RESEARCH ARTICLE Indian Journal of Animal Research, Volume 57 Issue 1: 40-43 (January 2023)

Probiotics for Augmenting Health

Rita Narayanan[1], B. Suresh Subramonian[2]

10.18805/IJAR.B-4246

ABSTRACT

Background: The probiotic microorganism consists mostly of the strains of the genera Lactobacillus and Bifidobacteria. Bifidobacterial species are common members of the infant gut where they form up to 91 per cent of the total micro flora in breast-fed babies and up to 75 per cent in formula fed infants.

Methods: Bifidobacterial species were isolated and identified from the faeces of breast fed infants by molecular techniques. Molecular tools like 16S rDNA targeted genus and species specific mPCR primers were used for confirmation. A total of 4 isolates were identified namely *B. longum* (IB_{10} and IB_{12}) *B. breve* (IB_{39}) and *B. bifidum* (IB_{42}). The identified species were screened for probiotic properties like bile tolerance, antimicrobial activity and tolerance to acidity. Ideal isolate of *B. longum* was incorporated in the preparation of whey based malted nutraceutical spray dried food and fed to weaned piglets.

Result: *B. longum* (IB_{10}) was the ideal probiotic when compared to other isolates as it had maximum antimicrobial activity and tolerance to bile and acidity. It was noted that isolate IB_{10} (*B. longum*) had an inhibitory zone ranging from 3.03 to 2.95 mm against *B. cereus, S. aureus*, *E. coli* and *P. aeruginosa* due to acid and antibacterial compounds. Isolate *B. longum* (IB_{10}) was incorporated in the preparation of whey based malted nutraceutical spray dried food. The influence of feeding five grams of the spray dried nutraceutical malt food containing *B. longum* at 10^7 cfu/g per day on animal production, gut health and gut architecture in young weaned piglets were also investigated. The probiotic regime had positive effects on the health of piglets when compared to the control group. A significant two log reduction in the coliform count was observed in the faeces of weaned piglets fed with *Bifidobacterial*nutraceutical powder than that of control. Thus, these probiotic bifidobacteria can be considered as safe adjuncts for preparation of dairy nutraceuticals.

Key words: Bifidobacterium, Health, Nutraceuticals, Probiotics.

INTRODUCTION

The concept of probiotics was introduced in the early 20th century by Elie Metchnikoff (Metchnikoff, 1907). The Food and Agriculture Organization of the United States and World Health Organization, describes them as: 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'.

The probiotic microorganism consists mostly of the strains of the genera Lactobacillus and Bifidobacteria. Bifidobacterial species are common members of the infant gut where they form up to 91 per cent of the total micro flora in breast-fed babies and up to 75 per cent in formula fed infants (Miloud Hadadji *et al*., 2005). Dairy products containing *Bifidobacteria* have potential benefits for infants and adults that are generally related to inhibition of pathogens, maintenance and restoration of normal intestinal flora. It has been reported that these organisms are able to exert beneficial effects including improvement of intestinal microflora by preventing colonization of pathogens, amelioration of diarrhoea or constipation, activation of the immune system and increasing protein digestion (Ishibashi and Shimamura, 1993). Owing to these properties, *Bifidobacteria* are now frequently used to prepare probiotic dietary adjuncts.

Dehydration is a common practice to preserve biological materials so that they are stable in the long run. Among the many cell preservation methods, spray drying is widely used in the food industry because it is economical especially on a large scale. In the current study the spray dried probiotic *B. longum* was fed to weaned piglets and its probiotic potential was evaluated in terms of weight gain and coliform count in the weaned treated pig.

[1]Department of Food Processing Technology, College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University, Koduvalli, Chennai-600 052, Tamil Nadu, India.
[2]Department of Dairy Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007, Tamil Nadu, India.

Corresponding Author: Rita Narayana, Department of Food Processing Technology, College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University, Koduvalli, Chennai-600 052, Tamil Nadu, India.
Email: ritanarayanan@yahoo.com

How to cite this article: Narayanan, R. and Subramonian, B.S. (2023). Probiotics for Augmenting Health. Indian Journal of Animal Research. 57(1): 40-43. doi: 10.18805/IJAR.B-4246.

