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Effect of Storage on the Incorporation of Solid State Fermented Selected Species of Bifdobacterium to Baby Food

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Abstract:- Probiotics are live microorganisms promoted with claims that they provide health benefits when consumed, generally by improving or restoring the gut flora. Probiotics are considered generally safe to consume. Bifidobacteria that predominate in guts of babies consuming mother's milk maintain good health compared to bottle fed babies. The guts of milk formula consuming babies suffer from gastro-intestinal disturbances due to delayed establishment of of bifdobacteria. Mother's milk contain bifdogenic factors that support bifidobacteria in the intestine of babies. But those babies who lack the facility of mother's milk due to various reasons need an alternative to combat the enteric pathogens and increase population of bifidobacteria. This can be achieved by incorporating viable large numbers of bifidobacteria obtained through solid state fermentation on sterile black gram dhal with additives like moisture and skim milk powder. An attempt has been made in this study by incorporating the dired form of cultured balck gram dhal powdered to baby foods suh as Lactogen I, II & cerelac with determination of viability during room temperature storage. Initial count of 9.00 log viable count on an average of Bifidobacterium longum F51, PF1; B.adolescentis F35 and B.bifidum S26 reduced to 6.50 at the end of 6 months of storage at 300C. Consumption of such baby foods may help babies lacking mother's milk to maintain balance of bifidobacteria thus promising the wellness of the gut free from intestinal disturbances caused by enteric pathogens.

Keywords:- *Bifidobacteria, Bifidogenic Factor, Probiotics, Solid State Fermentation.*

I. INTRODUCTION

The internationally endorsed definition of probiotics is live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [1]. The scientific and clinical evidence have progressed rapidly, as has the development of a number of robust probiotic products. At the same time, probiotic products have received the legitimate attention of regulatory authorities with an interest in protecting consumers from misleading claims. Many species of lactic acid bacteria, bifidobacteria and yeasts, representing most of the commercially available probiotic strains, are judged to be safe for use in foods and supplements. This is because they belong to genera and species with a documented history of safe use, either as probiotics or as starter cultures [2]. *Bifidobacterium adolescentis, animalis, bifidum, breve* and *longum* are the species of bifidobacteria used as probiotics, the concept that there are common health benefits to be derived from consuming (or delivering) at an adequate dose any safe strain of a species that is already known to include an effective probiotic [3]. Bifidobacteria dominate the stool microbiota regardless of whether the infants are fed human milk or formula based on ruminant milk (cow or goat). However, bifidobacteria have about 20% higher relative abundances in human milk-fed compared to formula-fed babies [4]. The greater abundance of bifidobacteria in human-milk-fed infants can, be explained by the fact that bifidobacterial species that are enriched in the infant bowel can utilize Human Milk Oligosaccharides (HMO) or their components as growth substrates [5]. Enriching bifidobacterial populations in the bowel tends to minimize the abundance of other bacterial species, so a competitive exclusion function could be ascribed to HMO. HMO may act as "decoys" in the bowel by binding to pathogens) and their toxins and thus limiting contact with mucosal surfaces [6]. The increased morbidity reported extensively in infants born by caesarean section is likely led by altered early gut colonization partially [7]. In Bottle fed babies bifidobacteria take time for the establishment in their intestine with lower numbers compared breast fed babies. This may account for intestinal disturbances by enteropathogens. *Bifidobacterium* and *Bacteroides* were significantly higher, while *Streptococcus* and *Enterococcus* were significantly lower in breast-fed group than they were in formula A-fed group [8]. Bifidobacteria supplemented baby foods have been formulated for the gut health maintenance of bottle fed babies by several scientists having good shelf life [9, 10]. Producing the concentrated cells through solid state fermentation on black gram dhal or soya bean paste as solid nutritive substrates may serve as an alternative in obtaining a good biomass of bifidobacteria that can be converted to powder and incorporated to baby foods so that healthy gut maintenance is possible with a good inoculum of bifidobacteria having various therapeutic benefits [11].

II. MATERIALS & METHODS

A. Preparation of SSF culture of selected species of Bifidobacterium:

Black gram dhal with skim milk powder of 1 % was added and soaked in potable water in the ratio of 1:0.8 for one hour at 37 ^{0}C. After soaking, dhal medium was sterilized at 121^{0}C for 20 min. After cooling, the solid medium was inoculated with 1 % of milk culture of *Bifidobacterium longum*

F 51, PF1; *B.adolescentis* F35 and *B. longum* S26 separately and incubated at 37 ^{0}C for 24 hours.

