for 48 h. The experiments were repeated three times.

Results and Discussion

The bile tolerance of dairy and human based *L. fermentum* strains in different concentrations of bile 0.3, 0.6, 0.9, 1.2, 1.5, 1.8% (w/v) and control without bile are shown in table 2 to 8. At 0.3% bile concentration, dairy origin strains except NCDC-400 shown reduction of counts up to 3 logs, whereas counts for faecal origin strains did not changed. Most of the dairy origin strains were notable to tolerate 0.6% bile and count reduced almost by 5 log cycles. At 0.9% bile concentration, the same trend continued in dairy cultures. But in faecal samples, only BIF-18 and BIF-19 showed better tolerance. At 1.2%, 1.5%, and 1.8% bile concentrations, C-9, NCDC-605 and NCDC-606 strains were not able to survive. Among dairy strains, NCDC-400 showed better tolerance than others and among faecal origin samples, BIF-18 BIF-19 and BIF-20 were tolerant to bile. The faecal origin strains showed better tolerance to bile than that of dairy origin. Although all the faecal origin samples showed good tolerance to bile, BIF-19 was found to be the most tolerant to bile salt. This strain may be used as putative probiotic and should be further evaluated for other *in vitro* probiotic parameters including *in vivo* tests.

Table 1: List of *L. fermentum* strainsused in the study

Strains	Source	Details	Culture conditions
NCDC-400 (V-10)	Dairy	Isolated from dahi sample (Vishal Dairy, Karnal)	37°C, MRS
C-6	Dairy	Isolated from Chilika curd (prepared from milk of chilikkabuffallo breed) Orissa	37°C, MRS
C-9	Dairy	Isolated from chilika curd (prepared from milk of Chilikkabuffallo breed) Orissa	37°C, MRS
NCDC-156	Dairy	National Collection of Dairy Cultures	37°C, MRS
NCDC-605	Dairy	isolated from BajraRaabadi	37°C, MRS
NCDC-606	Dairy	Isolated from Raabadi	37°C, MRS
MTCC-8711	Dairy	Isolated from yoghurt, Tamilnadu	37°C, MRS
KT-85	Human	Isolated from faecal sample, Haryana	37°C, MRS
BIF-18	Human	Isolated from faecal sample of infants from Government hospital, Karnal	37°C, MRS
BIF-19	Human	Isolated from faecal sample of infants from Government hospital, Karnal	37°C, MRS
BIF-20	Human	Isolated from faecal sample of infants from Government hospital, Karnal	37°C, MRS

Table 2 : Tolerance to 0.3% bile salt by *Lactobacillus fermentum* strains

Cultures used	0 hr *	3 hrs *	6 hrs *
L. fermentum NCDC - 400	8.88 ± 0.06	7.59 ± 0.06	5.65 ± 0.5
L. fermentum C-6	8.30 ± 0.16	5.51 ± 0.51	4.45 ± 0.58
L. fermentum C-9	8.58 ± 0.59	5.57 ± 0.08	3.33 ± 0.11
L. fermentum NCDC-156	8.50 ± 0.20	5.63 ± 0.41	3.67 ± 0.29
L. fermentum NCDC-605	8.49 ± 0.61	3.76 ± 0.19	2.78 ± 0.18
L. fermentum NCDC-606	8.50 ± 0.61	4.86 ± 0.06	4.04 ± 0.15
L. fermentum MTCC - 8711	8.52 ± 0.32	6.33 ± 0.16	5.47 ± 0.43
L. fermentum KT- 85	8.73 ± 0.21	7.79 ± 0.37	6.43 ± 0.37
L. fermentum BIF-18	9.37 ± 0.18	10.10 ± 0.71	9.0 ± 0.33
L. fermentum BIF- 19	9.24 ± 0.14	10.17 ± 0.05	9.28 ± 0.03
L. fermentum BIF- 20	8.72 ± 0.12	9.33 ± 0.15	8.58 ± 0.15

