FUNCTIONAL CHARACTERIZATION OF *PEDIOCOCCUS* SPP. FOR THEIR APPLICATION IN DAIRY FOODS

THESIS SUBMITTED TO THE
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

DAIRY MICROBIOLOGY

BY

VARSHA
M.Sc. (Microbiology)

DAIRY MICROBIOLOGY DIVISION
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE
(DEEMED UNIVERSITY)
KARNAL-132 001 (HARYANA), INDIA

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This is to certify that the thesis entitled **"Functional characterization of *Pediococcus* spp. for their application in dairy foods"** submitted by **Ms. Varsha** towards the partial fulfilment of the award of the degree of **Doctor of Philosophy (Dairy Microbiology)** of the **ICAR-National Dairy Research Institute (Deemed University)**, Karnal (Haryana), India, is a bonafide research work carried out by her under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

Dated:

(Surajit Mandal)
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Dedicated

To

My Faith in GOD

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Dated:
Place: Karnal ***(Varsha)***

CONTENTS

Chapter	Title		Page No.
1.0	**INTRODUCTION**		**1-3**
2.0	**REVIEW OF LITERATURE**		**4-27**
2.1	HISTORICAL BACKGROUND AND TAXONOMY OF *PEDIOCOCCUS* SPP.		4
2.2	ISOLATION AND CHARACTERIZATION OF *PEDIOCOCCUS* SPP.		6
	2.2.1	Abundance, habitat and source of isolation	6
	2.2.2	Morphological and biochemical characterization	7
	2.2.3	Genotypic characterization	9
2.3	TECHNOLOGICAL ATTRIBUTES OF *PEDIOCOCCUS* SPP.		10
	2.3.1	Acidification profile	11
	2.3.2	Flavor production	11
	2.3.3	EPS production	13
	2.3.4	Phytase activity	14
2.4	FUNCTIONAL ATTRIBUTES OF *PEDIOCOCCUS* SPP.		16
	2.4.1	Probiotics: an overview	16
	2.4.2	Survival in the GI tract	18
	2.4.3	Adhesion properties	19
	2.4.4	Antimicrobial activity	20
	2.4.5	β-galactosidase activity	21
	2.4.6	Hypo-cholesterolemic, antioxidative, immune-modulatory and other beneficial activities	22
2.5	SAFETY ASPECTS OF *PEDIOCOCCUS* SPP.		22
2.6	PEDIOCOCCI AS STARTER AND NON-STARTER/ADJUNCT CULTURES		25
3.0	**MATERIALS AND METHODS**		**28-46**
3.1	ISOLATION OF *PEDIOCOCCUS* SPP.		28
	3.1.1	Collection of samples and reference cultures	28
	3.1.2	Sample preparation	28
	3.1.3	Procedure for isolation	29

	3.1.4	Morphology and catalase test		29
	3.1.5	Physiological characterization of isolates		29
		3.1.5.1	Culture preparation	29
		3.1.5.2	Pseudocatalase test	30
		3.1.5.3	Oxidase test	30
		3.1.5.4	Heterofermentation test	30
		3.1.5.5	Arginine hydrolysis	30
		3.1.5.6	Growth at different temperatures	30
		3.1.5.7	Growth at different pH	31
		3.1.5.8	Growth at different salt concentrations	31
3.2	IDENTIFICATION OF *PEDIOCOCCUS* ISOLATES			31
	3.2.1	Biochemical identification using APi 50CH kit		31
	3.2.2	Genotypic identification by PCR		31
		3.2.2.1	Isolation of genomic DNA	32
		3.2.2.2	Genus and species specific primers	32
		3.2.2.3	PCR reaction conditions	34
		3.2.2.4	Analysis of PCR products by agarose gel electrophoresis	35
3.3	MAINTENANCE AND PRESERVATION OF *PEDIOCOCCUS* CULTURES			35
3.4	EVALUATION OF TECHNOLOGICAL ATTRIBUTES OF *PEDIOCOCCUS* CULTURES			35
	3.4.1	Acidification profile		35
	3.4.2	Phytase activity		36
	3.4.3	Exopolysaccharide production		36
	3.4.4	Flavor (Diacetyl) production		36
	3.4.5	Galactose fermentation		36
		3.4.5.1	Screening for galactose fermentation	36
		3.4.5.2	Quantification of galactose utilization	37
3.5	EVALUATION OF PROBIOTIC ATTRIBUTES OF *PEDIOCOCCUS* CULTURES			38
	3.5.1	*In-vitro* acid tolerance		38

	3.5.2	*In-vitro* bile tolerance			38
	3.5.3	Cell surface hydrophobicity			39
	3.5.4	Cell auto-aggregation			39
	3.5.5	Antimicrobial activity			40
	3.5.6	β-galactosidase enzyme production			40
	3.5.7	Prebiotic fermentation			41
	3.5.8	Bile salt hydrolase (BSH) assay			41
3.6	SAFETY ASSESSMENT OF *PEDIOCOCCUS* CULTURES				41
	3.6.1	Haemolytic activity			41
	3.6.2	DNase test			42
	3.6.3	Liquefaction of gelatin			42
	3.6.4	Assessment of biogenic amines production			42
	3.6.5	Antibiotic susceptibility test			43
3.7	APPLICATION OF SELECTED *PEDIOCOCCUS* CULTURES				44
	3.7.1	Preparation of synbiotic fermented dairy beverage			44
		3.7.1.1	Physico-chemical properties		44
			3.7.1.1 a	Titratable acidity	44
			3.7.1.1 b	pH measurement	44
		3.7.1.2	Microbiological analysis		45
			3.7.1.2 a	Total lactic count	45
			3.7.1.2.b	Coliform count	45
			3.7.1.2 c	Yeast and mold count	45
		3.7.1.3	Phytase activity		45
	3.7.2	Yoghurt culture formulation			45
		3.7.2.1	Physico-chemical properties		45
		3.7.2.2	Microbiological analysis		46
3.8	ANALYSIS OF DATA				46
4.0	**RESULTS AND DISCUSSION**				**47-62**
4.1	ISOLATION OF *PEDIOCOCCUS* SPP.				47
4.2	EVALUATION OF TECHNOLOGICAL ATTRIBUTES OF *PEDIOCOCCUS* CULTURES				50
	4.2.1	Acidification profile			50

	4.2.2	Phytase activity	51
	4.2.3	Exopolysaccharide production	52
	4.2.4	Flavor (diacetyl) production	53
	4.2.5	Galactose fermentation	53
4.3	EVALUATION OF PROBIOTIC ATTRIBUTES OF *PEDIOCOCCUS* CULTURES		54
	4.3.1	*In-vitro* acid tolerance	54
	4.3.2	*In-vitro* bile tolerance	55
	4.3.3	Cell surface hydrophobicity	56
	4.3.4	Cell auto-aggregation	56
	4.3.5	Antimicrobial activity	57
	4.3.6	β-galactosidase enzyme production	58
	4.3.7	Prebiotic fermentation	59
	4.3.8	Bile salt hydrolase (BSH) assay	59
4.4	SAFETY ASSESSMENT OF *PEDIOCOCCUS* CULTURES		60
	4.4.1	Haemolysis, DNase, Gelatinase activity and biogenic amine production	60
	4.4.2	Antibiotic susceptibility test	61
4.5	APPLICATION OF TECHNO-FUNCTIONALLY POTENT *PEDIOCOCCUS* CULTURES		62
	4.5.1	Preparation of synbiotic fermented dairy beverage	62
	4.5.2	Yoghurt preparation	62
5.0	**SUMMARY AND CONCLUSIONS**		**63-66**
BIBLIOGRAPHY			**i-xxvi**
APPENDICES			

LIST OF TABLES

Table	Title	On/After Page
3.1	Primers used for the identification of *Pediococcus* spp.	33
3.2	PCR master mix composition	34
3.3	PCR cycling steps used for genus and species specific primers	34
3.4	List of antibiotics with different concentrations mentioned on the disc	43
4.1	Biochemical and growth characteristics of *Pediococcus* spp.	48
4.2	Biochemical and genotypic identification of *Pediococcus* isolates	48
4.3	Acidification profile (change in pH) of *Pediococcus* cultures in different media	50
4.4	Technological attributes of *Pediococcus* cultures	54
4.5	Acid tolerance of *Pediococcus* cultures	54
4.6	Bile tolerance of *Pediococcus* cultures	56
4.7	Antimicrobial activity of *Pediococcus* cultures	58
4.8	Antibiotic susceptibility of *Pediococcus* cultures	62
4.9	Techno-functional attributes of selected *Pediococcus* cultures	62
4.10	Physico-chemical and microbiological characteristics of synbiotic fermented dairy beverage	62
4.11	Physico-chemical and microbiological characteristics of yoghurt	62

LIST OF FIGURES

Fig.	Title	On/After Page
2.1	Structure of phytic acid	15
2.2	Action of phytase on phytic acid	15
2.3	Mechanisms of action of probiotics	17
2.4	Characteristics of an ideal probiotic strain	18
3.1	Standard curve of galactose	38
4.1	Samples for isolation of *Pediococcus* spp.	48
4.2	Isolation of *Pediococcus* spp.	48
4.3	Physiological characterization of isolates	48
4.4	Electrophoretic profile of *Pediococcus* isolates by polymerase chain reaction with genus specific oligonucleotide primer	48
4.5	Sugar fermentation profile of *Pediococcus* isolates using APi 50CH Kit	48
4.6	Electrophoretic profile of *Pediococcus* isolates by polymerase chain reaction with species specific oligonucleotide prime	48
4.7	Distribution of *Pediococcus* isolates	50
4.8	Acidification by *Pediococcus* cultures in different media	50
4.9	Zone of clearance by *Pediococcus* cultures on sodium phytate MRS agar	52
4.10	EPS production by *Pediococcus* cultures on sucrose MRS agar	52
4.11	Diacetyl production (Ring test) by *Pediococcus* cultures	54
4.12	Galactose fermentation by *Pediococcus* cultures	55
4.13	Acid tolerance of *Pediococcus* cultures at pH 2.5 for 3 h	54
4.14	Cell surface hydrophobicity of *Pediococcus* cultures	56
4.15	Cell auto-aggregation of *Pediococcus* cultures	58

4.16	Antimicrobial activity of *Pediococcus* cultures against (a) *S. aureus* (b) *S. enterica* ssp. Typhi (c) *E. coli* (d) *L. monocytogenes*	58
4.17	Prebiotic fermentation by *Pediococcus* cultures	60
4.18	Bile salt hydrolase activity of *Pediococcus* cultures	60
4.19	Haemolytic activity of *Pediococcus* cultures	60
4.20	DNase activity of *Pediococcus* cultures	60
4.21	Gelatinase activity of *Pediococcus* cultures	60
4.22	Biogenic amine production by *Pediococcus* cultures	60
4.23	Antibiotic susceptibility of *Pediococcus* cultures	62
4.24	Distribution (%) of *Pedicoccus* cultures based on antibiotic susceptibility	62
4.25	Synbiotic fermented dairy beverage prepared using selected *Pediococcus* culture	62
4.26	Yoghurt prepared using selected *Pediococcus* culture as an adjunct culture	62

ABBREVIATIONS

MRS	de Man, Rogosa and Sharpe
NCDC	National Collection of Dairy Cultures
GRAS	Generally Recognized as Safe
HPLC	High Performance Liquid Chromatography
LAB	Lactic Acid Bacteria
WHO	World Health Organization
CLSI	Clinical Laboratory Standard Institute
API	Analytical Profile Index
UV	Ultra Violet
OD	Optical Density
CFU	Colony Forming Unit
PUM	Phosphate Urea Magnesium
TGE	Tryptone Glucose Extract
VRBA	Violet Red Bile Agar
PDA	Potato Dextrose Agar
DNA	Deoxyribonucleic acid
PCR	Polymerase Chain Reaction
TE	Tris EDTA
SET	Sodium dodecyl sulphate
TAE	Tris-Acetate EDTA
dNTP	Deoxy nucleotide triphosphate
EtBr	Ethidium Bromide
N	Normal
M	Molar
bp	Base Pair
µM	micromolar
spp.	Species
ssp.	Subspecies
g	gram
µg	microgram
mg	milligram
w	weight
L	litre
mL	millilitre

nm	nanometer
µL	microlitre
µg	microgram
ng	nanogram
mm	millimeter
nm	nanometer
sec	second
min	minute
h	hour
d	days

ABSTRACT

The present investigation was aimed to isolate and characterize the *Pediococcus* spp. for techno-functional attributes. A total of 115 samples (dairy as well as non-dairy) collected from different locations in India were processed for isolation of *Pediococcus* spp. *Pediococcus pentosaceus* NCDC 38, *P. pentosaceus* NCDC 273 and *P. acidilactici* NCDC 609 were also included. Identity of forty putative *Pediococcus* isolates (based on morphology and physiological tests) were confirmed as *P. pentosaceus* (39) and *P. acidilactici* (1) by APi 50CH sugar fermentation patterns, and genus and species-specific PCR. All the 40 *Pediococcus* cultures along with 3 NCDC cultures were evaluated for technological (acidification profile, phytase activity, diacetyl production, EPS production and galactose utilization) and probiotic (*In-vitro* acid and bile tolerance, cell surface hydrophobicity, auto-aggregation, β-galactosidase activity, antimicrobial activity, prebiotic utilization and bile salt hydrolase activity) attributes. Lactose fermentation was exhibited by *P. acidilactici* NCDC 609. Milk curdling was exhibited by some *Pediococcus* cultures only after supplementation of protein hydrolysates. Thirty four *Pediococcus* cultures along with NCDC 38 and 273 displayed phytase activity on sodium phytate MRS agar. Twelve *Pediococcus* cultures were found positive for diacetyl production. Five cultures (PD-1, PD-44, 42-PD, Sil-1 and Sil-2) along with NCDC 38 showed mucoid colony on sucrose MRS agar. All the cultures were galactose fermenter and 28 cultures along with 3 NCDC cultures utilized more than 90% galactose within 12 h. A total of 19 cultures along with NCDC 273 and 609 were able to survive at pH 2.5 up-to 3 h, and maximum survival was exhibited by 42-PD, Sil-1 and PD-28. Majority of the cultures survived in 1% bile salt up-to 4 h. Maximum survival was observed with Sil-2 followed by NCDC 609, PD-21 and PD-26. All the cultures were able to ferment fructo-oligosaccharide (FOS), while five cultures (PD-1, PD-7, PD-8, PD-10 and 8-PD) were positive for inulin fermentation. A wide variation in cell surface hydrophobicity (7.95-59.35%) and auto-aggregation (39.28-83.28%) was observed. Seven cultures (PD-3, PD-10, PD-29, PD-30, PD-41, NCDC 273 and 609) demonstrated intracellular β-galactosidase activity (0.035-0.597 IU/mg dry weight). All the *Pediococcus* cultures demonstrated appreciable antimicrobial activity against tested indicator organisms (*S. aureus, S. enterica* ssp. Typhi*, E. coli* and *L. monocytogenes*). *Pediococcus* cultures PD-2, PD-4, PD-8. PD-18, PD-21, PD-44, 42-PD, NCDC 38 and 273 were highly active against all the four indicator strains. *In-vitro* safety assessment showed that all *Pediococcus* cultures were negative for β-haemolysis (displayed α-haemolysis), DNase, gelatinase activity and biogenic amine production from histidine and tyrosine. Most of the cultures were sensitive to the important antibiotics and all were resistant to vancomycin (except PD-39) and nalidixic acid. On the basis of phytase activity, prebiotic fermentation, galactose utilization and probiotic attributes; *P. pentosaceus* PD-27, PD-32, PD-34 and 14-PD were selected for the preparation of synbiotic fermented dairy beverage (single strain starter) and yoghurt (along with low galactose utilizing *Streptococcus thermophilus* NCDC 428 and *Lactobacillus delbrueckii* ssp. *bulgaricus* NCDC 277). On the basis of phytase activity and quality, fermented synbiotic dairy beverages prepared with PD-32 and PD-34 were found better. Yoghurts prepared with PD-32 and PD-34 were comparable with control yoghurt. Characterized *Pediococcus* cultures can be exploited for various techno-functional applications as starter, adjunct culture and probiotic.

सारांश

वर्तमान जांच का उद्देश्य पीडीओकोकस जाति को तकनीकी-कार्यात्मक गुणों के लिए अलग करना और उसे चिह्नित करना था। भारत के विभिन्न स्थानों से पीडीओकोकस जाति के अलगाव के लिए कुल 115 नमूनों (डेयरी और अन्य-डेयरी) को एकत्रित किया गया। एनसीडीसी केन्द्र से पीडीओकोकस पैंटोसेशियस एनसीडीसी 38, पीडीओकोकस पैंटोसेशियस एनसीडीसी 273 और पीडीओकोकस असिडिलेक्टिसी एनसीडीसी 609 को मानक के रूप में लिया गया। API 50 सीएच्एल किण्वन कीटो, रूपात्मक तथा आनुवंशिक तत्यों के आधार पर 40 पीडीओकोकस जातियों में से पीडीओकोकस पैंटोसेशियस की 39 और पीडीओकोकस असिडिलेक्टिसी की एक जाति किस्म का अलगाव किया गया। सभी 40 पीडीओकोकस जातियों का तकनीकी-कार्यात्मक गुणों के लिए परीक्षण किया गया, जिसमें एसिडिफिकेशन प्रोफाइल, फाइटेज उत्पादन गतिविधि, डाईअसिटाइल उत्पादन, ईपीएस उत्पादन, गैलेक्टोज उपयोग, पित्त-नमक हाइड्रोलेज गतिविधि और सभी प्रोबायोटिक चिह्नित गुण शामिल हैं। लेक्टोस उपयोगी क्रिया केवल पीडीओकोकस असिडिलेक्टिकी एनसीडीसी 609 में पाई गई। 34 पीडीओकोकस जातियों (एनसीडीसी 38 और एनसीडीसी 273 सहित) में फाइटेज उत्पादन की क्षमता पाई गई। 12 पीडीओकोकस जातियों में डाईअसिटाइल उत्पादन की क्षमता पाई गई। एनसीडीसी 38 के साथ पांच जामन (पीडी-1, पीडी-44, 42-पीडी, सील-1 और सील-2) ने ईपीएस उत्पादन दिखाया। सभी पीडीओकोकस जातियों में गैलेक्टोज उपयोगी क्रिया पाई गई। 19 पीडीओकोकस जातियों (एनसीडीसी 273 और एनसीडीसी 609 सहित) में 3 घंटे तक की अम्ल सहनशीलता पाई गई जिनमें पीडी-28, 42-पीडी, सील-1 प्रमुख है। अधिकांश पीडीओकोकस 1% पित्त में 4 घंटे तक जीवित रहे। सील-2 के साथ अधिकतम अस्तित्व देखा गया, इसके बाद एनसीडीसी 609, पीडी-21 और पीडी-26 प्रमुख थे। सभी पीडीओकोकस जातियो में फ्रुक्टो-ऑलिगोसेकेराइड (फॉस) उपभोग करने की क्षमता, जबकि पांच पीडीओकोकस (पीडी-1, पीडी-7, पीडी-8, पीडी-10 और 8-पीडी) इनुलिन किण्वन के लिए सक्षम थे। पीडीओकोकस जातियो में सेल की सतह हाइड्रोफोबिसिटी (7.95-59.35%) और ऑटो-एकत्रीकरण (39.28-83.28%) में व्यापक विविधता देखी गई। सात जातियो (पीडी-3, पीडी-10, पीडी-29, पीडी-30, पीडी-41, एनसीडीसी 273 और 609) ने इंट्रासेल्युलर β-गलैक्टोसाइडेज़ गतिविधि (0.035-0.597 आईयू/एमजी) का प्रदर्शन किया। सभी पीडीओकोकस जातियो ने सूचक जीवों (एस ऑरियस, एस टाइफी, ई कोली और लिस्टिरिया) के खिलाफ सराहनीय रोगाणुरोधी गतिविधि का प्रदर्शन किया। पीडीओकोकस जातियो में पीडी-2, पीडी-4, पीडी-8, पीडी-18, पीडी-21, पीडी-44, 42-पीडी, एनसीडीसी 38 और एनसीडीसी 273 सभी चार सूचक जीवों के खिलाफ अत्यधिक सक्रिय थे। सभी पीडीओकोकस जाति β-हेमोलीसिस, (α-हेमोलीसिस देखी गई), डीएनएज़ उत्पादन, जिलेटिनेज गतिविधि और हिस्टीडीन और टाइरोसिन से बायोजेनिक अमाइन उत्पादन प्रदर्शित करने के लिए नकारात्मक पाए गए। पीडीओकोकस जातिया महत्वपूर्ण एंटीबायोटिक दवाओं के प्रति संवेदनशील थीं और ये सभी वैनकोमाइसिन (पीडी-39 को छोड़कर) और नालिडेक्सिक एसिड के प्रतिरोधी थे। ऊपर लिखित सभी गुणों के आधार पर पीडीओकोकस-27, पीडी-32, पीडी-34 और 14-पीडी को सिनबायोटिक किण्वित डेयरी पेय (एकल जामन) और दही (स्टैप्टोकोकस थर्मोफिलस एनसीडीसी 428 और लैक्टोबैसिलस डेलब्र्यूकेय एनसीडीसी 277) तैयार करने के लिए चुना गया। फाइटेज गतिविधि और गुणवत्ता के आधार पर पीडी-32 और पीडी-34 के मदद से निर्मित सिनबायोटिक किण्वित डेयरी पेय बेहतर पाए गए। पीडी-32 और पीडी-34 के साथ तैयार योग्हर्ट नियंत्रित दही के साथ तुलनीय थे। अन्तः वर्णित पीडीओकोकस का इस्तेमाल जामन, सहायक जामन और प्रोबायोटिक जामन के रूप में विभिन्न तकनीकी-कार्यात्मक अनुप्रयोगों के लिए किया जा सकता है।

CHAPTER –1

Introduction

INTRODUCTION

Lactic acid bacteria (LAB) are industrially important microorganisms because of long and safe history of their application to produce numerous fermented foods. The recent widespread use of LAB and bifidobacteria for elaboration of functional foods is attributable to the accumulating scientific evidence showing their beneficial effects on human health. Besides *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*; *Pediococcus* is one of the important LAB, belonging to the family *Lactobacillaceae*. Interestingly, it is the only acidophilic, homo-fermentative LAB that divides alternatively in two perpendicular directions in a single plane to form tetrads. Several species and strains of *Pediococcus* have been used either as starter, non-starter or adjunct cultures in the fermentation of various food products including vegetables, meat, sausages, silage, milk and milk products etc (Singla *et al*., 2017). *Pediococcus acidilactici* and *P. pentosaceus* are most widely used in food and dairy fermentations (Porto *et al*., 2017). Earlier production of fermented foods was the result of spontaneous fermentation due to the development of microflora naturally present in the raw material. However, in recent production facilities, where high level of control over fermentation processes and standard end products are expected, the artisanal starters are replaced with well characterized defined cultures. Therefore, a continuous need exists for the isolation of new strains with better functional attributes. Pediococci prefer to inhabit fermentable sugar rich niches such as plant materials, where they are generally associated with *Lactobacillus* and *Leuconostoc* species. Other habitats of pediococci include different varieties of cheese, idly batter, kadhi, sauerkraut, fermented beverages, silage and human sources such as breast milk, saliva, faeces, and vaginal samples (Osmanagaoglu *et al*., 2011; Saravanan *et al*., 2015).

Evaluation of technological attributes is crucial for comprehensive characterization of LAB for selection of strain(s) to be used for desired fermentation process(s). Several technological attributes to make pediococci cultures as industrially important microbes include acidification, phytase activity, galactose fermentation ability, flavor production, exopolysaccharide production, antimicrobial activity etc. Being homofermentative, pediococci produce only lactic acid as fermentation end

product from various sugars, and no any side product formation make them desirable in numerous food fermentations. Unfortunately, pediococci are typically unable to ferment lactose which restricts their direct application in milk fermentations. However, some *Pediococcus* strains are positive for β-galactosidase enzyme (Attri *et al*., 2015). Reports indicating that non-starter and adjunct *Pediococcus* spp. impart desirable attributes in cheese which suggest that pediococci may be good dairy starter bacteria if they possess the ability to utilize lactose. Pediococci are galactose fermenter, so lactose and galactose positive pediococci have the potential for the replacement of *Streptococcus thermophilus* in thermophilic dairy starter blends and may facilitate development of new strain rotation schemes to combat *S. thermophilus* galactose utilizing and bacteriophage sensitivity problems. Pediococci also exhibit strain specific ability to produce phytase enzyme, which degrades phytic acid, a potent chelator of cations. The presence of this particular enzyme can make bio-availability of minerals, thus, enabling phytase positive pediococci as potential starters in cereal based fermented products (Nuobariene *et al*., 2015). Exopolysaccharide production by LAB has been investigated because of growing interest in the use of cultures to confer textural and rheological attributes to foods *in-situ* through natural fermentations rather than by incorporation of additives. Some strains of *Pediococcus* are known to produce EPS with enhanced functionality (Ghany *et al*., 2016). The proteolytic activity of *Pediococcus* spp. has profound applications in the ripening of cheeses as an adjunct culture. Some strains of *P. acidilactici* and *P. pentosaceus* have proteolytic enzymes, so they are used as non-starter lactic acid bacteria (NSLAB) or secondary starter cultures in the development of flavor, body and texture in cheddar and other related cheese varieties. The pediococcal fermentations not only help in improving the sensory quality but also enhance the safety and extend the shelf life of foods by inhibiting the growth of pathogenic and spoilage microorganisms through elaboration of antimicrobial substances such as bacteriocins (Papagianni and Anastasiadou, 2009). Besides antibacterial, antifungal activity is also reported in some strains of pediococci (Sellamani *et al*., 2016). Hence, pediococci can be used as potent bio-protective cultures.

In recent time, there has been a wide spread change in consumer perception towards food. This shift has generated new concepts of functional foods and

probiotics. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Gallego and Salminen, 2016). Some *Pediococcus* strains are found to endure harsh physiological conditions of gastro-intestinal (GI) environment, making them an ideal probiotic candidate (Noohi *et al*., 2016). They also have the ability to de-conjugate bile acids and this activity is imperative in maintaining the equilibrium of gut microflora and also lowering of serum cholesterol level. Additionally, they can work as probiotics for animals and livestock. Strains of *P. acidilactici* have also been included in the commercial probiotic preparations for human use (Erkkila and Petaja, 2000).

Pediococci are generally considered as safe because they have long history of consumption in fermented foods (Ribeiro *et al*., 2014). Many pediococci strains are proven to be safe as they have been evaluated by assessing biogenic amines production, antibiotic susceptibility, haemolytic activity, DNase activity, gelatin liquefaction etc. At the same time, some pediococcal strains present an emerging potential of opportunistic pathogens for humans and are associated with potential virulence factors. Assurance of safety of pediococcal cultures before their use in food and probiotic preparations is important one.

Hence, detailed characterization of *Pediococcus* species is essential which provides complete knowledge of the species, their physiological, metabolic properties and also functional attributes of industrial interest. Although a lot of studies have been done on LAB both as probiotic and starter cultures, but among them, there is dearth of knowledge about *Pediococcus* spp. To get potent safe pediococcal cultures which possess promising techno-functional attributes, so that they can be exploited as starter or as adjunct cultures in various food fermentations, the present investigation was undertaken with the following objectives:

1. **Isolation and identification of *Pediococcus* spp. from dairy and non-dairy sources**
2. **Technological and probiotic evaluation of identified *Pediococcus* spp.**
3. **Safety assessment of techno-functionally potent *Pediococcus* spp.**

CHAPTER –2

Review of Literature

Lactic acid bacteria, one of the most numerous group of bacteria linked to humans, have been utilized in the production of dairy, plant and meat fermented products with desired taste and texture. They have also been used as probiotics for human and animals, and generally regarded as safe (GRAS) Food and Drug Administration (FDA) status. LAB are relatively heterogenous group that share several defining characteristics including low G+C ratio (<55 mol%), high acid tolerance, unable to synthesize porphyrins and demonstrating fermentative metabolism with lactic acid as their major metabolic end-product (Broadbent and Steele, 2005). The discovery and characterization of LAB has significantly modified the production way of fermented foods. Over the years, considerable efforts have been devoted to increase the knowledge about physiology, biochemistry and genetics of LAB. These studies allowed microbiologists in selecting better strains and improve productivity, quality and safety of the final products. The umbrella-term LAB is mainly restricted to the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc, Streptococcus, Carnobacterium, Enterococcus, Aerococcus, Tetragenococcus, Weissella, Vagococcus,* and *Pediococcus* is one amongst them (Prasirtsak *et al*., 2013). Despite of being phylogenetically unrelated, the genus *Bifidobacterium* is also included in LAB group because of its physiological, biochemical and ecological similarities (Vankerckhoven *et al*., 2004). Strains of genus *Pediococcus* have been used as starter, non-starter and adjunct culture in various food fermentations since long time. This genus is endowed with a great magnitude of techno-functionality, yet it is not exploited up-to its fuller potential. So, the isolation and detailed characterization of *Pediococcus* spp. is essential in fulfilling the knowledge gap of the species, their physiological, metabolic properties and also the techno-functional aspects of industrial interest so that a better strain can be selected for food fermentations.

2.1 HISTORICAL BACKGROUND AND TAXONOMY OF *PEDIOCOCCUS* SPP.

The taxonomy of genus "*Pediococcus*" had a very complicated and confusing past. Initially, the bacteria similar to the pediococci were included in the genera *Sarcina*, *Streptococcus, Micrococcus, Leuconostoc* and *Lactobacillus*. Different

nomenclatures were assigned to the genus *Pediococcus*. 'Balcke' was the first person to mention the name *Pediococcus*. Afterwards, this name received the generic recognition with the type species "*P. cerevisiae* Balcke" (Pederson, 1949). Later on, similarity between the species of the genus *Pediococcus* and *Aerococcus viridans* was identified (Whittenbury, 1965). In 1985, Bergey's Manual (Garvie, 1986) documented eight species; while, Pfannebecker and Frohlich (2008) enlisted the ten species of the genus *Pediococcus*. Phylogenetically, species are heterogeneous; however, type species *P. damnosus* is closely related to *P. acidilactici*, *P. pentosaceus* and *P. parvulus*. *Pediococcus dextrinicus* is now re-classified as genus *Lactobacillus* because it did not show any close phylogenetic relationship to other *Pediococcus* species and have only 4-8% of DNA homology (Haakensen *et al.*, 2009). Additionally, *P. halophilus*, *P. cerevisae* and *P. urinaeequi* have been re-grouped as *Tetragenococcus halophilus*, *P. acidilactici* and *Aerococcus urinaeequi*, respectively (Felis *et al*., 2005; Haakensen *et al*., 2009). Some novel *Pediococcus* strains are also proposed such as NGRI 0510QT (from ryegrass silage) which was initially classified as representative of a new *Pediococcus* species, and named as *P. lolii* (Doi *et al.*, 2009). Later on, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis failed to differentiate between *P. lolii* LMG 25667T and *P. acidilactici* strains (Wieme *et al.*, 2012).

These are Gram-positive cocci which peculiarly divide in two perpendicular directions in a single plane to form tetrads. Consequently, they occur in pairs or a cluster of four but never form chains. So, morphologically they are like *Tetragenococcus* spp. However, the two genera are only distantly related and the genus *Pediococcus* has a closer phylogenetic relationship to *Lactobacillus* (more closely resembles *Lb. casei* and *Lb. paracasei*). Pediococci also resemble aerococci, but the former can grow anaerobically and at pH 5.

Taxonomically, genus *Pediococcus* belongs to:

Kingdom: Bacteria

Division: Firmicutes

Class: Bacilli

Order: Lactobacillales

Family: *Lactobacillaceae*

Currently, *Pediococcus* species include *P. acidilactici*, *P. claussenii*, *P. cellicola*, *P. damnosus*, *P. ethanolidurans*, *P. inopinatus*, *P. parvulus*, *P. pentosaceus*, *P. stilesii*, *P. siamensis* and *P. argentinicus* (Lv *et al*., 2014). Phylogenetically, *Pediococcus* and *Lactobacillus* form a super-cluster that further alienated into two sub-clusters. All the *Pediococcus* species fall within the *Lactobacillus casei* - *Pediococcus* sub-cluster. Based on the comparisons of 16S ribosomal RNA (rRNA) catalogs and sequences of Gram-positive bacteria, the pediococcal evolutionary line descends within the clostridial lineage which includes spore forming genera: *Clostridium* and *Bacillus*, and non-spore forming genera: *Lactobacillus*, *Leuconostoc, Pediococcus* and *Streptococcus*. The clostridial lineage is characterized by a low G+C ratio (less than 50%) in resemblance to *Pediococcus* spp. having G+C ratio in the range of 32-42% (Dobson *et al*., 2002; Tanasupawat *et al*., 2007).

2.2 ISOLATION AND CHARACTERIZATION OF *PEDIOCOCCUS* SPP.

2.2.1 Abundance, habitat and source of isolation

Lactic acid bacteria are indigenous to food-related habitats including plants (fruits, vegetables and cereal grains) and dairy environments. In addition, they are naturally associated to mucosal surfaces of animals, e.g., small intestine, colon and vagina. Pediococci mainly inhabit fermentable sugar rich niches such as plant materials, and in plant environments they are generally associated with *Lactobacillus* and *Leuconostoc* species (Cai *et al*., 1999). A pediocin producing strain *P. pentosaceus* ST44AM from marula fruit (*Sclerocarya birrea*) was isolated and characterized by Todorov and Dicks (2009). Abriouel *et al*. (2012) reported occurrence of pediococci (although in low number; 7.64%) along with other LAB including lactobacilli (81.94%) and leuconostocs (10.42%) in *Manzanilla alorena* (or Alorena) table olives that are naturally fermented traditional green olives. Sequencing of pheS and rpo genes revealed the identification of lactobacilli as *Lb. pentosus*, pediococci as *P. parvulus* and leuconostocs as *Leuco. pseudomesenteroides*. A strain of *P. pentosaceus* T1 was isolated from Kimchi, a fermented vegetable (Jang *et al*., 2014). In China, a

bacteriocinogenic strain *P. pentosaceus* 05-10 was isolated from Sichuan pickle (Huang *et al*., 2009).

