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Comparative Evaluation of Commercial and Indigenous Bacterial Isolates for Antigenotoxic Potential

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Short communication

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Abstract

A total of four bacteria isolated from commercial food products viz., Amul Probiotic Ice-cream, Nestle Baby & Me and Yakult were compared against two potential indigenous probiotic isolates from traditional fermented foods of North-Western Himalayas along with reference strain Lactobacillus rhamnosus GG-ATCC 53103 for antigenotoxic potential. Indigenous isolate ADF10, Lactobacillus plantarum showed highest antigenotoxicity (>90% against 4-NQO and furazolidone) among all the isolates used in this study followed by commercial isolate L4, Bifidobacterium lactis from Nestle Baby & Me. This study showed that AdF10 can be used for specific enrichment of fermented foods to enhance their health potential benefits.

Keywords: Indigenous; Commercial; Antigenotoxic; Fermented food; North-Western Himalayas

Introduction

The gut microbiota is made up of diverse and complex microbial communities that plays a key role in the host overall health [1] through its metabolic activities and physiological regulation. Alteration of the microbiota may cause some direct or indirect digestive pathologies like infectious diseases and metabolic disorders. Probiotics are the health promoting viable microorganisms that exhibit a beneficial effect on the health of human being by improving the intestinal microbial balance. There is global scientific and commercial interest in probiotics due to a range of health promoting attributes [2,3]. The increased interest in probiotics has set the stage for expanded marketing of these products worldwide [4] including India. A lot of awareness among people about the use of probiotics during the last decade has been observed due to increased influx of probiotic foods with several strains of lactic acid bacteria (LAB) in the food market [5]. Today, Probiotics have been of scientific and commercial interest due to a range of health promoting attributes, including suppression of growth of pathogens, control of serum cholesterol level, modulation of the immune system, improvement of lactose digestion, synthesis of vitamins, ability to adhere to gut tissue, increase in bio-availability of minerals and possible anti-carcinogenic activity [2,3]. However, to generate the confidence level among people for these commercial probiotic isolates, a thorough examination is needed with respect to already existing indigenous strains with established probiotic potential. There has been much interest generated during the last few years in traditional fermented food products as they are the rich sources of probiotics or functional microorganisms. Himachal Pradesh is a rich repository of indigenous fermented foods which are traditionally prepared and consumed by the inhabitants. A variety of indigenous fermented food products and beverages of North-Western Himalayas have been documented with

respect to substrates and probiotic diversity [6,3,7]. A probiotics have been made available in the market which is fostered by the current trend of consuming healthy food in order to prevent illness. Each fermented food is associated with unique group of microflora which is responsible for elevating the nutritional quality of that product. The viability of probiotic bacteria within products is crucial for the beneficial effects they intend to offer to the consumer's health. Nevertheless, several studies have shown low viability of utilized probiotic strains within storage time of products [8,9]. It is necessary to identify suitable LAB's with good characteristics of probiotics to promote Public Health and to avoid confusion. Screening of probiotic characteristics of these bacterial strains by in vitro studies forms the basis for selection of functional probiotics for commercial use. Studies conducted on indigenous probiotic bacteria isolated from traditional fermented foods of North-wide range of commercial food products containing Western Himalayas have demonstrated these isolates as potential probiotic strains with proven protective and therapeutic traits against colon carcinogenesis [3,10,11]. Screening and comparison of protective attributes of these isolates with commercial strains by in vitro studies forms the basis for selection of functional probiotics for commercial use to promote public health.

Materials and Methods

Isolation of Microorganisms

Commercial probiotic bacteria used in this study were isolated from three commercial probiotic food products viz., Amul Probiotic Ice-cream, Nestle Baby & Me and Yakult available in the local market using spread plate technique on selective media (Table 1A).

S. No.	Food Sample	Isolated bacteria	Batch No.	Expiry Date	Sampling Time
1	Amul probiotic ice-cream (L1)	*Lactobacillus* spp.	GAH0383	14-08-2013	12-06-2013
2	Nestle baby & me (L2&L4)	*Lactobacillus rhamnosus*(GG) &*Bifidobacteriumlactis*	L2180029185	3/9/2013	14-06-2013
3	Yakult (L3)	*Lactobacillus casei*	2707	10/8/2013	18-06-2013

Table 1A: Commercial food products used for the isolation of probiotic bacteria.