*Average log cfu/ml± SD

Table 3 : Tolerance to 0.6% bile salt by *Lactobacillus fermentum* strains

Cultures used	0 hr *	3 hrs *	6 hrs *
L. fermentum NCDC - 400	8.26 ± 0.45	6.25 ± 0.12	4.77 ± 0.21
L. fermentum C-6	8.25 ± 0.13	4.41 ± 0.39	3.49 ± 0.46
L. fermentum C-9	6.98 ± 0.05	3.94 ± 0.02	2.35 ± 0.05
L. fermentum NCDC-156	8.23 ± 0.09	3.26 ± 0.02	3.0 ± 0.06
L. fermentum NCDC-605	7.28 ± 0.07	0	0
L. fermentum NCDC-606	7.51 ± 0.16	3.51 ± 0.155	2.32 ± 0.13
L. fermentum MTCC - 8711	8.40 ± 0.43	5.59 ± 0.37	4.79 ± 0.26
L. fermentum KT- 85	8.62 ± 0.24	5.55 ± 0.597	4.35 ± 0.35
L. fermentum BIF-18	9.32 ± 0.17	9.50 ± 0.50	8.92 ± 0.18
L. fermentum BIF- 19	9.34 ± 0.07	9.85 ± 0.45	9.13 ± 0.045
L. fermentum BIF- 20	8.26 ± 0.39	8.03 ± 0.13	7.32 ± 0.14

*Average log cfu/ml± SD

Table 4 : Tolerance to0.9% bile salt by *Lactobacillus fermentum* strains

Cultures used	0 hr *	3 hrs *	6 hrs *
L. fermentum NCDC - 400	7.89 ± 0.06	5.86 ± 0.07	4.17 ± 0.06
L. fermentum C-6	7.33 ± 0.07	3.17 ± 0.55	3.41 ± 0.43
L. fermentum C-9	6.45 ± 0.25	3.61 ± 0.08	2.10 ± 0.05
L. fermentum NCDC-156	6.63 ± 0.42	3.10 ± 0.09	2.93 ± 0.39
L. fermentum NCDC-605	6.18 ± 0.05	0	0
L. fermentum NCDC-606	6.17 ± 0.11	1.88 ± 0.06	1.31 ± 0.16
L. fermentum MTCC - 8711	8.35 ± 0.32	4.41 ± 0.32	3.94 ± 0.44
L. fermentum KT- 85	8.40 ± 0.07	5.33 ± 0.10	3.39 ± 0.09
L. fermentum BIF-18	9.64 ± 0.43	9.32 ± 0.52	8.28 ± 0.66
L. fermentum BIF-19	9.22 ± 0.16	9.75 ± 0.52	9.08 ± 0.04
L. fermentum BIF- 20	8.44 ± 0.05	7.89 ± 0.24	7.13 ± 0.04

*Average log cfu/ml± SD

Table 5 : Tolerance to1.2% bile salt by *Lactobacillus fermentum* strains

Cultures used	0 hr *	3 hrs *	6 hrs *
L. fermentum NCDC - 400	7.43 ± 0.26	4.295 ± 0.21	3.88 ± 0.07
L. fermentum C-6	6.79 ± 0.61	2.249 ± 0.09	2.23 ± 0.47
L. fermentum C-9	6.02 ± 0.16	2.75 ± 0.03	0
L. fermentum NCDC-156	6.42 ± 0.52	2.53 ± 0.26	2.24 ± 0.16
L. fermentum NCDC-605	5.48 ± 0.38	0	0
L. fermentum NCDC-606	5.66 ± 0.11	1.57 ± 0.30	0
L. fermentum MTCC - 8711	8.32 ± 0.10	4.26 ± 0.34	3.28 ± 0.33
L. fermentum KT- 85	8.31 ± 0.06	4.21 ± 0.02	3.11 ± 0.10
L. fermentum BIF-18	9.18 ± 0.25	9.288 ± 0.49	7.17 ± 0.09
L. fermentum BIF- 19	9.12 ± 0.03	9.71 ± 0.44	8.93 ± 0.14
L. fermentum BIF- 20	8.39 ± 0.06	7.82 ± 0.24	7.08 ± 0.02

