Journal of Advances in Microbiology

10(2): 1-7, 2018; Article no.JAMB.40269
ISSN: 2456-7116

Probiotic Properties of Lipolytic Bacteria Isolated from Fermented Food and Dairy Products

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Authors' contributions

This work was carried out in collaboration between both authors. Author MR designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author KS managed the analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/40269
Editor(s):
(1) Hung-Jen Liu, Distinguished professor, Institute of Molecular Biology, National Chung Hsing University, Taiwan.
Reviewers:
(1) Silvana Carro, University of the Republic, Uruguay.
(2) R. Prabha, Karnataka Veterinary, Animal and Fisheries Sciences University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/24497

Original Research Article

Received 22nd February 2018
Accepted 27th April 2018
Published 7th May 2018

ABSTRACT

Aims: Present study deals with the isolation and probiotic characterization of lactic acid bacteria purified from fermented food and dairy products.
Study Design: This study designed to isolate, identify, and *in vitro* characterization (low pH/high bile salt tolerance, antibacterial activity, and antibiotic susceptibility).
Place and Duration of Study: Microbial Research Laboratory, Department of Biotechnology, Mohanlal Sukhadia University, Rajasthan. Between November 2017 to January 2018.
Methodology: The ability of the isolates to survive in the presence of hydrochloric acid (*p*H 1.0 and *p*H 3.0), pepsin (3 mg/ml, *p*H 2.0), pancreatin (1 mg/ml, *p*H 8.0) and bile salts (0.3% w/v Ox Gall) was measured. The intrinsic antimicrobial activity of cell-free extract from selected LAB isolates against *E. coli, Pseudomonas aeruginosa, Bacillus subtilis, Proteus vulgaris and Staphylococcus aureus* were determined by agar well method.
Results: Among the 7 bacterial isolates purified, 6 were from dairy and fermented food samples whereas 1 isolates were used as standard purified from Yakult (known probiotic drink). Isolates were checked for the presence of lipid degrading principle, maximum activity was observed in *Lactobacillus helveticus* purified from pickle (a fermented food). Probiotic characterization profiling suggested that *Lactobacillus helveticus* (pickle), *Lactobacillus Plantarum* (unfermented camel milk) and *Pediococcus pentosacceos* (Dosa batter) showed a good potential of probiotic as compared to other isolates.

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Conclusion: Furthermore characterization of these isolates and subsequent clinical studies will pave the way to design a novel probiotic formulation based on fermented food and camel milk.

Keywords: Functional foods health status; probiotic; lipolytic; fermented food and dairy food; probiotic formulation.

1. INTRODUCTION

Probiotics are live microorganisms that have health benefits when consumed. Several probiotic items are available in the market. These products are very helpful in building up immunity as well as digestive health, reduce depression and promote heart health [1]. Among the microbes known as probiotic, Lactobacilli are one of the well-known probiotics. These microbes, like *Bifidobacterium,* are lactic-acid producing bacteria (LAB) and in fact, the two genera share a few common genes.

Fermented food and dairy products are an essential part of our diet and contain a diverse microbiota. Lactic acid bacteria (LAB) are the main players during dairy and food fermentation, which results in an increased acidity that makes growth conditions of microorganisms other than LAB increasingly inauspicious. The LAB involved in fermenting food as well as in dairy processing belong to various microbial groups that are characterized by diverse nutritional, metabolic, and culture requirements as well as different technological properties. The most common LAB present in milk includes species belonging to the genera *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Enterococcus,* and *Lactococcus* [2]. Lactobacillus bacteria are one of the members of Lactic Acid Bacteria, well known for probiotic properties.

The present study deals with the isolation of lipolytic Lactobacillus bacteria from fermented food and dairy samples. Prior to developing the probiotic formulation, isolates need to be checked for probiotic properties especially pH resistance, bile salt tolerance, antimicrobial activity and antioxidant activity. Probiotic microorganisms can be screened from non-intestinal sources, such as fermented food [3], fruit juices [4], grains [5], honey-comb [6] and soil [7]. LAB primarily *Lactobacillus plantarum* has been found in many types of fruit juices from both solid and citrus fruits whereas *Leuconostoc mesenteroides* is rarely found in these fruits but is the species that is most commonly found in tomatoes [4]. Literature suggested the presence of lipolytic lactic acid bacteria from camel milk and other dairy sources [8,9]. Findings are also available on cholesterol reducing properties of Lactobacillus bacteria [10]. HA, CHUL-GYU et al. also reported the cholesterol lowering effect of *Lactobacillus Plantarum* Isolated from Human Feces [11].

Lactobacillus from these samples regarded as safe. *Lactobacillus* isolates purified from food products are usually used to develop probiotic formulation. Thus developed probiotic formulation can be helpful to develop remedies to get rid of health implications associated with increased serum lipid and the consequent effect on other organs.

