



## Research Paper

---

# Probiotic properties of lipolytic lactic acid bacteria isolated from fermented food and dairy products

Accepted 26<sup>th</sup> February, 2018

### ABSTRACT

Functional foods are very good and healthy means to improve health status of humans. Food enriched with probiotic lipolytic bacteria can be a good means to reduce serum lipid level and reduce the risk associated with enhanced level of serum lipid. The present study deals with the isolation and probiotic characterization of lactic acid bacteria purified from fermented food and dairy products. Among the 7 isolates, 6 were purified from dairy and fermented food samples whereas 1 isolate was used as standard purified from yakult (known as probiotic drink). Isolates were checked for presence of lipid degrading principle and maximum activity was observed in *Lactobacillus helveticus* purified from pickle (a fermented food). Probiotic characterization profiling suggested that *L. helveticus* (pickle), *Lactobacillus plantarum* (unfermented camel milk) and *Pediococcus pentosaceus* (Dosa batter) showed good potential of probiotic as compared to other isolates. 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.

Madhu Rathore\* and Kanika Sharma

Department of Biotechnology, Mohanlal Sukhadia University, Udaipur 313001.

\*Corresponding author. E-mail: madhurathore25@gmail.com.

**Keywords:** Functional foods, health status, probiotic, lipolytic, fermented food, dairy food, probiotic formulation.

---

### INTRODUCTION

Probiotics are live micro-organisms that have health benefits when consumed. Several probiotic items are available in market. These products are very helpful in building up immunity as well as, digestive health, reduce depression and promote heart health (Rathore and Sharma, 2017). 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 micro-organisms 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* (Quigley et al., 2011).

The present study deals with isolation of lipolytic Lactobacillus bacteria from fermented food and dairy samples. Prior to develop probiotic formulation, isolates need to be checked for probiotic properties especially pH resistance, bile salt tolerance, antimicrobial activity and antioxidant activity. Probiotic micro-organisms can be screened from non-intestinal sources, such as fermented food (Mahasneh et al., 2010), fruit juices (Naeem et al., 2012), grains (Hamet et al., 2013), honey-comb (Tajabadi et al., 2013) and soil (Chen et al., 2005). 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 (Naeem et al., 2012). Literature suggested the presence of lipolytic lactic acid bacteria from camel milk and other dairy sources (Rathore et al., 2009; Gasmala et al., 2017).

*Lactobacilli* from these samples are 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 consequent effect on other organs.

## MATERIALS AND METHODS

### 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. Commercialized products positively containing 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 determinative biology (Haynes and Burkholder, 1957).

#### DNA extraction

For DNA extraction, single colonies were resuspended in 50  $\mu$ L of sterile deionized water. Next, 50  $\mu$ L 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  $\mu$ L of the upper aqueous phase was used as a source of DNA template for the PCR reaction.

#### 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: cttgtcgggcccctcaattc. The Polymerase Chain Reaction (PCR) was carried out by mixing 5  $\mu$ L of each extracted DNA with 25  $\mu$ L of 2X PCR Master kit (composition of 1X solution: 0.5 M Tris-HCl, 1.5 mM MgCl<sub>2</sub> – 200  $\mu$ M dATP, 200  $\mu$ M dCTP, 200  $\mu$ M dGTP and 200  $\mu$ M dTTP and 0.04 Units/ $\mu$ L Taq), 1  $\mu$ L Oligo forward (10 picomole/ $\mu$ L), 1  $\mu$ L Oligo

reverse (10 picomole/ $\mu$ L) and 18  $\mu$ L Sterile deionized water. The amplification was achieved by 40 PCR cycles. The Amplified product were examined using 1.5% (w/v) agarose gels in 0.5X Tris/Borate/Ethylenediaminetetraacetic acid (TBE) buffer at 75 V for 90 min with a DNA ladder. Product thus obtained was 875 bp.

### Detection of lipolytic activity

Purified isolates were checked for significant lipolytic activity using Agar spot method on selective media, that is, 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.

#### 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 (pH 1.0 and pH 3.0), pepsin (3 mg/ml, pH 2.0), pancreatin (1 mg/ml, pH 8.0) and bile salts (0.3% w/v Ox Gall) was measured. The reagents for these tests were obtained from HI-Media. Intrinsic antimicrobial activity of cell free extract from selected LAB isolates against *Escherichia 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.

## RESULTS

Several health problems like heart disorder, chronic kidney disease, gastro-intestinal problems and weak immunity etc can be fatal for human life. One of the reasons of these problems is food habits and sedentary life styles. Drug abuse is also a reason associated with kidney failure and consequent multiple organ disorder. There is need to develop functional food that not only meet nutritional requirement but also improve overall health status of humans.

