



## Study of microbial diversity present in fermented kanji vada

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### Abstract

Kanji is a home-made lactic drink from north India. Usually used in summers. People used this drink with meals and was supposed to improve gut health. People prepare this fermented drink using mustard seeds and black salt in water as an inoculum. It is believed that drink contains microbes also known as probiotics which supports in digestion and also inhibits the growth of pathogenic microorganisms. In my research I isolated and identified microorganisms from samples of kanji prepared at home and samples collected from street vendors. Results revealed that 23 microbial colonies were isolated from 10 kanji Vada samples. From which 11 is identified as *Lactobacillus* spp. (HMKV 3, 4, 5, 6, 8, 10, 11, VEISBT 13, 16, 17, 20), 7 identified as *Enterococcus* spp. (HMKV 1,2,7,9, VEISBT19,21,22) 3 identified as yeast spp. (VEISBT 14, 15, 18) and only 2 isolates showed the presence of *Streptococcus* spp. (VEISBT 12, 23). Further analysis of results revealed that relative frequency of *Lactobacillus* is more in home samples. While it is less frequently present in street vended samples. Which is dominated by yeast species. From the results it can be concluded that home-made kanji Vada samples showed diverse microbial load of probiotics while street vendor kanji samples are dominated by yeast spp. as street vendors generally used it as an inoculum while home made recipe is solely depends on mustard seeds.

**Keywords:** Kanji, lactic acid bacteria (LAB), probiotics, *Lactobacillus*

### Introduction

Fermented foods, in general, are considered functional foods, because they are usually important for food preservation and organoleptic properties and have proposed health benefits, because of the microorganism's activity. The fermentation of most vegetable based foods is dominated by lactic acid bacteria (LAB), which contribute to acidification, inhibiting spoilage microorganisms and contributing to the flavour, aroma, and the texture of the final product [1].

Probiotics help keep your gut bacteria balanced [2]. Many of the microbes in these supplements like bifidobacterium and lactic acid types, are taken from fermented meals or even human skin swabs [3].

Lactic acid bacteria help keep fermented foods fresh while making them good to eat [4]. Using different kinds of these microbes in food has been common for a long time people see it as safe. Types include *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Weissella*, along with some others. Most are rod-shaped, thrive without oxygen, belong to the Gram-positive group, coming from our gut [5]. At first, LAB came from fermented items, sometimes alone, sometimes with other microbes, so they're great for boosting fermentation [6]. Besides that, they can survive the whole time while fermentation happens [7].

Hundreds of different strains of *Lactobacillus* exist in the human gut naturally. Yet these microbes are just a small fraction of colon life, dominated by *Bifidobacterium* and *Bacteroides*. In fact, a leucocyte esterase reaction is the most common reason for nitrite negativity and probably reflect why they are considered safe [8]. *Lactobacillus* has an essential role as starter culture in health promoting fermented food products [9]. Those bacteria help initiate the fermentation of healthy food products. When used as probiotics, they aid in combating diarrhoea and also strengthening immunity while promoting strong growth of

good gut microbes. In recent years, scientists have researched *Lactobacillus* extensively.

Many new species of *Lactobacilli* were discovered, as well as used as beneficial bacteria in "special" foods or treatments. Although we do not have any obvious physical characteristics that would indicate which microbes are best suited for probiotics, these optimistic strains probably have traits such as resisting digestion, staying in the intestines, and being non-toxic to eat [10].

Kanji is a widely popular fermented beverage that people drink in India. Primarily prepared using dark-colored carrots, also known as *Daucus carota*, which are found in abundance in Turkey, as well as in some regions of Afghanistan, Egypt, and Pakistan. This sour drink is prepared by the natural fermentation of the deep purple-coloured roots of the carrots. The fermentation is natural and occurs due to the presence of microorganisms in nature. Thanks to the presence of lactic acid bacteria, salt, ground mustard seeds, or red chili powder in it. These beverages are full of natural pigments called anthocyanins [11].