2. MATERIALS AND METHODS

2.1 Collection of Sample

Various dairy samples and fermented food samples were collected from local vendors and villages and also prepared at home (Table no.1).

2.2 Isolation, Purification and Molecular Typing of Lipolytic Lactic Acid Bacteria Strains Purified from Fermented Food and Camel Milk

Six bacterial isolates were isolated and purified from dairy and fermented food samples. Isolation was done by using selective culture technique employing selective media i.e. MRS agar.

Commercialized products positively contained probiotic strains like Yakult (Japan) were also subjected to isolation process. Purified bacterial isolates were identified by direct microscopic examination, cultural characteristics and biochemical tests. In all the cases, identification was done following the Bergey's manual of systematic biology [12].

2.3 DNA Extraction

For DNA extraction, single colonies were resuspended in 50 µL of sterile deionized water. Next, 50 µL of chloroform/isoamyl alcohol (24:1) was added to the suspensions, and after

vortexing, the mixture was centrifuged at 16 000 g for five minutes at 4°C. Then, 5 µL of the upper aqueous phase was used as a source of DNA template for the PCR reaction.

2.4 Amplification of the Internally Transcribed Spacer (ITS) Region and Analysis of the Amplified Ribosomal DNA

The primers used for the amplification of the ITS region between the 16S and 23S rRNA genes were Forward primer: agagtttgatcctggctcag and Reverse primer: cttgtgcgggcccccgtcaattc. The Polymerase Chain Reaction (PCR) was carried out by mixing 5 µL of each extracted DNA with 25 µL of 2XPCR Master kit (composition of 1X solution: 0.5 M Tris-HCl, 1.5 mM $MgCl_2$ – 200 µM dATP, 200 µM dCTP, 200 µM dGTP and 200 µM dTTP and 0.04 Units/ul Taq), 1 µL Oligo forward (10 picomole/µL), 1 µL Oligo reverse (10 picomole/µL) and 18 µL Sterile deionized water. The amplification was achieved by 40 PCR cycles. The Amplified product was examined using 1.5% (w/v) agarose gels in 0.5X Tris/Borate/Ethylenediaminetet raacetic acid (TBE) buffer at 75 V for 90 minutes with a DNA ladder. The product thus obtained was 875 bp.

2.5 Detection of Lipolytic Activity

Purified isolates were checked for significant lipolytic activity by using Agar spot method on selective media i.e. Tributyrin Agar media. Quantitative estimation was made by titrimetric analysis method using tributyrin as a substrate. For this purpose cell-free extract was prepared from purified isolates and subjected to partial purification of lipid degrading principle by ammonium sulphate precipitation method.

2.5 *In vitro* Test to Screen Potentiality of Strains for Good Probiotic Properties

Tolerance to acid, pepsin, bile and pancreatin: The ability of the organisms to survive adverse conditions was assessed by incubating the organisms in MRS broth culture in presence of these adverse influences, and removing aliquots at specified times for plating on MRS agar plates to ascertain growth. The ability of the isolates to survive in the presence of hydrochloric acid (*p*H 1.0 and *p*H 3.0), pepsin (3 mg/ml, *p*H 2.0), pancreatin (1 mg/ml, *p*H 8.0) and bile salts (0.3% w/v Ox Gall) was measured. The reagents for these tests were obtained from HI- Media. The intrinsic antimicrobial activity of cell-free extract from selected LAB isolates against *E. coli, Pseudomonas aeruginosa, Bacillus subtilis, Proteus vulgaris and Staphylococcus aureus* were determined by agar well method. Antioxidant activity of selected lipolytic LAB was done by FRAP assay. All the data obtained were subjected to determine mean ±SD via formula given in MS Excel.

3. RESULTS

Several health problems like heart disorder, chronic kidney disease, gastrointestinal problems, weak immunity etc. can be fetal for human life. One of the reasons for these problems is food habits and sedentary lifestyles. Drug abuse is also a reason associated with kidney failure and consequent multiple organ disorder. The need of the hour is to develop functional food that not only meets the nutritional requirement but also improve overall health.

Dairy samples and fermented food samples were incorporated into a screening process to check the presence of lipolytic lactobacillus bacteria. On biotyping using analytical grade chemicals and API strep (Biometrics) and sequencing of 16S rDNA/D1/D2 domain of LSU rDNA or ITS region and BLAST analysis Lipolytic bacterial isolates were identified as *Lactobacillus Plantarum*, *Bacillus spp, Lactobacillus Plantarum*, *Pentococcus pentosacceos*, *Bacillus subtilis* and *Lactobacillus helveticus* (90% to 99% similarities) purified from Unfermented camel milk, fermented camel milk, curd, butter, fermented fish and pickle respectively. *Lactobacillus casei* from Yakult was used as a known standard of probiotic (Table 1). All the bacterial isolates were gram-positive and showed a catalase negative reaction. These isolates also checked for the existence of lipolytic activity. Maximum lipolytic activity was observed for *Lactobacillus helveticus* (pickle) i.e. 149.54 activity/mg (Tables 1 and 2).

