IJBPAS, October, 2025, 14(10): 5493-5510

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

# ISOLATION, CHARACTERIZATION, AND SCREENING OF *Lactobacillus* FROM VARIOUS SOURCES FOR PROBIOTIC ATTRIBUTES

**MAKADIYA DB[1] AND VISHWAKARMA NP[2*]**

**1:** Department of Microbiology, Atmiya University, Rajkot 360005, Gujarat, India

**2:** Department of Biotechnology, Atmiya University, Rajkot 360005, Gujarat, India

***Corresponding Author: Dr. Nutan Prakash Vishwakarma: E Mail:** nutan.vishwakarma@atmiyauni.ac.in

**Received 26th April 2024; Revised 29th Aug. 2024; Accepted 18th Oct. 2024; Available online 1st Oct. 2025**

https://doi.org/10.31032/IJBPAS/2025/14.10.9489

## ABSTRACT

The "friendly" bacteria Lactobacilli generally inhabit our gastrointestinal, urinary, and genital systems without causing any harmful diseases. In this research, *Lactobacillus* strains were isolated, characterized, and screened with potential probiotic attributes from sources like fruits & vegetables, dairy & non-dairy products, fermented foods, cow milk, pineapples waste, human breast milk, etc. Antimicrobial effect for both gram-positive & gram-negative pathogens was tested. The samples were collected from 10 different sources from the local areas of Rajkot, Gujarat, India. After growth on MRS agar medium, 42 bacterial isolates were obtained, and pure cultures were also obtained by subculturing them on the same MRS medium. The purity of the isolated Lactic acid bacteria was verified by morphological analysis, Gram staining, and further identified by some *Lactobacillus* specific biochemical tests. Considering the influence of pH, bile salt, and NaCl, isolated *Lactobacillus* strain exhibits tolerance at lower pH (2.0), bile salt (up to 4%) and NaCl (up to 6%). Finally, based on their phenotypic characteristics and molecular identification by 16S rDNA sequencing, two bacterial isolates were identified as *Lactobacillus* spp. The two isolated strains (*Limosilactobacillus fermentum* DBM2 and *Lactiplantibacillus plantarum* SDPW25) showed strong in vitro antibacterial activity along with potential probiotic properties. Therefore, the current study

represents the isolation of Lactobacilli from different sources, their proposed mechanism as probiotics, and the health benefits to human beings, along with their future aspects.

**Keywords:** ***Probiotics, Lactobacillus, isolation, screening, identification***

## 1. INTRODUCTION

Microorganisms play a vital role in the food industry, and among them, lactic acid bacteria (LAB) stand out as particularly significant. LAB is commonly employed as starter cultures in the production of a wide range of dairy products. The *Lactobacillus* genus, a subgroup of LAB, is characterized by its rod-shaped morphology, gram-positive nature, lack of spores, and non-pigmented appearance. *Lactobacillus* possesses several attributes that make it an ideal choice for various fermentation processes in the production of dairy products **[1]**. Firstly, it is catalase negative and thrives in microaerophilic to strictly anaerobic environments **[2]**. This feature contributes to its effectiveness in promoting fermentation. Furthermore, *Lactobacillus* is a member of the group of lactic acid bacteria extensively used in the manufacturing of fermented foods **[3]**. These LAB are generally recognized as safe (GRAS) organisms, allowing for their safe use in both veterinary and medical applications **[4]**.

According to FAO/WHO (2006), probiotics are live microorganisms that are not pathogenic and can provide health benefits to the host when administered in sufficient quantities **[5]**. The most prominent probiotics are members of the genus *Lactobacillus* and *Bifidobacterium* **[6]**. Probiotics are also known to improve nutritional absorption, reduce blood cholesterol level, manage lactose intolerance and reduce the need of antibiotics, along with other additional beneficial effects **[7]**. The use of probiotics in routine is considered beneficial for treating a number of clinical diseases, including inflammatory bowel disease, infantile diarrhea, and uro-genital infections **[8]**. It has been proposed that fruits and vegetables are the ideal medium for the growth of probiotics since they naturally contain vital nutrients, look good, and have a wonderful taste and flavor **[9,10]**. Considering the strain characteristics will help in the selection of new probiotics. The basic selection criteria for probiotics include a number of functional attributes, including phenol toxicity, bile tolerance, gastric acidity resistance, as well as the ability to de-conjugate bile salts **[11]**.

Traditional fermented foods are a rich source of live and active microbes, in fact, they are considered as a primary natural source of Lactic acid bacteria (LAB) **[12]**. Food born bacteria have the same ability as probiotics to survive in the GI tract and have a beneficial impact on the host. According to

some estimates, the consumption of fermented foods causes the ingestion of a large amount of live Lactic acid bacteria (approximately of $10^8$–$10^{11}$ CFU/d) **[13]**. Millets, legumes, fruits, and vegetables, as well as non -dairy fermented beverages, are commonly reported as beneficial probiotic-rich sources **[14]**. Various strains of *Pediococcus* spp. have been identified as potential probiotics and were isolated from different traditional fermented sources **[15]**. The most commonly available probiotic strains belong to the *Lactobacillus* spp., including *acidophilus*, *fermentum, gasseri, casei, johnsonii, reuteri, plantarum, paracasei, rhamnosus*, or *salivarius*. Additionally, the *Bifidobacterium* species, such as *bifidum, breve, adolescentis, longum*, or *animalis* are also commercially available probiotic strains **[16]**.

Numerous strains play a vital role in promoting gut health and overall well-being. Among these strains, *Leuconostoc mesenteroides* is commonly found in tomatoes, while *Lactobacillus Plantarum* has been detected in various types of fruit juices, including those from both solid and citrus fruits **[17]**. Probiotics can be isolated from the sources like pineapple waste using the same alternative growth medium utilized for producing lactic acid bacteria **[18]**. Various fruits and vegetables like dragon fruit, ginger, durian, papaya, star fruits, and guava can be examined to identify beneficial lactic acid bacteria (LAB) capable of producing antimicrobial substances to combat pathogenic bacteria **[19]**. Animal intestines are the most probable source of Lactic acid bacteria (LAB), other sources like fruit juice, flesh, long grass, and vegetables can also be tested for the presence of LAB **[20]**. Probiotic isolates, such as *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and *Staphylococcus arlettae*, exhibit inhibitory effects against pathogens like *S. aureus* and *Listeria monocytogenes* **[21]**.