B. Conversion of fermented dhal to powder:

In order to facilitate the incorporation of fermented dhal into baby foods, dhal was cooled to 20^{0}C and made into batter in sterile blender by adding sterile potable water, spread on sterile glass plate, dried at 35 ^{0}C for 20 hours in BOD incubator. After drying the dried flakes were made into powder in a sterile blender for 1 min. The powder form can be incorporated into baby foods.

➢ *Selection of baby foods:*

Three bay foods selected form local market were

- Lactogen – I (LI - 0 to 6 months)
- Lactogen – II (LII - 6 months onwards)
- Cerelac – wheat based weaning food

C. Incorporation of baby foods with SSF cultures of bifidobacteria:

The powder containing viable four species of *Bifidobacterium* F51, PF1, F35 & S26 were added separately to baby foods to obtain final count of 8.0 $\log_{10}$cfu/g, mixed and packed aseptically in laminar air flow in sterile laminated pouches of 25 g each and stored at ambient temperature (30^{0}C) for a period of 6 months and every month the viable counts were obtained by serial dilution and pour plate method using Modified Garche agar. Presence of coliforms and yeast & molds using Violet Red Bile Agar and Malt Extract Agar with pH 5.4 and adjusted to pH 3.5 using 10 % filter sterile lactic acid at the time of plating respectively were used as selective media to determine the safety of the bay foods in storage [12].

III. RESULTS AND DISCUSSION

The initial viable counts of four species of *Bifidobacterium*: *B.longum* F51 & PF1; *B.adolescentis* F35 and *B.bifidum* S26 when incorporated into baby foods that ranged from 8.00 to 8.10 $\log_{10}$cfu/g on 0 day. The storage temperature noted was 30^{0}C. Average of 0.20 log viable count decreased notice for every month and later at the end of 6th month around 6.85 log viable count was noticed. This indicated that room temperature storage slowly led to slow decrease in viability. Sensory parameters like colour smell was maintained the same in all the bifidobacteria incorporated baby foods. Coliforms and yeast and molds were absent till the 6th month of storage of baby foods indicating hygiene of the product. In case of SSF *B.longum* F51 incorporated baby foods, initial viable count of 8.05 reduced to 6.65, 6.60 and 6.95, in Lactogen I, II and cerelac at the end of 6th month storage at 30^{0}C with overall average reduction in viability by 0.20, 0.21 and 0.16 log, respectively. Similar trend was noticed in SSF *B.longum* PF1incorporated baby foods stored at room temperature (30^{0}C) with initial count of 8.00 reduced on 6th month of storage to 6.55, 6.60 and 6.75 with overall average reduction of 0.21, 0.20 and 0.19 log, in Lactogen I, II and cerelac, respectively (Table 1). SSF *B.adolescentis* F35 added baby foods showed initial viable count of 8.07 reduced to 6.85, 6.78 and 6.90, in Lactogen I, II and cerelac at the end of 6th month storage at 30^{0}C with overall average reduction in viability by 0.17, 0.20 and 0.17 log, respectively. Almost same trend was noticed in SSF *B.bifidum* S26 incorporated baby foods stored at room temperature (30^{0}C) with initial count of 8.00 reduced on 6th month of storage to 6.92, 6.80 and 6.96 with overall average reduction of 0.15, 0.17 and 0.15 log, respectively (Table 2). On the contrary with the present study Vanisri (1995) [13] showed reduction of one log viable countduring storage of fermented black gram dhal 91:1 moisture and 1 % skim milk powder) while Ramachandra *et.al* (2009) [14] also revealed the same results but substrate was paddy husk with *B.longum* F8. The present study justifies use of nutritive solid substrate in fermentation may help obtaining higher counts and dried form can thus be used in incorporation in baby foods to maintain gut health but hygienic practices needed to be followed at every step as it is for the vulnerable group.

REFERENCES

[1]. International Scientific Association for Probiotics and Prebiotics (ISAPP), 2013(0ct. 25th), Definition of Prebiotics & Probiotics, ISAPP News (Panel meeting held at London UK)., Pub: WS Design, California, USA.

[2]. Bourdichon, F., Alper, I., Bibiloni, R., Dubois, A., Laulund, S., Miks, M., et al. (2018).Inventory of microbial food cultures with safety demonstration in fermented food products. Bull. Int. Dairy Federat. 495, 5–71.

[3]. Sanders, M. E. 2009. How do we know when something called “probiotic” is really a probiotic? A guideline for consumers and health care professionals. Functional Food Rev.1: 3–12.