Fermented foods like idly batter, kadhi, sauerkraut and sausages are also prevalent sources of pediococci (Albano *et al*., 2009; Sukumar and Ghosh, 2010; Mandal *et al*., 2011). Saravanam *et al*. (2015) studied the diversity and functional characteristics of idli microflora. They analyzed 10 distinctive household idli batter samples which were taken at 3 h intervals from 0 to 15 h of natural fermentation. A total of 300 pure colonies were isolated, out of which, 40 strains were characterized and identified as: *Leuconostoc* spp., *Weissella* spp., *Pediococcus* spp., *Lactococcus* spp. and *Bacillus* spp. *Pediococcus pentosaceus* strains were obtained from 6 h old fermented idli batter. Similarly, Vidhyasagar and Jeevaratnam (2012) isolated *Pediococcus* strains from traditional fermented black lentil dish (idli) and found it 98-100% similar to *P. pentosaceus* strains. Papamanoli *et al*. (2003) isolated 147 LAB from two types of naturally fermented sausages at four different ripening stages. Identification of the isolates revealed 90% as lactobacilli, 4% enterococci, 3% pediococci along with sporadic isolates of *Leuco. pseudomesenteroides*, *Weissella viridescens*, and *Leuconostoc* spp.

Raw milk and milk products such as dried curd and different varieties of cheese are also inhabited by pediococci (Korasapati, 1998; Alonso-Calleja *et al*., 2002; Abbasiliasi *et al*., 2012; Martino *et al*., 2013). However, milk environment is not preferential niche for pediococci. Besides, pediococci have also been isolated from animals (Juven *et al*., 1991; Kurzak *et al*., 1998; Simpson *et al*., 2002) and human sources (Barros *et al*., 2001; Walter *et al*., 2001; Uymaz *et al*., 2009). Kaur *et al*. (2012) isolated a *Pediococcus* strain from faecal sample of healthy individual and identified it as *P. acidilactici* BA28. *Pediococcus pentosaceus* LI05 has been isolated from human GI tract and its whole genome has been sequenced (Lv *et al*., 2014).

2.2.2 Morphological and biochemical characterization

Pediococcus spp. exhibits variations in physiological and biochemical characteristics which can be used for their identification purpose (Papagianni and Anastasiadou, 2009). Microscopically, pediococci are identified by their

distinct characteristic formation of tetrads. They are round in shape, with colony diameter of 1-2.5 mm, non-spore forming, oxidase negative, facultative anaerobes to microaerophillic, and nutritionally fastidious microorganisms (Simpson and Taguchi, 1995). Generally, pediococci are catalase negative; however, specifically some strains may exhibit pseudocatalase reaction if grown in a low carbohydrate media (Korasapati, 1998)**.**

Usually, all species of pediococci grow at 30°C temperature. Their growth temperature ranges between 25-40°C. Dairy prevalent pediococci; *P. pentosaceus* and *P. acidilactici* are closely related species at phylogenetic level. Physiologically, *P. pentosaceus* is able to ferment maltose and cannot grow at 48 to 50°C, but opposite is the case for *P. acidilactici,* which can play a key role for presumptive identification of these two species (Holzapfel *et al*., 2006). Optimum pH for their growth is 6-6.5; however, they can also resist low pH. Most of the *Pediococcus* species are able to grow at 4% and 6.5% NaCl except *P. damnosus* (Abbasiliasi *et al*., 2012). Mandal *et al*. (2011) isolated a bacteriocinogenic *P. acidilactici* strain which can tolerate 10% NaCl concentration. Pediococci are chemo-organotrophs with homolactic fermentative metabolism and DL-lactate is the main fermentation product; the exception being *P. claussenii* which produces only L-lactic acid (Franz *et al*., 2006). On the basis of morphological, physiological and biochemical properties, Venkateshwari *et al*. (2010) characterized *P. pentosaceus* strain CFR B19 from beans. The isolate was catalase negative, tetra-coccus, and able to produce acid and gas from lactose, arabinose, sucrose, raffinose and maltose. It was unable to hydrolyze gelatin, starch and citrate.

APi 50CH is a test kit used for identification of the *Pediococcus* spp. which is based on carbohydrate fermentation pattern. Using APi 50CH, Mandal *et al*. (2011) characterized a pediocin NV5 producing *P. acidilactici* strain LAB 5 from vaccum packed meat product. Carbohydrate fermentation pattern of the isolate showed that it could ferment L-arabinose, galactose, glucose, fructose, ribose, N-acetyl glucosamine, esculine, cellobiose, tagatose, trehalose, gentibiose, arbutin, salicin, rhamnose, and D-xylose, but unable to ferment amygdalin, raffinose, maltose, lactose, melibiose, saccharose and inositol. Similarly,

biochemical identification of *Pediococcus* spp. using APi 50CH kit was carried by Abbasiliasi *et al*. (2012). They recorded two *P. acidilactici* strains with an accuracy of 99.6% similarity. Likewise, other researchers have also identified *Pediococcus* spp. using APi 50CH identification kit (Osmanagaoglu *et al*., 2011; Oh and Jung, 2015).

2.2.3 Genotypic characterization

Intra- and inter-species differentiations represent fundamental components of the appropriate selection of starter cultures, because most of the technological and probiotic properties are generally dependent on the strain type (Martino *et al*., 2013). Despite the commercial value of the genus *Pediococcus*, poor identification at the species and strains level is common (Santos *et al*., 2005). The routinely used phenotypic and biochemical methods for their identification are not completely discriminatory, having poor reproducibility, and are also time consuming (Calmin *et al*., 2008). Thus, a more comprehensive and pragmatic approach is required to furnish important information for complete characterization of these bacteria. Several molecular methods exhibiting various levels of inequitable power have been developed for LAB and these methods proved useful in terms of species and strain differentiation and characterization (Temmerman *et al*., 2003a; Ehrmann and Vogel, 2005; Cai *et al*., 2007; Singh *et al*., 2009).

For the identification of *Pediococcus* species, various molecular methods have been used. Fingerprinting methods like ribotyping (Barney *et al*., 2001), restriction analysis of the amplified 16S rDNA (16S-ARDRA) (Rodas *et al*., 2003), randomly amplified polymorphic DNA (RAPD)-PCR (Mora *et al*., 2000), and pulsed-field gel electrophoresis (PFGE) (Simpson *et al*., 2002) are useful for the discrimination of strains at the species and intra-species level. Day to day advancements and progress in molecular identification techniques are further believed to play a key role in peculiar characterization of *Pediococcus* spp. De Bruyne *et al*. (2008) isolated a novel *Pediococcus* species from Argentinean fermented wheat flour and identified as *P. argentinicus* spp. nov. through 16S rRNA gene sequencing which further revealed the phylogenetic position of this novel strain within the genus *Pediococcus*, with *P. stilesii*, *P. pentosaceus* and *P.*

acidilactici as its closest relatives (97.7, 97.3 and 96.9% gene sequence similarity, respectively). They found that sequence analysis of the genes which encode the alpha subunits of phenylalanyl-tRNA synthase (pheS), ATP synthase (atpA), and RNA polymerase (rpoA) is better and more reliable molecular identification methodology. Dobson *et al*. (2002) used DNA sequences of the first three variable regions of 16S rRNA gene, the 16S–23S rRNA internally transcribed spacer region sequence and a third of the 60 kDa heat-shock protein gene to reveal phylogenetic groupings within the genus *Pediococcus*. Phylogenetic trees were constructed with sequence data from 31 *Pediococcus* and 3 *Lactobacillus* strains. The phylogenetic analyses concluded that seven isolates, which were of brewing spoilage origin, belonged to a novel species, for which the name *Pediococcus claussenii* sp. *nov*. was proposed.

In another study by Pfannebecker and Frohlich (2008), large subunit rRNA gene sequences of nine *Pediococcus* type strains were analyzed and constructed genus and species specific PCR primers. They found that the constructed primers could be applied in a multiplex PCR assay for simultaneous identification of *Pediococcus* species. With this technique, the species *P. damnosus*, *P. inopinatus*, *P. parvulus*, *P. cellicola*, *P. pentosaceus*, *P. claussenii, P. acidilactici* and *P. stilesii* could be discriminated simultaneously in a single PCR run. Multilocus sequence typing (MLST) is a universal technique for characterizing bacteria based on sequence polymorphisms within internal fragments of multiple housekeeping genes. To clarify the taxonomic relationships among *P. pentosaceus* strains, Martino *et al*. (2013) developed a multilocus sequencing approach. They used a collection of 29 field strains, 1 type strain and 1 reference strain of *P. pentosaceus* and developed a MLST database for *P. pentosaceus* (http://pubmlst.org/ppentosaceus) for international communication purpose.

2.3 TECHNOLOGICAL ATTRIBUTES OF *PEDIOCOCCUS* SPP.

The role of pediococci in fermentation not only relates to the lactic acid production from sugar, but several other technologically important attributes such as production of bacteriocin (antimicrobial activity), phytase enzyme, exo-polysaccharides, flavor compounds, galactose fermentation etc make these cultures industrially important. The diverse technological performance represents

the degree of phenotypic diversity existing within the *Pediococcus* species. Diversity in technological properties is the main factor that makes bacteria essential component of numerous industrial processes (INRA, 2005).

2.3.1 Acidification profile

Among LAB, acidification is an important aspect for the selection of starter culture (De Vuyst, 2000). The souring of milk by microorganisms has been used for several years as a natural way of preservation. The major role of a starter culture is the production of lactic acid at a suitable rate to ensure a consistent and successful fermentation (Caplice and Fitzgerald, 1999). Lactate is the major end product of pediococcal carbohydrate fermentation. Reduction in milk pH leads to progressive solubilization of micellar calcium phosphate which causes demineralization and destabilization of casein micelles, resulting in precipitation of casein. The methods available for quantifying the starter activity are usually based on change in pH as a function of time, lactic acid production measured by titration (Accolas and Auclair, 1970) or the determination of lactic acid per se (Nielsen *et al*., 1989). Vidhyasagar and Jeevaratnam (2013) quantified the lactate production by *P. pentosaceus* using lactate dehydrogenase enzyme kit and found that the strain produces lactic acid in the range of 35-40 mM. In tune to this report, Sukumar and Ghosh (2010) reported lactate production by measuring titratable acidity at the rate of 2.60±0.01 (g/20 mL of supernatant) by *Pediococcus* spp. GS4. However, some *Pediococcus* species can produce acetic acid, acetoin, and CO_2 under certain conditions. In general, there is a huge strain variation in the ability of pediococci to ferment different carbohydrates.

2.3.2 Flavor production

Lactic acid bacteria are involved in several fermentation processes where characteristic flavor plays a significant role. They acidify the food, resulting in a tangy lactic acid taste. Also, as a result of their proteolytic and lipolytic activities, they produce a number of aromatic compounds. Homofermentative LAB ferment the sugar (as energy source) almost completely into lactic acid via pyruvate formation. Pyruvate itself can lead to the formation of many metabolites such as acetate, ethanol, diacetyl, and acetaldehyde. In this way, LAB produce volatile

substances that contribute to the typical flavor of fermented foods such as sourdough (determined by the lactate/acetate ratio), kefir and koumiss (ethanol), butter and buttermilk (diacetyl), and yoghurt (acetaldehyde) (Leroy and De Vuyst, 2004).

The production of acetaldehyde and diacetyl by LAB seems to be strain dependent. *Pediococcus* spp., either alone or in combination with *Lactobacillus* is involved in cheese ripening processes and reported to enhance the flavor development (Goodwins *et al*., 2008; Barouei *et al*., 2011). Diacetyl or 2,3 butanedione is a well-recognized flavoring compound which imparts desirable essence to the fermented dairy products such as dahi, butter, sour cream etc. Diacetyl is formed as an intermediate metabolite in the reductive decarboxylation of pyruvic acid to 2,3 butanediol in which pyruvic acid is derived from the metabolism of sugar, amino acid or citric acid. Diacetyl and Diketones in yoghurt is contributed only by pyruvate since thermophilic starter cultures are generally unable to metabolize citrate (Monnet and Corrieu, 2007). Similarly, pediococci usually do not utilize citrate, still strain specific capability to produce diacetyl and acetoin has been reported (Pasteris and de Saad, 2005). The deamination of serine is of great interest since this compound is liberated during cheese ripening as a result of proteolysis and acts as a precursor for flavor compounds. Irmler *et al*. (2013) correlated the diacetyl production with serine dehydratase (dsd A) activity in *P. pentosaceus*. They found that mutation in dsdA gene resulted in the loss of serine dehydratase activity, leading to no diacetyl formation.

The addition of NSLAB as adjunct cultures for cheese manufacturing increases the level of free amino acids, peptides, and free fatty acids, leading to flavor intensity and accelerated cheese ripening (Crow *et al*., 2001). The production of acetate and CO_2 by *P. pentosaceus* during lactate oxidation also contributes to the development of characteristic flavor and ripening of the cheddar cheese. Danisco has formulated a freeze-dried *P. acidilactici* culture marketed as 'CHOOZIT^{TM} Lyo. Flav 43, which is recommended to be used as an adjunct culture that accelerates and augments the flavor in cheddar and semi-hard cheeses (Papagianni and Anastasiadou, 2009). Some *Pediococcus* strains are reported to produce L-alanine; a flavor compound used in the food industry

(Irmler *et al*., 2013). *In-situ* production of L-alanine can lead to dairy products with an intrinsic sweetness.

2.3.3 EPS production

The *in-situ* elaboration of polysaccharides as valuable compounds by GRAS microorganisms is progressively holding the attention of food industries and researchers because of their potential health benefits and economic importance. LAB is one of the most common EPS producing bacteria because of their capabilities to elaborate EPS with wide diversity of structures without any health risk (Sanalibaba and Cakmak, 2016). EPS from LAB is either bounded to the bacterial surface as capsular polysaccharides (CPS) or secreted into the extracellular environment in the form of slime as free EPS (fEPS) (Fontana *et al*., 2015). Based on the composition, LAB may synthesize homo-polysaccharides or hetero-polysaccharides. Homo-polysaccharides consist of only one monosaccharide moiety such as glucose (glucans), fructose (fructans), or galactose (polygalactans) while, hetero-polysaccharides are somewhat complex polymers consisting of repeating subunits of 3-8 monosaccharides, their derivatives or substituted monosaccharides. Hetero-polysaccharides mainly consist of glucose, galactose and rhamnose with varying ratios (Torino *et al*., 2015). EPS as bio-thickener, gelling agent, emulsifier, and physical stabilizer can improve the rheology of fermented foods. Beyond sensory enhancer, other physiological benefit is that EPS remain for longer period in the GI environment, so that colonization of probiotic bacteria could be enhanced (Welman and Maddox, 2003). In addition, EPS from LAB have been claimed to show antitumor effects, antiulcer, antioxidant, immune-stimulatory activity, cholesterol lowering property etc (Torino *et al*., 2015). Bio-flocculants, drug delivery agents, bio-absorbents, heavy metal removal agents are some of the novel applications of EPS (Zajsek *et al*., 2013).

LAB strains belonging to the genera *Pediococcus*, *Lactobacillus* and *Oenococcus* isolated from cider and wine are found to produce β-glucan (Dols-Lafargue *et al*., 2008; Coulon *et al*., 2012). *Pediococcus parvulus* 2.6, a ropy strain, produces 2 substituted (1,3)-β-D-glucan and curing of its 35-kDa pPP2 plasmid resulted in the isogenic non-ropy strain (Fernandez *et al*., 1996). The production amount and composition of EPS vary considerably among strain to

strain (Song *et al*., 2013). Sabir *et al*. (2010) quantified EPS production as: 231±0.20, 276±0.01 and 230±0.00 mg/L by *P. dextrinicus* ZN 1P, *P. acidilactici* ZN 10P and *P. pentosaceus* ZN 13P strain, respectively. EPS production also differs with the growth phase as well as nutrient availability. Shukla and Goyal (2013) characterized a high mol wt. ($2.93x10^5$ Da) novel dextran (having solely glucose monomers) from *P. pentosaceus* CRAG3 strain. Generally, gene responsible for the EPS production was determined as plasmid borne gtf gene that encodes an enzyme GTF glycosyltransferase (Werning *et al*., 2006). However, they may also be located on chromosomes. Whole genome sequence analysis of *P. pentosaceus* strain LI05 and *P. pentosaceus* strain LP28 has revealed some novel EPS biosynthesis genes (Lv *et al*., 2014; Yasutake *et al*., 2016). de Palencia *et al*. (2009) depicted immune-modulatory activity of β-D-glucan producing *Pediococcus* strain compared to its non-ropy isogenic strain. The presence of EPS counteracts the proinflammatory activation of macrophages. EPS produced by pediococci is reported to: possess antitumor and antimicrobial activity, act as prebiotic and decrease serum cholesterol level (de Palencia *et al*., 2009; Ghany *et al*., 2016).

2.3.4 Phytase activity

Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexa kis dihydrogen phosphate) is the major storage form of phosphorous in cereals, legumes, nuts etc. Plant derived foods contain approximately 50-80% of their entire phosphorus as phytate. This molecule is heavily charged with six phosphate groups which extend from the central myo-inositol ring (Fig. 2.1). Phytate acts as an anti-nutritional factor for humans and animals as it chelates the cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+}. Besides, it also complexes the basic amino group of proteins, which hampers their absorption and dietary availability (Raboy, 2003). The phytate phosphorus is largely unavailable to mono-gastric animals i.e., chickens, swine, poultry animals, humans etc either due to lack/insufficient amount of phytate degrading enzymes. Since, phytic acid cannot be absorbed, feeds for pigs and poultry are commonly supplemented with inorganic phosphate. The excretion of undigested phytate along with supplementation with inorganic phosphorus imposes ecological problems (eutrophication) resulting in hypoxia, cyanobacterial blooms, and death of marine animals (Naqvi *et al*., 2000).

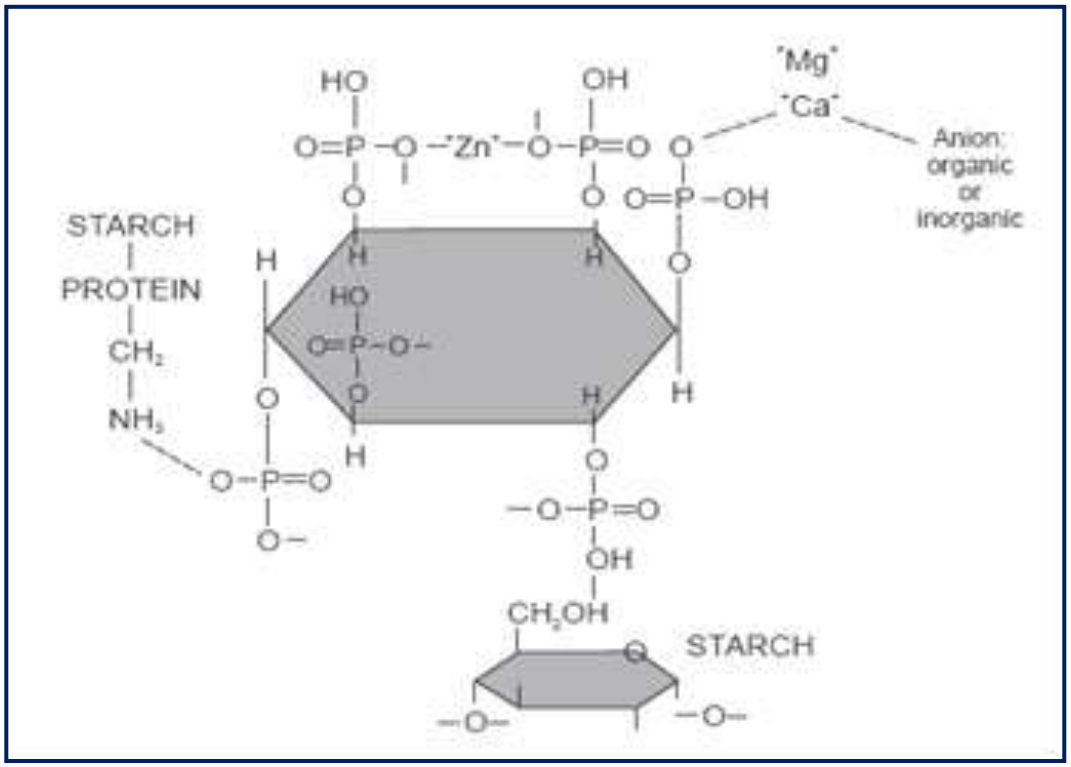


Fig. 2.1 Structure of phytic acid

Because of all these problems, there is a considerable interest in phytate-degrading enzyme i.e. phytase (myo-inositol hexakisphosphate phosphohydrolase), a monomeric enzyme which catalyzes the hydrolysis of phytic acid into myoinositol and phosphoric acid via penta- to mono-phosphate (Fig. 2.2). Phytase enzymes catalyze the hydrolysis of phosphate moieties from phytic acid, thereby, resulting in the loss of ability of phytic acid to chelate metal ions. Also, the supplementation of animal feed with phytase can increase the bio-availability of phosphorus in mono-gastric animals besides reducing the phosphorus problem in the areas of intensive livestock units (Boling *et al*., 2000).

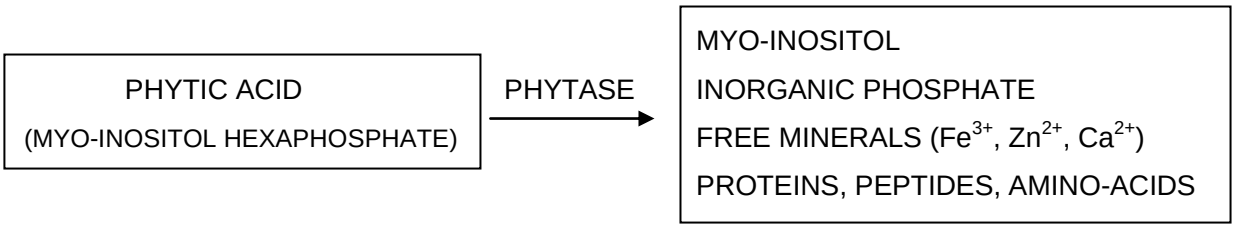


Fig. 2.2 Action of phytase on phytic acid

Phytase enzymes have been reported in cereals, legumes, oilseeds, animal tissues, and also produced by some microorganisms such as bacteria, yeast and fungi (Singh and Satyanarayana, 2015; Jain and Singh, 2016). Within GI environment, microbial phytases are more effective compared to plant phytases and are active over a wider pH range. Thus, microbial sources of phytase are most promising on a commercial level. Besides other LAB, *Pediococcus* spp. have strain specific phytase producing ability (Raghavendra and Halami, 2009; Nuobariene *et al*., 2015). Raghavendra *et al*. (2010) reported the phytate degrading ability (both sodium as well as calcium phytate) in *P. pentosaceus* CFR R123 strain which was isolated from cereal based fermented preparations. The isolate CFR R123 decreased the phytic acid levels and increased the Ca content during malted finger millet seed coat and soya milk fermentation. Thus, phytate degrading pediococci can be used as starter cultures and also as ingredient in the functional foods to increase the nutritive values. Phytase activity in pediococci has also been quantified. Cizeikiene *et al*. (2015) reported the phytase activity as 32 and 54 U/mL in *P. pentosaceus* strains KTU05-8 and KTU05-9, respectively under the bread dough leavening conditions; while, Raghavendra and Halami (2009) found it as 213 and 89 U by *P. pentosaceus* strains CFR R38 and CFR R35, respectively. Now, progress in recombinant DNA technology has resulted in higher production of phytase enzyme with enhanced functionality (Jain and Singh, 2016; Priyodip *et al*., 2017).

2.4 FUNCTIONAL ATTRIBUTES OF *PEDIOCOCCUS* SPP.

2.4.1 Probiotics: an overview

"Let medicine be your food and food be your medicine"

The worldwide market for probiotics has been increasingly growing in recent years guided by the rising consumers' demand for healthy diets and wellness. This has caused food industries and researchers to develop probiotic based food products as well as to explore specific characteristics of probiotics and their beneficial effects on the health. Probiotic is a Greek word means "prolife." The history of probiotics date back to 1907 when Russian scientist Ellie Metchnikoff postulated the idea of using LAB for modulating intestinal microflora. Since then intensive research was undertaken in the field of probiotics. The definition of a

probiotic has been evolving with increasing understanding of the mechanisms by which they influence human health (Fig. 2.3). Probiotics are well defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). Recently, 'The International Scientific Association for Probiotics and Prebiotics' on a consensus viewed the term probiotics as "live, single or mixed and concentrated form of bacterial cell threshold (10^9 CFU), which, when administered by different ways in humans and animals, grant cogent, aspired and site-targeted health benefit(s) of total safety" (Hill *et al*., 2014). Examination of each strain for their probiotic attribute(s) is essential as the mechanism of a probiotic action is strain specific (Kekkonen *et al*., 2007). Potential probiotic organisms are expected to have some desirable properties (FAO/WHO, 2002) such as resistance to gastric and intestinal juices, adhesion and aggregation capability, production of antimicrobial substances, β-galactosidase enzyme etc (Fig. 2.4). Ability to survive and grow in food systems would be an added technical advantage (Goldin, 2011; ICMR-DBT, 2011).

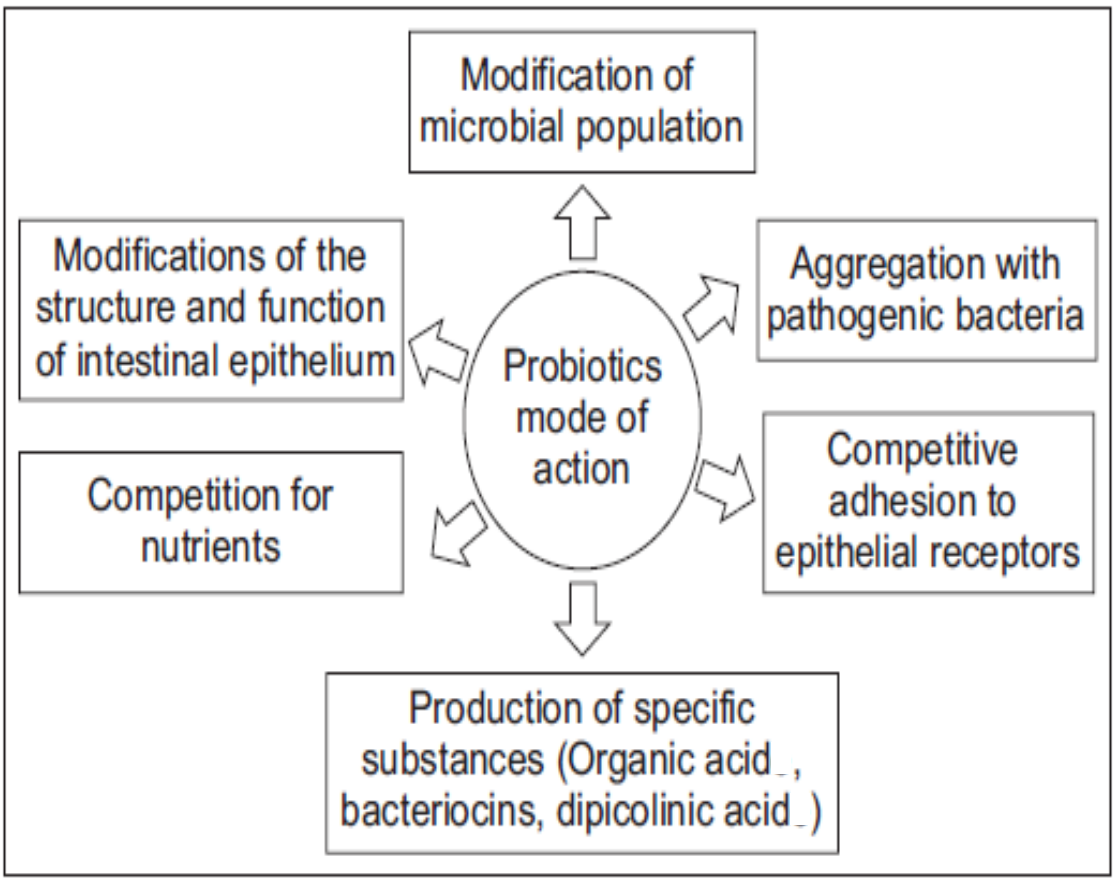


Fig. 2.3 Mechanisms of action of probiotics (Tiwari *et al*., 2012)

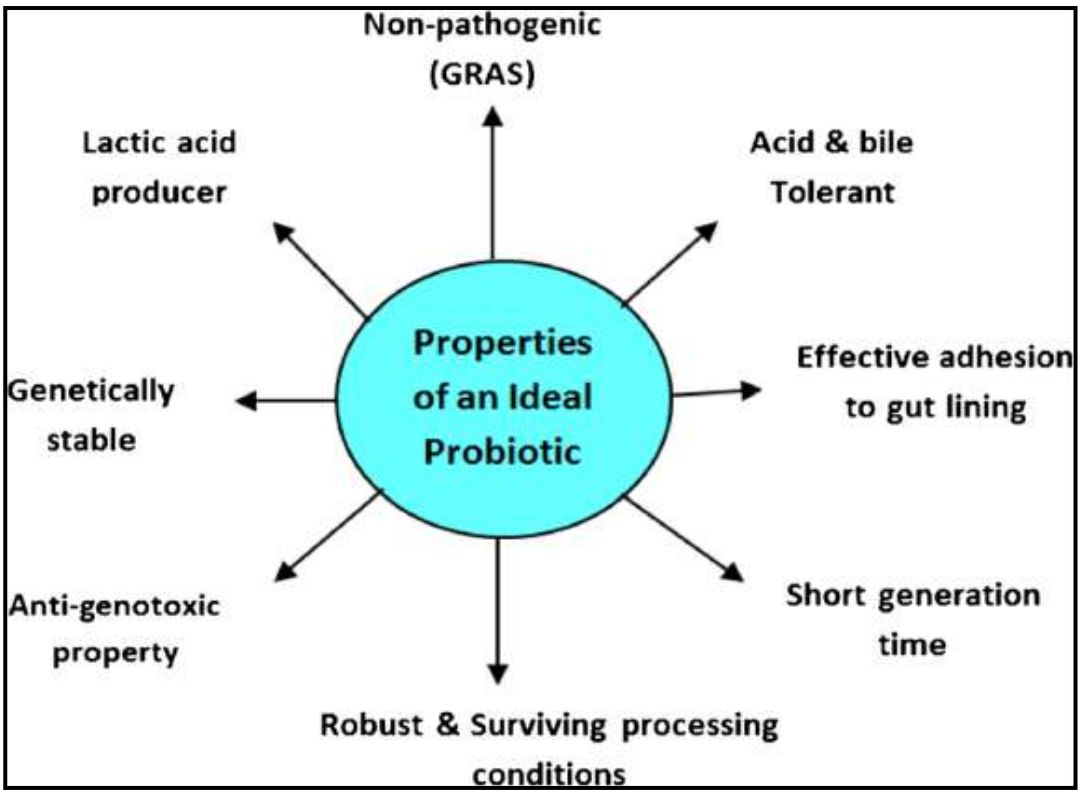


Fig. 2.4 Characteristics of an ideal probiotic strain (Pandey *et al*., 2015)

The term probiotic includes a large range of microorganisms, mainly bacteria but also yeasts (*Saccharomyces boulardii*) (Tiwari *et al*., 2012; Pandey *et al*., 2015). Members of LAB are among the major probiotic contributors as they have a long history of safe use and there are evidences to support their positive impacts. The widely-used LAB in probiotic preparations include: *Lactobacillus*, *Bifidobacterium*, *Streptococcus* and *Pediococcus* (FAO/WHO, 2002). New genera and strains of probiotics are continuously emerging with more advanced and focused research efforts.

2.4.2 Survival in the GI tract

The GI tract is the site where probiotics are believed to exert most health-modulating activities. Thus, probiotics should have the ability to survive the harsh conditions of GI environment such as low pH in the stomach, and bile of the small intestine (Mathara *et al*., 2008). Tolerance of LAB to acid and bile is strain dependent, hence, requires individual evaluation. Gastric pH of healthy human is about 2-2.5 (Fernandez *et al*., 2003), but it could be as low as 1.5, or as high as 6.5 after food intake. The gut transit time of food can vary between 2 to 4 h. The

physiological concentration of bile acids in the small intestine is between 5,000 to 20,000 µM (Pinto *et al*., 2006). Zavaglia *et al*. (1998) considered 0.3-0.5% ox-bile as a crucial concentration to evaluate a bile tolerant probiotic LAB.

Several studies revealed the tolerance of pediococci strains towards such harsh environments (Ruiz-Moyano *et al*., 2008; Badarinath *et al*., 2010; Osmanagaoglu *et al*., 2010; Jensen *et al*., 2012; Mukherjee *et al*., 2013; Federici *et al*. 2014; Noohi *et al*., 2016). A study conducted by Attri *et al*. (2015) depicted that *P. acidilactici* NCDC 252 could survive up-to 6 h at pH 2. Survival was declined with decrease in pH and increase in exposure time; however, the survival percentage was significant even at pH 2 up-to 6 h. Cao *et al*. (2016) studied the probiotic features of *P. pentosaceus* L1 strain isolated from artisan pickled radish. The strain retained its viability with a negligible reduction (<0.3 log CFU/mL) for up-to 3 h and a minor reduction (<0.3 log CFU/mL) after 4 h in simulated gastric juice and simulated intestinal juice, respectively.