Indigenous potential probiotic microorganisms isolated from different fermented food products of North-Western Himalayas [3] were procured from Department of Microbiology, CSK HPAU, Palampur (Table 1B). The reference strain Lactobacillus rhamnosus GG-ATCC 53103, used in the present study was procured from American Type Culture collection (ATCC), USA.

S. No.	Isolate	Isolation source	Identification using BLAST n algorithm	GenBank[a] Accession no.	NBAIM[b] Accession no.
1	AdF5	Fermented milk	*Lactobacillus plantarum*	GU396274	NAIMCC-B-01048
2	AdF10	Jan Chang	*Lactobacillus plantarum*	GU396278	NAIMCC-B-01051

[a]GenBank, National Centre for Biotechnology Information (NCBI), USA.

[b]National Bureau of Agriculturally Important Microorganisms, Mau NathBhanjan, Uttar Pradesh, India.

Table 1B: Indigenous probiotic bacterial isolates used for comparative studies.

Antigenotoxic Studies

Cell preparation and genotoxin-cell co-incubation: Overnight grown bacterial cultures at 37 °C in MRS broth were washed and resuspended in saline (108-109 cells

mL-1). Genotoxins (4-NQO and furazolidone) were added at a final concentration of 0.1m mol-1 and co-incubated activity was determined in supernatants after centrifugation (5000g, 15 min) and filtration through 0.45µm membrane.

Genotoxicity assay: SOS chromotest was used to evaluate genotoxicity by activation of SOS-response with target as prokaryotic tester strain E. coli PQ37. This strain is susceptible to genotoxins due to envelope permeability (rfa), no excision of DNA repair (uvrA) and sfiA::lacZfusion, which is responsible for β-galactosidase (BG) induction in the presence of DNA damage. Constitutive alkaline phosphatase (AP) production was used as an indicator of protein synthesis in the presence of genotoxins (Quillardet and Hofnung, 1985; Quillardet and Hofnung, 1993). PQ37 incubated overnight in LB medium plus ampicillin (20 µg mL-1), was transferred (100 µL) to 5 mL of the same medium and incubated for 2 h (OD520=0.3–0.4). One milliliter of this suspension was added to 9 mL of fresh LB without ampicillin and aliquots of 600 µL were mixed with 20 µL of either sample containing genotoxin (positive control) or supernatant of genotoxin co-incubated with cells. Negative control contained 20 µL of saline. After 2h incubation at 37°C, the activation of SOS DNA repair system was evaluated by with cell suspension for 150 min in an incubator shaker at 100 rpm. After co-incubation, the residual genotoxic induction of BG, and correction was done on the basis of AP (constitutive activity). SOS induction factor (IFSOS) was defined as BG to AP ratio of the sample under analysis, divided by the same ratio of negative control. Both enzyme activities were detected colorimetrically [12]. Results were expressed as per cent inhibition of genotoxicity in relation to IF of the genotoxin without cell co-incubation (positive control).

Results and Discussion

The ability of probiotic microorganisms to inhibit genotoxins is an important unconventional functional property. Probiotic metabolites alter the transcriptome and the metabolome of the host, affecting the intestinal mucosa, which may prevent carcinogenesis [13]. A high degree of survival of probiotic bacteria was reported after genotoxin exposure [14]. Similar effect was observed in our previous study [10] as well as in this study against a base intercalating agent (4-NQO) and a DNA binding agent (furazolidone).Cell viability of more than 80% was observed in presence of genotoxins for the commercial as well as indigenous bacteria (Figure 1).