Dairy samples and fermented food samples were incorporated to screening process to check the presence of

**Table 1:** Identified lipolytic lactic acid bacterial isolates.

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

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

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

**Table 3:** Quantitative estimation of lipolytic activity by titrimetric method.

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

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

lipolytic lactobacillus bacteria. On biotyping using analytical grade chemicals and API strep (Biomarius) and sequencing of 16S rDNA/D1/D2 domain of LSU rDNA or ITS region and BLAST analysis lipolytic bacterial isolates were identified as *L. plantarum*, *Bacillus spp*, *Pentococcus pentosaceos*, *B. subtilis* and *L. helveticus* (90 to 99% similarities) purified from Unfermented camel milk, fermented camel milk, curd, batter, 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 catalase negative reaction. These isolates were also checked for the existence of lipolytic activity. Maximum lipolytic activity was

observed for *L. helveticus* (pickle), that is, 149.54 activity/mg (Tables 1, 2 and 3).

Results of 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; *L. casei*), maximum acid tolerance ability was observed in *Lactobacillus helveticus* followed by *Pediococcus pentosaceos* and *L. plantarum*. As compared to control (Yakult; *L. casei*), Maximum bile salt tolerance ability was observed in *L. plantarum* followed by *L. helveticus* and *P. pentosaceos*. Results of intrinsic antimicrobial activity of cell free extract from selected LAB

**Table 4:** Acid and pepsin resistance.

| Source                            | Bacteria identified             | Absorbance at 600 nm |       |       |       |
|-----------------------------------|---------------------------------|----------------------|-------|-------|-------|
|                                   |                                 | 0                    | 1     | 2     | 3     |
|                                   |                                 | <b>h</b>             |       |       |       |
| Pickle                            | <i>Lactobacillus helveticus</i> | 0.983                | 0.983 | 0.982 | 0.981 |
| Curd                              | <i>Lactobacillus plantarum</i>  | 0.246                | 0.230 | 0.200 | 0.187 |
| Unfermented camel milk            | <i>Lactobacillus plantarum</i>  | 0.866                | 0.866 | 0.865 | 0.866 |
| Dosa batter                       | <i>Pediococcus pentosaceos</i>  | 0.912                | 0.911 | 0.910 | 0.911 |
| Fermented fish                    | <i>Bacillus subtilis</i>        | 0.253                | 0.233 | 0.200 | 0.098 |
| Fermented camel milk              | <i>Bacillus spp</i>             | 0.269                | 0.250 | 0.216 | 0.116 |
| Yakult (Known as probiotic drink) | <i>Lactobacillus casei</i>      | 0.998                | 0.998 | 0.997 | 0.997 |

**Table 5:** Bile and pancreatin tolerance.

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

isolates against *E. coli*, *Pseudomonas aeruginosa*, *B. subtilis*, *P. vulgaris* and *S. aureus* were determined by agar well method. As compared to control (Yakult (Known probiotic drink; *L. casei*)) *L. helveticus* inhibit the growth of all test pathogenic bacteria whereas *P. pentosaceos* was shown to inhibit growth of three test pathogens, that is, *P. aeruginosa*, *P. vulgaris* and *S. aureus*. Results of antioxidant activity suggested that as compared to control (Yakult (Known probiotic drink; *L. casei*)), Maximum Fe<sup>2+</sup> µM/L observed in *L. helveticus* was followed by *P. pentosaceos* (Tables 4, 5, 6 and 7).

## 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 (Mattila-Sandholm et al., 2002). Probiotics micro-organisms of the genera *Lactobacillus*, *Bifidobacterium* and *Saccharomyces*, these isolates not only find place in fermented dairy products and infant formula but also in pharmaceutical preparations have been recognized for their "generally recognized as safe (GRAS)" status (Salminen et al., 1998).

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. Activity might also be strain specific and depend on inherent factors of isolates. Probiotic micro-organisms have properties to improve gastro-intestinal health and enhancement of immunity. These are also known to fight against several diseases. Lactic acid bacteria possess lipolytic activity that can help to reduce serum triglyceride level and reduce the risk of heart disease.

Furthermore, all the lactic acid bacterial isolates were 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 purified sample and isolates. Probiotic micro-organism must have properties to withstand the environment to which they are exposed in gastro-intestinal tracts, that is, gastric pH and pepsin, presence of bile salt, microbial flora of intestine and oxidative stress etc.

Reports are available on study of probiotic properties in lactic acid bacteria. Turpin et al. (2011) 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. (2012) studied the antibacterial, acid and bile tolerance properties of *Lactobacilli* isolated from Koozeh cheese.

**Table 6:** Antimicrobial activity of lactic acid bacterial isolates.

| Source                         | Bacteria identified             | <i>Pseudomonas</i> | <i>E. coli</i> | <i>Bacillus</i> | <i>Proteus</i> | <i>Staphylococcus</i> |
|--------------------------------|---------------------------------|--------------------|----------------|-----------------|----------------|-----------------------|
| Pickle                         | <i>Lactobacillus helveticus</i> | +                  | +              | +               | +              | +                     |
| Curd                           | <i>Lactobacillus plantarum</i>  | -                  | -              | +               | -              | -                     |
| Unfermented camel milk         | <i>Lactobacillus plantarum</i>  | -                  | -              | -               | +              | -                     |
| Dosa batter                    | <i>Pediococcus pentosaceus</i>  | +                  | -              | -               | +              | +                     |
| Fermented fish                 | <i>Bacillus subtilis</i>        | -                  | -              | +               | +              | -                     |
| Fermented camel milk           | <i>Bacillus spp</i>             | +                  | -              | -               | +              | -                     |
| Yakult (Known probiotic drink) | <i>Lactobacillus casei</i>      | -                  | +              | -               | -              | -                     |