Therefore, the aim of this present study was to isolate, identify and screen *lactobacillus* strain from diverse food products and healthy human. Tolerance of the isolates demonstrates the survivability in the small intestine and colon to contribute in the balance of the intestinal microbiota. Moreover, their antimicrobial activity against pathogens and antibiotic susceptibility was also studied to screen potential probiotic isolates.

## 2. MATERIALS AND METHODS

### *2.1 Isolation of a Lactobacillus strain*

*Sample Collection*

Various fresh and preserved food products like cow milk, butter milk, curd, lassi, fermented batter, seasonal fruits, and vegetables were screened for the isolation of Lactic acid bacteria (LAB) with potential probiotic properties. Samples were collected from Gujarat, India. Additionally, a unique

sample of human breast milk was obtained. All samples were immediately transported under aseptic conditions in a chilled container (5±1°C) to the Microbiology laboratory at Atmiya University and were maintained at the same temperature until sample preparation started.

*Enrichment and isolation of Lactic acid bacteria*

For culture enrichment method of lactobacilli, a quantity of one gram was taken from each sample and combined with 9 ml of MRS (De man Rogosa Sharp) broth medium **[22]** and incubated at 37°C for 48 hrs, maintaining a static condition. For isolation, the method used was slight modified as described by Jimenez *et al.,* 2008 **[23]**. Each enriched culture was serially diluted to seventh dilution, and 1.0 ml of the suitable dilutions were mixed with molten MRS agar tubes and poured in sterile petri plates. To analyze lactic acid bacteria from unconventional sources like fruits and vegetables, the samples were washed with regular water, followed by final rinse with sterile N-saline (0.85%). Following the culture enrichment method, the samples were diluted appropriately and mixed with molten MRS agar on MRS agar plates. The plates were kept in anaerobic conditions and incubated at 37°C for duration of 24- 48 hrs. After incubation, the colonies with different morphologies were selected randomly, picked up and purified by re-plating on MRS agar. The purified colonies were stored at 4°C for further characterization **[24]**.

***2.2 Identification of bacterial isolates***

*Gram staining and catalase test*

The isolated bacteria were initially identified as belonging to the genus Lactobacillus based on their morphological, cultural, and biochemical characteristics **[25, 26]**. The morphological and phenotypic attributes of the isolates were confirmed through microscopic examination following Gram staining, using 1% (v/v) activated culture and a compound microscope with 100x magnification **[27]**. To assess the presence of enzyme catalase and their tolerance to oxygen, catalase test was performed for all isolates using the slide method, and 3% hydrogen peroxide ($H_2O_2$) was used as a substrate for the reaction **[28]**. Among all, isolates that exhibited gram positive rod morphology with varying lengths, (short to long), and arranged individually, in pairs, or in chains and that tested catalase negative were considered as potential candidates belonging to genus *Lactobacillus.*

***2.3 Phenotypic characterization of lactobacilli***

*Gas production from glucose and Temperature assay*

Gas production was evaluated in order to categorize Lactobacillus into two group homofermentative and heterofermentative. MRS medium containing glucose was

placed with an inverted Durham's vial and inoculated with activated cultures of *lactobacilli.* Observations are made by looking the presence of $CO_2$ production (air bubbles) that appeared in the Durham's tube after incubation for 24 hrs at 37°C **[28]**. To perform the temperature assay, a 1% (v/v) fresh, overnight-grown culture of the isolate was inoculated into MRS broth. The inoculated broth was then incubated at different temperatures, specifically 15°C and 45°C, under anaerobic conditions to evaluate the growth response. This measurement provides its potential for diverse application, from food preservation to probiotic development as well as suitability for various environments and industrial uses **[29]**.

*Carbohydrate fermentation profile*

Sugar fermentation was done by inoculating 1% of an activated culture in sterile basal MRS medium (without glucose and beef extract) containing 1% Andrade's indicator (Himedia, India) and 1% sugar. Utilization of carbohydrate was determined after incubation at 37°C for 48 hrs. A positive reaction was recorded by the development of pink color, indicating acid production. Appropriate controls were also included.

***2.4 Screening of probiotic lactobacilli***

Isolates that were catalase-negative and gram-positive underwent additional screening to assess their tolerance to low pH levels (pH 2 and 3), varying bile salt concentrations (2% and 4%), and different NaCl concentrations (4% and 6%).

*Tolerance to acidic conditions, bile salt, and NaCl*

Tolerance to low pH, bile salt and NaCl were assessed using the method described by Islam M.A. *et al* 2018 **[30]** with minor modifications. A 1% (v/v) fresh overnight culture of *Lactobacillus* was inoculated into MRS broth medium with varying pH concentration (pH 2 & pH 3) bile salt (2% & 4%) concentrations and NaCl (4% & 6%) concentrations, to evaluate optical growth and tolerance. The inoculated broths were then incubated under anaerobic conditions for 24 hours at 37°C. After 24 hrs, 100 µl of bacterial culture from above concentrations were plated on MRS agar plates and incubated at 37°C for 48 hrs and observed for growth.

***Antimicrobial activity of Cell- Free Extract Filtrates of lactobacilli***

*Preparation of CFE filterate*

To prepare the cell-free extract (CFE) filtrate, the overnight grown and activated cultures of *Lactobacillus* were subjected to centrifugation at 10,000 rpm for 20 minutes at 4°C. The resulting supernatant of the culture was then filtered through a 0.45 µ Millipore filter to remove any remaining bacterial cells or debris. The sterile filtrate thus obtained was used as the cell-free extract (CFE).

*Antimicrobial activity by well-diffusion agar assay*

The agar diffusion method was performed to assess the antimicrobial effects of presumptive *Lactobacillus* strains against foodborne and gastrointestinal pathogenic organisms **[31]**, as mentioned in Table 4. On nutrient agar slant (Hi media, India) all the pathogens were maintained at 4°C. A loopful of culture from the slant was added to nutrient broth and subjected to incubation at 37°C for 18-24 hrs for activation. Briefly, 100 µl of 18 hrs old cultures of test pathogens were inoculated into molten nutrient agar and poured into a sterile petri plate, allowing it to solidify. Using a sterile cup borer, wells with a diameter of 7 mm were created on the plate, and 100 µl of CFE filterate was introduced into these wells. The plates were then pre-incubated at 4°C for 2 hours, followed by incubation at 37°C for 24 hrs. Subsequently, the zone of inhibition was measured in mm after a 24 hours incubation period. Acetate buffer with a pH of 4.5 and a concentration of 10mM was used as control.