2.4.3 Adhesion properties

Adhesion to host gut epithelial cells and intestinal mucus is an important aspect of a probiotic strain for colonization of the GI tract and stimulation of beneficial health effects (Shah, 2001; Holzapfel and Schillinger, 2002). Bacterial surface properties have been associated with the attachment to a number of substrates, which in turn is linked with hydrophobicity i.e. Cell surface hydrophobicity (Aswathy *et al*., 2008). Bacterial cells exhibiting higher hydrophobicity may form strong interaction with mucus or epithelial cells (Shobharani and Agrawal, 2011). Microbial adhesion to hydrocarbons (MATH) test has been extensively used for measuring cell surface hydrophobicity in LAB and bifidobacteria (Gomez Zavaglia *et al*., 2002; Vinderola *et al*., 2004; Canzi *et al*., 2005). Various strains of probiotic bacteria possess different adhesive properties which may vary from 2 to 95% (Prakash *et al*., 1997; Schillinger *et al*., 2005).

Bacterial adhesion also determines the colonization ability of a microorganism. In order to manifest beneficial effects, probiotic bacteria need to achieve an adequate mass through aggregation. Aggregation between microorganisms of the same strain (auto-aggregation) or between genetically different strains (co-aggregation) is of considerable importance in several ecological niches,

especially in human gut, where probiotics need to be active (Jankovic *et al*., 2003). Hydrophobicity and auto-aggregation of microorganisms are phenotypically related to its adhesion capacity (Del *et al*., 2000). Various reports showed that pediococci have strain specific hydrophobicity and aggregation ability (Raghavendra *et al*., 2010; Cao *et al*., 2016; Ilavenil *et al*., 2016). Osmanagaoglu *et al*. (2010) depicted that *P. pentosaceus* OZF originated from human breast milk auto-aggregated at 85.71% after 5 h. In another report, 6 strains of *P. pentosaceus* isolated from idly batter exhibited significant auto-aggregation properties (≥42%) after 5 h (Vidhyasagar and Jeevaratnam, 2013). Attri *et al*. (2015) reported hydrophobicity by *P. acidilactici* NCDC 252 as 23.15% (for xylene) and 11.78% (for toluene); while with the same strain, Garsa *et al*. (2014) reported it as 17.71, 15.96 and 8.87% for xylene, n-octane and n-hexadecane, respectively.

2.4.4 Antimicrobial activity

One of the desirable properties of a probiotic strain is the ability to produce antimicrobial substances. Lactic acid bacteria produce several antimicrobial compounds like organic acids, hydrogen peroxide, bacteriocin etc which prevent the growth of pathogenic microorganisms (Herreros *et al*., 2005; Tiwari *et al*., 2012). Strains of pediococci are reported to exhibit broad spectrum of inhibition against a number of Gram-positive (*Staphylococcus aureus*, *Clostridium perfringens*, *Listeria monocytogenes*, *Bacillus* spp. etc) and Gram-negative (*Escherichia coli*, *Vibrio parahaemolyticus*, *Pseudomonas* spp., *Salmonella* enterica ssp. Typhi etc) spoilage and pathogenic microbes (Tadesse *et al*., 2005; Badarinath *et al*., 2010; Bajpai *et al*., 2016; Noohi *et al*., 2016). Their antifungal activity against *Aspergillus*, *Rhizopus* and *Penicillium* has also been reported (Garg and Kaur, 2015). Beside others, one of the most probable reason for their antagonism is production of bacteriocins. Bacteriocins are antimicrobial peptides which inhibit the microorganisms that are usually closely related to producer strain (Klaenhammer, 1993). A number of bacteriocins elaborated by LAB have been discovered and characterized (Galvez *et al*., 2007). Bacteriocins elaborated by *Pediococcus* spp. are known as pediocins which are characterized as small (36-48 residues), cationic, membrane active peptides, possessing YGNGVXC consensus motif (Papagianni and Anastasiadou, 2009; Porto *et al*.,

2017). Pediocins are also termed as 'Listeria active' because of their strong anti-listerial property (Jang *et al*., 2015). A number of pediocins along with their structure, mode of action and inhibition spectrum have been reported (Bennik *et al*. 1997; Rodriguez *et al*., 2002; Papagianni and Anastasiadou, 2009; Martino *et al*., 2013; Suganthi and Mohanasrinivasan, 2015; Turgis *et al*., 2016). The cultures with antagonistic properties may find their applications in food preservation system as bio-protective cultures.

2.4.5 β-galactosidase activity

Lactose, a predominant disaccharide in dairy products is hydrolyzed by β-galactosidase/lactase enzyme into its constituent monosaccharides, i.e. glucose and galactose. Deficiency of this enzyme lead to lactose intolerance which is characterized by abdominal discomfort, flatulence, diarrhea etc after intake of milk (Lactose Intolerance-Scientific status report, 2011). Lactose intolerance is very common among almost 70% of world adult population (Pal *et al*., 2013). So, probiotic bacteria with β-galactosidase activity may have the potential to alleviate the symptoms of lactose intolerance. The occurrence and importance of this enzyme in plants, animal tissues and in microbes including fungi, yeast and bacteria has been well documented (Kokkiligadda *et al*., 2016). However, the enzyme derived from LAB is more appealing because of their safety aspects and frequent usage in food fermentations.

Generally, most of the pediococci are unable to ferment lactose, however, some strains are found positive for β-galactosidase enzyme (Vidhyasagar and Jeevaratnam, 2013; Attri *et al*., 2015). Bhowmik and Marth (1990) reported the intracellular lactose-inducible β-galactosidase enzyme in *Pediococcus* strains. Similarly, Halami and Chandrashekar (2005) demonstrated the β-galactosidase activity in bacteriocinogenic *P. acidilactici* strain C20. The production of this enzyme is highly influenced by the environmental and fermentation parameters (Kamran *et al*., 2016). β-galactosidase activity has also been quantified and interestingly, Raghavendra *et al*. (2010) reported the enzyme activity in *Pediococcus* strain CFR R123 as 413 MU which was even higher than that of the reference strain *Lb. rhamnosus* GG (105 MU). Thus, β-galactosidase positive strains may find dual applications, that is, for their use as probiotics for lactose intolerance and as starter cultures in fermented dairy products.

2.4.6 Hypo-cholesterolemic, antioxidative, immune-modulatory and other beneficial activities

Besides satisfying the basic probiotic criteria, pediococci strains possess additional health promoting properties which make them even more attractive to study and explore. *Pediococcus* spp. are reported to have hypo-cholesterolemic effects, *in-vitro* (Banwo *et al*., 2013) as well as in animal models (Damodharan *et al*., 2015). Cholesterol lowering effect can occur through deconjugation of bile acids with the help of Bile salt hydrolase (BSH) enzyme or by the cholesterol assimilation mechanism. Bile salt hydrolase enzyme deconjugates the conjugated bile salts and thus helps in cholesterol removal (Patel *et al*., 2010). Strains of pediococci are found to elaborate BSH enzyme (Shukla and Goyal, 2013; Garsa *et al*., 2014; Banwo *et al*., 2013). However, microbial BSH is still a debatable issue as it is reported to exhibit deleterious effects in the host such as malabsorption of lipids in the intestine (Begley *et al*., 2006). Pediococci also exhibit the antioxidative and immune-modulatory property (Jonganurakkun *et al*., 2008; Osmanagaoglu *et al*., 2013; Kuda *et al*., 2014). Castex *et al*. (2009) found that feeding probiotic *P*. *acidilactici* to healthy shrimps significantly increased the total antioxidant status and glutathione peroxidase activity. Modulation of the immune system can occur through intrinsic adjuvanticity and cytokine inducing properties. Immune-modulatory effect of ropy pediococcal strain was observed through induced production of anti-inflammatory cytokine IL-10 (de Palencia *et al*., 2009). Further, pediococci are also believed to be associated with anti-stress effect (Jang *et al*., 2010), found to reduce inflammation, obesity and fatty liver (Kuda *et al*., 2014; Higashikawa *et al*., 2016).

2.5 SAFETY ASPECTS OF *PEDIOCOCCUS* SPP.

With the increase in consumer awareness towards nutrition and health, it is important that strains used in food preparations, should be safe for all potential consumers. Survival, colonization, and fate of active components produced by microbial cultures need to be evaluated to predict not only the beneficial health aspects but also for any deleterious side effects such as D-lactate production, biogenic amine production, transferable-antibiotic resistance, gelatin degradation, haemolytic activity, DNase activity etc (FAO/WHO, 2002). The

absence of these objectionable parameters approves the use of screened microbial cultures for numerous food and feed applications.

Pediococci are generally considered safe as they have long history of consumption in fermented foods (Erkkila and Petaja, 2000; Huys *et al*., 2006; Ruiz-Moyano *et al*., 2010; Ribeiro *et al*., 2014). *P*. *parvulus* DSM28875 recently granted QPS status due to the absence of virulence determinants and pathogenicity in humans and animals (EFSA, 2016). Although, cases of infection have been reported by several *Pediococcus* strains in immune-compromised patients. So, it is imperative novel probiotics to have their safety assured. With regard to haemolysis, LAB are fairly considered safe (Gao *et al*., 2012). A clear zone of hydrolysis around the colonies on blood agar indicates complete haemolysis i.e. β-haemolysis, a partial hydrolysis and greening zone indicates α-haemolysis while no reaction means γ-haemolysis. Beta-haemolysis is considered as the risk factor for the consumers. Pediococci are either non haemolytic (Vidhyasagar and Jeevaratnam, 2013) or some strains may show α-haemolysis after prolonged incubation on blood agar (Riebel and Washington, 1990; Oh and Jung, 2015). The presence of biogenic amines (BAs) in food is of great concern to the food industry and to the regulatory agencies. The occurrence of high amounts of BAs may undesirably alter the organoleptic properties of food products, and the consumption of these foods can cause several toxicological illnesses including respiratory distress, headache, hyper- or hypo-tension, allergies etc. The production of BAs in pediococci is either absent or in trace amount only, which is well within the regulatory limit (Bover-Cid and Holzapfel, 1999; Tome *et al*., 2008; Abriouel *et al*., 2012). Ruiz-Moyano *et al*. (2010) evaluated preselected pediococci for biogenic amine production. All the twelve isolated *P. acidilactici* strains showed a low capacity for biogenic amine production (<100 mg/L), since none of these isolates presented a clear activity on the decarboxylase medium.

Generally, pediococci produce either racemic mixture of lactic acid (DL-lactic acid) or some strains may produce L-lactate as their metabolic end product (Liu, 2003). Low level of D-lactate production (10%) is reported by Ruiz-Moyano *et al*. (2010) in *P. acidilactici* strain SP979, which is within the regulatory limit. Unlike

L-lactic acid, D-lactate is metabolized relatively slowly and can accumulate in the blood to cause neurotoxicity, and can also be responsible for metabolic acidosis. Albano *et al*. (2009) have reported L/D-lactic acid production in ratio of 3:1 from a bacteriocin-producing strain of *P. acidilactici*.

The absence of acquired antibiotic resistance is another safety criterion to be tested in potential probiotic candidates. Some probiotics are closely related to opportunistic bacteria and this may cause transfer of antimicrobial resistance genes in between microbes. Resistance to antibiotics among microorganisms is increasing rapidly (Davis *et al*., 2007). This is a result of widespread and uncontrolled consumption of antibiotics in both human and animals. Resistance of bacteria to antibiotics can be inherent or may be acquired (Sharma *et al*., 2014). Inherent resistance can be looked as a species trait and is an innate property. Acquired resistance to antibiotics, however, is caused by genetic alterations (Danielsen and Wind, 2003). Intrinsic resistance is not horizontally transferable and poses no risk in nonpathogenic bacteria (Ammor *et al*., 2007).

Generally, pediococci are found susceptible to the clinically important antibiotics and no acquired resistance to these antimicrobials has been reported (Noohi *et al*., 2016), and so is also for multidrug resistivity (Banwo *et al*., 2013). Penicillin G, chloramphenicol and erythromycin are usually active antibiotics against *Pediococcus* spp. (Danielsen *et al*., 2007). Ruiz-Moyano *et al*. (2010) found that all the studied *P. acidilactici* strains were sensitive or moderately sensitive to tetracycline, although strains of *P. acidilactici* have been described as having resistance to this antibiotic by Albano *et al*. (2009). Hummel *et al*. (2007) investigated the low-level of tetracycline resistance among *Pediococcus* strains used for the manufacture of fermented sausages, but no known resistance genes were detected. Resistance to vancomycin (a glycopeptide) is a common feature among LAB; fortunately, this resistance is non-transferable (intrinsic resistance) in case of *Pediococcus* spp. due to a modified peptidoglycan precursor ending in D-Ala-D-lactate (Barton *et al*., 2001). Similar resistance to aminoglycosides such as kanamycin, gentamicin and streptomycin is also an intrinsic property among *Pediococcus* species (Hummel *et al*., 2007).

2.6 PEDIOCOCCI AS STARTER AND NON-STARTER/ADJUNCT CULTURES

The earlier production of fermented food products was based on spontaneous fermentation due to the development of microflora naturally present in the raw material. Spontaneous fermentation was optimized through back-slopping, i.e., inoculation of the raw material with a small quantity of previously performed successful fermentation. But, today, the production of fermented foods by spontaneous fermentation and back-slopping represents a cheap preservation method in developing countries, whereas in Western countries the large-scale production of fermented foods has become an important branch of food industry. Direct addition of selected starter cultures to raw materials has been a breakthrough in the processing of fermented foods, resulting in a high degree of control over the fermentation process and standardization of the end product. Recently, new starter cultures of LAB with an industrially important functionality are being developed (Leroy and De Vuyst, 2004). A starter culture is defined as "microbial preparation of large numbers of cells of at least one microbe, deliberately added to a raw material with the aim to ferment the food by accelerating its fermentation process".

Pediococci play a significant role in the fermentation of meat, sausages, vegetables, bread sourdoughs, cereals, silage etc due to rapid acidification (Erkkila and Petaja, 2000; Kleinschmit and Kung, 2006; Yuksekdag and Aslim, 2010). Unfortunately, milk is not a suitable growth medium for pediococci as lactose is not readily hydrolyzed by them and secondly unavailability of required growth factors in milk; which limits their direct application as dairy starter (Somkuti and Steinberg, 2010). Pediococci are commonly used as secondary starter, non-starter or as adjunct cultures in cheese industry to accelerate cheese ripening (Franciosi *et al*., 2008). As non-starter and adjunct culture, *Pediococcus* spp. imparts desirable attributes to milk products which suggests that pediococci may be a good dairy starter if they possess the ability to ferment lactose. Genetic recombination techniques have facilitated the introduction of lactose-positive phenotypes into *Pediococcus* spp. Thus, the development of gene transfer system provides new dimensions to investigate applications of pediococci in dairy environment (Morita *et al*., 1991; Caldwell *et al*., 1996).

In yogurt fermentation, lactose in milk is hydrolyzed to glucose and galactose by the β-galactosidase enzyme present in both *Lb. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* that are used together as yoghurt starter culture. However, it has been shown that many *S. thermophilus* strains metabolize only glucose, but not galactose (Mora *et al*., 2002; De Vin *et al*., 2005). Lower levels of galactose in fermented dairy products can be beneficial for human health, but too high galactose consumption can lead to the accumulation of toxic galactitol in human cells (Novelli and Reichardt, 2000). Galactose is also a major contributor to the cheese browning that occurs during heat applications such as the manufacturing of pizzas or processed cheese (Mukherjee and Hutkins, 1994). Additionally*, S. thermophilus* is phage sensitive which causes a huge loss mainly to the dairy industry. Pediococci, being homofermentative, ability to utilize galactose and to grow at 45°C; lactose-positive pediococci can be a suitable replacement for *S. thermophilus* in Italian starter blends. This may enable the development of new strain rotation schemes to combat the bacteriophage sensitivity and galactose fermentation problems associated with *S. thermophilus* (Caldwell *et al*., 1998). Caldwell *et al*. (1996) successfully transformed two *Pediococcus* species with a naturally occurring 35-kb lactose plasmid pPN-1 from *Lc. lactis*. They found that both the lactose-positive transformants efficiently acidified the milk and neither of them was attacked by bacteriophages in whey medium.

Ensiling is a traditional way to preserve forages, worldwide. Pediococci have the potential to improve the silage production by influencing its fermentation characteristics such as reducing the pH, inhibiting the undesirable microbes, preserving the nutritive value and palatability of dry matter (Kleinschmit and Kung, 2006). Probiotic effects are well reported in ruminants fed with LAB fermented silages. As a starter, pediococci are commercially available for pickles, meat, and many other products. In many countries, pediococci are used in the preparation of several fermented vegetable products such as Burong mustala (Philippines), Dua muoi (Vietnam), Gundruk (Nepal, India), Inziangsang (India), Kimchi (Korea), Olive (Spain, Italy), Pak-Gard-Dong (Thailand), Sinki (India, Nepal, and Bhutan), Sunki (Japan) etc and have been reported to increase the shelf life of fruits and vegetables (Swain *et al*., 2014). *Pediococcus cerevisiae* was the first LAB meat starter culture used in USA (Erkkila and

Petaja, 2000). A few *Pediococcus* strains are officially approved as zootechnical feed additives (EFSA, 2012).

Industrialized food has experienced a boost in the usage of chemical additives in their formulations; however, consumers are increasingly echoing their concern about using these additives in their diet. This fact, associated with the increasing demand for minimally processed foods has encouraged the search for bio-preservatives. Pediococci as such or their formulations are used as bio-protective agents due to their antimicrobial nature. As food preservative, formulation containing pediocin by *P. acidilactici* is available in the market under the commercial name 'Alta 2341'. This formulation is known to extend shelf life of a variety of foods, particularly by inhibiting the growth of *L. monocytogenes* in ready-to-eat products (Papagianni and Anastasiadou, 2009; Garsa *et al*., 2014). As a silage additive, Pediocin SA-1 by *P*. *acidilactici* NRRL B-5627 is used as it inhibits the growth of pathogenic bacterium and does not affect the beneficial microflora (Amado *et al*., 2016).

Due to the probiotic potential of pediococci, they can also exploit as adjunct in functional food and feed formulations. A number of studies have shown the positive influences of *Pediococcus* species (mainly *P. acidilactici*) on the growth performance of chicken, lambs, trouts etc (Taheri *et al*., 2010; El-Katcha *et al*., 2016). These bacteria have the ability to induce healthy intestinal conditions in animals and livestock. Several commercial feeds containing probiotic pediococci (*P. pentosaceus* and *P. acidilactici*) are available in the market (Vanbelle *et al*., 1990). 'MitoMaxSUPER' is a probiotic product which contains high concentration of live *P. acidilactici* and *Saccharomyces boulardii*, along with prebiotic, mannanoligosaccharide (MOS). The pediococci alone or in combination as multispecies probiotics is also reported to act as alternative medicines against infectious pathogens (Ghareeb *et al*., 2012). Another probiotic containing preparation for aquaculture is 'Bactocell' having *P. acidilactici* MA 18/5M strain (Juskiewicz *et al*., 2007).

CHAPTER –3

Materials & Methods

The composition of all the media, buffers and reagents used are presented in Appendix I and II. The media, media ingredients, chemicals and reagents, used were procured from Sigma-Aldrich (CA, USA), HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SRL (Mumbai, India) and Qualigens (Mumbai, India). All reagents, enzymes and oligonucleotides used for molecular studies were purchased from Genetix Biotech Asia Pvt. Ltd. (India) and Sigma-Aldrich (CA, USA). Skim milk powder was procured from Modern Dairy Pvt. Ltd., Karnal.

3.1 ISOLATION OF *PEDIOCOCCUS* SPP.

3.1.1 Collection of samples and reference cultures

A total of 115 samples comprising fermented batter of dosa, idli, kadhi, jalebi, raabdi, uttapam; pickles; spoiled beer and wine; dairy products including cow milk, buffalo milk, cheese, curd, and cream were collected from different hostels of ICAR-NDRI, Karnal, Institute's Experimental Dairy Plant, local market, house hold preparations, rural and urban areas of Karnal, and also from Delhi, Hisar and Jind. Silage samples were collected from cattle yard, ICAR-NDRI, Karnal, human faeces and human milk, from voluntaries from ICAR-NDRI, Karnal, chicken sausage from Yummiez (Godrej, India), fermented vegetables (cabbage, carrot) and fermented grape juices were prepared in laboratory. Samples were collected in sterile sample collection bottles (HiMedia, India). MRS medium (de Mann *et al*., 1960) (Appendix I) was used for isolation of *Pediococcus* spp.

Pediococcus pentosaceus NCDC 38, 273; *P. acidilactici* NCDC 609, collected from National Collection of Dairy Cultures (NCDC), ICAR-NDRI, Karnal were included as reference cultures. Freeze dried cultures were activated in MRS broth (37°C/24 h) and stored under refrigerated conditions (4°C). Active culture was prepared by sub-culturing twice in MRS broth.

3.1.2 Sample preparation

For isolation of *Pediococcus* spp., samples were processed as follows:

a) Raw milk (cow, buffalo, human), butter milk, beer, wine and grape juice: Ten millilitre of sample was transferred to 90 mL of normal saline (Appendix I) and mixed well.

b) Cream, curd, silage, batter (dosa, idli, uttapam, jalebi, raabdi, kadhi): Ten gram of aseptically weighed sample was mixed with 90 mL of normal saline (warmed to 45°C for cream sample).

c) Cheese: Ten gram of aseptically weighed cheese sample was grounded to fine slurry with the help of sterilized mortar and pestle in 90 mL of sterile 2% (w/v) sodium citrate solution (warmed to 40-45°C).

d) Human faeces: One gram of faeces was mixed with 9 mL of normal saline.

e) Pickle, sausages, fermented vegetables: Ten gram of aseptically weighed sample was grounded to fine slurry with the help of sterilized mortar and pestle in 90 mL of normal saline.

3.1.3 Procedure for isolation

Serial dilutions of the processed samples were prepared in normal saline. Appropriate dilutions were pour plated using MRS agar and incubated at 37°C for 48-72 h. Typical smooth, round, greyish-white colonies were randomly picked up, transferred to MRS broth and incubated at 37°C for 24-48 h.

3.1.4 Morphology and catalase test

Morphology of isolates was examined under microscope (100X) by negative (Nigrosin stain) as well as Gram staining. Gram-positive cocci in pairs/tetrads were further subjected to catalase test. Catalase negative, Gram-positive cocci in tetrads were studied further.

3.1.5 Physiological characterization of isolates

3.1.5.1 Culture preparation

Pediococcus isolates were grown in MRS broth at 37°C for 18-24 h. The cell biomass was harvested by centrifugation at 10,000 rpm for 15 min at 4°C. Cell pellet was washed twice with normal saline and unless otherwise stated

resuspended in equal amount in normal saline. This was used as an inoculum (@ 1%) unless otherwise stated for various tests.

3.1.5.2 Pseudocatalase test

MRS broth active cultures were streaked on low glucose agar plates (Appendix I) and incubated at 37°C for 48-72 h. Plates were flooded with 5 mL of 3% hydrogen peroxide (Sigma-Aldrich, USA) and observed up-to 30 min. Presence of effervescence indicated positive for pseudocatalase reaction (Felton *et al*., 1953).

3.1.5.3 Oxidase test

MRS broth active cultures were streaked on MRS agar plates and incubated at 37°C for 48-72 h. Colonies were picked up and rubbed on the oxidase disc (HiMedia, India). Development of purple blue color indicated positive reaction.

3.1.5.4 Heterofermentation test

The ability of the cultures to ferment glucose with gas production was examined by inoculating cell suspension (section 3.1.5.1) in MRS broth with immersed, inverted Durham's tubes and incubating at 37°C for 24-48 h. Accumulation of gas in Durham's tubes indicates heterofermentation (Muller, 1990).

3.1.5.5 Arginine hydrolysis

Hydrolysis of arginine was examined by inoculating the cell suspension in 5 mL of arginine broth (Appendix I) and incubating at 37°C for 24-48 h. Release of ammonia from arginine was tested by adding 1 mL of Nessler's reagent (HiMedia, India). Appearance of orange-brown to brick-red color was taken as positive for arginine hydrolysis (Hitchener *et al*., 1982).

3.1.5.6 Growth at different temperatures

The ability of the cultures to grow at different temperatures was tested by inoculating the cell suspension in 5 mL of MRS broth tubes and incubating at 30, 37, 42 and 50°C for 24-72 h. The growth was assessed by observing the turbidity of broths.

3.1.5.7 Growth at different pH

The ability of the cultures to grow at different pH was tested by inoculating the cell suspension in 5 mL of MRS broth tubes with pH 4.2, 6.5, 8.5 and 9.6 adjusted with 3N HCl or 3N NaOH and incubating at 37°C for 24-72 h. The growth was assessed by observing the turbidity of broths.

3.1.5.8 Growth at different salt concentrations

The ability of the cultures to grow at different salt concentration was tested by inoculating cell suspension in 5 mL of MRS broth tubes with 4, 6.5, 10 and 15% NaCl and incubating at 37°C for 24-72 h. The growth was assessed by observing the turbidity of broths.

3.2 IDENTIFICATION OF *PEDIOCOCCUS* ISOLATES

3.2.1 Biochemical identification using APi 50CH kit

Putative *Pediococcus* isolates based on morphology and physiological characteristics were identified biochemically using APi 50CH kit (bioMerieux, France). Active isolates were streaked on MRS agar and incubated at 37°C for 24-48 h. Two to three loop full of streaked culture was mixed with APi 50 CHL medium and homogenized well. The tubes were filled with the inoculated APi 50 CHL medium, and overlaid with mineral oil to create anaerobic environment. Incubation chambers provided with the kit were filled with sterile distilled water/saline to create moist environment. Incubated the strips with incubation chamber at 37°C for 24-48 h. All the tests were recorded after 24-48 h of incubation. A positive reaction corresponds to the development of yellow color. For esculin test (tube no. 25), a change from purple to black was taken as positive. Observations were recorded in the result sheet and were analyzed with the help of bioMerieux identification software (www.apiweb.biomerieux.com)**.**

3.2.2 Genotypic identification by PCR

The biochemically identified *Pediococcus* isolates were subjected to genotypic characterization by genus and species specific PCR. The composition of all the reagents used in this study is given in Appendix II.

3.2.2.1 Isolation of genomic DNA

The genomic DNA of all the biochemically identified *Pediococcus* isolates was extracted following the method of Pospiech and Neumann (1995). Cells were harvested from actively grown cultures in 1.5 mL micro-centrifuge tubes by using refrigerated centrifuge (Sigma-Aldrich, USA) at 8,000 rpm for 10 min. Without disturbing the pellet, the supernatant was carefully discarded and the cell pellet was washed twice with sterile Milli Q water or TE buffer. The cell pellet was resuspended in 500 µL of SET buffer (pH 8.0). The lysozyme was added to the above suspension @ 1 mg/mL and incubated at 37℃ for 1 h. The above step was followed by addition of 1/10th (50 µL) volume of 10% SDS, and further incubated at 37℃ for 30 min with occasional inversions. After that, one third volume (150 µL) of 5M NaCl and one volume (750 µL) of chloroform: isoamyl alcohol (24:1) were added to the above mixture and incubated at room temperature for 30 min with frequent inversions. The samples were centrifuged at 4,000 rpm for 15 min at 4℃ and aqueous phase was transferred to new 1.5 mL micro-centrifuge tube. The DNA was precipitated by adding one and half volume of chilled isopropanol (600 µL) and the tubes were inverted gently to mix and incubated at -20℃ for either 30 min or overnight. The DNA pellet thus obtained was recovered after centrifugation at 12,000 rpm for 10 min at 4℃. The pellet was washed with 70% alcohol, air dried under vaccum for 10 min. The DNA pellet was finally dissolved in 50 µL of TE buffer (pH 8.0) and stored at -20℃. The concentration of DNA was estimated using Infinite® 200 PRO NanoQuant® (Tecan trading AG). The integrity of DNA was checked by running extracted genomic DNA on 0.8% agarose gel in electrophoretic unit (Thermo Scientific, Schwerte, Germany) at a constant voltage of 50 V (60 mA).

3.2.2.2 Genus and species specific primers

The isolated DNA of each culture was used to perform PCR using genus and species specific forward and reverse oligonucleotide primers (Table 3.1) (Pfannebecker and Frohlich, 2008). Firstly, genus specific PCR was performed. After the confirmation of *Pediococcus* genus, PCR was performed for species level identification based on APi results.

Table 3.1 Primers used for identification of *Pediococcus* spp.

Specificity (Genus/species)	Target gene	Primer	Sequence (5'-3')	Length (bp)	Product size (bp)
Genus *Pediococcus*	23S rRNA	Pedio23S_F	-GAACTCGTGTACGTTGAAAAGTGCTGA-	27	701
		Pedio23S_R	-GCGTCCCTCCATTGTTCAAACAAG-	24	
P. pentosaceus		PPE23S_F	-CCAGGTTGAAGGTGCAGTAAAAT-	23	1647
		P23S_R	-CTGTCTCGCAGTCAAGCTC-	19	
P. acidilactici		PAC23S_F	-GTTTCGGAGGAGGCGCAA-	18	213
		P23S_R	-CTGTCTCGCAGTCAAGCTC-	19	

3.2.2.3 PCR reaction conditions

The reaction mixture constituting 10X PCR buffer (containing $MgCl_2$), dNTPs and primers were prepared and distributed into reaction tubes as per the requirements (Table 3.2). The final volume of PCR mix was adjusted to 25 µL. PCR tubes were transferred to thermocycler (Eppendorf Master cycler, Germany) already adjusted to the conditions required for specific amplification (Table 3.3). Reaction was terminated by cooling the constituents to 4°C and PCR product was stored at -20°C.

Table 3.2 PCR master mix composition

Reagents	Concentration	Volume (µL)
Sterile nuclease free deionized water	----	18.7
10X reaction buffer with $MgCl_2$ (15 mM)	1 X	2.5
dNTP mix (2.5 mM)	0.2 mM	0.5
Primer forward	0.1-0.5 µM	0.5
Primer reverse	0.1-0.5 µM	0.5
Taq DNA polymerase	0.03-0.05 U/µL	0.3
Template DNA	50-100 ng/µL	2.0

Table 3.3 PCR cycling steps for genus and species specific primers

Steps		***Pediococcus* genus**	***P. pentosaceus*/*P. acidilactici***
1	Initial denaturation	95°C/5 min	95°C/5 min
2	Denaturation	94°C/30 sec	94°C/30 sec
3	Annealing	69.5°C/30 sec	66°C/1 min
4	Extension	72°C/30 sec	72°C/1 min
5	Final extension	72°C/5 min	72°C/10 min
	Number of cycles (Step 2-4)	32	22

3.2.2.4 Analysis of PCR products by agarose gel electrophoresis

PCR amplified products were electrophoresed on (1-2%) agarose gel (Sambrook *et al*., 1989). DNA ladder (Bangalore Genei, Bangalore, India) of 100 bp and 1 kb were used as molecular size marker depending on the PCR product size. Electrophoresis was carried out in 1X TAE buffer (pH 8) at 100 V (60 mA current) in electrophoretic unit (Thermo Scientific, Schwerte, Germany). After completion of electrophoresis, the gels were examined using gel Doc system (Alpha Innotech Corp, Santa Clara, CA, USA) and photographed using AlphaView SA software.

3.3 MAINTENANCE AND PRESERVATION OF *PEDIOCOCCUS* CULTURES

All the cultures were maintained in MRS broth under refrigerated conditions. Long term preservation of cultures was carried out in 30% glycerol stocks (Appendix I) and in lyophilized form in ampules.

3.4 EVALUATION OF TECHNOLOGICAL ATTRIBUTES OF *PEDIOCOCCUS* CULTURES

Various technological attributes viz. acidifying activity, phytase activity, EPS production, diacetyl production and galactose fermentation were evaluated for all the 40 isolated *Pediococcus* cultures along with 3 NCDC cultures.

3.4.1 Acidification profile

The acidification profile of *Pediococcus* cultures was evaluated in MRS broth, CHL broth containing 1% filter sterilized lactose (Appendix I), Skim milk (12%) and skim milk supplemented with 0.216% protein hydrolysate. The media were supplemented with bromocresol purple (BCP) dye @ 0.017%.

All the four media tubes were inoculated with cell suspension (@ 1%) and incubated at 37°C. Change in color from purple to yellow along with change in pH was determined at 0, 6, 12 and 24 h. The change in pH was measured using pH meter (Thermo Electron, USA).

3.4.2 Phytase activity

Method for detecting phytase activity rely on the disappearance of precipitated phytate (sodium phytate) as an indication of phytase activity. *Pediococcus* cultures were qualitatively characterized for phytase activity on MRS agar containing 1% sodium phytate (Sigma-Aldrich, USA) by spotting method (Bae *et al*., 1999). Activated cultures were aseptically spotted on sodium phytate MRS agar plates. Plates were incubated at 37°C for 48 h and flooded with 2% aq. cobalt chloride solution and kept at room temperature. After 5 min, cobalt chloride solution was replaced with a freshly prepared solution containing equal volume of 6.25% (w/v) aq. ammonium molybdate solution and 0.42% (w/v) ammonium vanadate solution. Phytase activity was observed as zone of clearance (mm) in opaque background.

3.4.3 Exopolysaccharide production

Active *Pediococcus* cultures along with positive control (*Lb. fermentum* NCDC 400) were streaked on 5% sucrose MRS agar plates (without dextrose) and incubated at 37°C for 24-48 h. Colonies with mucoid appearance or viscous growth on sucrose MRS agar were taken as positive for EPS production.