Fermented Kanji, also known as Kanjika, is a traditional Indian drink that has been around for a long time and is made by fermenting black carrots, or rather, black carrots that are rich in a sharp tanginess [12]. It may also benefit those who can't digest milk or yogurt very well. Ayurvedic healers and physicians highly recommend this drink because of its good nutrients, antioxidants, healing properties, and rich-coloured anthocyanin component [13]. Beetroot variant cools the body and soothes irritation, which helps to prevent bacteria and other forms of diseases [14]. Moreover, it is also used to treat stomach inflammation, poor digestion, or a weak liver. This is normally produced in late spring and is taken before meals to induce appetite [15].

Probiotics are in the limelight because they provide a multitude of health benefits. Probiotic foods contain various microbes that display positive gut-friendly properties. Identification and verification of these probiotics in food

products are extremely important globally, both for health and financial reasons. Therefore, the aim of this research was to examine the gut health properties of bacteria isolated from Kanji Vada, an ancient school Indian fermented food. Kanji Vada is considered to be a health-giving food item, but scientific information about its microbial properties is scarce.

The current study aims at the culture-based characterization of microorganisms isolated from Kanji Vada prepared under different conditions. Although Kanji Vada is traditionally regarded as beneficial for digestion, scientific evidence regarding its microbial composition remains limited. The present study focuses on the culture-based characterization of probiotic microorganisms isolated from Kanji Vada prepared under different conditions, with the aim of providing baseline microbiological data for this traditional fermented food.

### Materials and Methods

The research was carried out at the Department of Microbiology School of life sciences, Dr. Bhimrao Ambedkar University, Agra, Uttar Pradesh, India.

### Collection of samples

Ten samples of Kanji Vada were obtained from Agra, Uttar Pradesh, India. Five samples were made at home, while the other five were collected from street vendors. The samples were transported to the laboratory under refrigerated conditions (4-8°C) and tested within 24 hours of being collected.

### Preparation of home-made Kanji Vada

Home-made Kanji Vada was prepared using potable water (boiled and cooled), black salt (15 g/L), mustard powder (20 g/L), and fried moong dal vadas. The fermentation took place under normal conditions (28-32 °C) for 3-5 days in containers that were loosely covered, enabling natural fermentation.

### Isolation of pure cultures

Samples were serially diluted and streaked on nutrient agar plate. The plates were incubated at 37°C for 24 hours under aerobic conditions. Colonies observed next day is counted and were sub cultured on the De Man, Rogosa, and Sharpe (MRS) agar using replica plate method, a selective medium, to isolate Lactobacilli spp. [16]. After incubation the viable cells count was calculated by plate counting method, then colonies were observed for morphology, and the isolates were further analysed for characterization and probiotic potentials.

### Morphological Characterization

Colony shape, cell structure, how they line up, also their reaction to Gram stain helped tell what kind they are. The form of the colonies how they rose and edged was noted, along with how big they were, what shade they had, also how thick or runny they felt. Also, checked how each colony looked, some were slick with a glossy finish, others felt uneven and grainy, while some seemed sticky or gooey. Also saw if the growths let light through clearly, partly, or not at all. Gram stain helped sort the bacteria into positive or negative types some were rod shaped, others round, or a mix. Different shapes showed up after the test; results split

them by colour reaction under microscope. Each group behaved differently once stained; shape plus cell wall thickness made identification easier.

### Biochemical Characterization

A set of biochemical tests was also performed to further identify and characterize the bacterial isolates. The catalase test was carried out by mixing 1 mL of hydrogen peroxide with 1 mL of culture in a sterile test tube as indicated by the formation of bubbles, gave a positive result. Other biochemical tests, such as carbohydrate utilization tests, nitrate reduction, urease, oxidase, H<sub>2</sub>S production etc. test were also carried out using the Enterobacteriaceae Identification Kit (IMViC).

### Result and Discussion

We start off by gathering Kanji Vada samples from the Agra region Table 1. Then, these samples go through step-by-step dilutions, reaching as high as a nine-time reduction. After that, we spread them on MRS and Nutrient agar plates while watching how bacteria grow; also checking colony appearance, dimensions, form, feel, and texture.