***Antibiotic susceptibility profile***

Antibiotic discs (Hi Media) were used to determine the antibiotic susceptibility profile of isolated *Lactobacillus* strains. The methodology involved inoculating *Lactobacillus* cultures in MRS broth and incubating them at 37°C for 18-24 hrs to activate growth. A volume of 0.1 ml of the activated culture was then added to 25 ml of molten MRS agar (1.5%), which was subsequently poured into sterile petri plates. To assess antibiotic susceptibility, antibiotic discs were placed on the surface of MRS agar plates. The entire set up was then incubated for 24 hrs at 37°C **[32]**. Following the incubation period, the diameter of inhibition zone around each antibiotic disc was measured in millimetres (mm).

***2.5 Sequence analysis or Genome sequencing***

The PCR ampicon's DNA sequencing reaction was performed with the primer 1492R using the BDT v3.1 Cycle sequencing kit, targeting the 16s rRNA gene. The ABI 3730xl Genetic analyser was utilized for sequencing process. Subsequently, an obtained gene sequence was subjected to a BLAST search against the NCBI GenBank database. From the BLAST results, the first ten sequences with the highest identity scores were selected. These selected sequences were then aligned using multiple alignment software programs. This alignment allowed for a comprehensive comparison and analysis of the sequences, revealing common regions and variations.

**3. RESULTS**

***3.1 Isolation and identification of Lactic acid bacteria***

A total of 42 bacterial isolates were obtained from various sources and categorized based

on their isolation sources. The maximum number (8 isolates) was obtained from seasonal fruits, while the minimum number (2 isolates) was obtained from seasonal vegetables, fruit juice, and human breast milk **(Figure 1)**. Among all the bacteria isolated from different samples, only rod-shaped bacteria that are gram-positive and catalase-negative were further characterized. The isolates were tentatively recognized as Lactobacillus through the study of their colony morphology, as well as their physiological, cultural, and biochemical characterization. To examine colony traits, the growth patterns were examined on MRS medium. Colonies with attributes like creamy white appearance, circular shape, with raised elevation, and entire margin were considered indicative of the genus Lactobacillus. Colony attributes of the isolates are presented in **Table 1**.

### *3.2 Phenotypic characterization of lactobacilli*

Organisms are characterized into three groups namely 1) Thermobacteria, 2) Streptobacteria, and 3) Betabacteria based of their growth temperature. Looking at the results of the temperature assay, almost all isolates showed growth at 45°C, but only a few at 15°C. All the strains fermented Glucose, Lactose, Fructose, Galactose, Maltose and Ribose. Isolates exhibited diversity in their ability to ferment Mannitol, Mannose, and Arabinose. None of the strains fermented Sorbitol and Xylose **(Table 2)**. So, according to the sugar fermentation pattern and growth temperatures, the isolates were tentatively identified as *L. fermentum or L. plantarum* strains. The isolates DBM2 & SDPW25 were further characterized through the analysis by 16S rDNA sequencing.

### *3.3 screening of isolated lactobacillus strain*

**Table (3)** illustrates the growth of *Lactobacillus* strain in presence of low pH, Bile salt, and NaCl. Out of 42 isolates, isolate SDFK15, SDPW25, SDB21, SDL33, and DBM2 exhibited tolerance to all the prescribed conditions like lower pH (pH 2), bile salt (up to 4%) and NaCl (up to 6%). While not all isolates survived the harsh acidic environment of pH 2, a remarkable nine isolates demonstrated their potential for applications in acidic food preservation. Doubling the acidity to pH 3 further revealed 14 resilient isolates, showcasing their adaptability to diverse environments. Under varying salt and bile concentrations, all isolates tackled the lower bile concentration of 2% and higher salt concentration (6%), while only 18 demonstrated survivability against the higher bile 4% challenge, indicating their potential for probiotic use in overcoming the harsh gut environment.

### *3.4. Antimicrobial activity of cell - free Extract (CFE) filtrates of Lactobacilli*

The cell-free extract (CFE) filtrate of *Lactobacillus* strains grown in MRS medium demonstrated a broad antimicrobial spectrum, effectively inhibiting both Gram-positive and Gram-negative organisms (as shown in **Table 4**). However, the degree of activity varied among the different strains. The inhibition was particularly prominent against *B. Subtilis* and *B. megaterium*, followed by *B. cereus* and *S. aureus.*

### *3.5 Antibiotic susceptibility test*

Susceptibility to twelve different antibiotics was determined for *Lactobacillus* isolates. All the isolates were susceptible to Ampicillin, Cephalexin, Chloramphenicol, Ciprofloxacin, Cloxacillin, Co-trimoxazole, Erythromycin, Gentamicin, Lincomycin, Streptomycin, and Tetracycline. However, the same strains were not susceptible to Vancomycin **(Table 5)**.

### *3.6 Molecular identification of isolates*

Molecular identification of two isolates, DBM2 and SDPW25, with potential probiotic properties were carried out. The two strains were identified as *Limosilactobacillus fermentum* (DBM2) and *Lactiplantibacillus plantarum* (SDPW25) respectively, by 16S rDNA sequencing. The sequences that were identified were submitted to the National Centre for Biotechnology Information (NCBI). The Genbank accession numbers assigned to the sequences are OQ692404 for *Limosilactobacillus fermentum* DBM2 and OQ692416 for *Lactiplantibacillus plantarum* SDPW25. The phylogenetic tree of DBM2 **(Figure 2)** and SDPW25 **(Figure 3)** was constructed using BLAST.