Submitted: 11-07-2020 **Accepted:** 09-08-2021 **Online:** 29-09-2021

MATERIALS AND METHODS

Bifidobacteria longum (IB_{10}) and (IB_{12}), *Bifidobacteria breve* (IB_{39}) and *Bifidobacteria bifidum* (IB_{42}) were isolated and identified from breast fed infant faeces by phenotypic and molecular techniques.

Screening of *Bifidobacteria* for probiotic properties

Tolerance to bile salt

Tolerance to bile salts was carried out as per the method adopted by Jaya Prasad *et al*. (1999).

Inhibitory activity assay and tolerance to acidity

Inhibitory activity assay and tolerance to acidity was carried out as per the method adopted by Zinedine and Faid (2007).

The ideal isolate *B. longum* (IB_{10}) was incubated for 4 hours in a mixture of 4 per cent whey protein concentrate, 0.4 per cent inulin, 3 per cent honey and 9 percent malted ragi extract and spray dried.

Feeding trials with weaned piglets

Seventeen weaned large white Yorkshire piglets from the University Research Farm, were randomly chosen from 2 litters for the experimental study. The piglets were penned in individual sheds which permitted monitored feeding. The experimental group was administered a daily supplement of 5 gms of 10^7 cfu/g of the probiotic spray dried powder. The piglets were weighed at weekly intervals and the body weight was recorded.

Coliform was enumerated from freshly voided faeces collected from control and experimental group by pour plate method and compared.

The data obtained were analyzed statistically as per the procedure of Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Screening of bifidobacteria for probiotic properties

Tolerance of bifidobacterial isolates to bile salts

From Table 1, it is seen that the isolated bifidobacterial species had tolerance towards varying levels of bile. The isolate IB_{10} showed greater tolerance ($P \leq 0.01$) to 1 per cent bile when compared to other isolates. All the isolates showed more than 80 per cent survival rate in the presence of 1 per cent w/v bile which was affirmed as probiotic property by Jaya Prasad *et al.* (1999) who opined that an ideal probiotic candidate should have a survival threshold of 80 per cent in the presence of 1 per cent w/v. Zinedine and Faid (2007) also reported that various species of *Bifidobacterium* showed tolerance to bile salts. In the present study bile concentrations of up to 1 per cent w/v were used, though the mean intestinal bile concentration is believed to be 0.3 per cent w/v (Gilliland *et al.,* 1984). Goldin *et al.* (1992) also reported that a concentration of 0.3 per cent w/v of bile has consequently been used in most studies screening for bile tolerant strains. Interestingly all the four isolates of IB_{10} and IB_{12}, IB_{39} and IB_{42} survived and showed tolerance to bile concentrations of up to 1 per cent w/v. This property may provide these species with an advantage *in vivo*. Among all the isolates, IB_{10} showed maximum tolerance.

Inhibitory activity of bifidobacterial isolates against pathogenic bacteria

From Table 2 it is evident that the non-neutralized and neutralized cell free supernatants of isolate IB_{10} showed inhibition against all the four pathogenic species tested. The isolates in the present study showed inhibitory activity against selected pathogenic bacteria. On comparing the inhibitory assay of the four isolates, it was noted that isolate IB_{10} (*B. longum*) had an inhibitory zone ranging from 3.03 to 2.95 mm against *B. cereus, S. aureus*, *E.coli* and *P. aeruginosa* due to acid and antibacterial compounds which was similar to the work of Zinedine and Faid (2007).

Tolerance of bifidobacterial isolates to acidity at pH 3

Table 3 shows the tolerance of bifidobacterial isolates to acidity at pH 3 as in most *in vitro* assays pH 3 has been preferred (Garriga *et al.,* 1998). Isolate IB_{10} showed a maximum count of 10.375±0.174 ($\log_{10}$ cfu/ml) at 90 minutes

Table 1: Tolerance of bifidobacterial isolates# to bile salts.

Isolate code	Control	Percentage of Bile salt			F value
		0.4%	0.8%	1%	
IB_{10}	11.756^{cC}±0.023	11.685^{cC}±0.023	10.642^{bB}±0.115	9.974^{aB}±0.242	42.82**
IB_{12}	10.809^{cB}±0.027	10.706^{cB}±0.024	9.786^{bA}±0.040	9.116^{aA}±0.116	34.16**
IB_{39}	10.673^{cA}±0.029	10.487^{cA}±0.065	9.634^{bA}±0.127	9.000^{aA}±0.050	95.09**
IB_{42}	10.650^{cA}±0.043	10.550^{cA}±0.057	9.664^{bA}±0.016	9.000^{aA}±0.050	298.63**
F value	218.09**	133.58**	9.48**	9.24**	

Average of six trials #$\log_{10}$ cfu/ml. ** Statistically highly significant ($P \leq 0.01$). Small case superscript shows difference between replicates. Capital case superscript shows show difference between treatments.