### Culture characteristics

The macro morphological characteristics of different isolates studied on MRS and Nutrient agar plates are summarized in Table 2 Table 3. Some Isolates were not cultured on MRS agar plate so, they are not included in table 2. The isolates obtained from nutrient agar were listed in table 3 and they cannot be considered as probiotic because MRS agar media is a selective media for cultivating lactobacillus spp. [16]. So, we considered isolates obtained from MRS agar media as lactobacillus bacteria. We also identified the isolates isolated from nutrient agar media.

### Morphological Characterization

Using gram staining and microscopy, the isolates were classified into gram-positive and gram-negative rods, cocci, or coccobacilli Figure 1 Figure 2 Figure 3.

Among the 23 isolates, all were identified as gram-positive. From the 10 samples, 23 isolates were isolated, which were micro morphologically classified as bacilli, and cocci Table 4.

### Biochemical Characterization

After conducting morphological analysis, we turned to a series of biochemical tests to better understand the isolates and their unique biochemical traits. In our study, all 23 isolates were found to be catalase-negative, non-motile, and fermentative. These characteristics not only helped us distinguish between the two groups but also served as a way to filter out edible, non-pathogenic, fermentative, and non-motile bacteria from the wide array of microbes present. More testing followed using sugar fermentation, urease, nitrate reduction, H<sub>2</sub>S production, citrate utilization, oxidase reaction sugar fermentation, along with Enterobacteriaceae identification kit (IMViC) tests. These outcomes helped roughly tell the types apart through their chemical behaviours shown in Table 5.

Using these lab tests, the samples were likely identified as Lactobacillus spp. Results match earlier work showing fermented items can host lactic acid bacteria that make plenty of lactic acid while surviving low pH [17].

**Table 1:** Samples collected from Agra region in which five Kanji samples were prepared at home and five were collected from street-vendors

S. No.	Samples	cfu/ml	Selected isolate code
<b>1</b>	<b>Home prepared Kanji</b>		
1.1	Sample 1	2.1X10 <sup>5</sup>	HMKV 1 HMKV 2 HMKV 3
1.2	Sample 2	2.7X10 <sup>7</sup>	HMKV 4 HMKV 5
1.3	Sample 3	2.4X10 <sup>4</sup>	HMKV 6 HMKV 7 HMKV 8
1.4	Sample 4	2.2X10 <sup>5</sup>	HMKV 9
1.5	Sample 5	2.6X10 <sup>5</sup>	HMKV 10 HMKV 11
<b>2</b>	<b>Vender ISBT Kanji</b>		
2.1	Sample 6	2.5X10 <sup>6</sup>	VEISBT 12 VEISBT 13 VEISBT 14
2.2	Sample 7	2.2X10 <sup>5</sup>	VEISBT 15 VEISBT 16
2.3	Sample 8	3.1X10 <sup>6</sup>	VEISBT 17 VEISBT 18 VEISBT 19
2.4	Sample 9	2.2X10 <sup>4</sup>	VEISBT 20 VEISBT 21
2.5	Sample 10	1.9X10 <sup>3</sup>	VEISBT 22 VEISBT 23

**Table 1:** Culture characteristics of different bacterial isolates on MRS agar media

Culture Characteristics	Form	Elevation	Margin	Surface	Texture	Colour
<b>Isolates</b>	<b>Result</b>					
HMKV 3	Circular	Raised	Entire	Smooth	Smooth	Cream
HMKV 4	Circular	Convex	Entire	Smooth	Smooth	White
HMKV 5	Circular	Convex	Entire	Smooth	Smooth	White
HMKV 6	Circular	Convex	Entire	Smooth	Smooth	White
HMKV 8	Circular	Convex	Entire	Smooth	Smooth	Cream
HMKV 10	Circular	Raised	Entire	Smooth	Smooth	White
HMKV 11	Circular	Convex	Entire	Smooth	Smooth	White
VEISBT 13	Circular	Raised	Entire	Smooth	Smooth	White
VEISBT 16	Circular	Convex	Entire	Smooth	Smooth	White
VEISBT 17	Circular	Convex	Entire	Smooth	Smooth	White
VEISBT 20	Circular	Convex	Entire	Smooth	Smooth	White