3.4.4 Flavor (Diacetyl) production

Flavor producing ability of the cultures were qualitatively checked by Voges and Proskauer (VP) reaction (Westerfeld, 1945). Acetoin and diacetyl reacts with guanido groups of creatine in alkaline medium to give pink color. Cell suspensions of *Pediococcus* cultures along with positive control (*Dahi* culture NCDC 167) were inoculated @ 1% in 2 mL of citrate broth (Appendix I) and incubated for 24-48 h. To this, 1 mL of 0.5% aq. creatine solution and 1 mL of 5% α-napthol (freshly dissolved in 2.5 N NaOH) was added. Tubes were kept in dark for about 10 minutes. Appearance of pink color band indicates positive for diacetyl production.

3.4.5 Galactose fermentation

3.4.5.1 Screening for galactose fermentation

Galactose fermentation by *Pediococcus* cultures was carried out in CHL broth with 1% galactose and 0.017% BCP dye (Appendix I). CHL broth tubes were

inoculated @ 1% with cell suspension and incubated at 37°C for 24 h. Change in color from purple to yellow indicates positive for galactose fermentation (Giraffa *et al*., 2001).

3.4.5.2 Quantification of galactose utilization

The prepared cell suspension was inoculated (@ 1%) in CHL broth containing 1% galactose and incubated at 37°C for 12 h. One millilitre of CHL broth cultures were centrifuged @ 10,000 rpm for 15 min at 4°C. The collected supernatant was passed through a 0.22 µm membrane filter and the clear filtrate was analyzed for unutilized galactose by HPLC (Shimadzu Corporation, Japan).

Standard galactose solution (1% w/v) prepared in HPLC grade water was filtered through 0.22 µm membrane filter. Quantification of galactose was accomplished by comparing the peak height of the samples to that of the standard solution. The following analytical conditions were standardized for the separation of galactose.

Coloum stationary phase	:	NH_2 column (Luna 5 µ NH_2 100 A, 250 X 4.6 mm; Phenomenex, USA)
Flow	:	Isocratic flow
Phase	:	Reverse phase
Detector	:	Refractive index detector (RID-10A; Shimadzu Corporation)
Mobile phase	:	Acetonitrile and water (70:30)
Flow rate	:	1mL/min
Run time	:	15 min
Maximum pressure	:	102 kgf/min
Injection volume	:	20 µL

The unutilized galactose was detected with HPLC according to the standard curve obtained using external pure galactose in a concentration range of 1-10 mg/mL.

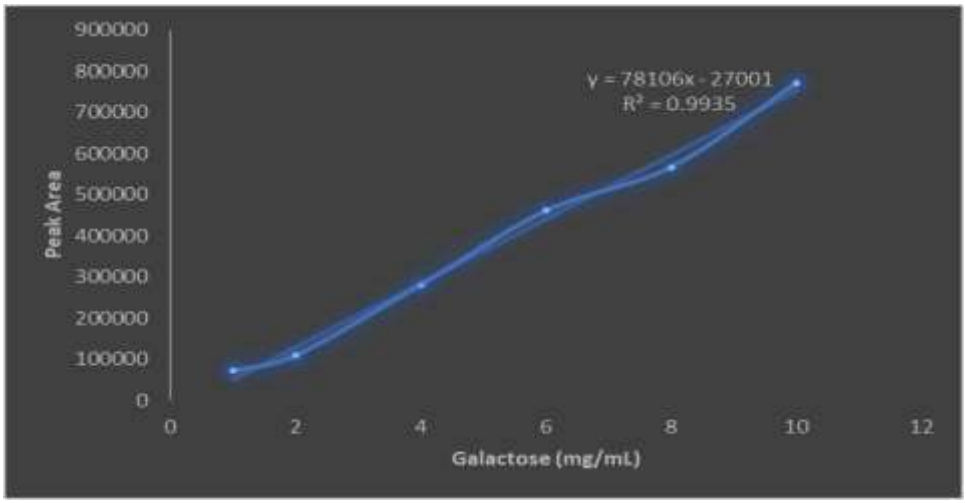


Fig. 3.1 Standard curve of galactose

3.5 EVALUATION OF PROBIOTIC ATTRIBUTES OF *PEDIOCOCCUS* CULTURES

All the 40 *Pediococcus* cultures along with 3 NCDC cultures were subjected to a series of *in-vitro* tests to explore various probiotic attributes as recommended by FAO/WHO (2002).

3.5.1 *In-vitro* acid tolerance

Tolerance to acidic condition was tested according to the method of Clark *et al*. (1993) with required modifications. *Pediococcus* cultures were grown in MRS broth at 37ºC for 12-16 h. The actively grown cells (~ 9 log CFU/mL) were harvested by centrifugation and resuspended in equal volume of MRS broth with pH adjusted to 2.5 with 1N HCl and in MRS broth with pH 6.5 (control). Survival was evaluated by determining the viable count (CFU/mL) at 0, 1.5 and 3 h on MRS agar plates (at 37ºC/48-72 h).

3.5.2 *In-vitro* bile tolerance

Tolerance to bile salts was tested according to the method of Gilliland *et al*. (1984). *Pediococcus* cultures were overnight grown in MRS broth at 37ºC. The actively grown cells (~ 9 log CFU/mL) were harvested by centrifugation and resuspended in equal volume of MRS broth with 0.5 and 1% (w/v) ox-bile (Himedia, India) and without bile salt (control). Survival was evaluated by viable count (CFU/mL) at 0, 2 and 4 h on MRS agar plates (37ºC/48-72 h).

3.5.3 Cell surface hydrophobicity

Ability of microorganisms to adhere hydrocarbons is a measure of their adherence to epithelial cells in the gut. Cell surface hydrophobicity of *Pediococcus* cultures was determined by microbial adhesion to hydrocarbons (MATH) assay (Rosenberg *et al*., 1980) with required modifications using n-hexadecane. Overnight grown MRS broth cultures were centrifuged (10,000 rpm/15 min at 4°C) and the cell pellet was washed twice with phosphate urea magnesium (PUM) buffer (pH 7.1) (Appendix I). The washed pellet was resuspended in PUM buffer and the OD (A_{610}) was adjusted to 0.7-0.8. Pediococci cell suspension (3.0 mL) and n-hexadecane (1.0 mL) were added in clean glass test tube, mixed by vortexing and pre-incubated at 37°C for 10 min for temperature equilibration. The mixture was again vortexed and incubated undisturbed at 37°C for phase separation. After 1 h, aqueous phase was taken carefully with pasteur pipette and absorbance (A_{610}) was measured using spectrophotometer (UV1800 Shimadzu). Cell surface hydrophobicity (H%) as calculated with the equation:

$$H\ (\%) = (A_i\text{-}A_f/A_i) \times 100$$

Where, A_i and A_f are initial and final absorbances at 610 nm.

3.5.4 Cell auto-aggregation

Cell auto-aggregation was determined following the method of Vandevoorde *et al*. (1992) with required modifications. Overnight grown MRS broth cultures were centrifuged at 10,000 rpm for 15 min at 4°C and the cell pellets were washed twice with phosphate buffer saline (PBS) and resuspended again in PBS (pH 6.0) (Appendix I) to a final absorbance of 0.5-0.6 at 600 nm (A_i). Cell suspension was taken in clean glass test tube, mixed by vortex for 10 sec and incubated undisturbed at 37℃ for 4 h. Thereafter, 1 mL of the upper suspension was taken to measure the absorbance at 600 nm (A_f) by using PBS as reference. The percent difference between initial and final absorbance was taken as an index of cell auto-aggregation (%) was expressed as follows:

$$\text{Cell auto-aggregation}\ (\%) = (A_i\text{-}A_f/A_f) \times 100$$

Where A_i and A_f are absorbances at 0 and 4 h.

3.5.5 Antimicrobial activity

Antimicrobial activity of *Pediococcus* cultures against indicator organisms (*E. coli* ATCC 11783, *S. enterica* ssp. Typhi NCDC 113, *S. aureus* NCDC 133, *L. monocytogenes* ATCC 15319) was evaluated by agar well assay (Tejero-Sarinena *et al*., 2012). Two hundred millilitres of molten tryptone glucose extract (TGE) (Appendix I) agar containing Tween 80 (0.1%) was seeded with 100 µL of brain heart infusion (BHI) broth (HiMedia, India) active indicator culture (10^6 CFU/mL) and poured into sterile petri plates. Wells (8 mm diameter) were cut using gel perforator after solidification of agar. Two hundred microlitres of cell free filtrate (obtained from actively growing *Pediococcus* cultures in MRS broth at 37ºC/16 h) was poured in respective wells. Plates were incubated at 37ºC for 24 h and examined for inhibition zone (mm) around the well. Ten percent lactic acid was taken as positive control while sterilized broth was negative control.

3.5.6 β-galactosidase enzyme production

β-galactosidase enzyme activity was determined by ONPG method. Both extracellular and intracellular enzyme activity was evaluated. Two millilitre of actively grown culture was collected in micro-centrifuge tube and centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was used for extracellular and the pellet was used for intracellular enzyme assay.

For intracellular enzyme assay, extraction of the enzyme was done by SDS chloroform method (Bansal *et al*., 2008). Cell pellet was suspended in an equal amount of chilled Z buffer (60 mM $Na_2HPO_4.7H_2O$, 40 mM $NaH_2PO_4.H_2O$, 10 mM KCl, 1 mM $MgSO_4.7H_2O$, 50 mM 2-mercaptoethanol). Hundred microlitre of the suspended solution was mixed with 900 µL of the Z buffer and the cells were permeabilized using 50 µL of 0.1% SDS and 100 µL of the chloroform. Mixed the content and kept at room temperature for 2 min. Then 200 µL of O-nitrophenyl β-D-galactopyranoside (ONPG) was added at a concentration of 4 mg/mL and waited until the development of yellow color. The reaction was stopped by adding 0.5 mL of 1M Na_2CO_3. For extracellular enzyme assay, instead of enzyme extract, one mL of the collected supernatant was mixed with 200 uL of ONPG substrate. The absorbance was measured at 420 nm using the double beam UV-Vis spectrophotometer (UV1800 Shimadzu) and enzyme activity was

expressed in IU/mg dry weight. One unit was defined as the quantity of the enzyme that catalyzed the release of 1 µ mol of orthonitrophenol per minute under standard assay conditions (Lima *et al*., 2013).

3.5.7 Prebiotic fermentation

Prebiotic fermentation by *Pediococcus* cultures was carried out in CHL broth containing 1% filter sterilized prebiotic sugar and 0.017% BCP dye. Test was done with two prebiotic sugars (fructo-oligosaccharide (FOS) and inulin). Cell suspension was inoculated @ 1% in sugar tubes and incubated at 37ºC for 24-48 h. Development of yellow color indicates fermentation of prebiotics. Inoculated tubes with sugar dextrose was taken as positive control while un-inoculated prebiotic sugar tubes were taken as negative control.

3.5.8 Bile salt hydrolase (BSH) assay

A direct plate assay of Singh *et al*. (2012) was employed for detection of BSH activity. A loopfull of active culture was streaked on MRS agar plates containing 0.5% (w/v) bile salts (sodium taurocholate, sodium tauro-deoxycholate) and 0.37 g/L $CaCl_2$. Petri plates were incubated at 37°C anaerobically (GasPak HiMedia, India) for 72 h. BSH activity was indicated when the hydrolyzed products of the salts, viz. cholic acid or deoxycholic acid, precipitated in the agar medium in and around the colonies giving the appearance of opaque zone.

3.6 SAFETY ASSESSMENT OF *PEDIOCOCCUS* CULTURES

All the 40 *Pediococcus* cultures along with 3 NCDC cultures were subjected to a series of *in-vitro* safety tests.

3.6.1 Haemolytic activity

MRS broth active *Pediococcus* cultures were streaked on blood agar plates (HiMedia, India) containing 5% fresh calf blood obtained from Animal Health Complex of the Institute, and incubated at 37°C for 48-72 h. The haemolytic reaction was recorded by observation of a clear zone of hydrolysis around the colonies (β-haemolysis), a partial hydrolysis and greening zone (α-haemolysis) or no reaction (γ-haemolysis) (Gupta and Malik, 2007). A strain of *S. aureus* NCDC 109 was used as positive control.

3.6.2 DNase test

Using heavy inoculum, MRS broth active *Pediococcus* cultures were line streaked on DNase agar plates without indicator dye (HiMedia, India), and incubated at 37℃ for 48-72 h. Plates were flooded with 1N HCl and left undisturbed for a few min. Excess HCl was decanted and plates were examined against a dark background. A clear zone around the bacterial growth indicates positive reaction (Gupta and Malik, 2007). A strain of *S. aureus* CRG-015 was used as positive control.

3.6.3 Liquefaction of gelatin

Using heavy inoculum, MRS broth active *Pediococcus* cultures were line streaked on gelatin agar plates (Appendix I), and incubated at 37℃ for 24-48 h. After incubation, the plates were flooded with saturated ammonium sulphate solution. Development of clear zones around the bacterial growth against opaque background indicated a positive reaction. A strain of *S. aureus* NCDC 109 was used as positive control.

3.6.4 Assessment of biogenic amines production

The ability of the *Pediococcus* cultures to produce biogenic amine i.e. histamine by decarboxylation of histidine was qualitatively evaluated in improved decarboxylase broth, while tyramine production from tyrosine was checked in improved decarboxylase broth as well as on agar plates (Bover Cid and Holzapfel, 1999). All the test cultures along with both positive and negative control (*E. faecalis* NCDC 114 and *Lc. lactis* ssp. *cremoris* NCDC 61, respectively) were sub cultured 5 times in MRS broth containing 0.1% amino acids (i.e. tyrosine and histidine) and 0.005% pyridoxal-5-phosphate to promote enzyme induction before the actual screening test. Further, cultures were inoculated in the improved decarboxylase medium (Appendix I) and incubated at 37ºC for 4 d under aerobic and anaerobic conditions in gas jar (GasPak, Himedia, India). The time to produce violet color in the broth tubes containing different amino acids was noted at an interval of 1 d for each culture. Cultures were considered amino acids decarboxylase positive if color of the indicator turned yellow to violet in improved medium.

As tyrosine was not soluble in the broth media, the tyrosine decarboxylase activity was also assayed in tyrosine plate medium (TPM) (Appendix I). MRS broth active cultures were spotted on preformed TPM plates and incubated at 37ºC for 4 d under aerobic and anaerobic conditions in gas jar. Plates were observed for solubilisation of tyrosine as indicated by clear zones below the grown cells.

3.6.5 Antibiotic susceptibility test

Antibiotic susceptibility of *Pediococcus* cultures was studied by disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI, 2007). Antibiotic susceptibility discs (HiMedia, India) with 20 antibiotics (Table 3.4) were used.

Table 3.4 List of antibiotics with different concentrations mentioned on the disc

Antibiotics	Concentration	Antibiotics	Concentration
Amikacin (AK)	30 mcg	Co-Trimoxazole (COT)	25 mcg
Ampicillin (AMP)	10 mcg	Erythromycin (E)	15 mcg
Amoxycillin (AMX)	10 mcg	Gentamicin (GEN)	10 mcg
Cefadroxil (CFR)	30 mcg	Nalidixic acid (NA)	10 mcg
Cefoperazone (CPZ)	75 mcg	Netillin (NET)	10 mcg
Ceftazidime (CAZ)	30 mcg	Nitrofurantoin (NIT)	300 mcg
Ceftriaxone (CTR)	30 mcg	Norfloxacin (NX)	10 mcg
Chloramphenicol (C)	30 mcg	Penicillin (P)	10 units
Ciprofloxacin (CIP)	5 mcg	Tobramycin (TOB)	10 mcg
Cloxacillin (COX)	1 mcg	Vancomycin (VA)	30 mcg

MRS agar plates were prepared and over laid with 50 mL of MRS soft agar tempered at 45ºC and seeded with 200 µL of MRS broth active cultures. Plates were allowed to stand at room temperature for 15 min and then the antibiotic discs were dispensed onto agar using forceps under aseptic conditions. The

agar plates were incubated at 37ºC aerobically for 24 h. The zone of inhibition (mm) was measured using antibiotic zone scale and results were expressed in terms of resistance (R), intermediate susceptible (IS) and susceptible (S) with zone diameter of ≤14, 15-19 and ≥20 mm respectively (CLSI, 2007)

3.7 APPLICATION OF SELECTED *PEDIOCOCCUS* CULTURES

Based on techno-functional attributes, *Pediococcus* cultures (PD-27, PD-32, PD-34, 14-PD) were selected for preparation of synbiotic fermented dairy beverages and yoghurts.

3.7.1 Preparation of synbiotic fermented dairy beverage

Skim milk supplemented with FOS was heat treated at 90-95°C for 10 min and cooled to 37°C. Cell suspensions of *Pediococcus* cultures (PD-27, PD-32, PD-34 and 14-PD) were inoculated (@ 5%) and was incubated at 37°C. Sample was analyzed after 24 and 48 h for physico-chemical, microbiological and qualitative sensory attributes (texture, taste and mouthfeel).

3.7.1.1 Physico-chemical properties

a) Titratable acidity

Ten millilitre of fermented beverage sample was taken in a clean 100 mL conical flask and diluted with 10 mL distilled water followed by proper mixing. Phenolphthalein (4-5 drops) was added as an indicator to the flask. Titration was carried out against N/10 NaOH with continuous stirring till pale pink color appeared for at least 30 sec. as end point. The volume of NaOH used for titration was recorded. The results were expressed as percent lactic acid using formula given below:

$$\text{Titratable acidity (\% lactic acid)} = \frac{9\times \text{Normality of NaOH} \times \text{Volume of NaOH used}}{\text{Volume of sample taken}} \times 100$$

b) pH measurement

The pH of fermented beverage was measured at 24 and 48 h at 25°C with the help of pH meter (Thermo Electron, USA) by mixing 30 mL of sample in a beaker.

3.7.1.2 Microbiological analysis

a) Total lactic count

The total lactic counts of fermented beverage was enumerated at 48 h. Well mixed sample (1 mL) was serially diluted in 9 mL normal saline and total lactic count was enumerated on MRS agar at 37°C/48-72 h.

b) Coliform count

Coliform count (CFU/mL) was obtained by plating on violet red bile agar (Hi-media, India) incubated at 37°C for 24 h.

c) Yeast and mold count

Yeast and mold count (CFU/mL) was enumerated on potato dextrose agar, (Hi-media, India) at 25°C for 3-5 days.

3.7.1.3 Phytase activity

Phytase activity of the fermented beverage (48 h) was checked as per the procedure described under section 3.4.2. Here, instead of culture broth, fermented product was spotted on the sodium phytate MRS agar.

3.7.2 Yoghurt culture formulation

Yoghurt was prepared using selected *Pediococcus* cultures in combination with low galactose utilizing *S. thermophilus* NCDC 428 (ST) and *Lb. delbrueckii* ssp. *bulgaricus* NCDC 277 (LB). Cow milk (4.5% fat and 8.5% SNF) was heat treated at 90-95°C for 10 min and cooled to 42°C. The culture cell suspensions were inoculated as @ 0.5% ST, 1.0% LB, and 1.0% of *Pediococcus* culture, and filled into 100 mL polystyrene cups. The cups were incubated at 42°C till curd setting. After that cups were transferred to refrigerator (6–8°C). Yoghurts prepared with selected pediococci were analyzed for physico-chemical, microbiological and qualitative sensory attributes, and compared with control yoghurt (ST and LB @ 1:1).

3.7.2.1 Physico-chemical properties

Titratable acidity and pH of the prepared yoghurt was evaluated as described under section 3.7.1.1.

3.7.2.2 Microbiological analysis

Microbiological analysis of the prepared yoghurt was evaluated as described under section 3.7.1.2. Here the total lactic count was evaluated on both MRS as well as M-17 agar.

3.8 ANALYSIS OF DATA

The experiment data were presented as the mean±SD (Standard Deviation). The mean±SD of different parameters studied were graphically presented using GraphPad prism3.02, 1999, GraphPad Software Inc., San Diego CA and Microsoft R Excel 2003 Software Package, Microsoft Corporation, USA.

CHAPTER –4

Results and Discussion

RESULTS AND DISCUSSION

Pediococcus is one of the important LAB used in numerous fermented food products. Due to long history of their safe use in food fermentations, they have got GRAS status. Large-scale industrial processes rely on the use of only a few selected cultures which led to a low variability in the microflora. Increasing information on the natural microbial population present in the products can help to prevent the loss of microbial biodiversity in typical foods and consequently the loss of indigenous microbial population. Indeed, the diversity of starters used in industrial dairy fermentation is low and there is an alarming demand for novel starter cultures that can exhibit valuable effects on product characteristics. Pediococci are endowed with a number of technological and functional attributes with assured safety parameters (Ruiz-Moyano *et al*., 2010; Singla *et al*., 2017). So, the techno-functionally potent *Pediococcus* cultures can be used as starter, adjunct or non-starter for the development of various food products. In view of this, the study was carried out to isolate, identify and characterize the *Pediococcus* spp. for important technological, probiotic and safety attributes. Techno-functionally potent *Pediococcus* cultures were then used for product preparation.

4.1 ISOLATION OF *PEDIOCOCCUS* SPP.

A total of 115 samples were processed for isolation of *Pediococcus* spp. (Fig. 4.1). Smooth, round, greyish-white colonies were randomly picked from MRS agar plates, transferred to MRS broth, and incubated at 37ºC for 24 h to get active cultures. The isolated cultures were examined for morphology and catalase. Gram-positive cocci occurring in pairs and tetrads with catalase negative reaction considered as tentative pediococci isolates (Fig. 4.2). Forty pure isolates were preliminary identified as *Pediococcus* spp. by an array of physiological tests (Fig. 4.3, Table 4.1). All the isolates were oxidase negative, homofermentative and positive for arginine hydrolysis. Ten isolates (PD-2, PD-3, PD-4, PD-25, PD-44, 14-PD, 25-PD, 42-PD, Sil-1 and Sil-2) were positive for pseudocatalase, while other 2 isolates (PD-24 and PD-28) exhibited weak

reaction. All the isolates showed appreciable growth at 30, 37 and 42°C temperature while only one isolate (PD-45) could grow at 50°C. All the isolates were capable to grow at pH 4.2, 6.5 and 8.5, and 4 and 6.5% NaCl; but not at pH 9.6, and 10% NaCl. As pediococci are the only LAB that divide in two perpendicular directions in a single plane to form tetrads, morphological characterization plays a significant role in their easy identification at very early stage of isolation procedure (Singla *et al*., 2017). Strain specific pseudocatalase activity has been reported in pediococci (Korasapati, 1998). Cai *et al*. (1999) isolated and characterized two *P. acidilactici* (LA 3 and LA 35) and one *P. pentosaceus* (LS 5) strain from forage crops. The cultures were tetrad in shape, Gram-positive and homolactic fermentative. All the cultures were able to grow at 45°C temperature, but at 50°C, only *P. acidilactici* strains showed growth. Kumar *et al*. (2017) isolated two *P. pentosaceus* strains (PKL-14 and PKL-17) from pickle and found that both the strains were able to grow at pH 6.5 and 8.5, at 6.5% NaCl, and at 30 and 45°C temperature, but not at 8.5% NaCl. These strains were positive for arginine hydrolysis.

The taxonomic status of the putative *Pediococcus* isolates was confirmed genotypically (PCR using genus and species specific primers) and biochemically (APi 50CH identification kit). All the isolates yielded 701 bp amplicon (Fig. 4.4) during genus specific PCR. This confirmed their identity as genus *Pediococcus*. *Pediococcus* isolates were further identified up-to species level using APi 50CH kit. Thirty nine *Pediococcus* isolates were identified as *P. pentosaceus* and one (PD-45) belonged to *P. acidilactici* (Fig. 4.5, Table 4.2, Appendix III). Complete fermentation of lactose was demonstrated by only *P. acidilactici* NCDC 609. All the tested isolates except *P. acidilactici* PD-45 and NCDC 609 were able to ferment maltose; while only these two isolates were able to grow at 50°. This is the key differentiating characteristic between the two most widely occuring *Pediococcus* spp. i.e. *P. pentosaceus* and *P. acidilactici* (Holzapfel *et al*., 2006). Strain specific sugar fermentation patterns of pediococci has been reported (Uymaz *et al*., 2009; Mandal *et al*., 2011; Abbasiliasi *et al*., 2012). To confirm the species identity at molecular level, PCR was carried out using specific primers, selected on the basis of APi identification (Fig. 4.6, Table 4.2). All the 39 *P.*

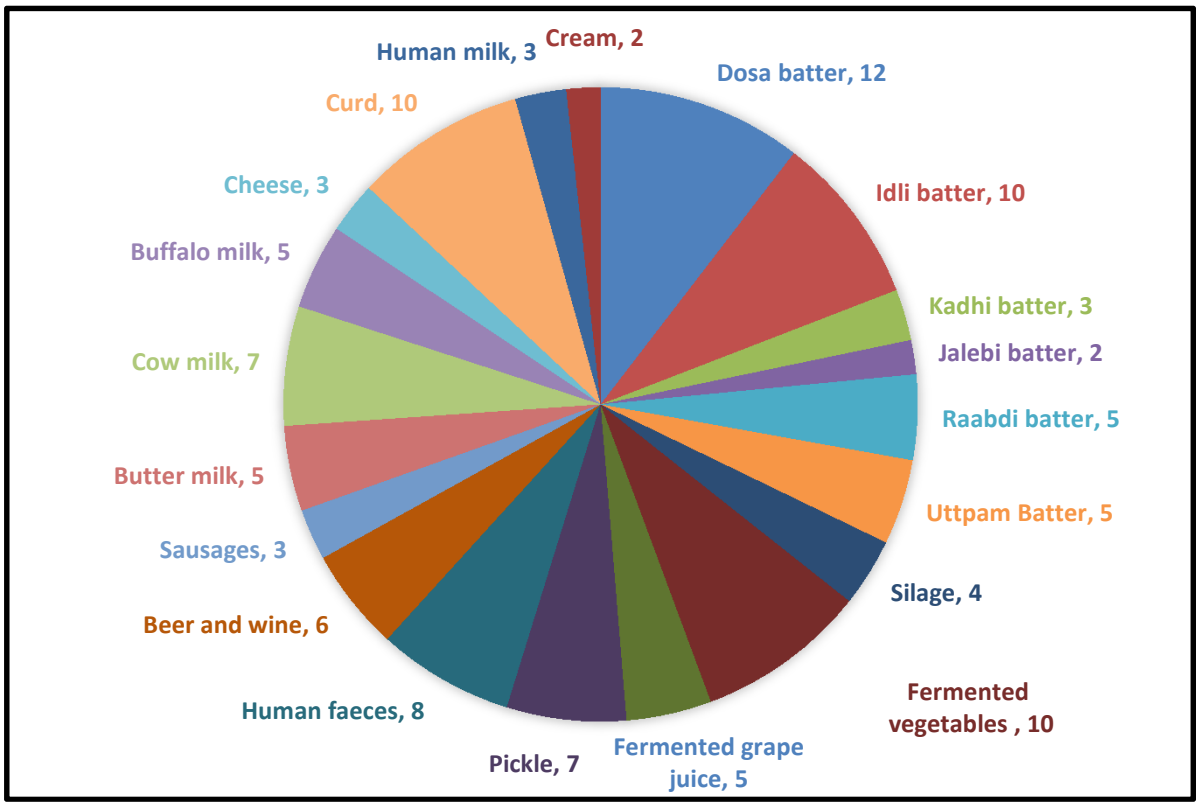


Fig. 4.1 Samples for isolation of *Pediococcus* spp.

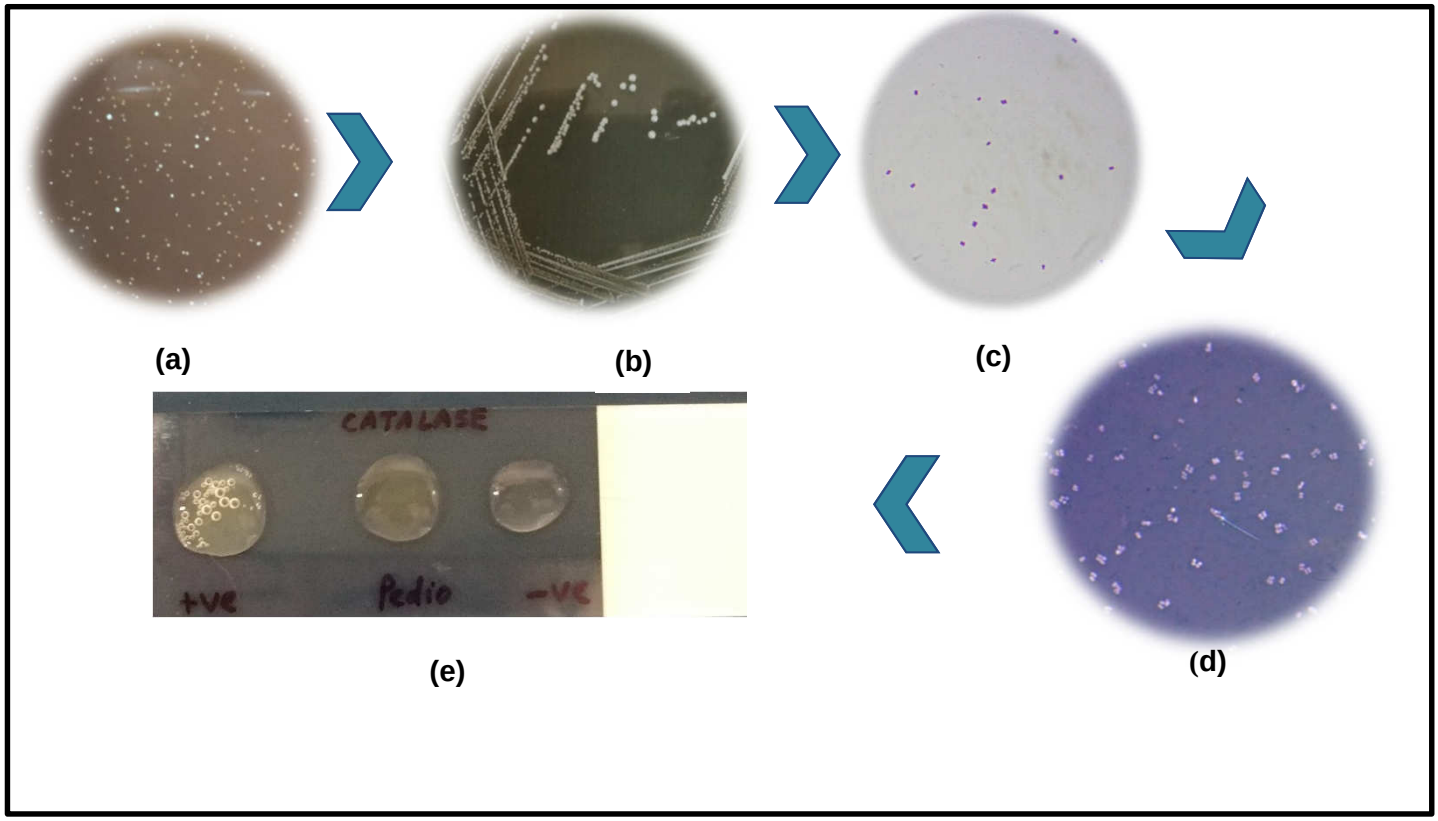
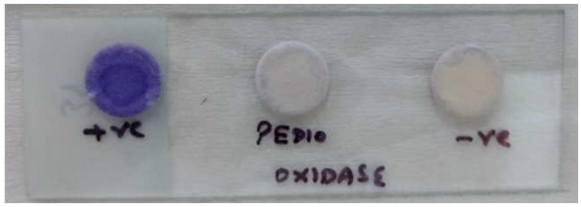


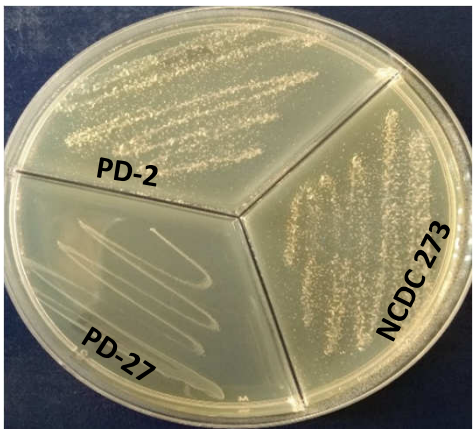
Fig. 4.2 Isolation of *Pediococcus* spp.