Results of the presence of probiotic properties such as acid and bile salt tolerance, antimicrobial properties and antioxidant properties, suggested that among the isolates few showed good probiotic potential. As compared to control (Yakult; *Lactobacillus casei*), Maximum acid tolerance ability was observed in *Lactobacillus helveticus* followed by *Pediococcus pentosacceos* and *Lactobacillus Plantarum* As compared to control (Yakult; *Lactobacillus casei*), Maximum bile salt tolerance ability was observed in *Lactobacillus plantarum* followed by *Lactobacillus helveticus* and *Pediococcus pentosacceos.* Results of intrinsic antimicrobial

activity of cell-free extract from selected LAB isolates against *E. coli, Pseudomonas aeruginosa, Bascillus subtilis, Proteus vulgaris and Stphylococcus aureus* were determined by agar well method. As compared to control (Yakult (Known probiotic drink; *Lactobacillus casei*) *Lactobacillus helveticus* inhibit the growth of all test pathogenic bacteria whereas *Pediococcus pentosacceos* shown to inhibit the growth of three test pathogens i.e. *Pseudomonas aeruginosa, Proteus vulgaris* and *Staphylococcus aureus*. Results of antioxidant activity suggested that as compared to control (Yakult (Known probiotic drink; *Lactobacillus casei*), Maximum Fe^{2+} µM/L observed in *Lactobacillus helveticus* followed by *Pediococcus pentosacceos*.

4. DISCUSSION

Since "healthy" foods and increasing consumer health consciousness increase the demand of functional foods hence the food industry associated with probiotic goods has a central role in facilitating consumer's health and represents a rapid growth within the global souk [13]. Probiotics microorganisms of the genera Lactobacillus, Bifidobacterium, and Saccharomyces, these isolates not only find a place in fermented dairy products and infant formula but also in pharmaceutical preparations have been recognized for their "generally recognized as safe (GRAS)" status [14].

Table 1. Identified Lipolytic lactic acid bacterial isolates

S. No.	Isolates	Sources	Bacteria identified
1.	MRL a	Pickle	*Lactobacillus helveticus*
2.	MRL b	Curd	*Lactobacillus Plantarum*
3.	MRL c	Unfermented camel milk	*Lactobacillus plantarum*
4.	MRL d	Dosa batter	*Pentococcus pentosacceos*
5.	MRL e	Fermented fish	*Bacillus subtilis*
6.	MRL f	Fermented camel milk	*Bacillus spp*
7.	MRL g	Yakult (Known probiotic drink)	*Lactobacillus casei*

Table 2. Qualitative estimation of lipase activity (Agar spot method)

S. No.	Source	Zone of hydrolysis (mm)			Mean± S.D.
		R1	R2	R3	
1.	Batter	11	10	11	10.66±0.57157
2.	Fermented camel milk	13	11	13	11.66±0.57735
3.	Curd	14	14	13	13.66±0.57735
4.	Yakult	12	11	12	11.66±0.57735
5.	Pickle	19	18	19	18.66±0.57735
6.	Unfermented camel milk	12	11	12	11.66±0.57735
7.	Fermented fish	11	10	11	10.66±0.57735

Table 3. Quantitative estimation of lipolytic activity by the titrimetric method

S. No.	Source	Micromole of FFAs/h				Specific activity (Total activity/mg)
		R1	R2	R3	Mean ± S.D.	
1.	Batter	1.927	1.925	1.883	1.911±0.20	18.92
2.	Fermented camel milk	2.45	2.46	2.39	2.43±0.03	30.37
3.	Curd	3.71	3.71	3.68	3.70±0.014	78.72
4.	Yakult	2.25	2.20	2.25	2.23±0.023	26.89
5.	Pickle	3.31	3.25	3.31	3.29±0.028	149.54
6.	Unfermented camel milk	2.70	2.68	2.70	2.69±0.009	29.88
7.	Fermented fish	1.48	1.42	1.48	1.46±0.028	14.6
8.	PC 1	1.083	1.041	1.083	1.069±0.019	13.362
9.	PC 2	0.833	0.958	0.833	0.874±0.058	10.925

PC1: Pseudomonas Lipase; PC 2: Pancreatic porcine lipase (Positive control)