*Average log cfu/ml± SD

Table 6 : Tolerance to 1.5% bile salt by *Lactobacillus fermentum* strains

Cultures used	0 hr *	3 hrs *	6 hrs *
L. fermentum NCDC - 400	6.86 ± 0.07	3.87 ± 0.07	3.80 ± 0.08
L. fermentum C-6	5.91 ± 0.93	1.81 ± 0.22	0
L. fermentum C-9	5.37 ± 0.23	1.42 ± 0.08	0
L. fermentum NCDC-156	5.36 ± 0.31	2.15 ± 0.09	1.65 ± 0.40
L. fermentum NCDC-605	5.42 ± 0.42	0	0
L. fermentum NCDC-606	5.51± 0.17	0	0
L. fermentum MTCC - 8711	7.67 ± 0.55	3.90 ± 0.10	2.33 ± 0.11
L. fermentum KT- 85	8.20 ± 0.15	3.85 ± 0.10	2.70 ± 0.06
L. fermentum BIF-18	8.68 ± 0.66	9.12 ± 0.03	6.41 ± 0.55
L. fermentum BIF- 19	8.55 ± 0.56	9.59 ± 0.54	8.10 ±0.14
L. fermentum BIF- 20	8.33 ± 0.09	6.96 ± 0.45	6.88 ± 0.06

*Average log cfu/ml± SD

Table 7 : Tolerance to 1.8% bile salt by *Lactobacillus fermentum* strains

Cultures used	0 hr *	3 hrs *	6 hrs *
L. fermentum NCDC - 400	6.53 ± 0.36	3.68 ± 0.10	2.92 ± 0.40
L. fermentum C-6	3.55 ± 0.58	1.72 ± 0.07	0
L. fermentum C-9	3.92 ± 0.05	1.12 ± 0.10	0
L. fermentum NCDC-156	3.64 ± 0.25	2.04 ± 0.16	1.14 ± 0.23
L. fermentum NCDC-605	4.37 ± 0.12	0	0
L. fermentum NCDC-606	4.92 ± 0.05	0	0
L. fermentum MTCC - 8711	7.40 ± 0.29	3.73 ± 0.05	1.25 ± 0.18
L. fermentum KT- 85	8.05 ± 0.05	3.37 ± 0.17	2.42 ± 0.21
L. fermentum BIF-18	7.70 ± 0.22	7.27 ± 0.36	5.61 ± 0.11
L. fermentum BIF- 19	8.32 ± 0.49	8.07 ± 0.69	7.82 ± 0.58
L. fermentum BIF- 20	8.20 ± 0.13	6.83 ± 0.40	6.53 ± 0.35

*Average log cfu/ml± SD

Table 8 : *Lactobacillus fermentum* counts without bile stress

Cultures used	0 hr *	3 hrs *	6 hrs *
L. fermentum NCDC - 400	9.08 ± 0.11	10.11 ± 0.17	10.43 ± 0.63
L. fermentum C-6	8.56 ± 0.09	10.08 ± 0.15	10.53 ± 0.29
L. fermentum C-9	9.10 ± 0.46	9.19 ± 0.01	10.21 ± 0.66
L. fermentum NCDC-156	8.93 ± 0.19	9.88 ± 0.09	10.43 ± 0.19
L. fermentum NCDC-605	9.28 ± 0.04	9.50 ± 0.63	10.25 ± 0.03
L. fermentum NCDC-606	9.14 ± 0.14	9.46 ± 0.14	10.08 ± 0.56
L. fermentum MTCC - 8711	8.57 ± 0.67	9.77 ± 0.52	10.40 ± 0.29
L. fermentum KT- 85	8.73 ± 0.25	9.74 ± 0.61	10.30 ± 0.22
L. fermentum BIF-18	9.48 ± 0.21	10.24 ± 0.16	10.60 ± 0.15
L. fermentum BIF- 19	9.28 ± 0.40	10.13 ± 0.24	10.57 ± 0.05
L. fermentum BIF- 20	8.69 ± 0.05	9.36 ± 0.15	10.09 ± 0.04

*Average log cfu/ml± SD

Conclusions

The faecal origin *Lactobacillus fermentum* strains showed better tolerance to bile than dairy origin. Among the dairy origin cultures, strain NCDC-400 (V-10) exhibited the most bile tolerance. BIF-18, BIF-19 and BIF-20 of faecal origin were tolerant up to 1.8% bile concentration. Although all the faecal origin samples showed good tolerance to bile, BIF-19 had shown the best result.

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