(a) Pour plating of sample on MRS agar
(b) Streaking of isolate on MRS agar
(c) Gram-positive cocci in pairs and tetrads (100X) (Gram staining)
(d) Cocci in pairs and tetrads (100X) (Negative staining)
(e) Catalase test of *Pediococcus* isolates

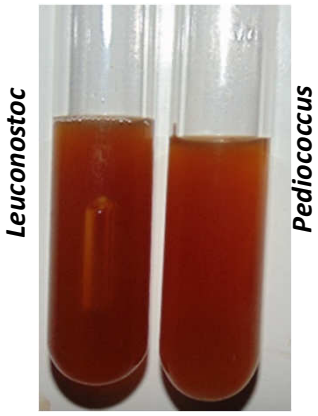
(a)



(b)



(c)



(d)

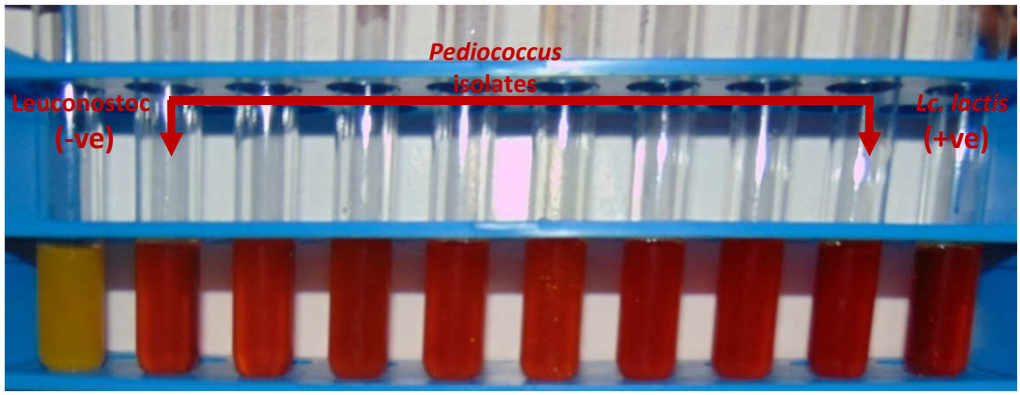


Fig. 4.3 Physiological characterization of isolates

(a) Oxidase test of *Pediococcus* isolates
(b) Pseudocatalase activity of *Pediococcus* isolates
(c) Homo/Heterofermentation test of *Pediococcus* isolates
(d) Arginine hydrolysis test of *Pediococcus* isolates

Table 4.1 Biochemical and growth characteristics of *Pediococcus* isolates

S. No.	Isolates	Source of isolation	Microscopic observation	Catalase test	Pseudo-catalase test	Oxidase test	Gas production from glucose	Arginine hydrolysis	Growth at different temperatures (^{0}C)				Growth at different pH				Growth at different salt concentration (%)				Putative identification
									30	37	42	50	4.2	6.5	8.5	9.6	4	6.5	10	15	
1	PD-1	Dosa batter, Girls hostel, NDRI Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
2	PD-2	Dosa batter, Girls hostel, NDRI Karnal	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
3	PD-3	Dosa batter, Boys hostel, NDRI, Karnal	Gram -positive Cocci in P&T	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
4	PD-4	Dosa batter, Boys hostel, NDRI, Karnal	Gram- positive Cocci in P&T	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
5	PD-7	Butter milk, household, Kamal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
6	PD-8	Idli batter, Boys hostel, NDRI, Kamal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
7	PD-10	Idli batter, Girls hostel, NDRI, Kamal	Gram- positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
8	PD-13	Pickle, household, Hisar	Gram- positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
9	PD-14	Dosa batter, Local market, Kamal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
10	PD-16	Pickle, house hold, Karnal	Gram- positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
11	PD-18	Dosa batter, Local market, Kamal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
12	PD-19	Dosa batter, Local market Kamal	Gram- positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.

S. No.	Isolates	Source of isolation	Microscopic observation	Catalase test	Pseudo-catalase test	Oxidase test	Gas production from glucose	Arginine hydrolysis	Growth at different temperatures (^{0}C)				Growth at different pH				Growth at different salt concentration (%)				Putative identification
									30	37	42	50	4.2	6.5	8.5	9.6	4	6.5	10	15	
13	PD-20	Dosa batter, Girls Hostel, NDRI Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
14	PD-21	Dosa batter, Local market, Jind	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
15	PD-22	Dosa batter, Local market, Jind	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
16	PD-24	Idli batter, Local market, Karnal	Gram-positive Cocci in P&T	-	+ (weakly)	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
17	PD-25	Dosa batter, Local market, Delhi	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
18	PD-26	Idli batter, Local market, Hisar	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
19	PD-27	Idli batter, Local market, Hisar	Gram- positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
20	PD-28	Fermented vegetable prepared in lab NDRI, Karnal	Gram-positive Cocci in P&T	-	+ (weakly)	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
21	PD-29	Fermented vegetable, prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
22	PD-30	Fermented vegetable, prepared in lab, NDRI, Karnal	Gram- positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
23	PD-31	Fermented vegetable, prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
24	PD-32	Fermented vegetable, prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.

S. No.	Isolates	Source of isolation	Microscopic observation	Catalase test	Pseudo-catalase test	Oxidase test	Gas production from glucose	Arginine hydrolysis	Growth at different temperatures (^{0}C)				Growth at different pH				Growth at different salt concentration (%)				Putative identification
									30	37	42	50	4.2	6.5	8.5	9.6	4	6.5	10	15	
25	PD-33	Fermented vegetable, prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
26	PD-34	Fermented vegetable, prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
27	PD-35	Fermented Grape juice, prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
28	PD-36	Fermented Grape juice, prepared in lab, NDRI, Karnal	Gram- positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
29	PD-37	Fermened Grape juice, prepared in lab, NDRI, Karnal	Gram- positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
30	PD-38	Dosa batter, Local market, Delhi	Gram- positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
31	PD-39	Fermented Grape juice, prepared in lab, NDRI, Karnal	Gram- positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
32	PD-41	Cow milk, Cattle yard Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
33	PD-44	Buffalo milk, Local market, Kamal	Gram- positive Cocci in P&T	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
34	PD-45	Human Faeces, voluntaries from NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	-	+	+	-	-	*Pediococcus* spp.

S. No.	Isolates	Source of isolation	Microscopic observation	Catalase test	Pseudo-catalase test	Oxidase test	Gas production from glucose	Arginine hydrolysis	Growth at different temperatures (^{0}C)				Growth at different pH				Growth at different salt concentration (%)				Putative identification
									30	37	42	50	4.2	6.5	8.5	9.6	4	6.5	10	15	
35	8-PD	Dosa batter, Boys hostel, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
36	14-PD	Dosa batter, Boys hostel, NDRI, Karnal	Gram- positive Cocci in P&T	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
37	25-PD	Idli batter, Girls hostel, NDRI, Karnal	Gram- positive Cocci in P&T	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
38	42-PD	Idli batter, Girls hostel, NDRI, Karnal	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
39	Sil-1	Silage, Cattle yard, NDRI, Karnal	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
40	Sil-2	Silage, Cattle yard, NDRI, Karnal	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*Pediococcus* spp.
	P. pentosaceus NCDC 38	-	Gram- positive Cocci in P&T	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*P. pentosaceus*
	P. pentosaceus NCDC 273	-	Gram- positive Cocci in P&T	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-	*P. pentosaceus*
	P. acidilactici NCDC 609	-	Gram- positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	-	+	+	-	-	*P. acidilactici*

* P & T: Pairs and Tetrads

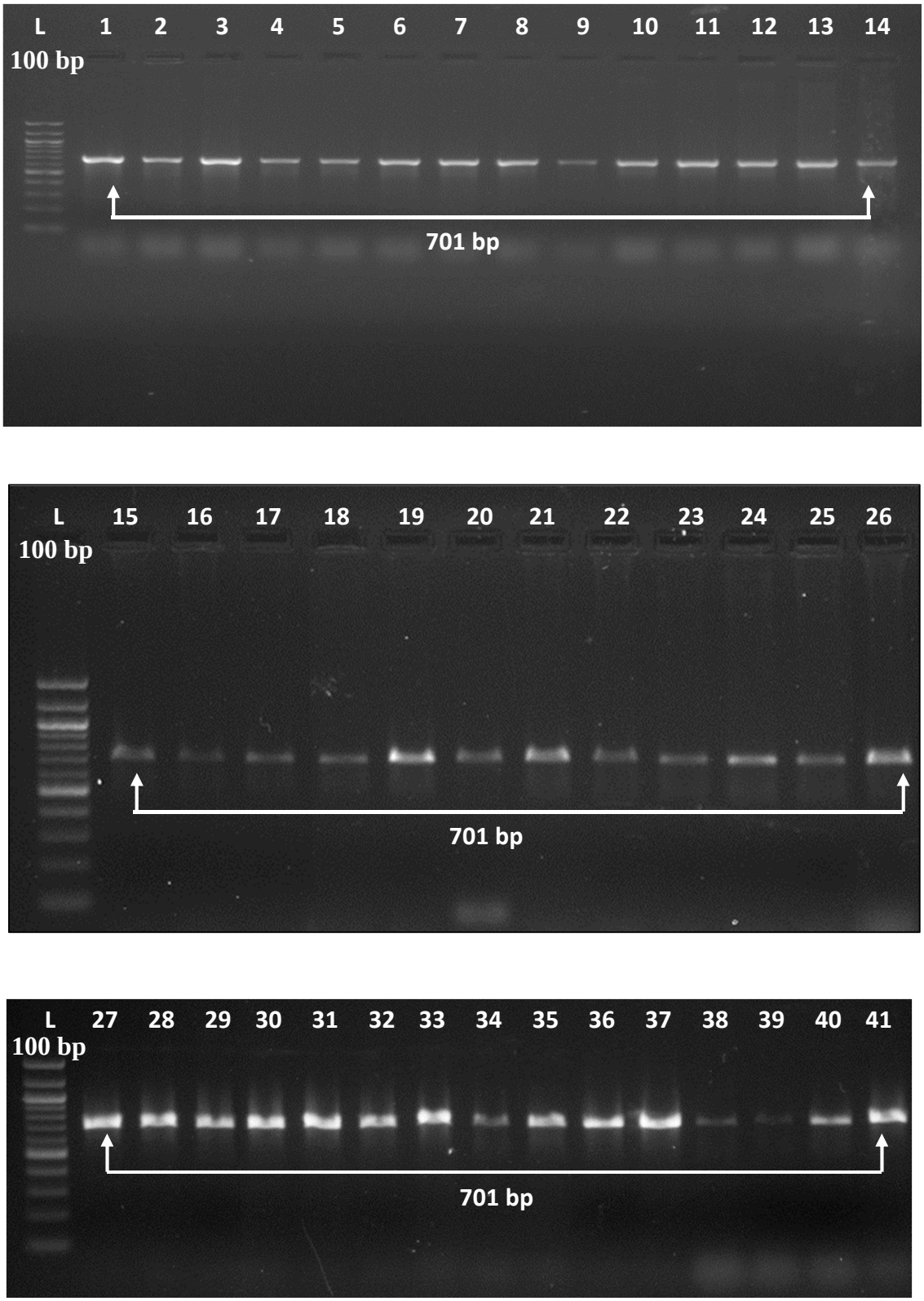
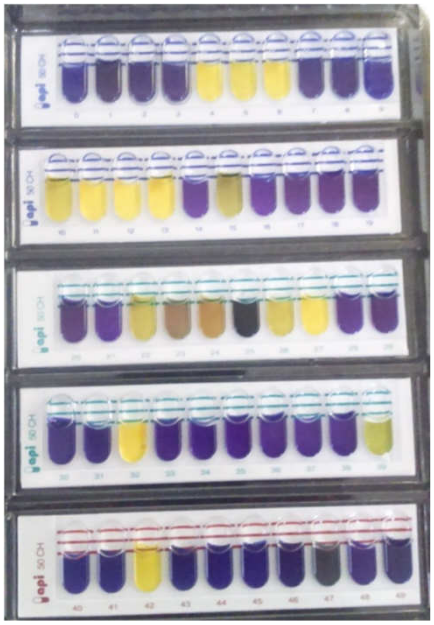


Fig. 4.4 Electrophoretic profile of *Pediococcus* isolates by polymerase chain reaction with genus specific oligonucleotide primer

Lane L : Ladder

Lane 1 : NCDC 273 (Positive control)

Lane 2-41: *Pediococcus* isolates (PD-1, PD-2, PD-3, PD-4, PD-7, PD-8, PD-10, PD-13, PD-14, PD-16, PD-18, PD-19, PD-20, PD-21, PD-22, PD-24, PD-25, PD-26, PD-27, PD-28, PD-29, PD-30, PD-31, PD-32, PD-33, PD-34, PD-35, PD-36, PD-37, PD-38, PD-39, PD-41, PD-44, PD-45, 8-PD, 14-PD, 25-PD, 42-PD, Sil-1, Sil-2)



Strip	API 50 CHL V5.2
Profile	+---+++---++++--------+++++++---+------?----------
Note	

Significant taxa	% ID	T	Tests against	
Pediococcus pentosaceus 1	99.6	0.56	0	0%

PD-27: *P. pentosaceus*



Strip	API 50 CHL V5.2
Profile	+---+++---++++----------+-+----+---------+-------
Note	

Significant taxa	% ID	T	Tests against					
Pediococcus acidilactici	99.9	0.21	0	0%	RHA	75%	NAG 100%	SAL 75%
			GEN 100%					

PD-45: *P. acidilactici*

Fig. 4.5 Sugar fermentation profile of *Pediococcus* isolates using APi 50CH Kit

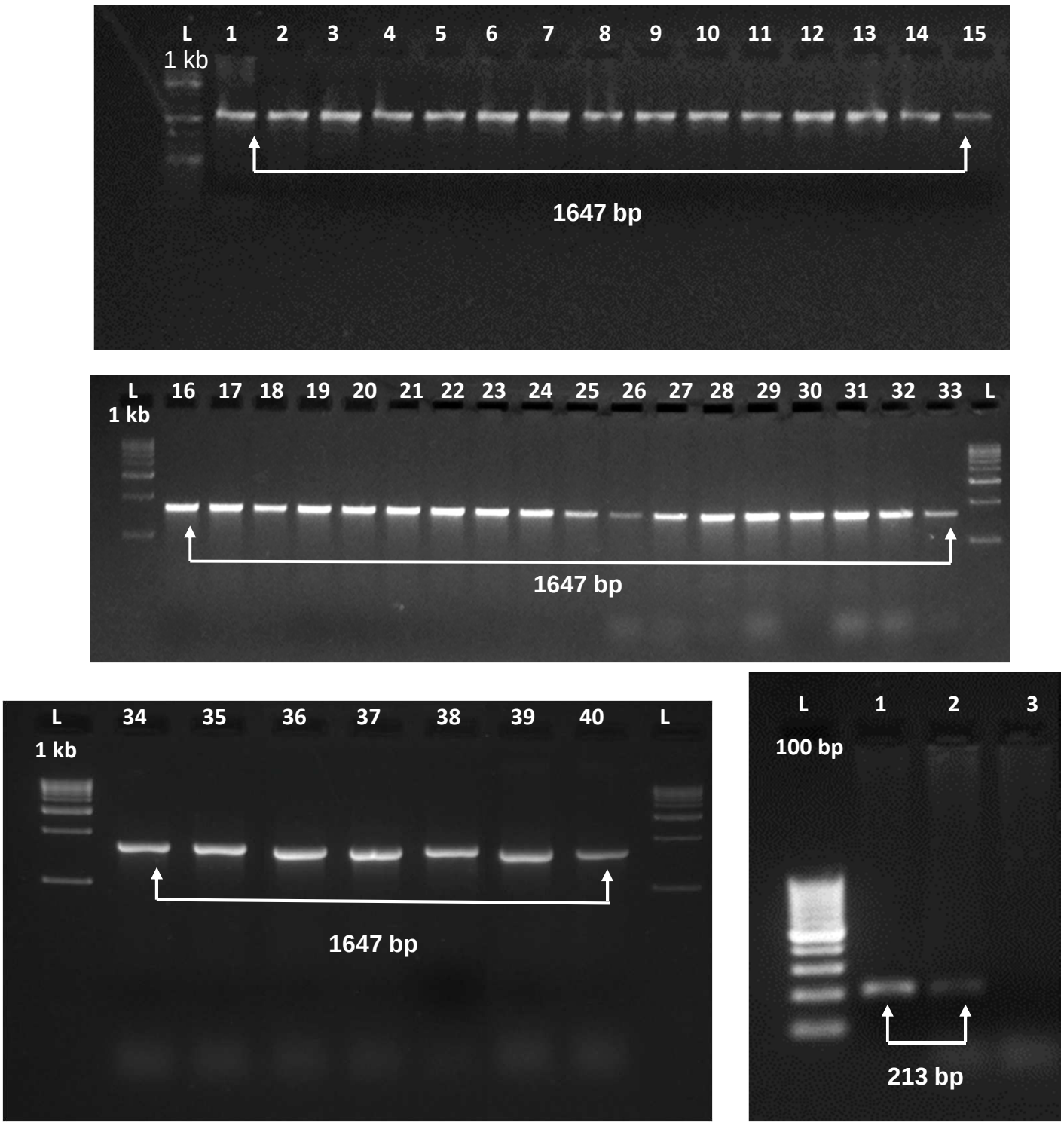


Fig. 4.6 Electrophoretic profile of *Pediococcus* isolates by polymerase chain reaction with species specific oligonucleotide primer

(a) *P. pentosaceus* identification
Lane L : Ladder
Lane 1 : NCDC 38 (Positive control)
Lane 2-40: *Pediococcus* isolates (PD-1, PD-2, PD-3, PD-4, PD-7, PD-8, PD-10, PD-13, PD-14, PD-16, PD-18, PD-19, PD-20, PD-21, PD-22, PD-24, PD-25, PD-26, PD-27, PD-28, PD-29, PD-30, PD-31, PD-32, PD-33, PD-34, PD-35, PD-36, PD-37, PD-38, PD-39, PD-41, PD-44, 8-PD, 14-PD, 25-PD, 42-PD, Sil-1, Sil-2)

(b) *P. acidilactici* identification
Lane L : Ladder
Lane 1 : NCDC 609 (Positive control)
Lane 2 : PD-45
Lane 3 : NCDC 273 (Negative control)

Table 4.2 Biochemical and genotypic identification of *Pediococcus* isolates

S. No.	Isolates	Genotypic identification	Biochemical identification	Genotypic identification
		Genus specific PCR	APi 50CH	Species specific PCR
1	PD-1	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
2	PD-2	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
3	PD-3	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
4	PD-4	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
5	PD-7	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
6	PD-8	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
7	PD-10	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
8	PD-13	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
9	PD-14	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
10	PD-16	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
11	PD-18	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
12	PD-19	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
13	PD-20	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
14	PD-21	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
15	PD-22	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
16	PD-24	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
17	PD-25	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
18	PD-26	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
19	PD-27	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
20	PD-28	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
21	PD-29	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
22	PD-30	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*

23	PD-31	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
24	PD-32	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
25	PD-33	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
26	PD-34	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
27	PD-35	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
28	PD-36	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
29	PD-37	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
30	PD-38	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
31	PD-39	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
32	PD-41	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
33	PD-44	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
34	PD-45	*Pediococcus*	*P. acidilactici*	*P. acidilactici*
35	8-PD	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
36	14-PD	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
37	25-PD	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
38	42-PD	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
39	Sil-1	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
40	Sil-2	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
	NCDC 38	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
	NCDC 273	*Pediococcus*	*P. pentosaceus*	*P. pentosaceus*
	NCDC 609	*Pediococcus*	*P. acidilactici*	*P. acidilactici*

pentosaceus (amplicon size: 1647 bp) and one *P. acidilactici* (amplicon size: 213 bp) were confirmed.

Several reports are available in which biochemically characterized pediococci were confirmed using different modern molecular tools (De Bruyne *et al*., 2008; Pfannebecker and Frohlich, 2008; Martino *et al*., 2013; Saravanan *et al*., 2015; Gupta and Sharma, 2017). Osmanagaoglu *et al*. (2011) isolated *Pediococcus* spp. from human breast milk which was identified as *P. pentosaceus* by APi 50CH and was confirmed by PCR using 16S rRNA gene sequence analysis. Oh and Jung (2015) isolated 7 LAB from Omegisool, a fermented beverage and identified them as *Lactobacillus* and *Pediococcus* spp. (*P. pentosaceus* SW 01 and *P. acidilactici* SW 05) on the basis of APi 50CH and subsequently by PCR using primer pairs 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). APi profiling depicted that out of two *Pediococcus* strains, only *P. acidilactici* SW 05 could able to ferment maltose.

Out of 40 *Pediococcus* isolates*,* 37 were from non-dairy sources and 3 were from dairy sources (Fig. 4.7). Relatively lesser occurrence of pediococci in milk and milk products may be due to their poor growth in milk (Somkuti and Steinberg, 2010). Plant materials and fermented products are predominant habitats of pediococci (Cai *et al*., 1999; Kleinschmit and Kung, 2006; Nout *et al*., 2007; Sukumar and Ghosh, 2010; Yuksekdag and Aslim, 2010; Vidhyasagar and Jeevaratnam, 2012; Swain *et al*., 2014; Narayanan *et al*., 2017). Majority of the *Pediococcus* isolates were from fermented products such as idli, dosa, fermented vegetables, fermented grape juices and silage. Badis *et al*. (2004) characterized 725 LAB from goat milk; which were classified as: *Lactobacillus* (31.6%), *Lactococcus* (28.4%), *Leuconostoc* (22.2%), *Streptococcus* (13.7%) and *Pediococcus* (4.1%). Yuksekdag and Aslim (2010) isolated 5 strains of pediococci out of 35 LAB isolates obtained from the Turkish-type fermented sausage (sucuk) samples and were identified as *P. pentosaceus* (3 strains), *P. acidilactici* (1), and *P. dextrinicus* (1). Sukumar and Ghosh (2010) isolated *Pediococcus* spp. from kadhi batter while Vidhyasagar and Jeevaratnam (2012) from idli batter. Belhadj *et al*. (2014) isolated 10 *Pediococcus* strains (*P. pentosaceus* and *P. acidilactici*) along with other LAB from pollen grains.

4.2 EVALUATION OF TECHNOLOGICAL ATTRIBUTES OF *PEDIOCOCCUS* CULTURES

Assessment of technologically important traits of LAB is crucial for selection of potent strain for their application in food fermentations. Several technological attributes such as acidification profile, phytase activity, exopolysaccharide production, flavor production and galactose fermentation were evaluated for 40 isolated *Pediococcus* cultures along with 3 NCDC *Pediococcus* cultures.

4.2.1 Acidification profile

The acidification profile of *Pediococcus* cultures was evaluated in MRS broth (control), CHL broth containing 1% lactose, skim milk and skim milk supplemented with protein hydrolysate. Acidification was evaluated from decrease in pH of media (Fig. 4.8, Table 4.3). After 6 h, pH of different media ranged between 4.82 (NCDC 38) to 5.92 (PD-19), 6.16 (42-PD) to 6.57 (PD-29), 5.77 (42-PD) to 6.26 (PD-13) and 5.24 (PD-18) to 5.98 (NCDC 609) in MRS, CHL broth with lactose, skim milk and skim milk with supplement, respectively at 37°C. After 12 h, the pH ranged between 4.53 (NCDC 38) to 5.90 (PD-7), 5.19 (NCDC 609) to 6.51 (PD-29, PD-32), 5.41 (PD-18) to 6.16 (PD-26) and 4.95 (Sil-2, NCDC 38) to 5.82 (NCDC 609) in MRS, CHL broth with lactose, skim milk and skim milk with supplement, respectively at 37°C. After 24 h, the pH ranged between 4.20 (PD-3) to 5.06 (PD-7), 4.37 (NCDC 609) to 6.51 (PD-26), 5.25 (PD-18) to 6.10 (PD-26) and 4.32 (NCDC 273) to 5.67 (PD-45) in MRS, CHL broth with lactose, skim milk and skim milk with supplement, respectively at 37°C.

Overall, maximum drop in pH was shown by PD-3 in MRS broth after 24 h. *Pediococcus acidilactici* NCDC 609 was able to acidify CHL broth with lactose with a considerable drop in pH. There is more acidification in skim milk than CHL broth with lactose which was observed by drop in pH values. Supplementation of protein hydrolysates in skim milk affected the growth and curdling property of *Pediococcus* cultures. Hence, milk curdling (although loose) was exhibited by PD-1, PD-8, PD-10, PD-44, 8-PD, 14-PD, 25-PD, 42-PD, Sil-1, Sil-2, NCDC 38 and NCDC 273 only after addition of protein hydrolysate. It depicted that lactose is not the only factor responsible for making milk unfavorable for pediococci, lack of hydrolyzed protein in milk could also be the additional cause. Acidification

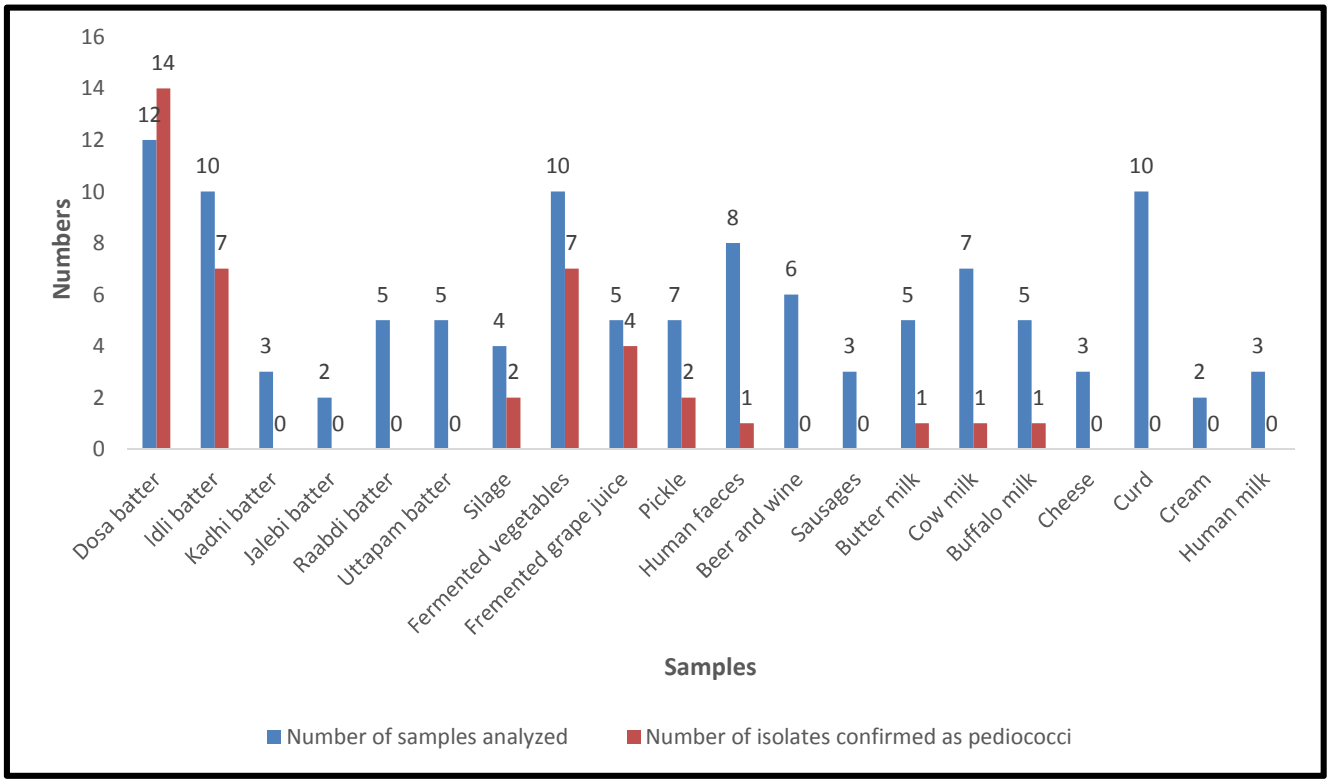


Fig. 4.7 Distribution of *Pediococcus* isolates

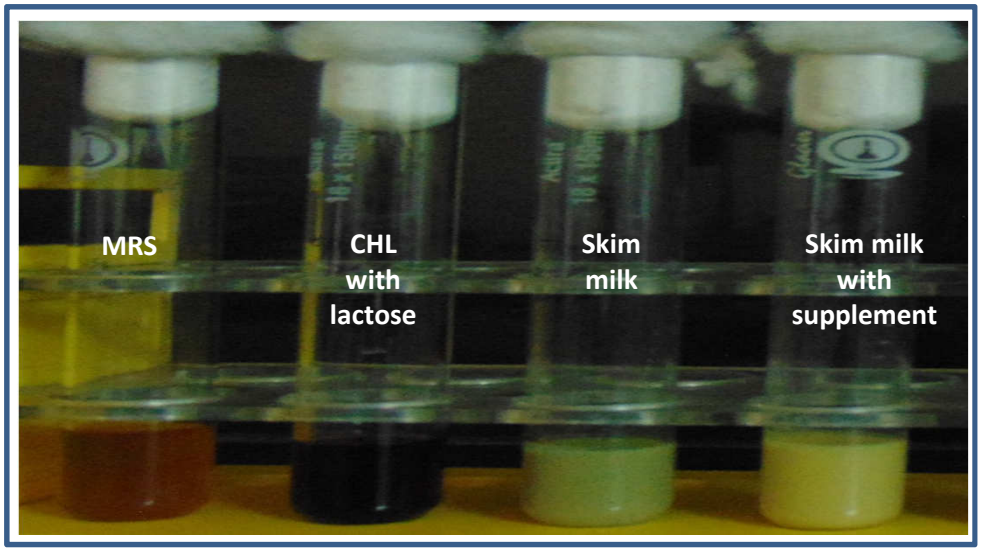


Fig. 4.8 Acidification by *Pediococcus* cultures

Table 4.3: Acidification profile (change in pH) of *Pediococcus* cultures in different media

S. No.	Cultures	MRS broth				CHL broth with lactose				Skim milk				Skim milk with supplement			
		Incubation time (h)															
		0	6	12	24	0	6	12	24	0	6	12	24	0	6	12	24
		pH															
1	*P. pentosaceus* PD-1	5.88	5.72	4.59	4.21	6.51	6.27	6.25	6.03	6.35	6.12	6.00	5.72	6.03	5.80	5.51	4.68
2	*P. pentosaceus* PD-2	5.93	5.75	4.59	4.45	6.53	6.31	6.25	6.24	6.38	6.13	6.00	5.70	6.05	5.70	5.32	4.79
3	*P. pentosaceus* PD-3	5.92	5.62	4.78	4.20	6.52	6.32	6.29	6.18	6.36	6.15	6.04	5.80	6.09	5.78	5.41	4.90
4	*P. pentosaceus* PD-4	5.97	5.75	4.57	4.40	6.51	6.32	6.26	6.25	6.37	6.12	6.03	5.72	6.05	5.72	5.34	4.80
5	*P. pentosaceus* PD-7	5.94	5.91	5.90	5.06	6.52	6.48	6.35	6.26	6.45	6.24	6.13	5.94	6.09	5.87	5.69	4.94
6	*P. pentosaceus* PD-8	5.88	5.52	5.50	4.21	6.52	6.34	6.33	6.10	6.4	6.16	6.00	5.68	6.09	5.74	5.34	4.60
7	*P. pentosaceus* PD-10	5.92	5.34	4.58	4.44	6.64	6.30	6.22	5.81	6.41	6.17	5.96	5.69	6.03	5.79	5.18	4.55
8	*P. pentosaceus* PD-13	5.95	5.51	4.81	4.41	6.55	6.38	6.36	6.34	6.45	6.26	6.05	5.74	6.12	5.94	5.56	4.85
9	*P. pentosaceus* PD-14	5.98	5.70	4.75	4.33	6.55	6.35	6.34	6.33	6.42	6.22	6.08	5.82	6.13	5.92	5.57	4.92
10	*P. pentosaceus* PD-16	5.94	5.78	4.80	4.39	6.58	6.40	6.38	6.38	6.47	6.22	6.09	5.83	6.15	5.92	5.56	4.87
11	*P. pentosaceus* PD-18	6.00	5.03	4.63	4.28	6.66	6.25	6.20	5.97	6.44	5.78	5.41	5.25	6.18	5.24	5.00	4.75
12	*P. pentosaceus* PD-19	6.01	5.92	4.72	4.39	6.65	6.37	6.32	6.27	6.56	6.23	6.09	5.85	6.19	5.80	5.25	4.70
13	*P. pentosaceus* PD-20	6.21	5.27	4.72	4.45	6.75	6.40	6.40	6.33	6.48	6.12	6.01	5.73	6.13	5.75	5.22	4.76
14	*P. pentosaceus* PD-21	6.22	5.28	4.72	4.38	6.72	6.40	6.34	6.28	6.5	6.11	6.00	5.74	6.12	5.76	5.23	4.75
15	*P. pentosaceus* PD-22	6.24	5.35	4.81	4.50	6.72	6.47	6.40	6.40	6.42	6.15	6.10	5.78	6.09	5.80	5.32	4.80
16	*P. pentosaceus* PD-24	6.29	5.47	4.88	4.56	6.75	6.49	6.44	6.41	6.42	6.18	6.06	5.74	6.15	5.84	5.35	4.78
17	*P. pentosaceus* PD-25	6.24	5.34	4.78	4.49	6.72	6.48	6.44	6.40	6.43	6.14	6.10	5.74	6.13	5.81	5.32	4.79
18	*P. pentosaceus* PD-26	6.39	5.35	4.94	4.55	6.79	6.48	6.48	6.51	6.53	6.23	6.16	6.10	6.21	5.81	5.64	5.61
19	*P. pentosaceus* PD-27	6.21	5.22	4.71	4.40	6.62	6.38	6.35	6.32	6.37	6.10	5.98	5.76	6.07	5.64	5.14	4.76
20	*P. pentosaceus* PD-28	6.24	5.24	4.77	4.48	6.71	6.44	6.44	6.44	6.45	6.13	6.00	5.67	6.11	5.71	5.10	4.70
21	*P. pentosaceus* PD-29	6.30	5.19	4.81	4.63	6.76	6.57	6.51	6.49	6.48	6.18	6.04	5.75	6.16	5.84	5.25	4.72

S. No.	Cultures	MRS broth				CHL broth with lactose				Skim milk				Skim milk with supplement			
		Incubation time (h)															
		0	6	12	24	0	6	12	24	0	6	12	24	0	6	12	24
22	*P. pentosaceus* PD-30	6.25	5.28	4.86	4.42	6.69	6.55	6.45	6.39	6.39	6.18	5.99	5.60	6.09	5.72	5.17	4.52
23	*P. pentosaceus* PD-31	6.26	5.30	4.90	4.42	6.80	6.51	6.43	6.37	6.40	6.10	5.98	5.60	6.12	5.80	5.19	4.76
24	*P. pentosaceus* PD-32	6.30	5.40	5.10	4.51	6.76	6.53	6.51	6.40	6.42	6.08	5.96	5.65	6.37	5.93	5.49	5.08
25	*P. pentosaceus* PD-33	6.25	5.46	4.96	4.50	6.80	6.45	6.42	6.40	6.37	6.02	5.91	5.51	6.39	5.91	5.42	5.02
26	*P. pentosaceus* PD-34	6.25	5.45	4.98	4.49	6.82	6.47	6.45	6.39	6.38	6.07	5.96	5.55	6.37	5.94	5.45	5.06
27	*P. pentosaceus* PD-35	6.37	5.20	5.16	4.53	6.80	6.52	6.50	6.40	6.40	6.06	5.92	5.61	6.35	5.90	5.47	5.10
28	*P. pentosaceus* PD-36	6.28	5.37	5.00	4.60	6.82	6.53	6.47	6.41	6.43	6.10	6.00	5.66	6.36	5.95	5.49	5.06
29	*P. pentosaceus* PD-37	6.13	5.25	4.71	4.45	6.61	6.33	6.32	6.30	6.16	5.86	5.74	5.48	6.21	5.73	5.19	4.90
30	*P. pentosaceus* PD-38	6.23	5.22	4.80	4.54	6.70	6.38	6.37	6.31	6.27	6.01	5.86	5.57	6.3	5.89	5.42	5.07
31	*P. pentosaceus* PD-39	6.27	5.39	4.88	4.61	6.69	6.40	6.37	6.34	6.32	6.06	5.93	5.56	6.29	5.89	5.43	5.01
32	*P. pentosaceus* PD-41	6.13	5.41	4.69	4.44	6.70	6.26	6.25	6.25	6.20	5.92	5.70	5.33	6.20	5.78	5.23	4.81
33	*P. pentosaceus* PD-44	6.19	4.99	4.77	4.41	6.68	6.26	6.24	6.05	6.21	5.86	5.78	5.56	6.23	5.44	5.02	4.55
34	*P. acidilactici* PD-45	6.18	5.07	4.67	4.60	6.66	6.48	6.41	6.38	6.21	6.08	5.99	5.94	6.17	5.86	5.76	5.67
35	*P. pentosaceus* 8-PD	6.13	4.97	4.68	4.42	6.69	6.41	6.35	5.96	6.26	5.96	5.70	5.45	6.24	5.53	5.13	4.67
36	*P. pentosaceus* 14-PD	6.18	5.05	4.64	4.45	6.72	6.38	6.38	6.38	6.33	5.99	5.97	5.78	6.27	5.71	5.27	4.79
37	*P. pentosaceus* 25-PD	6.19	5.08	4.78	4.54	6.72	6.42	6.38	6.36	6.26	6.02	5.97	5.75	6.29	5.72	5.70	4.60
38	*P. pentosaceus* 42-PD	6.00	4.85	4.69	4.33	6.55	6.16	6.06	5.43	6.08	5.77	5.71	5.42	6.10	5.43	4.99	4.44
39	*P. pentosaceus* Sil-1	6.24	5.08	4.72	4.38	6.72	6.42	6.38	6.16	6.38	6.07	6.00	5.86	6.09	5.40	4.98	4.58
40	*P. pentosaceus* Sil-2	6.29	5.04	4.76	4.46	6.72	6.46	6.41	6.19	6.39	6.03	5.94	5.67	6.11	5.42	4.95	4.50
	P. pentosaceus NCDC 38	6.05	4.82	4.53	4.31	6.56	6.22	6.13	6.08	6.12	5.81	5.62	5.49	6.12	5.34	4.95	4.56
	P. pentosaceus NCDC 273	6.14	5.06	4.66	4.38	6.62	6.55	6.30	5.41	6.20	5.87	5.62	5.34	6.18	5.35	5.02	4.32
	P. acidilactici NCDC 609	6.18	5.25	4.88	4.57	6.72	6.19	5.19	4.37	6.24	6.21	6.13	5.98	6.26	5.98	5.82	5.56

profile showed that lactose is not generally utilized by pediococci which is also supported by APi results and previous literature (Mandal *et al*., 2011; Abbasiliasi *et al*., 2012). However, Sukumar and Ghosh (2010), Venkateshwari *et al*. (2010) and Martino *et al*. (2013) reported lactose positive pediococci from different food environments. These findings demonstrated that lactose utilization by pediococci is although not common, but is a strain dependent property.