Table 2: Inhibitory activity of bifidobacterial isolates (zone in mm) against pathogenic bacteria.

Isolate code	*Bacillus cereus*		*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*	
	CFS	CFS pH7	CFS	CFS pH7	CFS	CFS pH7	CFS	CFS pH7
IB_{10}	3.03±0.019	3.00±0.020	3.00±0.031	2.95±0.037	2.98±0.043	2.97±0.051	3.03±0.051	3.00±0.032
IB_{12}	-	-	4.00±0.052	3.98±0.071	-	-	2.46±0.074	2.01±0.081
IB_{39}	4.03±0.030	3.99±0.022	2.10±0.052	2.00±0.061	-	-	1.58±0.028	-
IB_{42}	2.95±0.031	2.94±0.029	-	-	-	-	3.13±0.030	-

Average of six trials. CFS- Cell free supernatant. CFS pH7- Cell free supernatant adjusted to pH7.

Table 3: Tolerance of bifidobacterial isolates# to acidity at pH 3.

Isolate code	Incubation period in minutes			F value
	0	90	180	
IB_{10}	11.756^{b}±0.023	10.375±0.174	9.698^{aB}±0.254	37.51**
IB_{12}	10.809^{b}±0.027	9.375±0.275	8.895^{aA}±0.216	39.63**
IB_{39}	10.673^{b}±0.029	9.291±0.148	8.553^{aA}±0.219	49.83**
IB_{42}	10.650^{b}±0.043	9.359±0.272	8.742^{aA}±0.302	17.81**
F value	218.09**		4.17*	

Average of six trials. #$\log_{10}$ cfu/ml. Small case superscripts shows difference between replicates. Capital case superscripts shows difference between treatments.

Table 4: Performance (weight) of pigs fed with nutraceutical powder (BMW).

Days	Control (kg)	Experimental (kg) (BMW)	t-Test
0	13.00±1.064	12.83±1.194	0.10^{NS}
7	14.00±1.064	14.83±1.194	0.52^{NS}
14	15.58±1.019	19.25±1.435	2.08^{NS}
21	17.78±1.128	23.40±1.381	3.16*
28	20.28±1.128	26.68±1.694	3.14*
35	22.37±1.082	28.67±1.406	3.55**
42	25.57±1.082	37.50±1.586	6.21**
49	29.62±0.440	41.83±1.515	7.74**
56	32.30±0.791	45.90±2.391	5.40**
63	35.70±1.018	51.67±2.488	6.08**
70	38.88±0.631	55.00±2.363	6.59**

Average of six trails. NS: Non significant. * Statistically significant (P≤0.05) ** Statistically highly significant (P≤0.01).

Table 5: Enumeration of faecal coliform# post feeding in pigs fed control and nutraceutical whey based malt food (BMW).

Days	Control	BMW	t-Test
0	6.76±0.058	6.80±0.038	0.65^{NS}
7	6.76±0.058	6.72±0.040	0.60^{NS}
14	6.82±0.048	6.70±0.228	2.37*
21	6.87±0.036	6.53±0.108	2.92*
28	6.94±0.029	6.42±0.074	6.47**
35	6.82±0.048	6.30±0.109	4.34**
42	6.82±0.048	6.22±0.022	11.24**
49	7.01±0.029	5.85±0.050	19.84**
56	6.97±0.035	5.40±0.149	10.23**
63	6.97±0.035	5.05±0.050	31.29**
70	6.97±0.035	5.00±0.002	55.74**

Average of six trails. #$\log_{10}$ cfu/ml. NS: Non significant. * Statistically significant (P≤0.05). ** Statistically highly significant (P≤0.01).

and 9.698±0.254 ($\log_{10}$ cfu/ml) at 180 minutes post incubation as when compared to the observation of Jaya Prasad *et al.* (1999) who reported a viable count of 8.1 ($\log_{10}$ cfu/ml) at 3 hours incubation at pH 3. This increase in tolerance in the present study may be due to strain variability.