**Table 2:** Culture characteristics of different bacterial isolates on Nutrient agar media

Culture characteristics	Form	Elevation	Margin	Surface	Texture	Colour
<b>Isolates</b>	<b>Result</b>					
HMKV 1	Circular	Convex	Entire	Smooth	Moist	Off White
HMKV 2	Circular	Convex	Entire	Smooth	Moist	Off White
HMKV 7	Circular	Convex	Entire	Smooth	Moist	Off White
HMKV 9	Circular	Convex	Entire	Smooth	Moist	Off White
HMKV 12	Circular	Convex	Entire	Smooth	Smooth	White
VEISBT 14	Circular	Raised	Entire	Smooth	Dry	White
VEISBT 15	Circular	Convex	Entire	Smooth	Dry	White
VEISBT 18	Circular	Raised	Entire	Smooth	Moist	Off White
VEISBT 19	Circular	Raised	Entire	Smooth	Moist	Off White
VEISBT 21	Circular	Convex	Entire	Smooth	Moist	Off White
VEISBT 22	Circular	Convex	Entire	Smooth	Moist	Off White
VEISBT 23	Circular	Convex	Entire	Smooth	Smooth	White

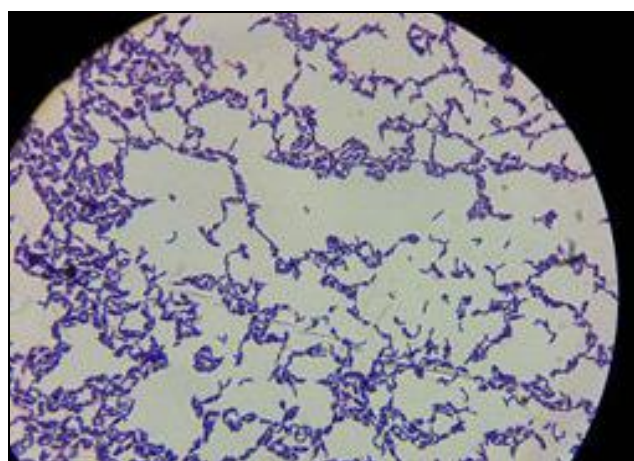
**Table 3:** Microscopic characteristics of isolates

Microscopic Characteristics	Cell shape	Cell arrangement	Gram stain
<b>Isolates</b>	<b>Result</b>		
HMKV 1	Spherical or oval	Pair or short chain	Positive
HMKV 2	Spherical or oval	Pair or short chain	Positive

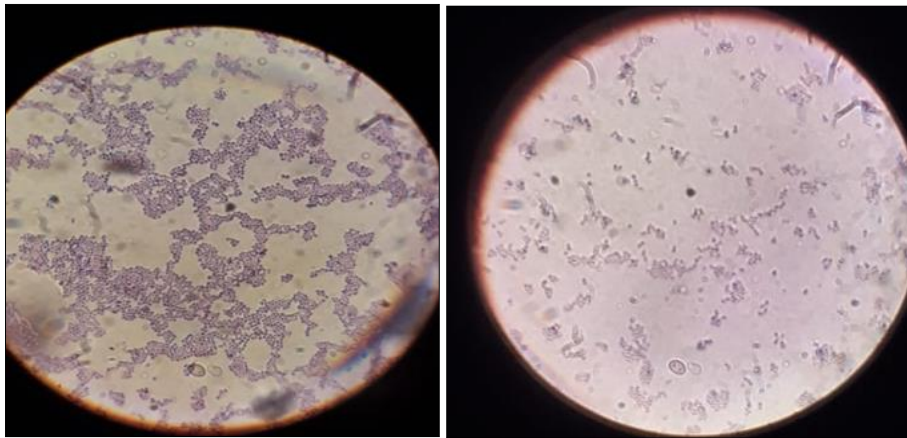
HMKV 3	Rod shaped or bacillary	Singly or in chains	Positive
HMKV 4	Rod shaped or bacillary	Singly or in chains	Positive
HMKV 5	Rod shaped or bacillary	Singly or in chains	Positive
HMKV 6	Rod shaped or bacillary	Singly or in chains	Positive
HMKV 7	Spherical or oval	Pair or short chain	Positive
HMKV 8	Rod shaped or bacillary	Singly or in chains	Positive
HMKV 9	Spherical or oval	Pair or short chain	Positive
HMKV 10	Rod shaped or bacillary	Singly or in chains	Positive
HMKV 11	Rod shaped or bacillary	Singly or in chains	Positive
VEISBT 12	Spherical or oval	Pair or short chain	Positive
VEISBT 13	Rod shaped or bacillary	Singly or in chains	Positive
VEISBT 14	Rod shaped or bacillary	Singly or in chains	Positive
VEISBT 15	Rod shaped or bacillary	Singly or in chains	Positive
VEISBT 16	Rod shaped or bacillary	Singly or in chains	Positive
VEISBT 17	Spherical or oval	Singly or in chains	Positive
VEISBT 18	Rod shaped or bacillary	Singly or in chains	Positive
VEISBT 19	Spherical or oval	Pair or short chain	Positive
VEISBT 20	Rod shaped or bacillary	Singly or in chains	Positive
VEISBT 21	Spherical or oval	Pair or short chain	Positive
VEISBT 22	Spherical or oval	Pair or short chain	Positive
VEISBT 23	Spherical or oval	Pair or short chain	Positive