**Table 7:** Antioxidant properties of LAB isolates.

| Source                            | Bacteria identified             | Fe <sup>2+</sup> µM/100 ml |
|-----------------------------------|---------------------------------|----------------------------|
| Pickle                            | <i>Lactobacillus helveticus</i> | 380                        |
| Curd                              | <i>Lactobacillus plantarum</i>  | 320                        |
| Unfermented camel milk            | <i>Lactobacillus plantarum</i>  | 310                        |
| Dosa batter                       | <i>Pediococcus pentosaceus</i>  | 375                        |
| Fermented fish                    | <i>Bacillus subtilis</i>        | 322                        |
| Fermented camel milk              | <i>Bacillus spp</i>             | 360                        |
| Yakult (Known as probiotic drink) | <i>Lactobacillus casei</i>      | 372                        |

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

## REFERENCES

- Chen YS, Yanagida F, Shinohara T (2005). Isolation and identification of lactic acid bacteria from soil using an enrichment procedure. *Lett. Appl. Microbiol.* 40(3): 195-200.
- Gasmala MAA, Teesema HA, Salaheldin A, Kamal-Alahmad, Hassanin HAM, Aboshora W (2017). Health benefits of milk and functional dairy products. *MOJ Food Process Technol.* 4(4): 00099.
- Haynes WH, Burkholder GI (1957). *Pseudomonas*. In *Bergey's manual of determinative bacteriology*, R. S. Breed, E.G.D. Murray, N.R. Smith (Eds., The Williams & Wilkins Co. Baltimore, Md), pp. 89-152.
- Hamet MF, Londero A, Medrano M, Vercammen E, Van Hoorde K, Garrote GL, Huys G, Vandamme P, Abraham AG (2013). Application of culture-dependent and culture-independent methods for the identification of *Lactobacillus kefirifaciens* in microbial consortia present in kefir grains. *Food Microbiol.* 36(2): 327-334.
- Hassanzadazar H, Ehsani A, Mardani K, Hesari J (2012). Investigation of antibacterial, acid and bile tolerance properties of lactobacilli isolated from Koozeh cheese. *Summer.* 3(3): 181-185.
- Mahasneh AM, Hamdan S, Mahasneh SA (2010). Probiotic properties of *Lactobacillus* species isolated from local traditional fermented products. *Jordan J. Biol. Sci.* 8(2): 81-87.
- Mattila-Sandholm T, Myllarinen P, Crittenden R, Mogensen G, Fonden R, Saarela M (2002). Technological challenges for future probiotic foods. *Int. Dairy J.* 12(2-3): 173-183.
- Naeem M, Ilyas M, Haider S, Baig S, Saleem M (2012). Isolation characterization and identification of lactic acid bacteria from fruit juices and their efficacy against antibiotics. *Pak. J. Bot.* 44: 323-328.

- Quigley L, O'Sullivan O, Beresford TP, Ross RP, Fitzgerald GF, Cotter PD (2011). Molecular approaches to analyzing the microbial composition of raw milk and raw milk cheese. *Int. J. Food Microbiol.* 150(2-3): 81-94.
- Rathore M, Sharma K (2017). Lipolytic *Lactobacillus* Species from Camel Milk. *Microbiol. Res. J. Int.* 22(1): 1-5.
- Rathore M, Sharma N, Sharma A, Kumari J, Sharma K. (2009). Camel milk: A potential source of therapeutic lipolytic lactic acid bacteria. *Biosci. Biotechnol. Res. Asia.* 6(1): 251-256.
- Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM, Fondén R, Saxelin M, Collins K, Mogensen G, Birkeland SE, Mattila-Sandholm T (1998). Demonstration of safety of probiotics - a review. *Int. J. Food Microbiol.* 44(1-2): 93-106.
- Tajabadi N, Mardan M, Manap MYA, Mustafa S (2013). Molecular identification of *Lactobacillus* spp. isolated from the honey comb of the honey bee (*Apis dorsata*) by 16S rRNA gene sequencing. *J. Apic Res.* 52(3): 235-241.
- Turpin W, Humblot C, Guyot J (2011). Genetic Screening of Functional Properties of Lactic Acid Bacteria in a Fermented Pearl Millet Slurry and in the Metagenome of Fermented Starchy Foods. *Appl. Environ. Microbiol.* 77(24): 8722-8734.

### Cite this article as:

Rathore M and Sharma K (2018). Probiotic properties of lipolytic lactic acid bacteria isolated from fermented food and dairy products. *Acad. J. Biotechnol.* 6(1): 016-020.

### Submit your manuscript at

<http://www.academiapublishing.org/ajb>