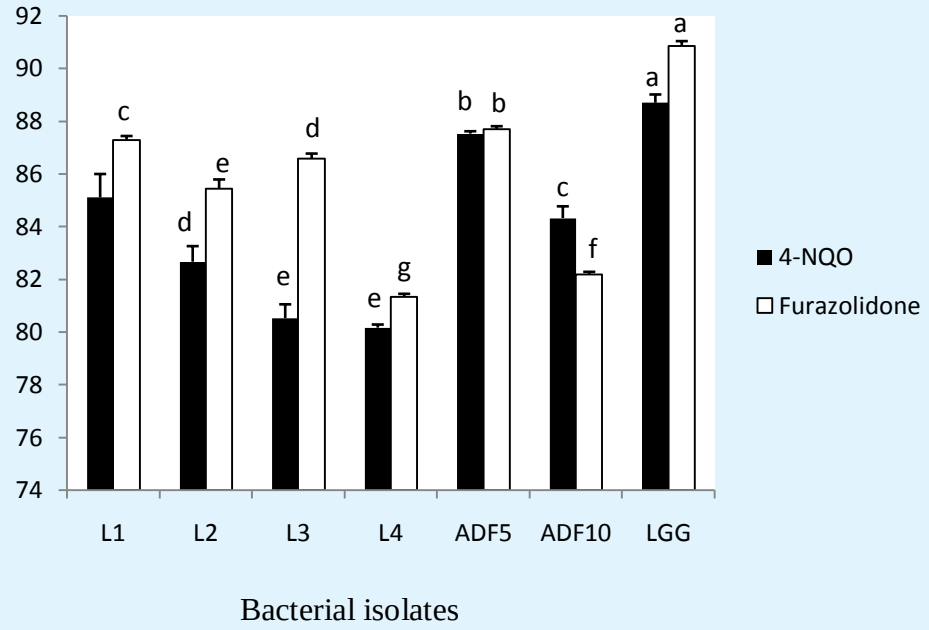

Figure 1: Percent viability of probiotic bacteria in the presence of 4-NQO(■) and furazolidone (□). Values are expressed as mean ± SD.

Thegenotoxic effect of 4-NQO and furazolidone in SOS chromotest was strongly reduced under in vitro co-incubation with all the probiotic bacteria (Table 2). All the isolates were effective upto different levels against these two genotoxins. Only L. plantarum (AdF10) exhibited more than 90% inhibition of 4-NQO and furazolidone which was higher than the antigenotoxicity expressed by the reference strain *L. rhamnosus* GG 53103. These results are in corroboration with the study of [11], where L. plantarum (AdF10) was reported to have proven anticarcinogenic effect against colon cancer by suppressing the COX-2 expression as one of the protective mechanisms. All other isolates showed more than 80% of antigenotoxicity except for *L. plantarum* (AdF 5). Reduction in genotoxicity may be a consequence of binding of genotoxins to the probiotics as reported earlier by other workers [15-17]. Simple physical binding followed by subsequent degradation by probiotics of potential dietary carcinogens has been responsible for their anticarcinogenic action, and thereby reducing the bioavailability of carcinogens in the gastrointestinal tract [18]. There are a large number of reports describing the adsorption or binding of mutagens and pro-mutagens, as well as food-borne carcinogens to LAB under in vitro conditions [19-22].

S.No.	Isolates	4-NQO (%antigenotoxicity)	Furazolidone (% antigenotoxicity)
1	L1	87.23^{c}	89.68^{c}
2	L2	83.49^{e}	89.93^{c}
3	L3	81.57^{f}	89.51^{d}
4	L4	88.44^{b}	90.46^{b}
5	AdF5	76.28^{g}	79.47^{e}
6	AdF10	91.32^{a}	93.59^{a}
7	LGG	85.02^{d}	89.45^{d}
CD (5%)		0.269	0.124

Results shown are means, number of replications=3, different lower case letters denote significant differences among values of various traits ($P<0.05$).

Table 2: Percent antigenotoxicity of probiotic bacteria in the presence of genotoxins.

Conclusion

The information generated in this study on indigenous microorganisms would be helpful in enriching the traditional fermented foods for imparting health benefits and also in conservation of the valuable microbial resource. This study also showed that the indigenous isolates are more promising than the commercial isolates in protective attributes, suggesting their significance in commercial exploitation with enhanced nutritional and therapeutic potential.

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