Table 4. Acid and pepsin resistance

Source	Bacteria identified	Absorbance at 600 nm			
		0 hr	1 hr	2hrs	3hrs
Pickle	*Lactobacillus helveticus*	0.983	0.983	0.982	0.981
Curd	*Lactobacillus plantarum*	0.246	0.230	0.200	0.187
Unfermented camel milk	*Lactobacillus plantarum*	0.866	0.866	0.865	0.866
Dosa batter	*Pediococcus pentosacceos*	0.912	0.911	0.910	0.911
Fermented fish	*Bacillus subtilis*	0.253	0.233	0.200	0.098
Fermented camel milk	*Bacillus spp*	0.269	0.250	0.216	0.116
Yakult (Known probiotic drink)	*Lactobacillus casei*	0.998	0.998	0.997	0.997

Table 5. Bile and pancreatin tolerance

Source	Bacteria identified	Absorbance at 600 nm			
		0 hr	1 hr	2 hrs	3 hrs
Pickle	*Lactobacillus helveticus*	1.650	1.650	1.649	1.649
Curd	*Lactobacillus plantarum*	1.020	1.019	0.997	0.910
Unfermented camel milk	*Lactobacillus plantarum*	1.168	1.168	1.165	1.165
Dosa batter	*Pediococcus pentosacceos*	1.390	1.389	1.388	1.388
Fermented fish	*Bacillus subtilis*	1.011	0.944	0.943	0.939
Fermented camel milk	*Bacillus spp*	0.967	0.965	0.955	0.933
Yakult (Known probiotic drink)	*Lactobacillus casei*	0.960	0.954	0.950	0.911

Table 6. Antimicrobial activity of lactic acid bacterial isolates

Source	Bacteria identified	*Pseudomonas*	*E. coli*	*Bacillus*	*Proteus*	*Staphylococcus*
Pickle	*Lactobacillus helveticus*	+	+	+	+	+
Curd	*Lactobacillus plantarum*	–	–	+	–	–
Unfermented camel milk	*Lactobacillus plantarum*	–	–	–	+	–
Dosa batter	*Pediococcus pentosacceos*	+	–	–	+	+
Fermented fish	*Bacillus subtilis*	–	–	+	+	–
Fermented camel milk	*Bacillus spp*	+	–	–	+	–
Yakult (Known probiotic drink)	*Lactobacillus casei*	–	+	–	–	–

Table 7. Antioxidant properties of LAB isolates

Source	Bacteria identified	Fe^{2+} µM/100mL
Pickle	*Lactobacillus helveticus*	380
Curd	*Lactobacillus Plantarum*	320
Unfermented camel milk	*Lactobacillus plantarum*	310
Dosa batter	*Pediococcus pentosacceos*	375
Fermented fish	*Bacillus subtilis*	322
Fermented camel milk	*Bacillus spp*	360
Yakult (Known probiotic drink)	*Lactobacillus casei*	372

Results suggested the presence of lipid degrading principle in all the isolates purified from dairy and fermented food samples. However, optimum lipolytic activity varies according to sample from which isolates were purified. The activity might also be strain specific and depend on inherent factors of isolates. Probiotic microorganisms have properties to

improve gastrointestinal health and enhancement of immunity. These are also known to fight well against several diseases. Lactic acid bacteria possess lipolytic activity can help to reduce serum triglyceride level and reduce the risk of heart disease.

Furthermore, all the lactic acid bacterial isolates checked for the presence of probiotic properties. All the isolates showed acid and bile acid tolerance and give antioxidant properties, however, it varies according to sample and isolates purified from the same. The probiotic microorganism must have properties to withstand the environment to which they exposed in gastrointestinal tracts i.e. gastric pH and pepsin, the presence of bile salt, microbial flora of intestine and oxidative stress etc.

Reports are available on the study of probiotic properties in lactic acid bacteria. Turpin et al. [15] studied that *in vitro* tests showed that only a limited set of isolates, mainly those belonging to L. fermentum, could tolerate a low pH and high frequency of tolerance to bile salts observed. Hassanzadazar et al. [16] studied the antibacterial, acid and bile tolerance properties of *Lactobacilli* isolated from Koozeh cheese. Maragkoudakis et al. [17] reported the probiotic potential of *L. casei* Shirota ACA-DC 6002, *L. Plantarum* ACA-DC 146 and *L. paracasei* subsp. *tolerance* ACA-DC 4037strains isolated from dairy products. Jamal et al. [18] studied the probiotic potential of *Lactobacillus* strains isolated from known popular traditional Moroccan dairy products.

After clinical trials, these isolates can be used to enrich camel milk and fermented food or can be used to develop a fortified formulation which can be helpful for peoples suffering from high serum lipid level and their consequences.

5. CONCLUSION

Lipolytic *Lactobacillus* bacteria isolated from dairy and fermented food products can be used as probiotics. Most of the isolates showed good probiotic potential hence further quality assurance and subsequent clinical trials will be helpful to develop probiotic formulation confers several health benefits.

ACKNOWLEDGEMENT

The authors acknowledge Department of Health Research and ICMR for providing a fund for this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24497