**Table 1: Colony characteristics of the *Lactobacillus* strains growing on MRS**

| Parameters | Results |
|---|---|
| Size | Medium |
| Shape | Circular |
| Opacity | Opaque |
| Texture | Smooth |
| Margin | Entire |
| Elevation | Raised |
| Pigment | White – Creamy |

**Table 2: Phenotypic characteristics of *Lactobacillus* strains**

| Isolates | Glucose | Lactose | Fructose | Galactose | Maltose | Mannitol | Mannose | Sorbitol | Xylose | Ribose | Arabinose | Gas Production | Temperature (15ºC) | Temperature (45ºC) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SDFB1** | + | + | + | + | + | - | + | - | - | + | + | + | - | + |
| **SDFB2** | + | + | + | + | + | - | - | - | - | + | + | - | + | + |
| **SDFB3** | + | + | + | + | + | - | + | - | - | + | - | - | - | - |
| **SDFB4** | + | + | + | + | + | - | + | - | - | + | + | - | - | - |
| **SDFB5** | + | + | + | + | + | - | - | - | - | + | - | + | + | + |
| **SDFS8** | + | + | + | + | + | - | + | - | - | + | + | + | + | + |
| **SDFK15** | + | + | + | + | + | + | + | - | - | + | - | + | - | + |
| **SDFK16** | + | + | + | + | + | - | + | - | - | + | + | - | + | + |
| **SDAW19** | + | + | + | + | + | - | - | - | - | + | + | - | + | + |
| **SDAW20** | + | + | + | + | + | - | - | - | - | + | - | + | + | + |
| **SDOW24** | + | + | + | + | + | + | + | - | - | + | + | + | + | + |
| **SDPW25** | + | + | + | + | + | - | + | - | - | + | - | + | + | + |
| **SDAJ29** | + | + | + | + | + | - | - | - | - | + | + | - | + | + |
| **SDOJ31** | + | + | + | + | + | - | + | - | - | + | - | - | - | + |
| **SDVC2** | + | + | + | + | + | - | - | - | - | + | - | - | - | + |
| **SDVC3** | + | + | + | + | + | - | - | - | - | + | + | - | + | + |
| **SDM1** | + | + | + | + | + | - | + | - | - | + | + | - | + | + |
| **SDM3** | + | + | + | + | + | + | + | - | - | + | - | - | + | - |
| **SDM8** | + | + | + | + | + | - | + | - | - | + | + | + | - | + |
| **SDM12** | + | + | + | + | + | - | + | - | - | + | + | + | - | + |
| **SDM13** | + | + | + | + | + | - | + | - | - | + | - | - | + | + |
| **SDM19** | + | + | + | + | + | - | + | - | - | + | - | - | + | + |
| **SDM20** | + | + | + | + | + | - | + | - | - | + | + | - | - | + |
| **SDB1** | + | + | + | + | + | - | - | - | - | + | - | - | - | + |
| **SDB3** | + | + | + | + | + | - | + | - | - | + | + | + | + | + |
| **SDB4** | + | + | + | + | + | - | + | - | - | + | + | - | - | + |
| **SDB9** | + | + | + | + | + | - | + | - | - | + | + | + | + | + |
| **SDB11** | + | + | + | + | + | - | + | - | - | + | + | - | + | + |
| **SDC3** | + | + | + | + | + | - | + | - | - | + | - | + | - | - |
| **SDC7** | + | + | + | + | + | + | - | - | - | + | + | + | - | + |
| **SDC8** | + | + | + | + | + | - | + | - | - | + | + | - | - | + |
| **SDC11** | + | + | + | + | + | - | + | - | - | + | + | - | - | + |
| **SDB13** | + | + | + | + | + | - | + | - | - | + | - | - | - | + |
| **SDB17** | + | + | + | + | + | - | + | - | - | + | - | - | - | + |
| **SDB18** | + | + | + | + | + | - | + | - | - | + | + | + | + | + |
| **SDB21** | + | + | + | + | + | - | + | - | - | + | + | - | - | + |
| **SDL26** | + | + | + | + | + | + | + | - | - | + | - | - | - | + |
| **SDL29** | + | + | + | + | + | - | - | - | - | + | - | + | + | - |
| **SDL33** | + | + | + | + | + | + | + | - | - | + | + | + | + | + |
| **SDL34** | + | + | + | + | + | - | + | - | - | + | + | - | + | + |
| **DBM2** | + | + | + | + | + | - | - | - | - | + | - | - | - | + |
| **DBM3** | + | + | + | + | + | - | - | - | - | + | - | - | - | + |

**(+) fermenter of sugar, (-) non fermenter of sugar**

**Table 3: Screening of *Lactobacillus* strains for their probiotic properties (growth determination at lower pH and in presence of bile salt and NaCl**

| Isolates | pH | | Bile salt (%) | | NaCl (%) | | Isolates | pH | | Bile salt (%) | | Nacl (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 2 | 4 | 4 | 6 | | 2 | 3 | 2 | 4 | 4 | 6 |
| SDFB1 | - | - | + | - | + | + | SDM19 | - | - | + | - | + | + |
| SDFB2 | - | - | + | - | + | + | SDM20 | - | - | + | - | + | + |
| SDFB3 | - | - | + | - | + | + | SDB1 | - | - | + | - | + | + |
| SDFB4 | - | - | + | - | + | + | SDB3 | - | + | + | + | + | + |
| SDFB5 | + | + | + | - | + | + | SDB4 | - | + | + | + | + | + |
| SDFS8 | - | - | + | - | + | + | SDB9 | - | - | + | - | + | + |
| SDFK15 | + | + | + | + | + | + | SDB11 | - | - | + | - | + | + |
| SDFK16 | - | - | + | + | + | + | SDC3 | - | - | + | - | + | + |
| SDAW 19 | - | - | + | - | + | + | SDC7 | - | - | + | + | + | + |
| SDAW 20 | - | - | + | - | + | + | SDC8 | - | - | + | - | + | + |
| SDOW 24 | + | + | + | - | + | + | SDC11 | - | - | + | + | + | + |
| SDPW 25 | + | + | + | + | + | + | SDB13 | - | - | + | - | + | + |
| SDAJ29 | - | - | + | - | + | + | SDB17 | - | - | + | - | + | + |
| SDOJ31 | - | - | + | + | + | + | SDB18 | + | + | + | - | + | + |
| SDVC2 | - | - | + | + | + | + | SDB21 | + | + | + | + | + | + |
| SDVS3 | - | - | + | - | + | + | SDL26 | - | - | + | + | + | + |
| SDM1 | - | - | + | + | + | + | SDL29 | - | + | + | + | + | + |
| SDM3 | - | - | + | - | + | + | SDL33 | + | + | + | + | + | + |
| SDM8 | - | + | + | + | + | + | SDL34 | + | + | + | - | + | + |
| SDM12 | - | - | + | + | + | + | DBM2 | + | + | + | + | + | + |
| SDM13 | - | - | + | - | + | + | DBM3 | - | + | + | + | + | + |