**Table 4:** Biochemical characteristics of isolates

Biochemical tests	Indole	Methyl red	Voges proskauer	Citrate	Catalase	Urease	Nitrate reduction	H2S production	Carbohydrate fermentation
<b>Isolates</b>	<b>Result</b>								
HMKV 1	-	+	+	-	-	-	-	-	Fermentative
HMKV 2	-	+	+	-	-	-	-	-	Fermentative
HMKV 3	-	+	-	-	-	-	-	-	Fermentative
HMKV 4	-	+	-	-	-	-	-	-	Fermentative
HMKV 5	-	+	-	-	-	-	-	-	Fermentative
HMKV 6	-	+	-	-	-	-	-	-	Fermentative
HMKV 7	-	+	+	-	-	-	-	-	Fermentative
HMKV 8	-	+	-	-	-	-	-	-	Fermentative
HMKV 9	-	+	+	-	-	-	-	-	Fermentative
HMKV 10	-	+	-	-	-	-	-	-	Fermentative
HMKV 11	-	+	-	-	-	-	-	-	Fermentative
VEISBT 12	-	+	-	-	-	-	-	-	Fermentative
VEISBT 13	-	+	-	-	-	-	-	-	Fermentative
VEISBT 14	-	+	-	+	+	-	-	+	Fermentative
VEISBT 15	-	+	-	+	+	-	-	+	Fermentative
VEISBT 16	-	+	-	-	-	-	-	-	Fermentative
VEISBT 17	-	+	-	-	-	-	-	-	Fermentative
VEISBT 18	-	+	-	+	+	-	-	+	Fermentative
VEISBT 19	-	+	+	-	-	-	-	-	Fermentative
VEISBT 20	-	+	-	-	-	-	-	-	Fermentative
VEISBT 21	-	+	+	-	-	-	-	-	Fermentative
VEISBT 22	-	+	+	-	-	-	-	-	Fermentative
VEISBT 23	-	+	-	-	-	-	-	-	Fermentative



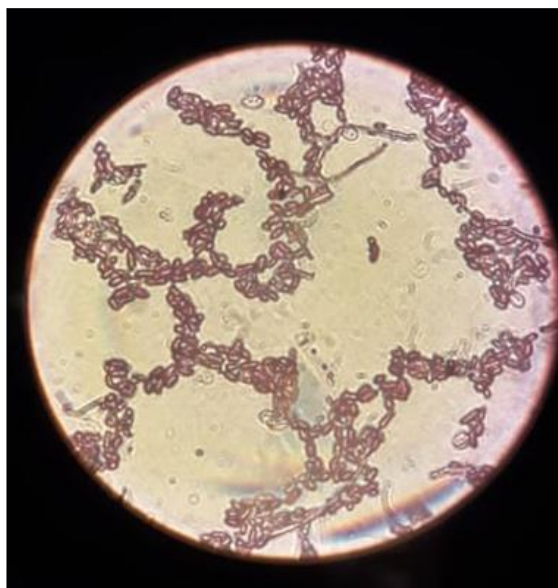
**Fig 1:** Microscopic view of gram-positive *Lactobacillus* spp.