Pediococci are generally used as starter cultures in various food products including meat, fermented vegetables, silage etc. due to their rapid acidification ability (Kleinschmit and Kung, 2006; Laitila *et al*., 2006; Filya *et al*., 2007; Yuksekdag and Aslim, 2010; Piasecka-Jozwiak *et al*., 2013; Swain *et al*., 2014). In dairy, they are generally used as non-starter, adjunct or secondary starter cultures (Bhowmik and Marth, 1990; Franciosi *et al*., 2008; Barouei *et al*., 2011). However, gene transfer system finds the application of pediococci in milk environment. Lactose plasmid has been successfully transferred in pediococci cultures and application of the lactose positive phenotype in milk fermentation has been reported (Morita *et al*., 1991; Caldwell *et al*., 1996).

4.2.2 Phytase activity

Out of 40 *Pediococcus* cultures, 34 along with *P. pentosaceus* NCDC 38 and 273 showed phytase activity (zone of clearance around the colony) on sodium phytate MRS agar (Fig. 4.9, Table 4.4). The diameter of zones varied from 11 to 26 mm. *P. pentosaceus* 25-PD resulted the largest zone of clearance (26 mm) followed by 14-PD (22 mm) and PD-25 (21 mm). Six *Pediococcus* cultures were potent phytase producers (≥20 mm zone diameter).

Phytase activity has been documented in various LAB strains (De Angelis *et al*., 2003; Palacios *et al*., 2008; Songre-Ouattara *et al*., 2008; Basu *et al*., 2015). Pediococci possessed strain specific ability of phytate degradation. Three best phytase positive cultures belonged to fermented idli (*P. pentosaceus* 25-PD) and dosa (*P. pentosaceus* 14-PD and PD-25) batter. Raghavendra and Halami (2009) screened 40 LAB isolates for phytase activity by cobalt chloride staining method. Interestingly, all the 40 isolates were able to degrade calcium phytate. However, only two of them i.e. *P. pentosaceus* strains (CFR R38 and CFR R35) were capable to degrade sodium phytate. *Pediococcus pentosaceus* CFR R123, an isolate from cereal based fermented preparation was reported to degrade

both sodium as well as calcium phytate with potential probiotic characteristics (Raghavendra *et al*., 2010). *Pediococcus pentosaceus* CFR R123 strain was also evaluated for the fate of phytate and calcium solubility during fermentation (Raghavendra *et al*., 2011). Under standard conditions, the strain CFR R123 degraded 43% of sodium phytate in 15 minutes in modified MRS containing sodium phytate. Also, fermentation of malted finger millet seed coat (MFSC) and soya milk (SM) with CFR R123 strain for 12 h resulted in 5.6-12% phytate degradation and an appreciable increase in calcium availability. Abriouel *et al*. (2012) found phytase activity in 27% of the total pediococci strains from fermented Manzanilla alorena green table olives. Nuobariene *et al.* (2015) tested the phytate degrading activity in 168 LAB isolates from Lithuanian sourdoughs. Out of 168, 15 isolates were phytase positive which belonged to different species of *Lactobacillus* and *P. pentosaceus*. Shivanna and Venkateswaran (2015) screened 24 probiotic LAB strains for phytase activity by qualitative plate assay. Ten strains were phytase active belonging to 2 *Pediococcus* strains (*P. acidilactici* K_7 and *P. acidilactici* C_{20}) along with *Lactobacillus* and *Lactococcus* strains.

4.2.3 Exopolysaccharide production

Exopolysaccharide production was qualitatively evaluated on sucrose MRS agar. Five *Pediococcus* cultures (PD-1, PD-44, 42-PD, Sil-1 and Sil-2) and *P. pentosaceus* NCDC 38 showed mucoid colony. Other 18 *Pediococcus* cultures and *P. acidilactici* NCDC 609 displayed viscous growth which were considered weak EPS producers (Fig. 4.10, Table 4.4). Five potent EPS producer strains belonged to different sources i.e. buffalo milk (PD-44), silage (Sil-1 and Sil-2), dosa batter (PD-1) and idli batter (42-PD).

Polysaccharides produced by LAB vary considerably in composition and reported to display a multitude of techno-functionality (Patel and Prajapati, 2013, Fontana e*t al*., 2015; Torino *et al*., 2015; Sanalibaba and Cakmak, 2016). Sabir *et al*. (2010) quantified EPS production by pediococci from kefir (a fermented dairy beverage) as 231±0.20, 276±0.01 and 230±0.00 mg/L by *P. dextrinicus* ZN 1P, *P. acidilactici* ZN 10P and *P. pentosaceus* ZN 13P strain, respectively. Shukla and Goyal (2013) characterized a high mol. wt. (2.93×10^5 Da.) novel dextran by *P. pentosaceus* CRAG3 from fermented cucumber. The EPS

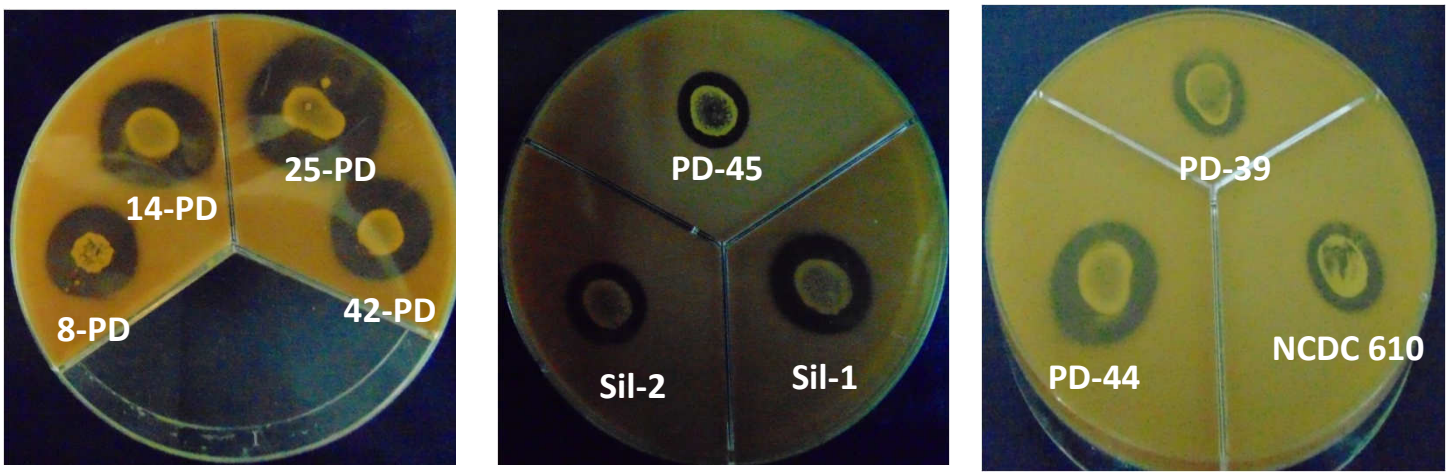


Fig. 4.9 Zone of clearance by *Pediococcus* cultures on sodium phytate MRS agar

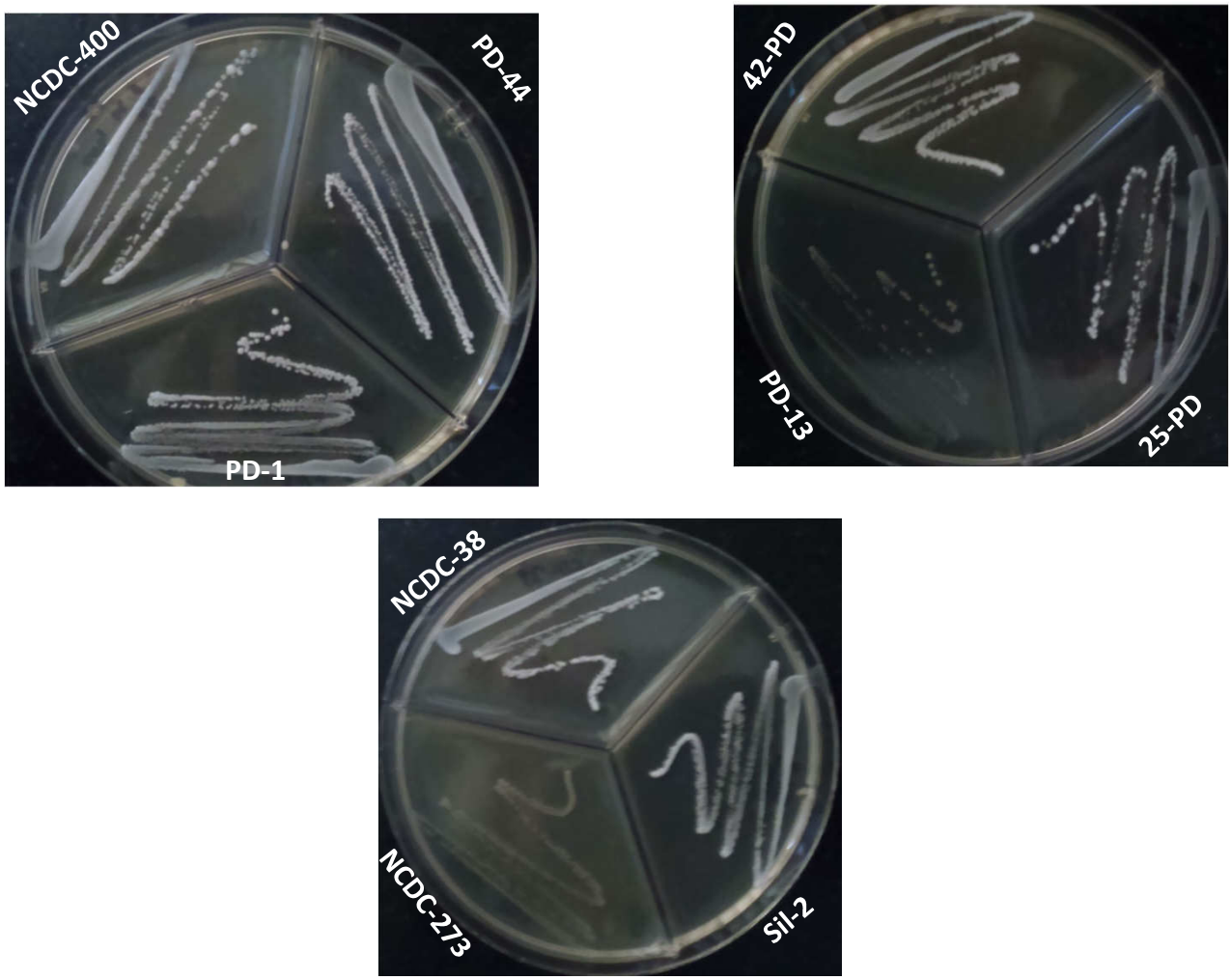


Fig. 4.10 EPS production by *Pediococcus* cultures on sucrose MRS agar

produced was solely consisted of glucose units. Similarly, Song *et al*. (2013) isolated dextran producing *P. acidilactici* M76 from Korean fermented rice wine (makgeolli) and reported the anti-oxidative activity of EPS. Mostefaoui *et al*. (2014) screened 82 LAB isolates from Algerian raw camel milk for EPS production. Based on the appearance of mucoid colony on customized MRS agar, 30 LAB isolates (including 2 *Pediococcus* strains) were found EPS producers.

4.2.4 Flavor (diacetyl) production

Diacetyl is one of the major flavor compound in fermented dairy products (Beshkova *et al*., 2003). A pathway well-known for producing diacetyl is through degradation of citrate by LAB (McSweeney and Fox, 2004), where pyruvate is formed as an intermediate compound that is converted via acetolactate to diacetyl. Pediococci cultures were screened for diacetyl production by Voges and Proskauer (VP) ring test where development of pink colored band indicates positive reaction. Twelve cultures were positive for diacetyl (Fig. 4.11, Table 4.4). However, the intensity of the band/ring color was comparatively less as compared to the positive control (*Dahi* culture NCDC 167). Out of 12 diacetyl positive cultures, 4 were from dosa batter, 2 from idli batter, 2 from fermented vegetable, 1 from pickle, 1 from human faeces, 1 from fermented grape juice and 1 from butter milk.

Although, pediococci are generally citrate negative (Venkateshwari *et al*., 2010; Gupta and Sharma, 2017), the production of diacetyl by *Pediococcus* strains is reported (Litopoulou-Tzanetaki *et al*., 1989). Pasteris and de Saad (2005) reported that through glycerol catabolism, *P. pentosaceus* N5p, an isolate from wine was capable of producing diacetyl. Irlmer *et al*. (2013) studied various strains of *P. pentosaceus* and *P. acidilactici* from cheese to produce diacetyl from pyruvate and L-serine. *P. pentosaceus* strains were able to produce diacetyl from both the substrates and the property was correlated with serine dehydratase activity.

4.2.5 Galactose fermentation

Pediococcus cultures were screened for galactose fermentation in CHL broth with galactose as sole carbon source. All the 40 *Pediococcus* cultures and 3 NCDC cultures were able to ferment galactose (Fig. 4.12a, Table 4.4). After

qualitative screening, the cultures were subjected for measuring the extent of galactose utilization. The amount of galactose utilized by *Pediococcus* cultures were evaluated by measuring the residual sugar present in CHL broth through HPLC after 12 h at 37°C (Fig. 4.12b and 4.12c). Galactose utilization by *Pediococcus* cultures ranged between 57.58% (PD-8) to 95.95% (PD-33). Twenty eight cultures utilized >90% galactose from the medium. Nine cultures utilized galactose in the range of 60-90%, while 3 cultures utilized <60% galactose. Galactose fermentation ability by *Pediococcus* cultures were also reported previously (Garvie, 1986; Shukla and Goyal, 2014; Singla *et al*., 2017).

4.3 EVALUATION OF PROBIOTIC ATTRIBUTES OF *PEDIOCOCCUS* CULTURES

A total 40 *Pediococcus* cultures along with 3 NCDC cultures were evaluated for probiotic attributes (FAO/WHO, 2002). The colonization of intestinal tract by microorganisms is prerequisite to be a successful probiotic. During transit in GI tract, microorganisms are subjected to several stresses and therefore probiotics must survive these harsh environments i.e. acidic condition of stomach and high bile concentration of intestine to colonize and exert health benefits therein. So, the cultures were tested for acid and bile tolerance, adhesion, bile salt deconjugation, antimicrobial and β-galactosidase activity. The standard probiotic culture of *Lb. rhamnosus* GG (LGG) was used as reference strain. All experiments were conducted under *in-vitro* conditions, anticipating that the qualified isolates may also mimic their activity *in-vivo*, when ingested by host.

4.3.1 *In-vitro* acid tolerance

The acid tolerance of *Pediococcus* cultures were evaluated by determining the viability (log CFU/mL) at pH 2.5 up-to 3 h. Viable counts were decreased with exposure time. Out of 40, 27 *Pediococcus* cultures along with *P. pentosaceus* NCDC 273 and *P. acidilactici* NCDC 609 were able to survive up-to 1.5 h; while, after 3 h, only 19 of them could survive (Fig. 4.13, Table 4.5). After 1.5 h, reduction in viable counts were least with 3 *Pediococcus* cultures such as Sil-1, PD-29 and Sil-2. *P. pentosaceus* 42-PD showed maximum survival with 1.24 log CFU reduction in viable counts from an initial count of 9.09±0.18 log CFU/mL, followed by Sil-1 with 1.53 log CFU reduction (initial count 9.13±0.26 log

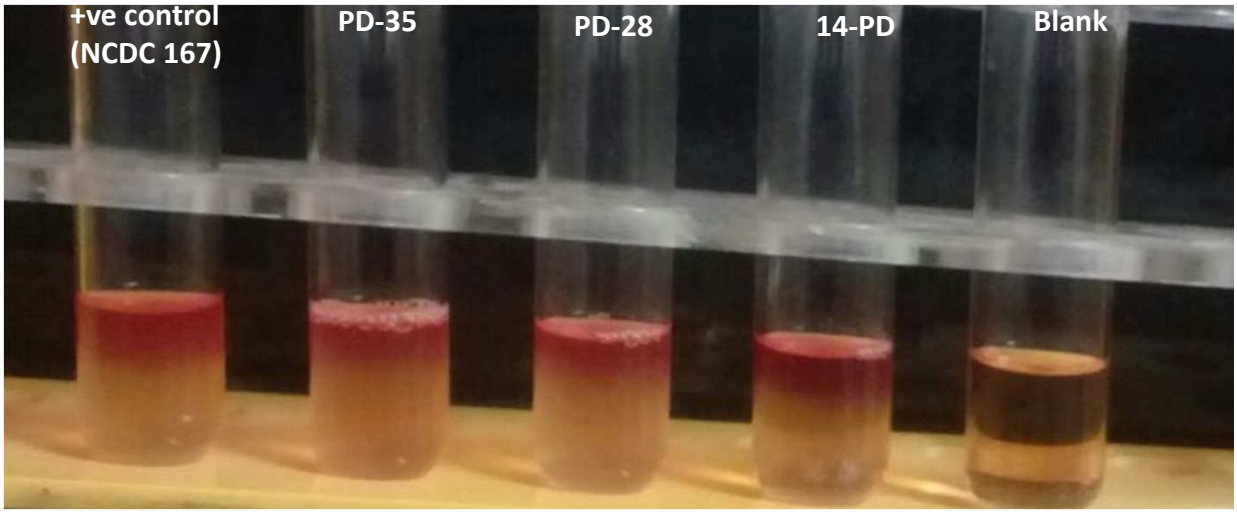
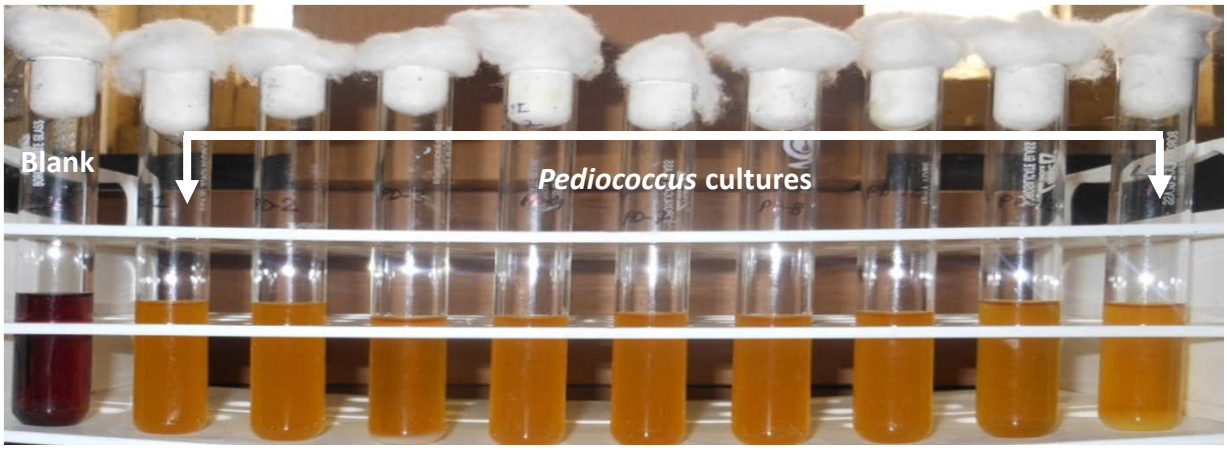
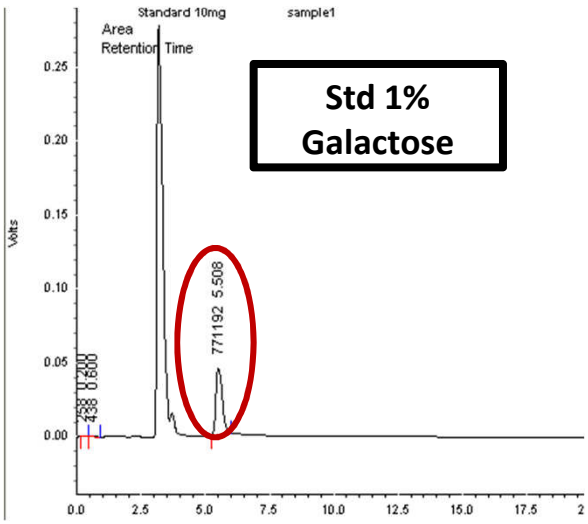
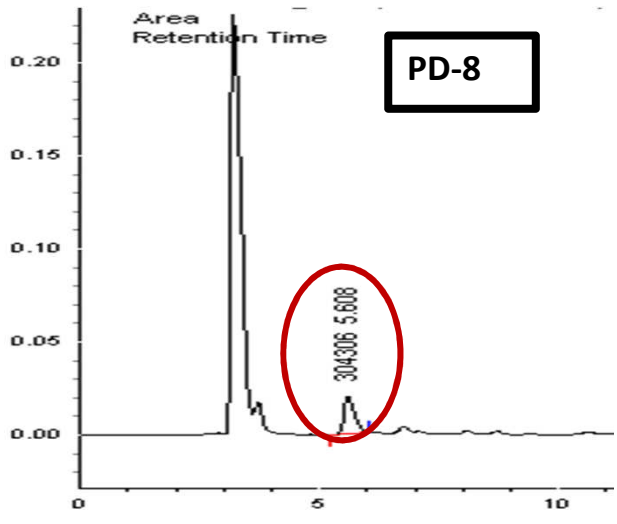


Fig. 4.11 Diacetyl production by *Pediococcus* cultures

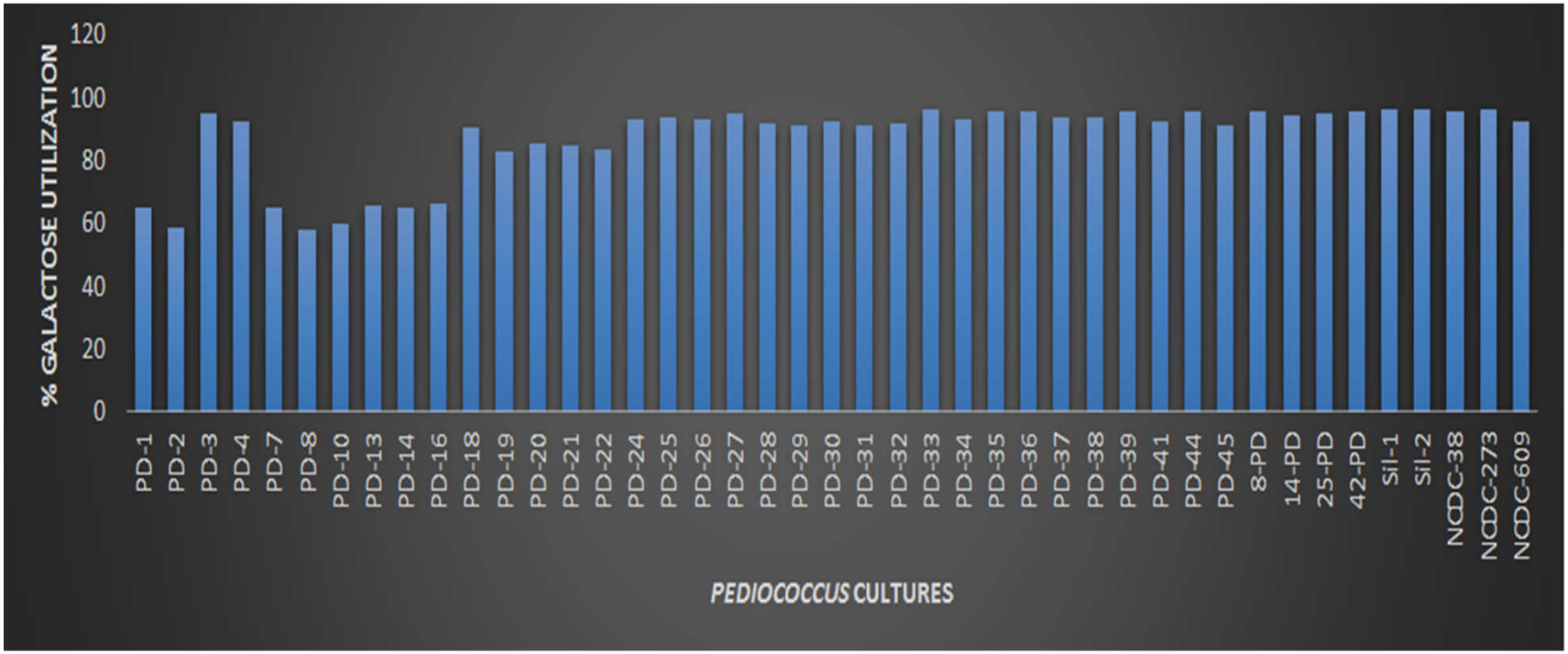


(a)





(b)



(c)

Fig. 4.12 Galactose fermentation by *Pediococcus* cultures

(a) Galactose fermentation by *Pediococcus* cultures in CHL broth containing 1% galactose

(b) HPLC chromatographs of galactose utilization by *Pediococcus* cultures (CHL broth, 1% filter sterilized galactose)

(c) Galactose utilization (%) by *Pediococcus* cultures (CHL broth containing 1% galactose)

Table 4.4 Technological attributes of *Pediococcus* cultures

S. No.	Cultures	Phytase activity (mm)	EPS production	Diacetyl production	Galactose fermentation
1	*P. pentosaceus* PD-1	11	++	+	+
2	*P. pentosaceus* PD-2	18	-	-	+
3	*P. pentosaceus* PD-3	17	-	-	+
4	*P. pentosaceus* PD-4	15	+	+	+
5	*P. pentosaceus* PD-7	15	+	+	+
6	*P. pentosaceus* PD-8	19	+	+	+
7	*P. pentosaceus* PD-10	-	+	+	+
8	*P. pentosaceus* PD-13	-	-	-	+
9	*P. pentosaceus* PD-14	-	-	-	+
10	*P. pentosaceus* PD-16	15	+	+	+
11	*P. pentosaceus* PD-18	14	+	-	+
12	*P. pentosaceus* PD-19	13.5	+	-	+
13	*P. pentosaceus* PD-20	11.5	+	+	+
14	*P. pentosaceus* PD-21	-	+	-	+
15	*P. pentosaceus* PD-22	14.5	+	-	+
16	*P. pentosaceus* PD-24	14.5	+	-	+
17	*P. pentosaceus* PD-25	21	+	-	+
18	*P. pentosaceus* PD-26	19	+	-	+
19	*P. pentosaceus* PD-27	19	+	-	+
20	*P. pentosaceus* PD-28	19	+	+	+
21	*P. pentosaceus* PD-29	-	-	-	+
22	*P. pentosaceus* PD-30	17.5	-	+	+

S. No.	Cultures	Phytase activity (mm)	EPS production	Diacetyl production	Galactose fermentation
23	*P. pentosaceus* PD-31	11	-	-	+
24	*P. pentosaceus* PD-32	20	-	-	+
25	*P. pentosaceus* PD-33	13	+	-	+
26	*P. pentosaceus* PD-34	17	+	-	+
27	*P. pentosaceus* PD-35	18	-	+	+
28	*P. pentosaceus* PD-36	13.5	-	-	+
29	*P. pentosaceus* PD-37	16	-	-	+
30	*P. pentosaceus* PD-38	-	-	-	+
31	*P. pentosaceus* PD-39	16	-	-	+
32	*P. pentosaceus* PD-41	15	-	-	+
33	*P. pentosaceus* PD-44	20	++	-	+
34	*P. acidilactici* PD-45	14	-	+	+
35	*P. pentosaceus* 8-PD	18	-	-	+
36	*P. pentosaceus* 14-PD	22	-	+	+
37	*P. pentosaceus* 25-PD	26	+	-	+
38	*P. pentosaceus* 42-PD	20	++	-	+
39	*P. pentosaceus* Sil-1	19	++	-	+
40	*P. pentosaceus* Sil-2	16	++	-	+
	P. pentosaceus NCDC 38	14	++	-	+
	P. pentosaceus NCDC 273	14	-	-	+
	P. acidilactici NCDC 609	-	+	-	+