**(+) Growth observed, (-) no growth**

**Table 4: Antimicrobial activity against some Gram - Positive food borne and Gram - Negative Gastrointestinal Tract Pathogens**

| | Zone of inhibition in (mm) Gram positive pathogens | | | | Zone of inhibition in (mm) Gram negative pathogens | | | |
|---|---|---|---|---|---|---|---|---|
| | *Bacillus Subtilis* | *Bacillus megateriu m* | *Bacillus Cereus* | *Staph. aureus* | *Escherichia Coli* | *Ent. Aerogenes* | *Pseudo. aeruginosa* | *Salmonella Typhi* |
| SDFB5 | 20 | 20 | 20 | 19 | 13 | 16 | 16 | 14 |
| SDFK15 | 16 | 16 | 15 | 16 | 15 | 13 | 14 | 14 |
| SDOW 24 | 14 | 14 | 14 | 14 | 14 | 13 | 15 | 15 |
| SDPW 25 | 18 | 15 | 18 | 16 | 14 | 14 | 15 | 15 |
| SDM8 | 16 | 16 | 17 | 16 | 16 | 14 | 14 | 13 |
| SDB3 | 17 | 16 | 15 | 17 | 14 | 13 | 14 | 14 |
| SDB4 | 17 | 16 | 16 | 16 | 15 | 14 | 15 | 14 |
| SDB18 | 18 | 19 | 18 | 18 | 15 | 14 | 15 | 15 |
| SDB21 | 17 | 18 | 18 | 16 | 15 | 13 | 14 | 16 |
| SDL29 | 19 | 17 | 19 | 17 | 13 | 13 | 16 | 16 |
| SDL33 | 19 | 18 | 16 | 17 | 14 | 13 | 16 | 15 |
| SDL34 | 18 | 18 | 14 | 17 | 17 | 16 | 16 | 15 |
| DBM2 | 16 | 17 | 17 | 18 | 15 | 15 | 16 | 14 |
| DBM3 | 17 | 16 | 16 | 18 | 14 | 15 | 15 | 15 |

*** Including 7 mm bore diameter**

**Table 5: Results of Antibiotic susceptibility testing of *Lactobacillus* strains**

| Antibiotics (µg) | Zone of inhibition (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | SDFK15 | SDPW25 | SDB3 | SDB21 | SDL29 | SDL33 | SDL34 | DBM2 | DBM3 |
| Ampicillin (20) | 24 | 25 | 20 | 20 | 23 | 25 | 20 | 22 | 25 |
| Cephalexin (30) | 20 | 22 | 15 | 20 | 20 | 20 | 17 | 21 | 18 |
| Chloramphenicol (25) | 21 | 23 | 20 | 18 | 19 | 18 | 16 | 21 | 20 |
| Ciprofloxacin (5) | 15 | 15 | 14 | 13 | 15 | 16 | 10 | 13 | 12 |
| Cloxacillin (1) | 25 | 24 | 22 | 20 | 23 | 25 | 24 | 23 | 23 |
| Co-trimoxazole (25) | 12 | 20 | 16 | 16 | 17 | 15 | 15 | 20 | 15 |
| Erythromycin (15) | 18 | 18 | 17 | 20 | 20 | 17 | 21 | 22 | 18 |
| Gentamicin (10) | 15 | 23 | 16 | 22 | 22 | 20 | 16 | 22 | 15 |
| Lincomycin (2) | 21 | 19 | 21 | 22 | 20 | 22 | 18 | 20 | 20 |
| Sreptomycin (25) | 20 | 22 | 13 | 25 | 20 | 14 | 15 | 23 | 12 |
| Tertracycline (30) | 25 | 24 | 20 | 25 | 21 | 23 | 21 | 24 | 22 |
| Vancomycin (30) | - | - | - | - | - | - | - | - | - |