In the present study a marginal decrease was noticed in the viable count of *Bifidobacteria* of all isolates at 90 and 180 minutes. However all the isolates crossed the threshold level of 80 per cent survivability as recommended by Jaya Prasad *et al.* (1999).

The findings of this study met the recommendation of the FAO/WTO draft guidelines of 2002 and ICMR-DBT guidelines for evaluation of probiotics in food 2011.

Biological trials

Performance (weight) of pigs fed with nutraceutical powder (BMW)

Table 4 presents a comparison of weight of pigs between control and experimental group fed with nutraceutical powder containing *B. longum* (BMW). The mean values (in kg) of experimental pigs and control on the 70th day were 55±2.363 and 38.88±0.631 respectively. The inference observed indicated a highly significant difference (P≤0.01) in the assimilation of nutrient leading to enhanced weight gain in BMW fed piglets.

The findings in this study correlated with this theory of Peter Kurti (2000) who reported that probiotics have shown beneficial effects on the intestinal balance and in improving feed utilization and weight gain. He also opined that better digestibility meant better absorption of nutrients and thus better weight gain than probiotic deprived piglets.

Enumeration of faecal coliform ($\log_{10}$ cfu/g) post feeding in control piglets and nutraceutical whey based malt food (BMW) fed piglets

From Table 5 significant difference (P≥0.05) was observed in the faecal coliform count up to 7 days of feeding of piglets in control and experimental groups. Significant difference (P≤0.05) was noticed from the 14th to the 21st day between control (basal diet) and experimental group (nutraceutical whey based malt food with *B.longum*). A highly significant difference (P≤0.01) was noticed in the reduction of faecal coliform counts between control and experimental group from 28th day till 70th day. There was no reduction in the coliform numbers in the control group till the end of the experiment.

The findings of the present study is in accordance to Boomba *et al.* (2002) who reported a one log reduction in the *E. coli* after administration of *L. paracasei* and maltodextrin. In the present study there was a significant (from 6.80±0.038 to 5.00±0.000) two log reduction in the coliform count in *Bifidobacterial* fed piglets than the controls.

REFERENCES

Boomba Nemcova, A.R., Gancarcikona, S., Gerich, R. and Guba, P. (2002). Improvement of probiotics effect of microorganism by their combination with maltodextrins, fructo oligosaccharides and polyunsaturated fatty acid. Brit. J. of Nutr. 88 (Suppl.): 595-599.

FAO/WHO Working Group Report, (2002). Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada.

Garriga, M., Pascual, M., Montford, J.M. and Hugas, M. (1998). Selection of Lactobacilli for chicken probiotic adjuncts. J. of Appl. Microbiol. 84: 125-132.

Gilliland, S.E., Staley, T.E. and Bush, L.J. (1984). Importance of bile tolerance of *L. acidophilus* used as dietary adjunct. J. of Dairy Sci. 67: 3045- 3051.

Goldin, B., Gorbach, S., Saxelin, M., Barakat, S., Gualtieri, L. and Salminen, S. (1992). Survival of *Lactobacillus* GG in human gastrointestinal tract. Dig. Dis. and Sci. 37: 121-128.

Ishibashi, N. and Shimamura, S. (1993). Bifidobacteria. Research and Development in Japan. Food Technol. 46: 126-135.

Jaya, P., Harsharanjit, G., John, S. and Gopal, P.K. (1999). Selection and characterization of *Lactobacillus* and *Bifidobacterium* strains for use as probiotics. Int. Dairy J. 8: 993-1002.

Metchnikoff, E. (1907). Lactic Acid as Inhibiting Intestinal Putrefaction. In: The Prolongation of Life: Optimistic Studies. W. Heinemann, London: 161-183.

Miloud, H., Rabha, B., Noureddine, S., Djamal Eddine, H. and Mebrouk, K. (2005). Identification of cultivable *Bifidobacterium* species isolated from breast-fed infants faeces in West Algeria. African J. of Biotechnol. 4(5): 422-430.

Peter Kurti (2000). Microbial balance and optimal digestion in pigs. Intern. Pig Topics. (16): 7.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical Analytical Methods. Oxford and IBM Publishers Co., New Delhi, India.

Zinedine, A. and Faid, M. (2007). Isolation and characterization of strains of *Bifidobacteria* with probiotic properties. World J. Dairy and Food Sci. 2(1): 28-34.