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Determination Of Survival And Resistance To Acidity As Probiotic Potential Of Infant And Calf Faecal Bifidobacteria

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Abstract: *Viability and survive of bifidobacteria strains under acidic environnement are the most important criteria for selection as probiotic. Twelve bifidobacterial strains isolated from calves faecal and eight from infant faecal during the milk-feeding period were tested for viability and resistance to acidity. These viability of bifidobacteria were examined at 0,7,14 and 21 days when the counts were variable, ranging between 5 to 6.5 log cfu/ml.Specific growth rate(μ) and generation time of Bifidobacterium strains were determined. Acid tolerance was determined by introducing the strains of Bifidobacterium in skimmed milk at pH=4.3 and enumerating during storage at 4°C.All strains showed ability to resist under SGF and bile salt but BC are more resistance and showed superior survival abilities and resistance to acidity than BI strains. Our result suggest that strains resistant to acidity seem to be suitable for food and biotechnological industry.*

Keywords : *Bifidobacteria , viability, acidity, probiotic*

I. Introduction

Probiotics are defined as 'live micro-organisms' which confer a health benefit on the host when administered in adequate amounts [1].Fermented dairy products containing probiotic bacteria such as Bifidobacteria, are generally considered as functional foods**[2]**. Production of fermented milks containing bifidobacteria is increasing because of their beneficial effects on health **[3].** The reported health benefits of bifidobacteria include stabilizing the gut mucosal barrier, modulation of immune response, modulation of intestinal microbiota, prevention of traveller's diarrhoea in children,reduction of necrotizing endocarditis in neonates, alleviation of atopic dermatitis symptoms in children, improvement of constipation, and antibacterial and anticarcinogenic activities[4],[5],[6].In order to provide health beneficets,Bifidobacteria must tolerante and survive gastrointestinal tract conditions during transit and must resist during manufacturing, storage and transport of the fermented dairy products .**[7],[8].**

fermented dairy product should contain a minimum level of life and active cultures at the time of consumption.It has been argued that a minimum level of 10^8 cfu/ml should be present in order to promote human health(Shah, 2000)[9].The international Standard of International Dairy federation**(IDF)** require 10^6 cfu/ml of Bifidobacteria in fermented milks containing Bifidobacteria at the time of sale**[10].** Several factors affect the survival of Bifidobacterium spp. in fermented products. These include strains of probiotic bacteria, pH, hydrogen peroxide, storage atmosphere, concentration of metabolites such as lactic acid and acetic acids, dissolved oxygen, and buffers such as whey proteins **[4].**

The aim of our work was to determine the viability and resistance to acidity of bifidobacteria isolates from calves and infants faecal.

II. Materials And Methods

Samples : Twenty feacal samples from five breast-fed infants bothes sexes in the age between 5 to 180 days and ten feacal samples from five Holstein calves both sexes between 5 to 60 days were investigated.

Bacterial cultures:

Total of twenty strains were isolated, 12 (BI) from infant faecal and 8 (BC)from calves faecal . Strains were cultured on MRS-raffinose and TPY agar supplemented with 0.5g/l cyeteine HCl and incubation in a 20% CO_2 at 37 °C for 72 h**[11].**Strains were identified on the basis of the colony morphology, Gram positif , pleomorphic fermentative rods often Y-shaped ,negative reaction in biochemical test (catalase, urease, oxydase) **. [12],[13]** and sugar fermentation**[14].** And by the detection of fructose-6- phosphate phospoketolase (F6PPK) activity**.[15]** .