*** - no zone of inhibition**

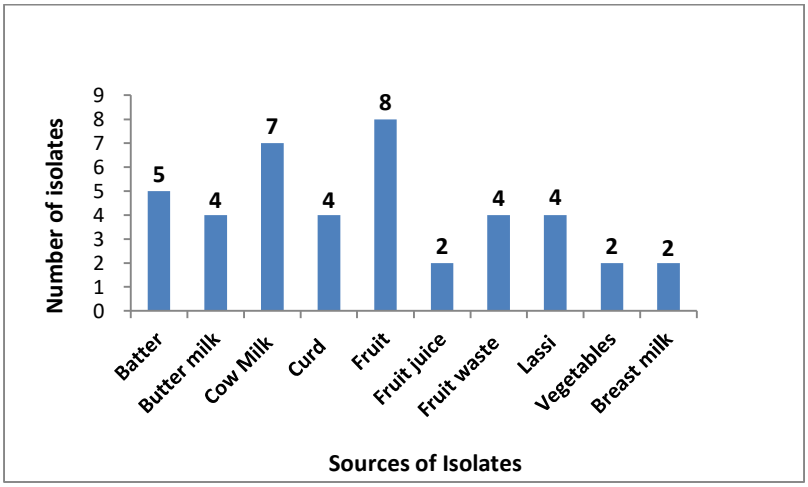


**Figure 1: Number of bacterial isolates from different sources**

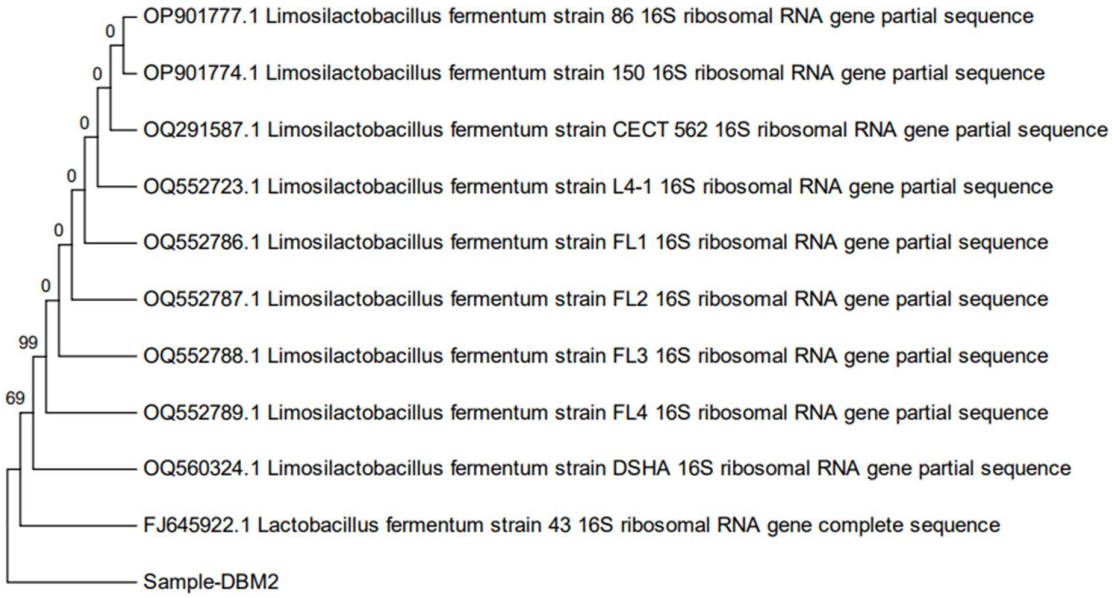


**Figure 2: Phylogenetic tree of *Limosilactobacillus fermentum* DBM2 (OQ692404) showing relationship of the strain nearest in BLAST based on 16 S rDNA gene sequences analysis and constructed using neighbor-joining method [39]**

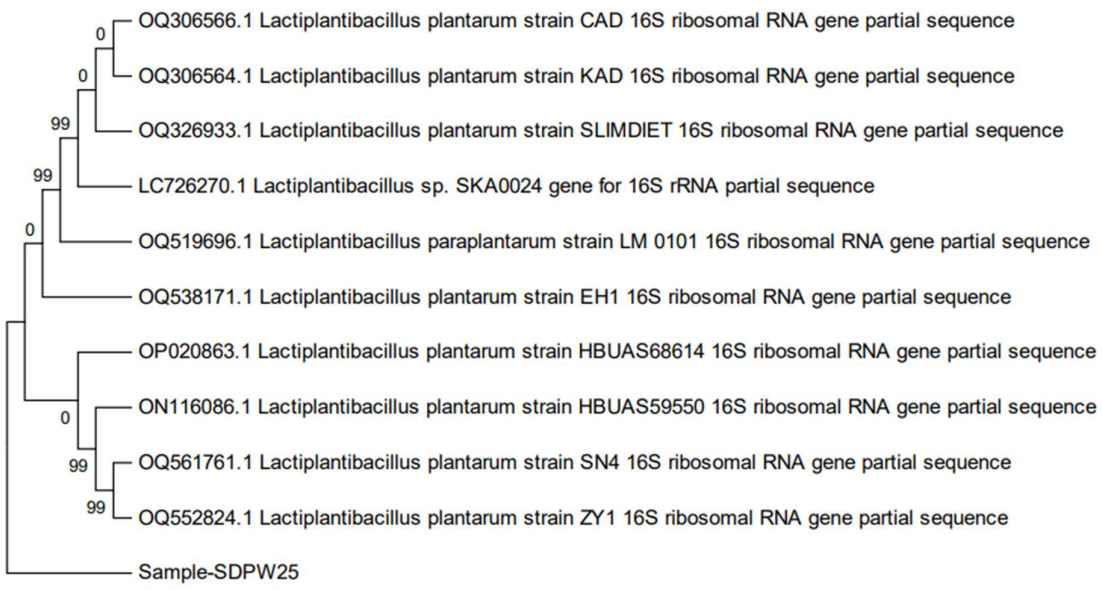


**Figure 3: Phylogenetic tree of *Lactiplantibacillus plantarum* SDPW25 (OQ692416) showing relationship of the strain nearest in BLAST based on 16S rDNA gene sequences analysis and constructed using neighbor-joining method [39]**

## 4. DISCUSSION

Probiotics are mostly obtained from *Lactobacilli* present in fruits, fermented foods, from the gastrointestinal tract, and oral cavity of humans. Therefore, the primary objective of this study was to examine *Lactobacillus* strains derived from diverse sources, including human-derived strains, fruits and fermented foods. Forty-two isolates that were catalase negative and Gram –positive rods arranged singly or in chains, were considered as Lactic acid bacteria. By performing phenotypic characterization, including growth patterns at temperatures 15°C and 45°C and sugar fermentation profiles, all the strains were further analyzed for biochemical characterization. The potent probiotic strains were further analyzed for molecular identification by 16S rDNA Sequencing. Potent probiotic strains were obtained from different origins such as breast milk (human origin) and Pineapple waste (food origin) belonging to the species *Limosilactobacillus fermentum* DBM2 and *Lactiplantibacillus plantarum* SDPW25 respectively.

The isolated strain is of human origin and food origin, so it can lead to a probiotic candidate eventually targeted for human consumption. Considering the history of *Lactobacillus* being safe for human consumption and their established GRAS (Generally recognized as safe) status, it is mandatory that the chosen probiotic strain originates from a health-promoting source and demonstrates nonpathogenic characteristics. Invitro assessments were conducted to evaluate the probiotic

attributes of the isolates, like resistance to acidic conditions, bile salts, NaCl as well as their antagonistic activities. Out of 42 isolates, only 5 isolates were able to tolerate all the prescribed conditions, even after exposure of 24 hrs. Such survival proves beneficial, as probiotic bacteria are not subjected to direct exposure to these low pH levels **[33]**.