*EPS: ++ denotes mucoid colonies; + viscous growth/low EPS producer

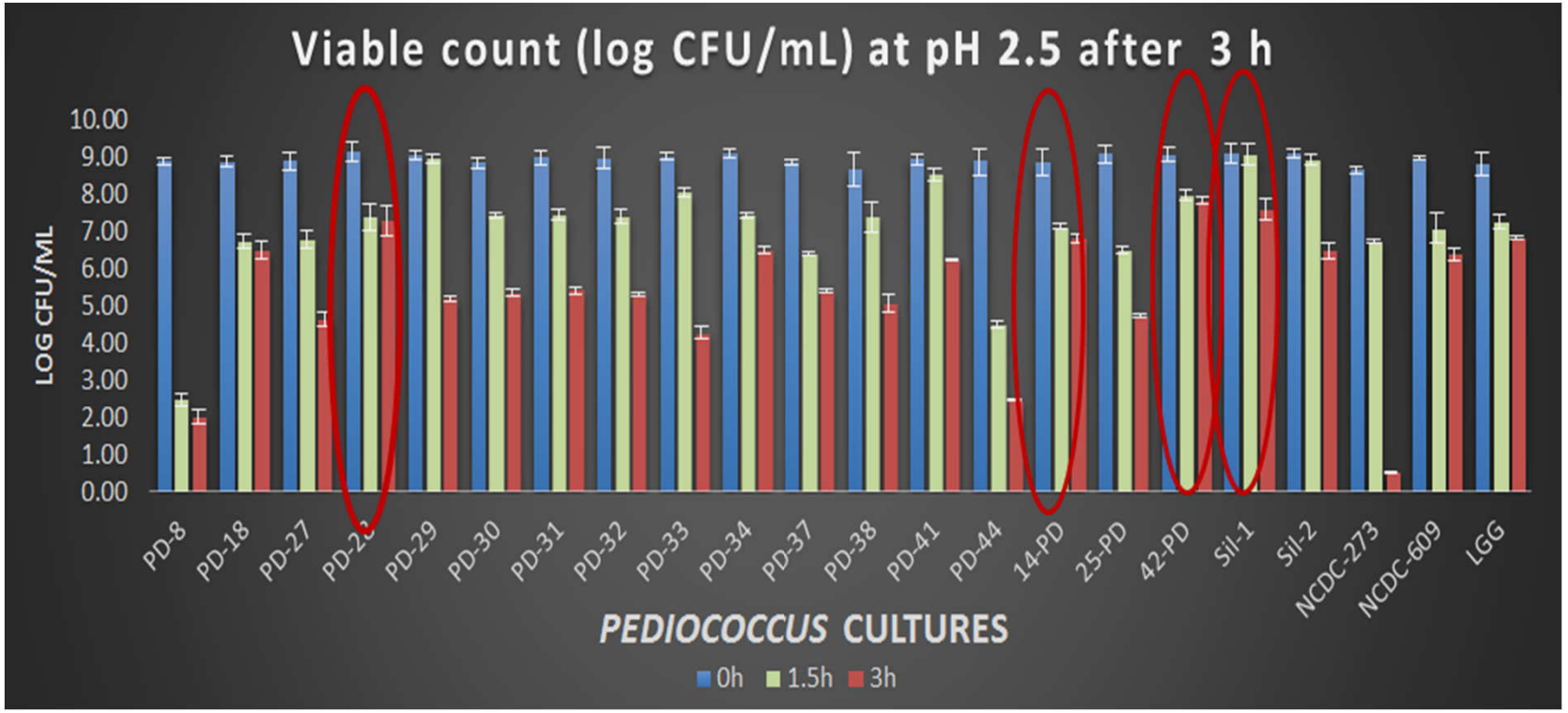


Fig. 4.13 Acid tolerance of *Pediococcus* cultures at pH 2.5 for 3 h

Table 4.5 Acid tolerance of *Pediococcus* cultures

S. No.	Cultures	pH					
		6.5			2.5		
		Time (h)					
		0	1.5	3	0	1.5	3
		Viable count (log CFU/mL)					
1	*P. pentosaceus* PD-1	8.59 ± 0.06	8.90 ± 0.04	9.00 ± 0.05	8.69 ± 0.04	-	-
2	*P. pentosaceus* PD-2	8.79 ± 0.15	8.91 ± 0.06	9.01 ± 0.06	8.76 ± 0.12	-	-
3	*P. pentosaceus* PD-3	8.66 ± 0.31	8.83 ± 0.26	9.03 ± 0.25	8.69 ± 0.22	-	-
4	*P. pentosaceus* PD-4	8.81 ± 0.24	8.95 ± 0.18	9.20 ± 0.14	8.73 ± 0.19	3.34 ± 0.31	-
5	*P. pentosaceus* PD-7	8.83 ± 0.06	9.26 ± 0.07	9.32 ± 0.07	8.77 ± 0.21	-	-
6	*P. pentosaceus* PD-8	8.93 ± 0.21	9.29 ± 0.20	9.34 ± 0.19	8.91 ± 0.09	2.50 ± 0.16	2.02 ± 0.18
7	*P. pentosaceus* PD-10	9.00 ± 0.04	9.11 ± 0.06	9.23 ± 0.06	8.92 ± 0.11	-	-
8	*P. pentosaceus* PD-13	9.15 ± 0.03	9.40 ± 0.05	9.48 ± 0.01	8.61 ± 0.29	-	-
9	*P. pentosaceus* PD-14	9.10 ± 0.15	9.13 ± 0.16	9.22 ± 0.15	8.95 ± 0.18	6.42 ± 0.37	-
10	*P. pentosaceus* PD-16	9.18 ± 0.13	9.22 ± 0.14	9.27 ± 0.17	8.45 ± 0.33	-	-
11	*P. pentosaceus* PD-18	9.12 ± 0.07	9.37 ± 0.08	9.40 ± 0.08	8.89 ± 0.14	6.75 ± 0.21	6.52 ± 0.25
12	*P. pentosaceus* PD-19	9.10 ± 0.18	9.27 ± 0.15	9.30 ± 0.16	9.07 ± 0.19	2.40 ± 0.09	-
13	*P. pentosaceus* PD-20	9.00 ± 0.05	9.10 ± 0.02	9.34 ± 0.05	8.96 ± 0.21	2.46 ± 0.05	-
14	*P. pentosaceus* PD-21	8.96 ± 0.13	9.18 ± 0.10	9.23 ± 0.07	8.89 ± 0.29	-	-
15	*P. pentosaceus* PD-22	8.98 ± 0.04	9.04 ± 0.07	9.23 ± 0.09	8.52 ± 0.35	-	-
16	*P. pentosaceus* PD-24	8.89 ± 0.06	8.96 ± 0.04	9.04 ± 0.05	8.72 ± 0.34	-	-
17	*P. pentosaceus* PD-25	8.80 ± 0.16	9.18 ± 0.13	9.22 ± 0.16	8.95 ± 0.18	4.49 ± 0.16	-
18	*P. pentosaceus* PD-26	8.78 ± 0.21	9.01 ± 0.23	9.08 ± 0.21	8.63 ± 0.44	-	-
19	*P. pentosaceus* PD-27	8.96 ± 0.18	9.16 ± 0.19	9.28 ± 0.17	8.91 ± 0.22	6.81 ± 0.26	4.65 ± 0.20
20	*P. pentosaceus* PD-28	8.99 ± 0.21	9.16 ± 0.19	9.24 ± 0.19	9.18 ± 0.26	7.42 ± 0.36	7.30 ± 0.38

21	*P. pentosaceus* PD-29	9.11 ± 0.10	9.22 ± 0.14	9.31 ± 0.13	9.08 ± 0.14	8.98 ± 0.11	5.20 ± 0.08
22	*P. pentosaceus* PD-30	8.81 ± 0.08	9.09 ± 0.13	9.24 ± 0.19	8.88 ± 0.15	7.44 ± 0.06	5.36 ± 0.09
23	*P. pentosaceus* PD-31	9.12 ± 0.18	9.18 ± 0.15	9.32 ± 0.07	9.00 ± 0.19	7.47 ± 0.15	5.44 ± 0.09
24	*P. pentosaceus* PD-32	9.18 ± 0.12	9.20 ± 0.11	9.41 ± 0.09	9.00 ± 0.27	7.43 ± 0.20	5.33 ± 0.06
25	*P. pentosaceus* PD-33	8.83 ± 0.25	8.85 ± 0.24	9.16 ± 0.16	9.03 ± 0.10	8.07 ± 0.13	4.28 ± 0.17
26	*P. pentosaceus* PD-34	9.28 ± 0.05	9.39 ± 0.06	9.44 ± 0.06	9.11 ± 0.12	7.46 ± 0.07	6.51 ± 0.08
27	*P. pentosaceus* PD-35	9.05 ± 0.20	9.26 ± 0.16	9.28 ± 0.16	9.14 ± 0.58	5.12 ± 0.33	-
28	*P. pentosaceus* PD-36	9.19 ± 0.14	9.29 ± 0.10	9.40 ± 0.10	8.84 ± 0.10	-	-
29	*P. pentosaceus* PD-37	9.12 ± 0.17	9.18 ± 0.14	9.30 ± 0.10	8.89 ± 0.07	6.43 ± 0.04	5.43 ± 0.04
30	*P. pentosaceus* PD-38	8.94 ± 0.23	9.01 ± 0.22	9.16 ± 0.22	8.70 ± 0.44	7.40 ± 0.39	5.08 ± 0.24
31	*P. pentosaceus* PD-39	8.99 ± 0.15	9.09 ± 0.18	9.27 ± 0.18	8.99 ± 0.07	6.67 ± 0.04	-
32	*P. pentosaceus* PD-41	8.81 ± 0.06	9.06 ± 0.13	9.29 ± 0.12	8.95 ± 0.13	8.55 ± 0.18	6.26 ± 0.03
33	*P. pentosaceus* PD-44	8.75 ± 0.07	9.15 ± 0.06	9.28 ± 0.06	8.91 ± 0.36	4.52 ± 0.09	2.48 ± 0.01
34	*P. acidilactici* PD-45	9.09 ± 0.04	9.35 ± 0.04	9.47 ± 0.02	9.04 ± 0.16	-	-
35	*P. pentosaceus* 8-PD	8.92 ± 0.26	9.05 ± 0.29	9.15 ± 0.30	9.04 ± 0.03	5.15 ± 0.02	-
36	*P. pentosaceus* 14-PD	9.05 ± 0.05	9.10 ± 0.06	9.21 ± 0.07	8.87 ± 0.37	7.15 ± 0.07	6.83 ± 0.11
37	*P. pentosaceus* 25-PD	9.01 ± 0.04	9.28 ± 0.07	9.37 ± 0.08	9.12 ± 0.24	6.52 ± 0.12	4.76 ± 0.04
38	*P. pentosaceus* 42-PD	9.13 ± 0.05	9.22 ± 0.06	9.35 ± 0.07	9.09 ± 0.18	8.00 ± 0.15	7.85 ± 0.09
39	*P. pentosaceus* Sil-1	9.15 ± 0.06	9.18 ± 0.06	9.36 ± 0.05	9.13 ± 0.26	9.09 ± 0.27	7.60 ± 0.28
40	*P. pentosaceus* Sil-2	9.19 ± 0.03	9.28 ± 0.04	9.30 ± 0.04	9.12 ± 0.11	8.95 ± 0.15	6.49 ± 0.23
	P. pentosaceus NCDC 38	8.97 ± 0.08	9.07 ± 0.08	9.15 ± 0.11	8.88 ± 0.46	-	-
	P. pentosaceus NCDC 273	8.41 ± 0.06	8.48 ± 0.04	8.57 ± 0.03	8.67 ± 0.11	6.77 ± 0.04	0.54 ± 0.02
	P. acidilactici NCDC 609	8.30 ± 0.08	8.35 ± 0.06	8.43 ± 0.05	8.99 ± 0.04	7.10 ± 0.40	6.40 ± 0.18
	Lb. rhamnosus GG (LGG)	8.52 ± 0.33	8.60 ± 0.34	8.73 ± 0.36	8.83 ± 0.33	7.28 ± 0.20	6.86 ± 0.05

All values are represented as mean±SD (n=3)

CFU/mL), and PD-28 with 1.88 log CFU reduction (initial count 9.18±0.26 log CFU/mL).

In-vitro acid tolerance of *Pediococcus* cultures was studied widely (Badarinath *et al*., 2010; Oh and Jung, 2015; Noohi *et al*., 2016). Jonganurakkun *et al*. (2008) studied acid tolerance of 5 LAB strains and reported that *P. pentosaceus* strain NB-17 (from fermented vegetable) exhibited the highest survival in simulated gastric juice at pH 3 up-to 3 h. Moreover, at pH 2.5, the strain resist the acid environment with 88.9% survival during 1 h, then decreased to about 50% after 3 h. Yuksekdag and Aslim (2010) studied the survival of *Pediococcus* strains at pH 1 to 3 and found that only two *P. pentosaceus* strains Z12P and Z13P were able to survive. Vidhyasagar and Jeevaratnam (2013) studied six bacteriocinogenic *Pediococcus* strains (from idly batter) for gastric acid tolerance at pH 2 and found that all the strains were able to survive up-to 4 h with considerable difference in survival. *P. acidilactici* Ch-2, a plant derived strain showed resistance at pH 2 and 3 up-to 3 h with 72.69 and 98.62% survival, respectively (Gupta and Sharma, 2017). Narayanan *et al*. (2017) tested 7 LAB strains for acid tolerance at pH 2.5 and found that only *P. pentosaceus* ID 9 (from idli batter) could tolerate the low pH stress.

4.3.2 *In-vitro* bile tolerance

Bile tolerance of *Pediococcus* cultures were evaluated by determining the viability (log CFU/mL) in 0.5 and 1% bile salt concentration up-to 4 h (Table 4.6). All the tested *Pediococcus* cultures tolerated 1% bile concentrations up-to 4 h. Viable counts were decreased with increase in bile concentration and exposure time. Most tolerant strain was Sil-2 with 1.05 log reduction in viable count (initial count 8.48±0.11 log CFU/mL) followed by NCDC 609 with 1.21 log reduction (initial count 8.70±0.15 log CFU/mL) and PD-21 with 1.41 log reduction (initial count 8.94±0.15 log CFU/mL). Ramirez-Chavarin *et al*. (2013) studied LAB strains for tolerance to different bile concentrations (0.1, 0.3, 0.5, 1 and 2%) and found that 3 out of 4 pediococci strains grow abundantly at all the bile concentrations while, one *Pediococcus* strain exhibited lower growth at 1 and 2% bile concentrations. Shukla and Goyal (2013) studied the probiotic potential of *P. pentosaceus* CRAG3 (from fermented cucumber) and observed that the strain tolerated 0.3, 0.5 and 1% bile salts with 73.33±4.2, 63.3±1.5 and 50.0±2.1%

survival, respectively. Attri *et al*. (2015) studied the probiotic property of *P. acidilactici* NCDC 252 and reported the survival as 15.7±1.1% at 0.5% bile, and it declined to 6.4±1.2% at 1% bile concentration.

4.3.3 Cell surface hydrophobicity

The hydrophobic nature of the outermost surface of microorganisms has been implicated in the attachment of bacteria to host tissue as this property could confer a competitive advantage, important for bacterial maintenance in human GI tract (Schillinger *et al*., 2005). All the *Pediococcus* cultures were evaluated for cell surface hydrophobicity towards n-hexadecane. Cell surface hydrophobicity varied considerably ranging from 7.95 to 59.35% (Fig. 4.14). Six out of 40 studied cultures showed >30% cell surface hydrophobicity (PD-21, 59.35±1.76; PD-4, 39.09±0.51; PD-22, 38.01±1.26; PD-13, 32.75±1.25; PD-37, 31.76±0.38; PD-30, 31.46±0.19%). Tamang *et al*. (2009) evaluated 94 LAB strains (including pediococci) for their hydrophobicity potential in the presence of n-hexadecane. They concluded a high variability among studied strains towards hydrophobicity. A total 77 strains out of 94 exhibited <30% hydrophobicity, 10 strains ranged between 30-70% and 7 strains showed >70% hydrophobicity. However, none of the pediococci strains showed more than 14% cell surface hydrophobicity. Interestingly, Raghavendra *et al*. (2010) has reported high hydrophobicity (62.8%) towards xylene by *Pediococcus* strain. Shukla and Goyal (2014) reported 33% hydrophobicity by *P. pentosaceus* strain using n-hexadecane; while, using the same hydrocarbon (i.e. n-hexadecane), Garsa *et al*. (2014) reported 14.13% and 8.87% hydrophobicity by *Pediococcus* strains. The wide variation in cell surface hydrophobicity shown by the cultures could be due to differences in cell surface composition and expression of the cell proteins as these varies among different species as well as among different strains of the same species (Kaushik *et al*., 2009).

4.3.4 Cell auto-aggregation

Testing of auto-aggregative ability as an indicator of adhesion ability (Collado *et al*., 2008; Rahman *et al*., 2008) and tolerance of GI environmental conditions (Mercenier *et al*., 2008) are considered as prerequisite for screening intestinal bacterial cultures for their functional properties. The cellular auto-aggregation of probiotic bacteria is considered a desirable characteristic, as they can potentially inhibit adherence of pathogenic bacteria to intestinal mucosa by forming a barrier via self-aggregation

Table 4.6 Bile tolerance of *Pediococcus* cultures

S. No.	Cultures	Bile concentration (% w/v)								
		0			0.5			1		
		Time (h)								
		0	2	4	0	2	4	0	2	4
		Viable count (log CFU/mL)								
1	*P. pentosaceus* PD-1	8.86 ± 0.13	8.98 ± 0.15	9.10 ± 0.11	8.94 ± 0.11	5.02 ± 0.10	5.04 ± 0.11	8.93 ± 0.25	4.99 ± 0.22	4.48 ± 0.29
2	*P. pentosaceus* PD-2	9.18 ± 0.04	9.27 ± 0.03	9.31 ± 0.02	8.74 ± 0.20	8.52 ± 0.22	8.60 ± 0.26	9.15 ± 0.15	6.40 ± 0.13	6.36 ± 0.09
3	*P. pentosaceus* PD-3	9.00 ± 0.15	9.20 ± 0.13	9.34 ± 0.1	8.77 ± 0.11	8.46 ± 0.15	8.41 ± 0.16	8.95 ± 0.14	7.05 ± 0.05	6.90 ± 0.04
4	*P. pentosaceus* PD-4	8.78 ± 0.04	9.13 ± 0.05	9.22 ± 0.07	8.78 ± 0.18	8.58 ± 0.18	8.48 ± 0.17	8.75 ± 0.18	6.30 ± 0.16	6.23 ± 0.15
5	*P. pentosaceus* PD-7	9.05 ± 0.03	9.27 ± 0.01	9.47 ± 0.04	9.04 ± 0.09	8.83 ± 0.11	8.46 ± 0.14	9.02 ± 0.08	8.04 ± 0.04	7.34 ± 0.02
6	*P. pentosaceus* PD-8	9.01 ± 0.09	9.22 ± 0.1	9.34 ± 0.04	8.98 ± 0.02	7.08 ± 0.07	6.54 ± 0.10	9.00 ± 0.09	5.78 ± 0.03	5.60 ± 0.14
7	*P. pentosaceus* PD-10	8.72 ± 0.16	9.3 ± 0.03	9.38 ± 0.05	8.72 ± 0.35	4.48 ± 0.44	2.45 ± 0.35	8.86 ± 0.09	3.32 ± 0.08	2.30 ± 0.07
8	*P. pentosaceus* PD-13	8.88 ± 0.05	9.18 ± 0.02	9.27 ± 0.03	8.83 ± 0.08	7.08 ± 0.03	6.88 ± 0.05	8.85 ± 0.13	6.30 ± 0.14	6.26 ± 0.16
9	*P. pentosaceus* PD-14	8.63 ± 0.09	9.08 ± 0.08	9.28 ± 0.05	8.63 ± 0.23	6.54 ± 0.28	6.38 ± 0.27	8.86 ± 0.08	6.61 ± 0.12	6.41 ± 0.12
10	*P. pentosaceus* PD-16	8.65 ± 0.01	8.89 ± 0.06	8.96 ± 0.05	8.65 ± 0.19	6.61 ± 0.27	6.30 ± 0.18	8.3 ± 0.1	5.70 ± 0.07	4.76 ± 0.02
11	*P. pentosaceus* PD-18	9.31 ± 0.03	9.45 ± 0.01	9.48 ± 0.05	9.34 ± 0.04	8.04 ± 0.04	8.00 ± 0.05	9.32 ± 0.10	7.97 ± 0.07	7.48 ± 0.07
12	*P. pentosaceus* PD-19	9.17 ± 0.17	9.30 ± 0.13	9.40 ± 0.11	9.15 ± 0.07	7.04 ± 0.06	6.95 ± 0.06	9.11 ± 0.18	6.75 ± 0.16	6.65 ± 0.16
13	*P. pentosaceus* PD-20	9.03 ± 0.04	9.17 ± 0.05	9.46 ± 0.03	8.94 ± 0.10	7.30 ± 0.08	7.30 ± 0.06	8.99 ± 0.04	5.46 ± 0.09	5.32 ± 0.10
14	*P. pentosaceus* PD-21	8.92 ± 0.23	9.19 ± 0.2	9.29 ± 0.16	8.92 ± 0.18	8.00 ± 0.13	7.70 ± 0.10	8.94 ± 0.15	7.75 ± 0.09	7.53 ± 0.16
15	*P. pentosaceus* PD-22	8.48 ± 0.1	8.85 ± 0.17	8.99 ± 0.13	8.48 ± 0.10	6.86 ± 0.08	6.62 ± 0.12	8.73 ± 0.20	5.60 ± 0.18	5.30 ± 0.13
16	*P. pentosaceus* PD-24	8.72 ± 0.12	8.99 ± 0.09	9.18 ± 0.05	8.72 ± 0.19	6.48 ± 0.16	6.30 ± 0.17	9.09 ± 0.07	7.60 ± 0.06	6.34 ± 0.02
17	*P. pentosaceus* PD-25	9.05 ± 0.02	9.34 ± 0.04	9.42 ± 0.01	9.15 ± 0.03	6.95 ± 0.05	6.90 ± 0.07	9.18 ± 0.04	6.88 ± 0.03	6.76 ± 0.04
18	*P. pentosaceus* PD-26	9.13 ± 0.04	9.30 ± 0.02	9.42 ± 0.04	9.13 ± 0.09	8.34 ± 0.07	8.28 ± 0.07	9.05 ± 0.04	7.48 ± 0.07	7.37 ± 0.05
19	*P. pentosaceus* PD-27	8.60 ± 0.09	8.94 ± 0.09	9.01 ± 0.10	8.94 ± 0.13	7.44 ± 0.06	6.63 ± 0.03	9.05 ± 0.08	7.39 ± 0.07	5.37 ± 0.06
20	*P. pentosaceus* PD-28	9.06 ± 0.07	9.28 ± 0.08	9.33 ± 0.10	8.41 ± 0.41	7.55 ± 0.41	7.37 ± 0.33	8.32 ± 0.21	6.33 ± 0.20	5.54 ± 0.16
21	*P. pentosaceus* PD-29	8.85 ± 0.08	8.99 ± 0.02	9.11 ± 0.03	8.85 ± 0.17	5.75 ± 0.21	5.48 ± 0.21	9.35 ± 0.03	5.09 ± 0.06	5.02 ± 0.06
22	*P. pentosaceus* PD-30	8.85 ± 0.16	9.13 ± 0.14	9.31 ± 0.12	8.85 ± 0.11	7.41 ± 0.05	6.44 ± 0.04	9.19 ± 0.03	5.45 ± 0.02	4.54 ± 0.04

S. No.	Cultures	Bile concentration (% w/v)								
		0			0.5			1		
		Time (h)								
		0	2	4	0	2	4	0	2	4
23	*P. pentosaceus* PD-31	8.61 ± 0.32	8.91 ± 0.3	9.18 ± 0.24	8.61 ± 0.30	6.38 ± 0.32	4.54 ± 0.45	9.15 ± 0.05	6.38 ± 0.04	4.32 ± 0.14
24	*P. pentosaceus* PD-32	8.78 ± 0.16	9.03 ± 0.17	9.23 ± 0.14	8.78 ± 0.04	6.30 ± 0.07	5.78 ± 0.09	8.83 ± 0.08	5.95 ± 0.24	4.66 ± 0.12
25	*P. pentosaceus* PD-33	8.81 ± 0.10	8.96 ± 0.08	9.16 ± 0.05	8.81 ± 0.18	6.65 ± 0.20	5.15 ± 0.28	8.65 ± 0.04	5.19 ± 0.06	2.85 ± 0.03
26	*P. pentosaceus* PD-34	8.73 ± 0.33	8.92 ± 0.2	9.01 ± 0.29	8.76 ± 0.15	6.70 ± 0.10	6.65 ± 0.08	8.98 ± 0.08	6.41 ± 0.29	6.18 ± 0.31
27	*P. pentosaceus* PD-35	8.25 ± 0.14	8.45 ± 0.14	8.59 ± 0.13	8.20 ± 0.09	5.18 ± 0.24	4.11 ± 0.37	8.20 ± 0.06	4.18 ± 0.16	3.34 ± 0.37
28	*P. pentosaceus* PD-36	8.95 ± 0.05	9.28 ± 0.09	9.39 ± 0.10	8.95 ± 0.15	5.97 ± 0.17	5.54 ± 0.08	8.83 ± 0.15	4.08 ± 0.12	4.04 ± 0.13
29	*P. pentosaceus* PD-37	9.00 ± 0.1	9.13 ± 0.11	9.24 ± 0.11	9.18 ± 0.07	6.23 ± 0.07	5.95 ± 0.11	9.21 ± 0.06	4.18 ± 0.12	3.95 ± 0.05
30	*P. pentosaceus* PD-38	8.83 ± 0.26	9.04 ± 0.27	9.22 ± 0.23	8.83 ± 0.15	7.34 ± 0.29	6.15 ± 0.18	8.93 ± 0.08	6.93 ± 0.14	3.85 ± 0.07
31	*P. pentosaceus* PD-39	8.60 ± 0.27	8.85 ± 0.25	9.08 ± 0.25	8.60 ± 0.11	4.52 ± 0.21	3.78 ± 0.09	8.51 ± 0.17	4.08 ± 0.14	3.49 ± 0.27
32	*P. pentosaceus* PD-41	8.71 ± 0.35	8.82 ± 0.39	8.96 ± 0.37	8.70 ± 0.09	5.90 ± 0.08	5.70 ± 0.06	8.87 ± 0.09	5.12 ± 0.12	5.06 ± 0.1
33	*P. pentosaceus* PD-44	8.76 ± 0.08	9.19 ± 0.05	9.34 ± 0.04	8.95 ± 0.12	7.75 ± 0.14	5.30 ± 0.21	8.89 ± 0.09	4.66 ± 0.16	4.63 ± 0.15
34	*P. acidilactici* PD-45	8.98 ± 0.03	9.33 ± 0.04	9.50 ± 0.02	8.99 ± 0.05	7.29 ± 0.05	7.05 ± 0.03	8.70 ± 0.11	6.63 ± 0.06	5.65 ± 0.13
35	*P. pentosaceus* 8-PD	8.69 ± 0.4	9.07 ± 0.39	9.20 ± 0.40	8.67 ± 0.15	7.10 ± 0.08	4.76 ± 0.11	8.83 ± 0.11	3.53 ± 0.09	2.60 ± 0.04
36	*P. pentosaceus* 14-PD	8.95 ± 0.07	9.05 ± 0.09	9.24 ± 0.11	8.73 ± 0.12	7.13 ± 0.13	6.26 ± 0.15	8.83 ± 0.09	6.11 ± 0.10	4.38 ± 0.13
37	*P. pentosaceus* 25-PD	8.72 ± 0.09	9.00 ± 0.14	9.11 ± 0.14	8.72 ± 0.11	6.65 ± 0.14	5.83 ± 0.18	8.84 ± 0.13	4.83 ± 0.18	3.73 ± 0.18
38	*P. pentosaceus* 42-PD	9.10 ± 0.04	9.28 ± 0.02	9.35 ± 0.03	9.47 ± 0.05	6.85 ± 0.10	4.76 ± 0.15	9.35 ± 0.04	4.88 ± 0.12	3.81 ± 0.06
39	*P. pentosaceus* Sil-1	9.11 ± 0.04	9.24 ± 0.03	9.39 ± 0.03	9.13 ± 0.08	5.06 ± 0.05	5.00 ± 0.08	9.05 ± 0.11	4.08 ± 0.11	4.00 ± 0.09
40	*P. pentosaceus* Sil-2	8.13 ± 0.21	8.28 ± 0.2	8.34 ± 0.21	8.18 ± 0.17	8.04 ± 0.21	7.51 ± 0.10	8.48 ± 0.11	7.51 ± 0.08	7.43 ± 0.10
	P. pentosaceus NCDC 38	8.90 ± 0.05	9.08 ± 0.08	9.23 ± 0.07	8.41 ± 0.22	7.19 ± 0.22	6.53 ± 0.30	8.46 ± 0.17	5.93 ± 0.13	4.34 ± 0.15
	P. pentosaceus NCDC 273	8.48 ± 0.23	8.61 ± 0.25	8.81 ± 0.23	8.46 ± 0.04	7.53 ± 0.07	7.08 ± 0.07	8.37 ± 0.05	6.65 ± 0.13	5.95 ± 0.08
	P. pentosaceus NCDC 609	8.67 ± 0.35	8.78 ± 0.36	9.01 ± 0.39	9.02 ± 0.08	8.95 ± 0.10	7.54 ± 0.11	8.70 ± 0.15	7.54 ± 0.20	7.49 ± 0.18
	Lb. rhamnosus GG (LGG)	8.57 ± 0.21	8.72 ± 0.23	8.94 ± 0.27	8.46 ± 0.08	7.75 ± 0.05	7.41 ± 0.08	8.49 ± 0.10	7.08 ± 0.11	6.69 ± 0.16

All values are represented as mean±SD (n=3)

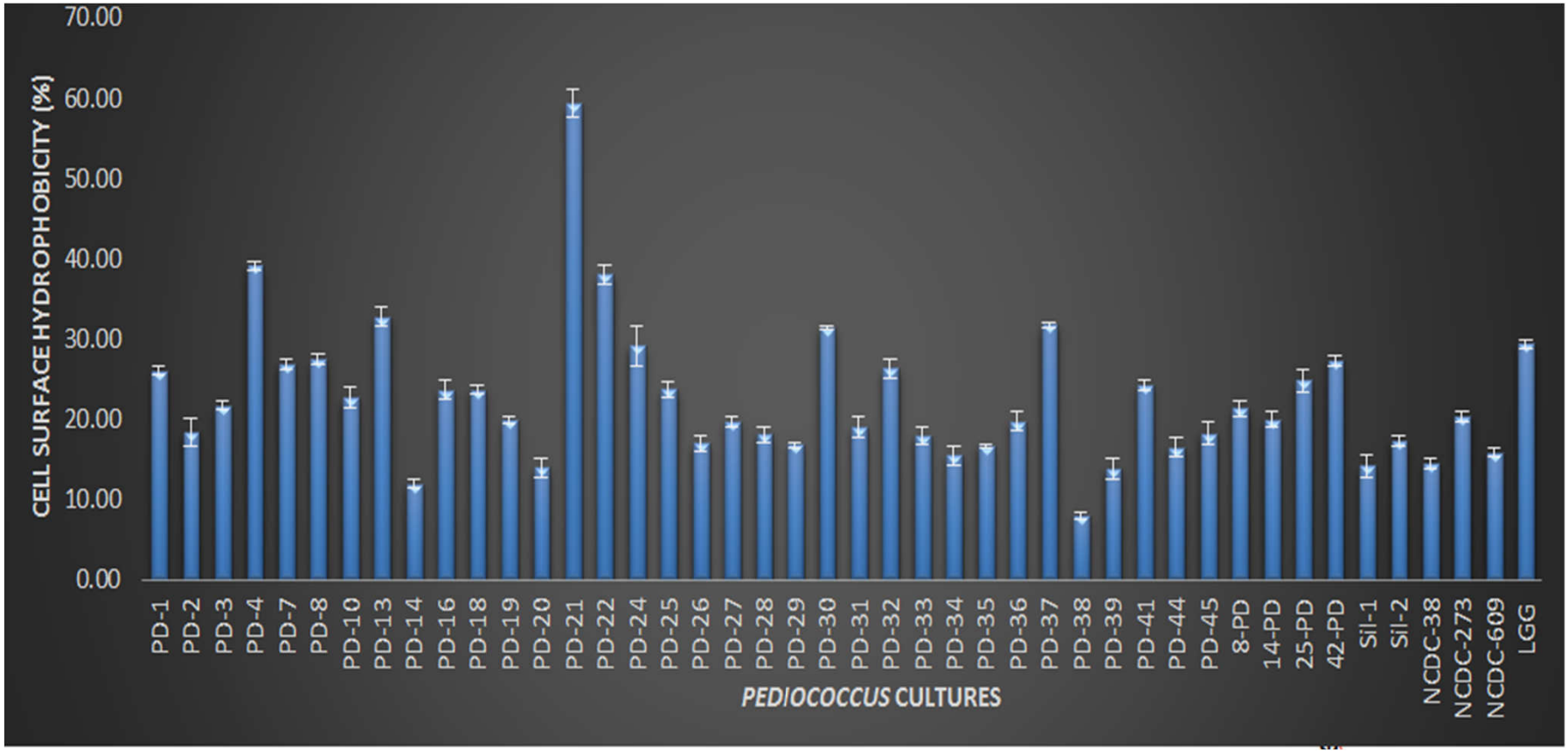


Fig. 4.14 Cell surface hydrophobicity of *Pediococcus* cultures

(Bujnakova and Kmet, 2002; Kaushik *et al*., 2009). Cellular auto-aggregation potential of *Pediococcus* cultures was evaluated by measuring the sedimentation rate over a period of 4 h. All the *Pediococcus* cultures showed the ability to auto-aggregate and auto-aggregation values were ranged from 39.28 to 83.28% (Fig. 4.15). Six out of 40 *Pediococcus* cultures along with *P. pentosaceus* NCDC 273 (61.21±0.35) exhibited >60% cellular auto-aggregation i.e. PD-4 (83.28±2.90) followed by PD-7 (73.13±0.51), 42-PD (65.78±2.82), PD-10 (61.98±0.19), PD-3 (60.77±0.77) and PD-18 (60.43±0.60). Thirty three cultures along with *P. pentosaceus* NCDC 38 and *P. acidilactici* NCDC 609 depicted 40-60% while only 1 culture i.e. PD-28 (39.28±0.51) showed <40% cellular auto-aggregation.

Wide variation in auto-aggregation ability among *Pediococcus* strains has been reported (Osmanagaoglu *et al*., 2010; Sabir *et al*., 2010; Vidhyasagar and Jeevaratnam, 2013). Gupta and Sharma (2017) depicted a very high auto-aggregation percentage (97%) by *P. acidilactici* strain Ch-2. Contrary to this, Cao *et al*. (2016) reported only 18.3% auto-aggregation by *P. pentosaceus* strain. Interestingly, *P. pentosaceus* PD-4 which was among the best culture with respect to cell surface hydrophobicity, also exhibited highest cell auto-aggregation potential. So, this type of culture might have good adhesive ability, as hydrophobicity and auto-aggregative potential are reported to be phenotypically related to the adhesion capacity (Collado *et al*., 2008).