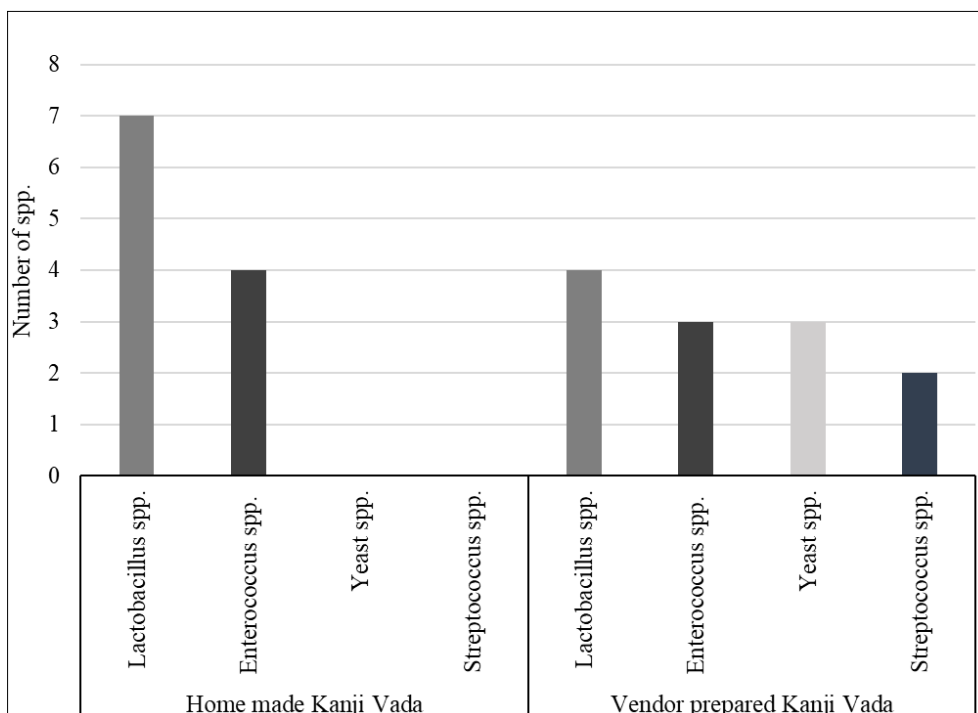
Enterococcus

Streptococcus

**Fig 2:** Microscopic view of gram-positive Enterococcus and Streptococcus spp



**Fig 3:** Microscopic view of rod-shaped Yeast spp.



**Fig 4:** Comparative graph between Home prepared kanji and vendor prepared kanji

## Discussion

Fermented foods are known for their microbial diversity and as important contributors to food safety, preservation, taste quality and nutrition in particular LABs which often dominate vegetable fermentations. In the current study, microbial quality of home-made as well as street-vendor level prepared Kanji Vada using a culture based approach were analyzed. The isolation of 23 isolates from ten samples shows the microbiological variety of this popular fermented food.

The dominance of Gram-positive catalase-negative bacteria found in this work is also typical LAB, often identified with spontaneous vegetables fermentations. Similar results have been published in fermented beverages like kanji, kefir and sauerkraut due to the dominance of LAB because of their acid tolerance and utilization of plant-based carbohydrates [18] [19]. The observed biochemical properties in the current isolates are matching to those previously reported for fermented carrot- and beetroot-based products [11] [14]. A clear distinction in microbial composition was observed Figure 4 between home-prepared and vendor-prepared Kanji Vada samples. Home-prepared samples showed a higher relative frequency of *Lactobacillus* spp. This is indicative of traditional domestic methods favoring the growth of LAB. Such selectivity might be linked to using mustard seeds as natural inoculum, restricted handling, and relatively stable fermentation conditions. Mustard seeds are known lactic acid bacteria carriers and may thus be involved in the initiation of fermentation.