Specific growth rate (µ) and generation time of strains:

Samples of UHT skim milk were inoculated with strain B1,B2,B3 and B4 (c.10^8 CFU/ml for each strain).During 6 h of incubation, sampling and dilutions in sterile Ringer solution with 0.05% cysteine HCl were made. Strains of bifidobacteria were cultured on MRS- agar with 0.05% cysteine HCl**.** [16].incubation in a 20% CO2 at 37°c for 72h.Specific growth rate (µ) for each culture was calculated using this equation $\mu = (\log_{10} X_t - \log_{10} X_{t0})/ (t_1 - t_0)$ where X_t and X_0 are counts (cfu/ml) at time t_t and t_0.Generation time (Tg) was calculated as $Tg = \ln(2/\mu)$**[17].**

Viability of bifidobacteria strains at 4°C in skimmed milk:

The UHT skimmed milk was used as the survival medium. Strains of bifidobacteria was introduced into 500 ml portion of sterile skimmed milk in Durran bottles to have a final population of c. 10^8 cfu/ml.The pH was adjusted to 4.3 using 88% lactic acid.the bottles were stored at 4°C for 21 days.The viability of Bifidobacteria was determined in samples at 0,7,14 and 21 days.The counts were made on MRS-agar with 0.05% cysteine Hcl after anaerobic incubation at 37°C for 72h [18].

Tolerance of Bifidobacterium strains to acidity:

The stability of the strains in gastric conditions was evaluated using simulated gastric fluid (SGF). SGF was comprised of 3.2 mg ml^{-1} pepsin (Sigma-Aldrich, Oakville, Ontario, Canada) in 0.2% (wt/vol) NaCl and pH was adjusted to 2, 2.5 and 3 by addition of HCl (5mol.L^{-1}) pH 2.0 and 2.4 **.** [19]. A volume of 1 mL of an overnight MRS broth culture of bifidobacteria was added to 9 mL of SGF for 30 min at 37 °C in anaerobic conditions for 15, 30 and 45 min. 1mL for each tubes was mixed in 9 mL of sterile phosphate buffer saline (PBS pH 7.4). the survival cell was determinate by cfu on MRS agar plates with cysteine under anaerobic conditions at 37°C after 72h.

Resistance of Bifidobacteria strains to bile salt:

To determine the effects of bile salts on bifidobacteria strains the plates of MRS agar with bile salt in concentration between 0 and 100 g.l^{-1} , was inoculated with 100 µl of each strain at 37 °C for 72h under anaerobic conditions**[20].**

Results: Results of specific growth rates and generation time (Tg) are shown in tables 1

Table 1:Specific growth rates(µ) and generation time(Tg) of four strains of bifidobacteria

Strains	µ(h-1)	Tg
BI1	0.45	1.45
BI2	0.40	1.50
BI3	0.45	1.55
BI4	0.38	1.43
BI5	0.35	1.40
BI6	0.30	1.25
BI7	0.38	1.45
BI8	0.45	1.51
BI9	0.35	1.40
BI10	0.32	1.25
BI11	0.20	1.10
BI12	0.35	1.42
BC1	0.45	1.54
BC2	0.40	1.54
BC3	0.40	1.50
BC4	0.40	1.50
BC5	0.45	1.50
BC6	0.45	1.54
BC7	0.45	1.54
BC8	0.45	1.54

The specific growth rates were ranged between 0.45 and 0.20 for strains isolates from infant faecal BI, and between 0.40 to 0.45 for strains isolate from calves faecal.

All BC strains survived for more than 21 days above 10^6 cfu/ml (figure 1),BI strains declined numbers and decreased below 10- cfu/ml (figure 2) All Bacteria tested can survive at an acidic environnement at 30 min, reduction in bacterial population was observed at pH 2.5 for BI strains and for all bacteria at pH 2 Table 2

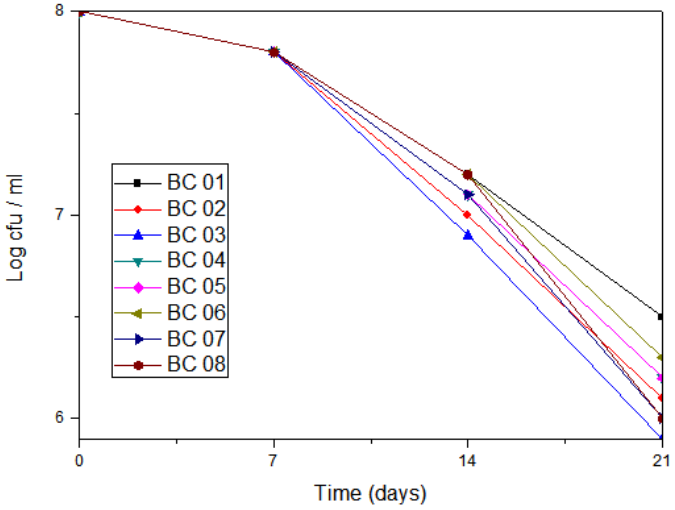

Figure 1 Viability of bifidobacteria strains(BC) at 4°C with pH=4.3 in skimmed milk