Acid tolerance is not only crucial for their potential as dietary supplements in acidic food preparation but also for their ability to withstand the gastro intestinal environment **[34]**. Strains DBM2 and SDPW25 were further chosen for comprehensive studies to assess additional probiotic attributes. Ingested bacteria must endure various extreme conditions to survive in human gastrointestinal tract. The duodenum receives bile acids released by gall bladder, which aids in the breakdown of fat. In the first hour of digestion, the concentration of bile salt varies between 1.5 to 2% (w/v) and then drops to 0.3% **[35]**. In the present study, all isolates are able to tolerate 2 % bile salt, while 18 isolates withstand 4% bile salt concentration. The ability of the strain to tolerate NaCl is fundamental for its viability during the processing of fermented vegetables, a procedure typically conducted in the presence of 6-8 % NaCl. Regardless to habitat differences, the benefits of the isolated strain include potential attributes such as tolerance to acid, bile, and NaCl. Such successful merits of the isolated strain make it possible for their viable transit through gastro intestinal tract. *Lactobacilli* showed a wide range of antimicrobial spectrum against food spoilage organisms and human pathogens. The isolated *Lactobacillus* strain shows a broad antimicrobial spectrum against *Bacillus* spp*., S. aureus*, *E. coli, E. aerogenes*, *P. aeruginosa*, and *S. typhi*, which are main gram-positive food spoilage agents and gram-negative human pathogens. The antimicrobial spectrum of the *Lactobacillus* strain against humans has been previously documented as well **[36]**. By preventing the growth of harmful bacteria, lowering cholesterol level, enhancing immune system, and producing vitamins, supplements containing probiotics (*Bifidobacterium spp*. and *lactobacillus*) are known for their potential to enhance gut resistance against infections **[37]**.

The antimicrobial property of the *Lactobacillus* strain is useful in treating fermented foods to prevent the growth of the organisms that causes food spoilage. The production of acid during fermentation may prevent the growth of microbes that causes food spoilage. The isolated *Lactobacillus* strains possess potential in the development of functional foods, as a starter culture in food fermentation, and in food ensiling. Verification of antibiotic resistance and

antibiotic susceptibility profiles is required to ensure the safety of the strains intended for probiotic usage. With just a few exceptions, our isolates exhibited a similar antibiotic susceptibility pattern. All the isolates were susceptible to Ampicillin, Cephalexin, Chloramphenicol, Ciprofloxacin, Cloxacillin, Co-trimoxazole, Erythromycin, Gentamicin, Lincomycin, Sreptomycin, and Tetracycline, but none of them were susceptible to Vancomycin. The ability of some probiotic strains to resist certain antibiotics may be used for both preventive and therapeutic purposes, contributing to the management of intestinal infections. Additionally, their ability to resist antibiotics shows their efficiency to reduce the harmful effects of antibiotic treatment on the host bacterial ecosystem **[38]**. The in vitro characterization shows that some *Lactobacillus* strains fulfill many functional features to be considered as suitable probiotics. These strains have been selected as they belong to different habitats. After preliminary screening, further in vitro studies have been carried out with control strain *Lactobacillus Plantarum* MTCC 2941 for the probiotic potential of the isolated strains.

## 5. CONCLUSION

Finally, it can be concluded that the *Lactobacillus* strains isolated from natural sources, such as human breast milk (human origin) and pineapple waste (food origin), exhibited promising probiotic attributes, as demonstrated by their capacity to withstand low pH conditions (pH 2.0), thrive in presence of bile salts (up to 4%) and tolerate NaCl concentrations (up to 6%). These attributes could potentially facilitate the successful colonization of the isolated strains within the gastrointestinal environment, enabling them to effectively compete with the pathogens. The isolated strain showed strong antagonistic activities against both food - borne organisms (Gram positive) and gastrointestinal pathogens (Gram negative), which make it applicable for both food industry and the medical sector. Based on the findings regarding antibiotic activity, suggest that this strain holds promise for screening purposes, aiding in the identification of potential probiotic bacteria that are suitable for both human and animal use.

## ACKNOWLEDGEMENTS

I extend my gratitude to the Department of Microbiology and Biotechnology at Atmiya University, Rajkot (Gujarat), for generously providing research facilities for this study.

## FUNDING

The research leading to these results received funding from The Scheme of Developing High-Quality Research (SHODH) Fellowship from the Government of Gujarat under Grant Agreement No KCG/SHODH/2022-23/Ref No: 202101243.

**REFERENCES**

**[1]** Hasan NA, Frank JF, Applied Dairy Microbiology (Second Edition). (E.H. Marth and J.L. Steele, Ed.), New York: Marcel Dekker, Inc., Chap. 6 (Starter Cultures and Their Use), 2001, 152-155.

**[2]** Vernoux JP, Coeuret V, Dubernet S, Bernardeau M, Gueguen M, Isolation, characterization and identification of lactobacilli focusing mainly on cheeses and other dairy products. INRA, EDP Sciences, 83, 2003, 269-306.

**[3]** Azcarate-Peril A.M, Raya RR, Food Microbiology Protocols. (J.F.T. Spencer and A.L.R.D. Spencer, Ed.). Totowa, New Jersey: Humana Press Inc., Chap. 17(Methods for Plasmid and Genomic DNA Isolation from Lactobacilli), 2001, 135.

**[4]** Fuller R, Probiotics in man and animals: A review, Journal of Applied Bacteriology, 66, 1989, 365-378.

**[5]** FAO/WHO (2006) Probiotics in food, health and nutritional properties and guidelines for evaluation. FAO food and nutritional paper. No. 85. WHO/FAO, Rome

**[6]** Sorokulova I (2013) Modern status and perspectives of Bacillus bacteria as probiotics. J Prob Health, 1

**[7]** Guo XH, Kim JM, Namb HM, Park SY, Kim JM (2010) Screening lactic acid bacteria from swine origins for multistrain probiotics based on in vitro functional properties. Anaerobe 16:321–326

**[8]** Reid G, Jass J, Sebulsky MT, McCormick JK (2003) Potential use of probiotics in clinical practice. Clin Microbiol Rev 16:658–672 .

**[9]** Luckow T, Delahunty C. 2004. Which juice is 'healthier'? A consumer study of probiotic non-dairy juice drinks. Food Qual Pref 15:751–759. doi: 10.1016/j.foodqual.2003.12.007.

**[10]** Sheehan VM, Ross P, Fitzgerald GF. 2007. Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices. Innov Food Sci Emerg Technol 8:279–284. doi: 10.1016/j.ifset.2007.01.007.