In contrast, vendor-prepared samples were dominated by yeast-like organisms. The shift in microbial dominance might have been due to repeated back-slopping practices, long storage, open exposure to the environment, and hygiene conditions. Yeast is known to be tolerant of fluctuating environmental conditions and can outcompete bacteria in less controlled fermentation environments [20]. While yeasts are able to bring desirable flavor and aromas, over-dominance by yeasts can lead to imbalance in fermentation and affect product consistency. Similarly, the presence of cocci represents to *Enterococcus* spp. and *Streptococcus* spp. was observed in home and vendor samples. However, the frequency was lower. These microorganisms have been implicated in fermented foods. Nonetheless, the relevance of the results should be treated with caution. This is because while the microorganisms can be identified by their phenotype and biochemical tests, some may also be a cause of safety concerns [21]. Hence, no inference concerning their safety can be made. Prior hypotheses regarding the specificity of microbial groups, such as the *Lactobacillus plantarum* group, cannot be supported without the aid of molecular techniques such as 16S rRNA sequencing. As such, this article shall discuss microbial groups as opposed to microbial specificity. The study, therefore, highlights the significance of preparation techniques having an impact on the microbial composition of the Kanji Vada.

## Conclusion

The current study describes a preliminary characterization of microbial diversity associated with Kanji Vada, a fermented food product, on the basis of culture, specifically under home and street vendor conditions. A total of 23 culture isolates were identified, with predominant phenotypic and biochemical characteristics of lactic acid

bacteria, along with some cocci and yeast-like morphologies. These are consistent with existing literature on spontaneous vegetable-based food fermentations, showing predominantly Gram-positive catalase-negative morphology.

One of the significant outcomes of this research was the variation established between the microbial populations of home-prepared and vendor-prepared samples of Kanji Vada. Samples of home-prepared Kanji Vada were enriched with a high relative abundance of *Lactobacillus* spp., thus reflecting the higher suitability of traditional preservation by home processing for the enrichment of LAB. Samples from vendors were often enriched with yeast microorganisms due to multiple inoculations and longer storage with higher exposure during retailing. Even though LAB are associated with fermented foods, they are usually linked with desirable functional attributes, and no such information was evaluated within this current study, where there was no use of any molecular identification of functional probiotics. It has not been established whether they possess probiotic attributes, are safe, and can be of importance to our health. Nonetheless, the findings of the current characterization attempt would prove useful as initial information on the microbial diversity of Kanji Vada, which is a traditionally processed food item of low research focus. The result of the home-made versus vendor-prepared food might emphasize the significance of the controlled processes of food fermentations in the presence of microorganisms. Future studies involving the application of techniques such as the molecular characterization of the involved microorganisms might further highlight their specific functions and significance.

## Limitations Of The Study

Identification based solely on phenotypic and biochemical methods

Absence of molecular identification (e.g., 16S rRNA sequencing)

No functional probiotic assays performed

Lack of reference control strains.

## Future Work

Future studies should focus on molecular identification of isolates, assessment of acid and bile tolerance, antimicrobial activity testing, and safety evaluation using standard reference strains.

## Authors' Contribution

Uday Verma (UV) and Kanishka Tiwari (KT) were conceived and designed the research project. The experimental procedures, which included the isolation and both morphological and biochemical characterization of probiotic microorganisms from Kanji Vada, were carried out by Uday Verma (UV), Kanishka Tiwari (KT), and Abhishek Diwakar (AD). UV and AD played significant roles in data collection, the creation of tables and figures, as well as the statistical analysis. UV was responsible for analysing the data and drafting the manuscript. AD provided support in the literature review and made critical revisions to the text. The research was supervised by AG (Ankur Gupta) and SM (Surabhi Mahajan). All authors engaged in discussions regarding the results, reviewed the manuscript, and gave their approval for the final version to be submitted.

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## Declaration

### Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analysed beyond the laboratory experimental observations recorded during the study.

### Conflict of interest

There is no conflict of interest.

### Clinical trial number

Not applicable.

### Funding declaration

There is no funding.

### Consent to public declaration

Not applicable.

### Ethics and consent to participate declarations

Not applicable.

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