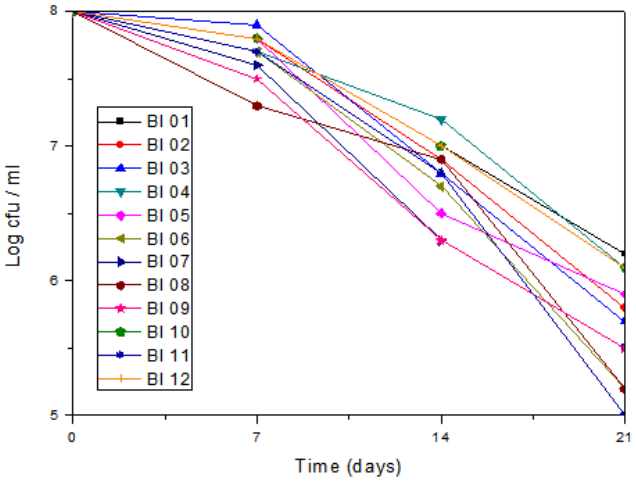

Figure 2 Viability of bifidobacteria strains(BI) at 4°C with pH=4.3 in skimmed milk

Table 2. survival of bifidobacteria strains after an incubation of 45 min at 37°C in simulated gastric fluid pH 2 to 3

		Time(min)			
pH	Bifidobacteria strains*	0	15	30	45
pH 3	BI(1-12) BC(1-8)	8.56 8.50	8.1 8.2	7,5 7,8	7 7,2
pH 2,5	BI(1-12) BC(1-8)	8.65 8.57	7,5 7 ,8	6,8 7	5,9 6
pH 2	BI(1-12) BC(1-8)	8.60 8.55	5,9 6	5 5,5	 4,5

*Average survivor log cfu of strains from same source

The tolerance of Bifidobacteria strains to bile salt, shown that the minimal inhibitory concentration was 50 g.l for almost of bacteria, except for BC1 and BC5 who growth at 100 g.l (data not shown).

III. Discussion And Conclusion

Specific growth rates (μ) and generation time of four strains of bifidobacteria ranged from 0.38 to 0.45 and 1.45 to 1.54 respectively .Similar results were found with B. lactis and B.breve **[21]**.The acid tolerance of bifidobacteria is important as the organism has to withstand the acidity in yogurts and gut, it has been reported to have health benefits.For digestion of food, the pH of gastric juice has to be around 2-4. **[22].** and the pH of yogurt varies from 3.8 to 3.36 **[23].**The results in the present study showed that Bc survived longer in high acid environements over other strains BI. **[24]** reported that of 17 Bifidobacterium strains, B.animalis spp lactis had highest acid tolerance.In their study,Hcl was used as the acidulent. A same result were found by [25]but they

used lactic acid as the acidulent.In our study the acid lactic was used to simulate the acidic environment in the product. Ability of probiotics to resist, survive the GST conditions are the most important criteria for the selection of the probiotics. Adhesion to intestinal mucosea is one of the major selection attribute for probiotics as it is required for intestinal colonization and also important for modulation of the immune system and antagonism against pathogens.[26].

All strains have a resistance under simulated gastric fluid at pH $\geq$ 2.5 , stomach pH .[27], but strains isolates from calves faecal shown more resistance than BI (infants feacal), this is the first study to shown the difference between Bifidobacteria isolate from humans and animals feacals.The resistance of probiotic to acidity have been shown on general Bifidobacteria and LAB without specificity of source.[28],[29] . Results obtained from this study can help the food and pharmaceutical industry to develop new technologies to ensure consumers receive quality and beneficial products.

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