**[11]** Guarner F, Schaafsma GJ. Probiotics. Int JFood Microbiol 2008; 39: 237–8.doi: 10.1016/S0168-1605(97)00136-0

**[12]** Pasolli, E.; De Filippis, F.; Mauriello, I.E.; Cumbo, F.; Walsh, A.M.; Leech, J.; Cotter, P.D.; Segata, N.; Ercolini, D. Large-scale genome-wide analysis links lactic acid bacteria from food with the gut microbiome. Nat. Commun. 2020, 11, 2610.

**[13]** Marco, M.L.; Hill, C.; Hutkins, R.; Slavin, J.; Tancredi, D.J.; Merenstein, D.; Sanders, M.E. Should There Be a Recommended Daily Intake of

Microbes? J. Nutr. 2020, 150, 3061–3067.

**[14]** Krishnamoorthy, M.; Arjun, P. Probiotic and antimicrobial activity of bacteria from fermented toddy of Cocus nucifera. J. Acad. Indus. Res. 2012, 1, 127–131

**[15]** Vidhyasagar, V.; Jeevaratnam, K. Evaluation of Pediococcus pentosaceus strains isolated from Idly batter for probiotic properties in vitro. J. Funct. Foods 2013, 5, 235–243.

**[16]** Panghal, A.; Janghu, S.; Virkar, K.; Gat, Y.; Kumar, V.; Chhikara, N. Potential non-dairy probiotic products—A healthy approach. Food Biosci. 2018, 21, 80–89.

**[17]** Naeem M, Ilyas M, Haider S, Baig S, Saleem M. Isolation characterization and identification of lactic acid bacteria from fruit juices and their efficacy against antibiotics. Pak J Bot. 2012;44:323–8.

**[18]** Patel A, Prajapati JB, Holst O, Ljungh A. Determining probiotic potential of exopolysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented food products. Food Biosci. 2014;5:27–33.

**[19]** El-Mabrok ASW, Hassan Z, Mokhtar AM, Hussain KMA, Kahar FKSBA. Sceening of lactic acid bacteria as biocontrol against Colletotrichum capsici on chilli Bangi. Res J Appl Sci. 2012;7:466–73.

**[20]** Siddiqee MH, Sarker H, Shurovi KM. Assessment of probiotic application of lactic acid bacteria (LAB) isolated from different food items. Stamford J Microbiol. 2013;2:10–4.

**[21]** Sim KY, Chye FY, Anton A. Probiotic potential and antimicrobial activities of micro- organisms isolated from an indigenous fish sauce. Borneo Science. 2012;31:57–63.

**[22]** De- Man, J.C. ; M. Rogosa and E. Sharpe (1960). Medium of lactobacilli. J. Appl. Bacteriol., 23:130-135.

**[23]** Jimenez, E. ; Fernandez, L. ; Maldonado, A. ; Martín, R. ; Olivares, M. ; Xaus, J., and Rodríguez, J. M. (2008). Oral administration of Lactobacillus strains isolated from breast milk as an alternative for the treatment of infectious mastitis during lactation. Appl. Environ. Microbiol., 74(15), 4650-4655.

**[24]** 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), 8.

**[25]** Kandler O, N Weiss.Bergey'sManual of Systematic Bacteriology In: P. H. A. Sneath, N. S. Mair, M. E. Sharpe, J. G.

Holt editors. Baltimore: Williams and Wilkins; 1986. p. 1209-1234.

**[26]** Sharpe ME. Identification of the lactic acid bacteria. In: Skinner, FA and Lovelock, Editors Identification methods for microbiologists. London: Academic Press; 1979. p. 233-259.

**[27]** Kale P.S.(2014) .Isolation and identification of bacteria from curd and its application in probiotic chocolate .European Journal of Experimental Biology, 2014, 4(6):95-97.

**[28]** Irza, D. A., Lister, I. N. E., Sihotang, S., & Fachrial, E. (2021, April). Isolation, Characterization, Molecular Identification of Probiotic Bacteria from Meconium. In IOP Conference Series: Earth and Environmental Science (Vol. 755, No. 1, p. 012041).

**[29]** Chakraborty A, Bhowal J. Isolation, Identification and Analysis of Probiotic Properties of Lactobacillus Spp. from Selected Regional Dairy Product. Int. J. Curr. Microbiol. App. Sci. 2015; 4(6):621-628.

**[30]** Islam MA, Akter F, Aziz MG, Uddin MB. 2018. Development of probiotic milk drinks using probiotic strain isolated from local yogurt. Fundam Appl Agric 3(2): 446–452.

**[31]** Flemming HP, Etchells JL, Costilow RL. Microbial inhibition by an isolate of Pediococcus from cucumber brines. Appl Microbiol 1985; 30:1040-1042.

**[32]** Reid G.The importance of guidelines in the development and application of Probiotics. Curr. Pharm. Des 2005; 11:11-16.

**[33]** Conway PL, Gorbach SL, Goldin BR. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. J Dairy Sci 1987; 70:1-12.

**[34]** Minelli EB, Benini A, Marzotto M, Sbarbati A, Ruzzenente O, Ferrario R et al. Assessment of novel probiotic Lactobacillus casei strains for the production of functional dairy foods. Int Dairy J 2004; 14:723-736.

**[35]** Noriega L, Gueimonde M, Sanchez B, Margolles A, de los ReyesGavilan C. Effect of the adaptation to high bile salts concentrations on glycosidic activity, survival at low pH and cross-resistance to bile salts in Bifidobacterium. Int J Food Microbiol 2004; 94:79-86

**[36]** Pithva SP, Shekh S, Dave JM, Vyas BRM. Probiotic attributes of autochthonous Lactobacillus rhamnosus strains of human origin. Appl Biochem Biotechnol 2014; 173:259-277.

**[37]** Chauhan Pooja, Mehta Naveen, Rathore M S, Jain Anurekha, Jain Amit Kumar. Probiotic assisted colon targeted drug delivery system: Research scope. Asian J Pharm Clin Res 2011; 4 suppl 2:12-15.

**[38]** El-Naggar MYM. Comparative study of probiotic cultures to control the growth of Escherichia coli O157:H7 and Salmonella typhimurium. Asian Network for Scientific Information Biotechnol 2004; 3:173-180

**[39]** Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.