[4]. Gerald W. Tannock, G.W., Pheng Soon Lee, P.S., Khai Hong Wong, k. H and Lawley B. 2016. Why Don't All Infants Have Bifidobacteria in Their Stool? Front Microbiol. **7:** 834 doi: **10.3389/fmicb.2016.00834**

[5]. Garrido D., Dallas D. C., Mills D. A., 2013. Consumption of human milk glycoconjugates by infant-associated bifidobacteria: mechanisms and implications. Microbiology. 159:649– 664.

[6]. Kunz C., Rudloff S., Baier W., Klein N., Strobel S., 2000. Oligosaccharides in human milk and metabolic aspects. *Annu. Rev*. Microbiol. 20, 699–722.

[7]. Rautava, S. 2016. Early microbial contact, the breast milk microbiome and child health. J. Dev. Orig. Health Dis. 7: 5–14.

[8]. Ma J., Li Z., Zhang W., Zhang C., Zhang Y., Mei H., Zhuo N., Wang H., Wang L and Wu D., 2020. Comparison of gut microbiota in exclusively breast-fed and formula-fed babies: a study of 91 term infants. Scientific Reports. 10:15792

[9]. Nagawa m., Nakabayashi A. and Fujino S., 1988. Preparation of the bifidus milk powder. J. Dairy Sci., 71: 1777-1782.

[10]. Nagendra R., Vshwanatha S., Venkat Rao S and Ravish S.R., 1992. Effect of feeding infant formula containing lactulose on intestinal flora in the infant. Indian J. paediatrics. 59: 763-766.

[11]. Olmos A.R., Maria Alejandra Correa Deza M A C and Garro M.s., 2014. Selected lactobacilli and bifidobacteria development in solid state fermentation using soybean

paste. Revista Argentina de Microbiología. 46 (S1): 1-144. DOI: 10.1016/j.ram.2016.08.007
[12]. Harrigan W.F., 1998. Laboratory Methods in Food Microbiology. Elsevier, Netherlands
[13]. Vanisri D.S., 1995. Preparation and performance of dairy starter cultures using solid state fermentation technique. M.Sc. Thesis, University of Agricultural Sciences, Bangalore
[14]. Ramachandra B., Manjunath H., Prabha R. and Krishna R., 2009. Performance evaluation of *Bifidobacterium longum* F8 in milk grown on Solid State Fermentation (SSF) medium. J. Dairying, Foods and Home Sciences, 28 (3 & 4):194-197

Storage period (months)	***Bifidobacterium longum***					
	F51			**PF1**		
	Baby foods incorporated with SSF bifidobacteria					
	Lactogen I	**Lactogen II**	**Cerelac**	**Lactogen I**	**Lactogen II**	**Cerelac**
	Viable count (log_{10}cfu/g)					
0	8.05	8.05	8.05	8.00	8.00	8.00
1	7.80	7.85	7.88	7.70	7.65	7.75
2	7.60	7.62	7.65	7.50	7.40	7.50
3	7.42	7.40	7.50	7.32	7.30	7.38
4	7.20	7.25	7.30	7.00	7.10	7.16
5	6.95	6.90	7.05	6.85	6.85	6.90
6	6.65	6.60	6.95	6.55	6.60	6.75
Average Reduction	0.20	0.21	0.16	0.21	0.20	0.19

Table 1: Effect of storage on the viable counts of *Bifidobacterium longum* F51 and PF1 in baby foods at 30^0C

Storage period (months)	**Species of *Bifidobacterium***					
	***B. adolescentis* F35**			***B. bifidum* S26**		
	Baby foods incorporated with SSF bifidobacteria					
	Lactogen I	**Lactogen II**	**Cerelac**	**Lactogen I**	**Lactogen II**	**Cerelac**
	Viable count (log_{10}cfu/g)					
0	8.07	8.07	8.07	8.00	8.00	8.00
1	7.90	7.85	7.92	7.80	7.72	7.88
2	7.78	7.70	7.85	7.70	7.50	7.68
3	7. 52	7.50	7.70	7.62	7.35	7.49
4	7.30	7.35	7.56	7.48	7.15	7.29
5	7.00	6.95	7.15	7.28	6.98	7.10
6	6.85	6.78	6.90	6.92	6.80	6,96
Average Reduction	0.17	0.20	0.17	0.15	0.17	0.15

Table 2: Effect of storage on the viable counts of *Bifidobacterium adolescentis* F35 and *B. bifidum* S26 in baby foods at 30^0C