4.3.5 Antimicrobial activity

Pediococcus cultures were examined for their antimicrobial activity by agar well diffusion assay against Gram-positive (*S. aureus* NCDC 113 and *L. monocytogenes* ATCC 15319) and Gram-negative (*S. enterica* ssp. Typhi NCDC 113 and *E. coli* ATCC 11783) indicator organisms. All the pediococci cultures demonstrated discernible antibacterial activity against the tested indicator strains with zones of inhibition from 17.67 to 24.67 mm (Fig. 4.16, Table 4.7). *Pediococcus pentosaceus* NCDC 38 (23.67 mm), PD-4 (24.67 mm), 25-PD (23.33) and PD-8 (24.33) were the most active cultures towards the indicator strains i.e. *S. aureus*, *S. enterica* ssp. Typhi, *E. coli* and *L. monocytogenes*, respectively. *Pediococcus* cultures PD-2, PD-4, PD-8. PD-18, PD-21, PD-44, 42-PD, NCDC 38 and NCDC 273 were highly active against all the four indicator strains.

Wide variations in antagonistic activities of pediococci against Gram-positive as well as Gram-negative spoilage and pathogenic organisms has been reported. The broad spectrum antimicrobial activity of *Pediococcus* cultures has been reported (Tadesse *et al*., 2005; Cosansu *et al*., 2007; Badarinath *et al*., 2010; Hladíkova *et al*., 2012; Cizeikiene *et al*., 2013; Cao *et al*., 2016). Bajpai *et al*. (2016) showed the antibacterial activity of *P. pentosaceus* 4I1 against both Gram-positive and Gram-negative bacteria with inhibition zone dia. from 16.5 to 20.4 mm. Noohi *et al*. (2016) evaluated antibacterial activity of 20 pediococci strains (from chicken) against Gram-positive and Gram-negative pathogenic bacteria. They found that some of the strains were highly active against all the indicator bacteria but the degree of antagonism varied among pediococci cultures. Probiotics mediate their antimicrobial effects by different mechanisms. LAB are known for the production of various antimicrobial compounds such as organic acids, hydrogen peroxide, bacteriocins or other inhibitory substances from metabolites which are probably responsible for their antagonism (Papagianni and Anastasiadou, 2009, Dalie *et al*., 2010; Hladíkova *et al*., 2012; Porto *et al*., 2017).

4.3.6 β-galactosidase enzyme production

The ability of *Pediococcus* cultures to produce extracellular as well as intracellular β-galactosidase enzyme was evaluated. No extracellular enzyme activity was exhibited by any of the tested cultures. Five cultures along with 2 NCDC cultures demonstrated intracellular β-galactosidase activity in the range of 0.035-0.597 IU/mg dry weight. Maximum enzyme was produced by *P. acidilactici* NCDC 609 (0.597±0.031) followed by *P. pentosaceus* NCDC 273 (0.074±0.005), PD-29 and PD-30 (0.054±0.002), PD-41 (0.042±0.004), PD-10 (0.040±0.005) and PD-3 (0.035±0.004). Vidhyasagar and Jeevaratnam (2013) studied the β-galactosidase activity in 6 pediococci strains and observed the activity in the range of 114 to 5,990 Miller unit (MU). Garsa *et al.* (2014) reported the production of β-galactosidase by *P. pentosaceus* NCDC 273 (0.086±0.001 U/mL) and *P. acidilactici* NCDC 252 (0.120±0.001 U/mL). Attri *et al*. (2015) depicted 65.08±0.9 MU β-galactosidase activity by *P. acidilactici* NCDC 252. Generally, pediococci are unable to utilize lactose, but strain specific ability to produce β-galactosidase enzyme has been reported. Inability to utilize lactose

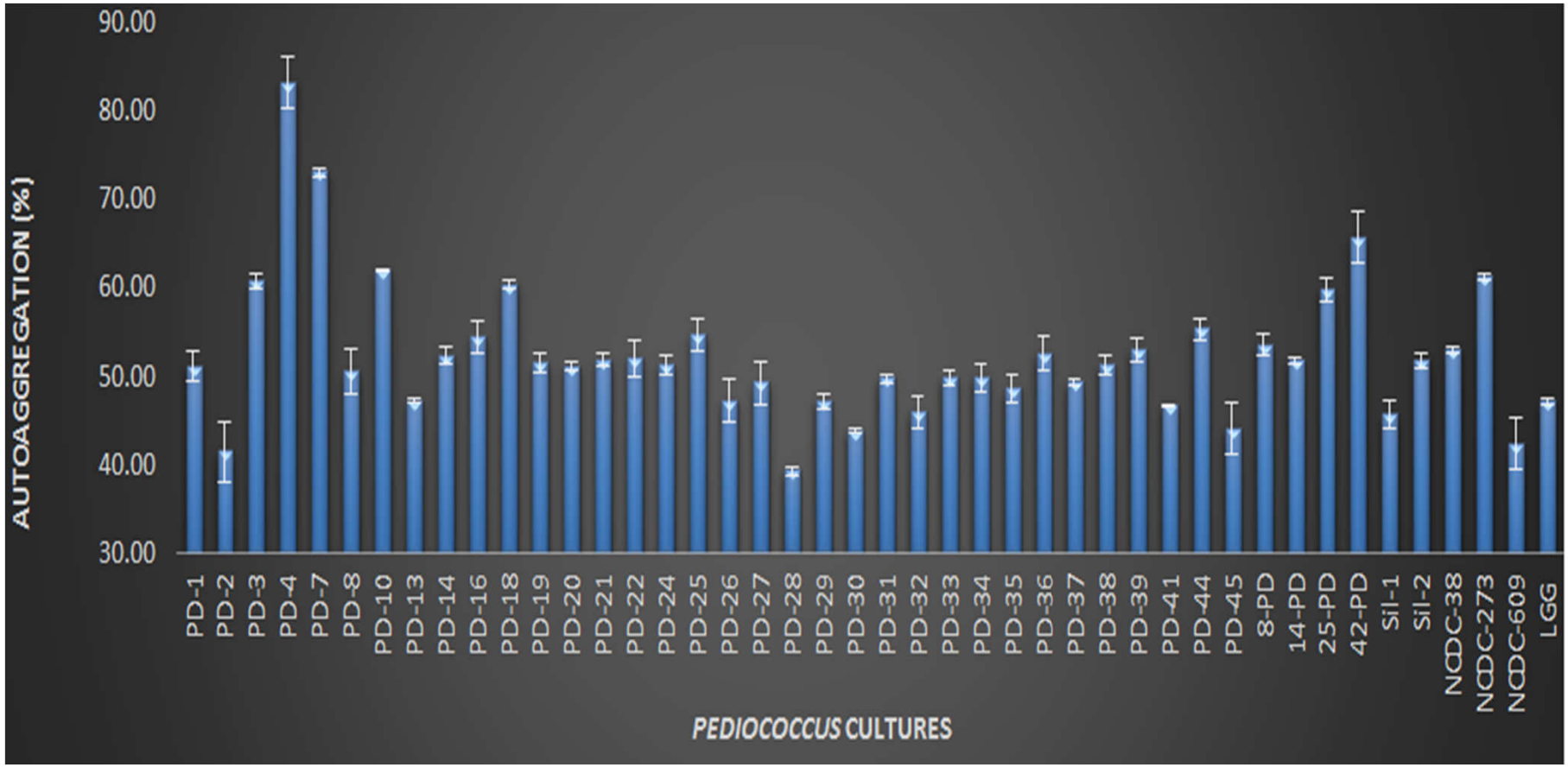


Fig. 4.15 Cell auto-aggregation of *Pediococcus* cultures

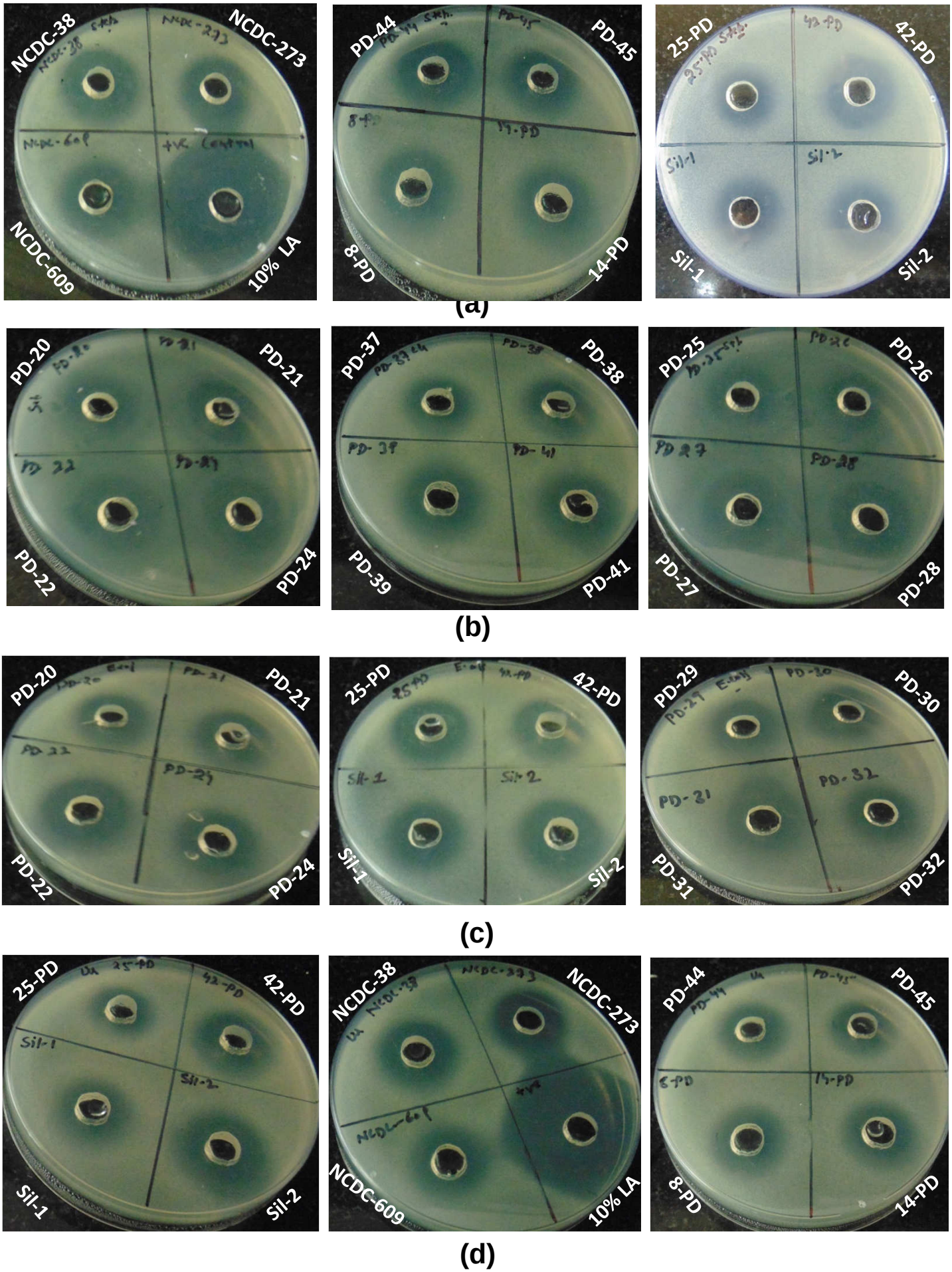


Fig. 4.16 Antimicrobial activity of *Pediococcus* cultures against (a) *S. aureus* (b) *S. enterica* ssp. Typhi (c) *E. coli* (d) *L. monocytogenes*

Table 4.7 Antimicrobial activity of *Pediococcus* cultures

S. No.	Cultures	Zone of inhibition in mm including well diameter of 8 mm			
		S. aureus	***S. enterica* ssp. Typhi**	***E. coli***	***L. monocytogenes***
1	*P. pentosaceus* PD-1	18.00 ± 1.00	15.67 ± 0.58	19.33 ± 0.58	18.67 ± 1.15
2	*P. pentosaceus* PD-2	21.33 ± 0.58	22.00 ± 1.00	20.33 ± 1.15	21.67 ± 0.58
3	*P. pentosaceus* PD-3	20.33 ± 1.15	21.33 ± 1.15	20.33 ± 0.58	21.33 ± 0.58
4	*P. pentosaceus* PD-4	21.67 ± 0.58	24.67 ± 0.58	19.00 ± 1.00	19.00 ± 1.00
5	*P. pentosaceus* PD-7	21.00 ± 1.00	20.00 ± 2.00	18.67 ± 1.15	17.67 ± 0.58
6	*P. pentosaceus* PD-8	20.67 ± 0.58	21.00 ± 1.00	21.33 ± 1.15	24.33 ± 0.58
7	*P. pentosaceus* PD-10	19.33 ± 0.58	20.67 ± 1.53	20.00 ± 1.00	20.00 ± 1.00
8	*P. pentosaceus* PD-13	20.67 ± 1.15	18.67 ± 0.58	17.00 ± 1.00	21.33 ± 1.53
9	*P. pentosaceus* PD-14	19.67 ± 1.53	22.33 ± 0.58	19.67 ± 0.58	19.67 ± 0.58
10	*P. pentosaceus* PD-16	18.67 ± 1.15	21.33 ± 0.58	18.33 ± 1.53	22.67 ± 1.15
11	*P. pentosaceus* PD-18	19.33 ± 0.58	21.33 ± 1.53	19.67 ± 0.58	21.00 ± 2.00
12	*P. pentosaceus* PD-19	18.67 ± 1.15	22.33 ± 1.53	21.33 ± 1.53	19.33 ± 1.15
13	*P. pentosaceus* PD-20	20.33 ± 0.58	21.00 ± 2.00	20.00 ± 2.00	18.33 ± 0.58
14	*P. pentosaceus* PD-21	21.67 ± 1.53	21.67 ± 1.15	20.00 ± 1.73	19.33 ± 1.15
15	*P. pentosaceus* PD-22	18.67 ± 0.58	21.67 ± 1.53	21.67 ± 1.53	21.00 ± 1.00
16	*P. pentosaceus* PD-24	20.67 ± 0.58	20.67 ± 0.58	19.33 ± 0.58	20.67 ± 0.58
17	*P. pentosaceus* PD-25	19.33 ± 1.15	19.67 ± 0.58	20.33 ± 0.58	17.67 ± 0.58
18	*P. pentosaceus* PD-26	19.00 ± 1.00	19.67 ± 0.58	19.33 ± 1.53	18.67 ± 1.15
19	*P. pentosaceus* PD-27	18.00 ± 1.73	22.33 ± 1.53	18.67 ± 0.58	20.67 ± 0.58
20	*P. pentosaceus* PD-28	17.67 ± 1.15	23.67 ± 1.15	21.67 ± 1.15	20.00 ± 1.00
21	*P. pentosaceus* PD-29	17.00 ± 1.00	19.33 ± 0.58	19.00 ± 2.00	18.67 ± 0.58
22	*P. pentosaceus* PD-30	18.67 ± 1.15	19.67 ± 1.15	21.00 ± 1.00	19.33 ± 0.58
23	*P. pentosaceus* PD-31	16.67 ± 0.58	23.67 ± 1.15	19.67 ± 1.53	18.00 ± 1.00

S. No.	Cultures	Zone of inhibition in mm including well diameter of 8 mm			
		S. aureus	***S. enterica* ssp. Typhi**	***E. coli***	***L. monocytogenes***
24	*P. pentosaceus* PD-32	17.67 ± 0.58	17.00 ± 1.00	17.67 ± 0.58	19.67 ± 1.15
25	*P. pentosaceus* PD-33	19.00 ± 1.00	20.67 ± 1.15	17.67 ± 1.15	19.33 ± 0.58
26	*P. pentosaceus* PD-34	19.33 ± 0.58	19.33 ± 0.58	17.33 ± 1.53	18.33 ± 1.53
27	*P. pentosaceus* PD-35	19.33 ± 0.58	18.33 ± 0.58	17.67 ± 1.53	19.00 ± 1.73
28	*P. pentosaceus* PD-36	18.67 ± 1.53	18.67 ± 0.58	18.33 ± 0.58	18.33 ± 0.58
29	*P. pentosaceus* PD-37	18.67 ± 0.58	17.67 ± 0.58	19.33 ± 1.53	17.67 ± 0.58
30	*P. pentosaceus* PD-38	18.33 ± 0.58	19.33 ± 1.15	17.67 ± 0.58	19.33 ± 0.58
31	*P. pentosaceus* PD-39	19.33 ± 1.15	18.33 ± 2.52	17.67 ± 0.58	19.33 ± 1.53
32	*P. pentosaceus* PD-41	18.00 ± 2.00	21.67 ± 0.58	20.67 ± 0.58	19.67 ± 2.08
33	*P. pentosaceus* PD-44	21.67 ± 0.58	19.33 ± 1.15	21.33 ± 1.15	19.67 ± 1.15
34	*P. acidilactici* PD-45	19.33 ± 1.15	21.33 ± 0.58	19.33 ± 1.53	17.67 ± 0.58
35	*P. pentosaceus* 8-PD	20.33 ± 0.58	20.67 ± 0.58	19.67 ± 0.58	18.33 ± 0.58
36	*P. pentosaceus* 14-PD	19.33 ± 0.58	19.00 ± 1.00	20.67 ± 1.53	18.00 ± 1.00
37	*P. pentosaceus* 25-PD	18.00 ± 1.00	17.67 ± 1.15	23.33 ± 0.58	18.00 ± 2.00
38	*P. pentosaceus* 42-PD	21.33 ± 0.58	20.33 ± 0.58	21.67 ± 1.53	21.33 ± 0.58
39	*P. pentosaceus* Sil-1	18.67 ± 0.58	18.67 ± 0.58	21.33 ± 1.53	18.00 ± 1.00
40	*P. pentosaceus* Sil-2	17.67 ± 1.53	23.67 ± 1.15	22.33 ± 0.58	21.67 ± 0.58
	P. pentosaceus NCDC 38	23.67 ± 1.15	21.67 ± 1.53	21.67 ± 0.58	21.33 ± 1.53
	P. pentosaceus NCDC 273	21.00 ± 1.00	22.00 ± 1.00	23.33 ± 1.53	19.67 ± 0.58
	P. acidilactici NCDC 609	21.33 ± 0.58	19.00 ± 1.00	20.33 ± 0.58	19.00 ± 1.00
	Positive control (10% LA)	40.33 ± 1.53	35.67 ± 2.08	39.33 ± 1.15	39.67 ± 2.08

even in the presence of enzyme may be due to the lack of lactose transport system in pediococci (Bhowmik and Marth, 1990; Halami and Chandrashekar, 2005; Badarinath *et al*., 2010; Sukumar and Ghosh, 2010).

4.3.7 Prebiotic fermentation

Utilization of prebiotics (fructo-oligosaccharide (FOS) and Inulin) by *Pediococcus* cultures were evaluated in CHL broth with filter sterilized prebiotics (1%). All the tested cultures were able to ferment FOS after 24 h while, only 5 cultures (*P. pentosaceus* PD-1, PD-7, PD-8, PD-10 and 8-PD) were positive for inulin fermentation after 48 h (Fig. 4.17). Shukla and Goyal (2014) depicted the utilization of prebiotic inulin by *P. pentosaceus* CRAG3 with 37.5% relative cell growth as compared to glucose within 24 h. Similarly, Siripornadulsil *et al*. (2014) reported the utilization of inulin by *Pediococcus* strains from fermented foods. Prebiotics are mostly fibers that are non-digestible food ingredients and beneficially affect the host's health by selectively stimulating the growth and/or activity of some genera of microorganisms in the colon (Kaur and Gupta, 2002; De Vrese and Schrezenmeir, 2008). Prebiotics like FOS, GOS, XOS and inulin are the most commonly used fibers which when used together with probiotics are termed synbiotics and are able to improve the viability of the probiotics (Pandey *et al*., 2015). The combination of a pre- and probiotic in one product has been shown to confer benefits beyond those of either on its own (Holzapfel and Schillinger, 2002).

4.3.8 Bile salt hydrolase (BSH) assay

Pediococcus cultures were evaluated for BSH activity using two bile salts i.e. sodium taurocholate and sodium tauro-deoxycholate. The ability of *Pediococcus* cultures to hydrolyze bile salts was indicated by the precipitation along with formation of opaque granular white colonies. All the studied pediococci cultures exhibited negligible zone of precipitation but good growth on both the bile salt plates (Fig. 4.18). Thus, although cultures may possess low deconjugation ability but they are tolerant to the bile salts. Strain specific ability of pediococci cultures to deconjugate and tolerate bile salts have been reported (Banwo *et al*., 2013; Shukla and Goyal, 2013; Garsa *et al*., 2014). It is generally considered that strains with BSH activity come from intestinal environment in which they are exposed to bile salts. However, the fact that not all strains of GI origin have BSH

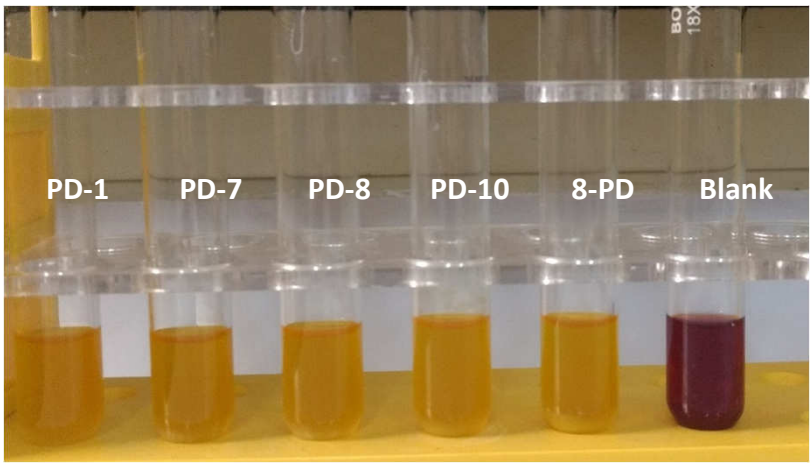
activity shows that bacteria without this enzyme can either survive in this environment or survive the passage through it. Also, the fact that some strains were able to grow in presence of conjugated bile salts while they were not able to deconjugate them is in accordance with the hypothesis that the capacity to express BSH is not related to the capacity to resist the toxicity of conjugated bile salts (Moser and Savage, 2001).

4.4 SAFETY ASSESSMENT OF *PEDIOCOCCUS* CULTURES

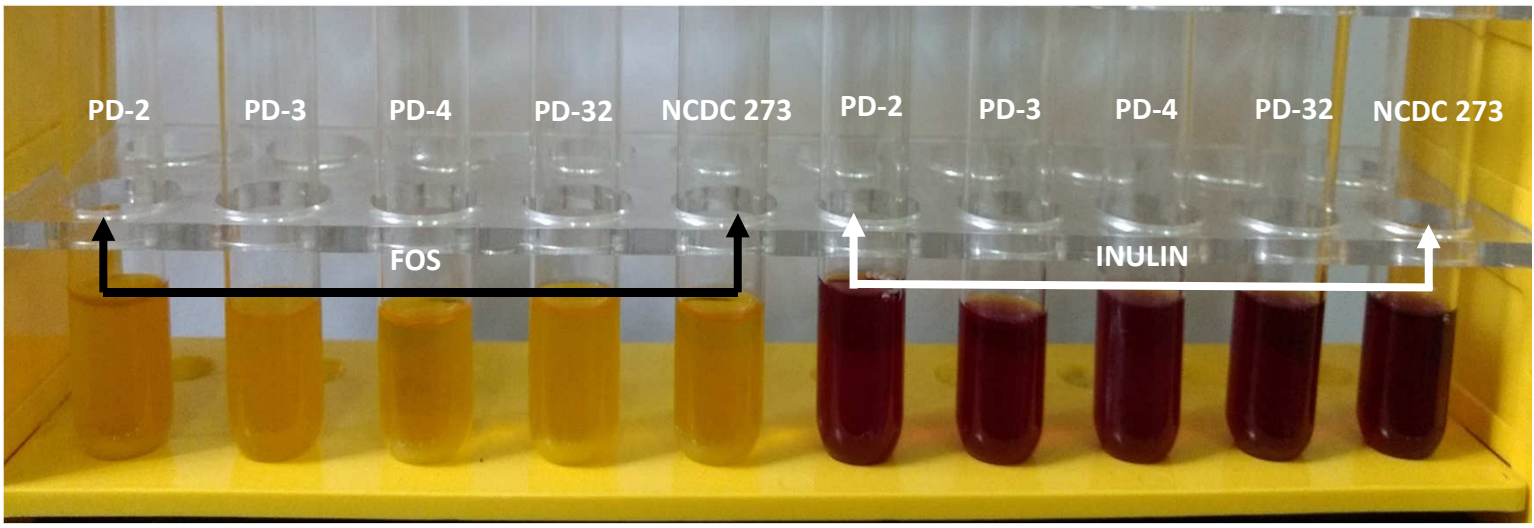
A total of 40 *Pediococcus* cultures along with 3 NCDC cultures were evaluated for *in-vitro* haemolysis, DNase activity, gelatinase enzyme activity, biogenic amine production and antibiotic susceptibility.

4.4.1. Haemolysis, DNase, Gelatinase activity and biogenic amine production

Lactic acid bacteria are generally regarded as safe. All the tested *Pediococcus* cultures showed greenish reaction/partial haemolysis on blood agar (α-haemolysis) (Fig. 4.19). None of the culture was positive for DNase (Fig. 4.20), gelatinase activity (Fig. 4.21) and did not produce biogenic amines from tyrosine and histamine (Fig. 4.22). Pediococci are generally reported as non-haemolytic (Uymaz *et al*., 2009; Osmanagaoglu *et al*., 2010; Ryu and Chang, 2013; Vidhyasagar and Jeevaratnam, 2013). However, they are also documented to exhibit α-haemolysis (Riebel and Washington, 1990; Siripornadulsil *et al*., 2014). Oh and Jung (2015) studied 2 pediococci strains for haemolytic reaction and observed that one of the strain exhibited α-haemolysis while another one demonstrated γ-haemolysis. The absence of gelatinase and DNase activity has also been reported in pediococci (Mandal *et al*., 2011; Attri *et al*., 2015). Borges *et al*. (2013) studied the safety aspects of *P. pentosaceus* SB83 for the use of the culture as vaginal probiotic. They found that the strain was devoid of virulence factors (haemolysis, DNase and gelatinase activity) and the corresponding virulence genes. Similarly, Gupta and Sharma (2017) reported *P. acidilactici* strain Ch-2 as avirulent with regard to haemolysis, DNase and gelatinase activity. Biogenic amines; histamine and tyramine have vasoactive and psychoactive properties. Moreover, they are also known to be potential precursors of carcinogenic agents (Bover-Cid and Holzapfel, 1999). Earlier reports demonstrated that pediococci do not produce biogenic amines or they



(a)

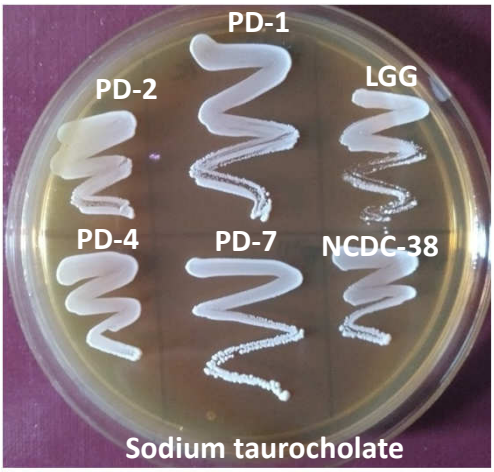


(b)

Fig. 4.17 Prebiotic fermentation by *Pediococcus* cultures

(a) Inulin fermentation by *Pediococcus* cultures

(b) Comparative FOS and Inulin fermentation by *Pediococcus* cultures



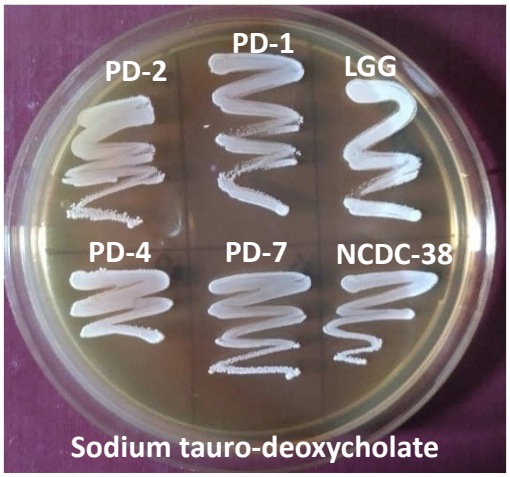


Fig. 4.18 Bile salt hydrolase activity of *Pediococcus* cultures



Fig. 4.19 Haemolytic activity of *Pediococcus* cultures

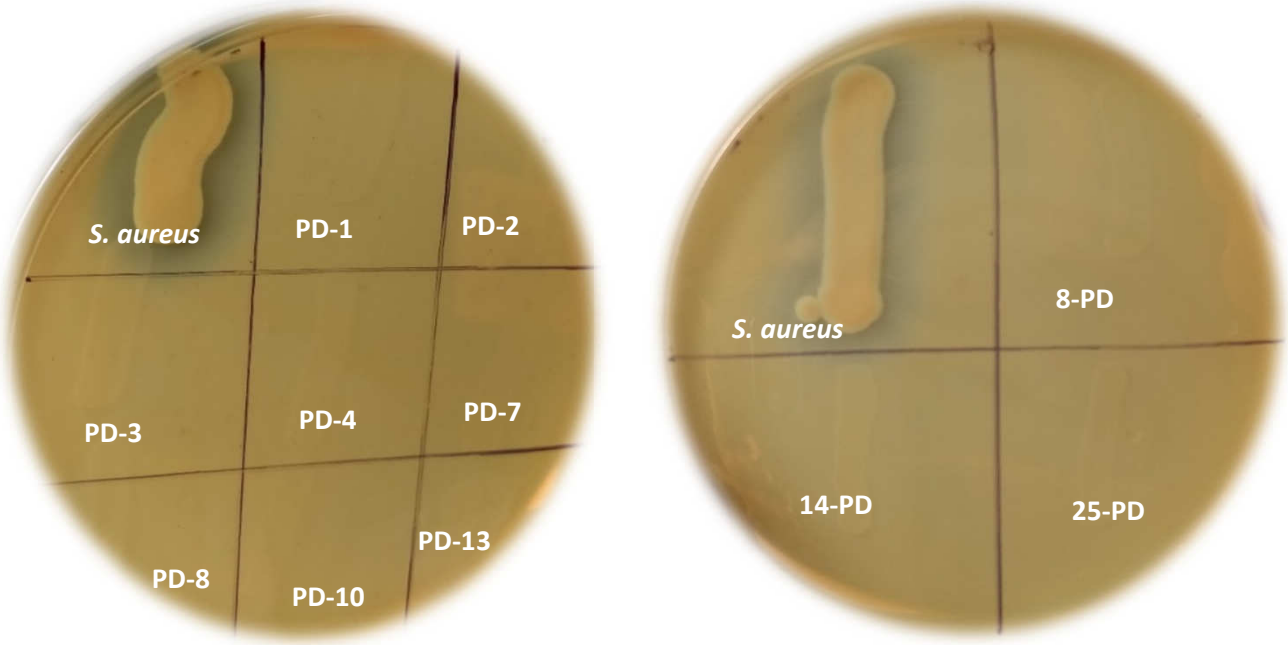


Fig. 4.20 DNase activity of *Pediococcus* cultures

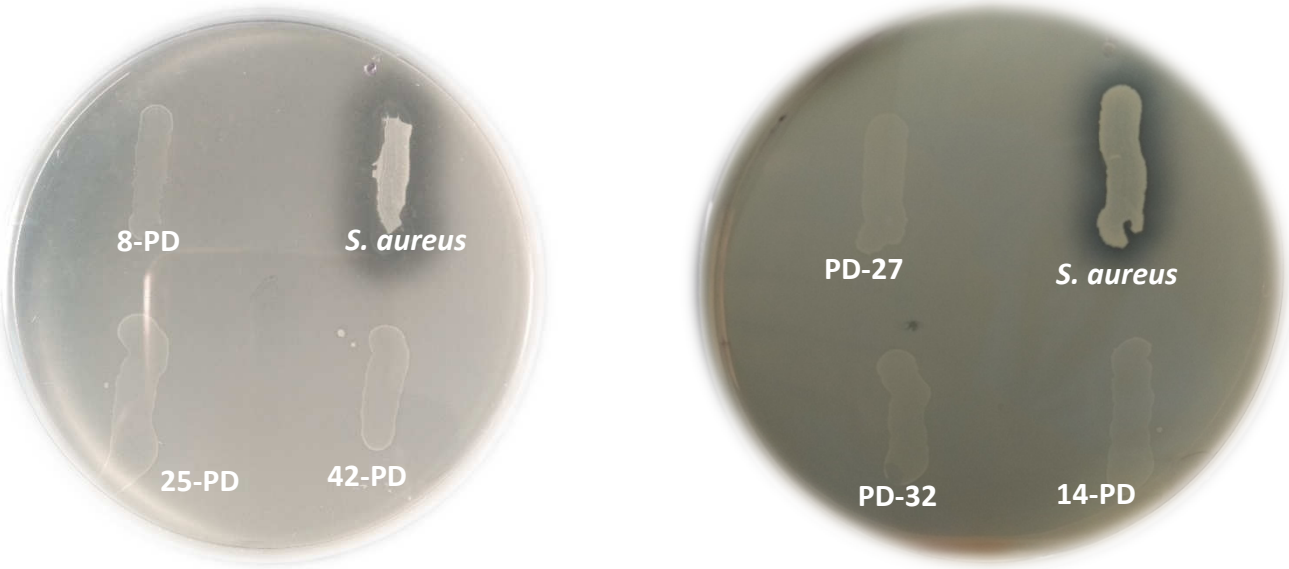
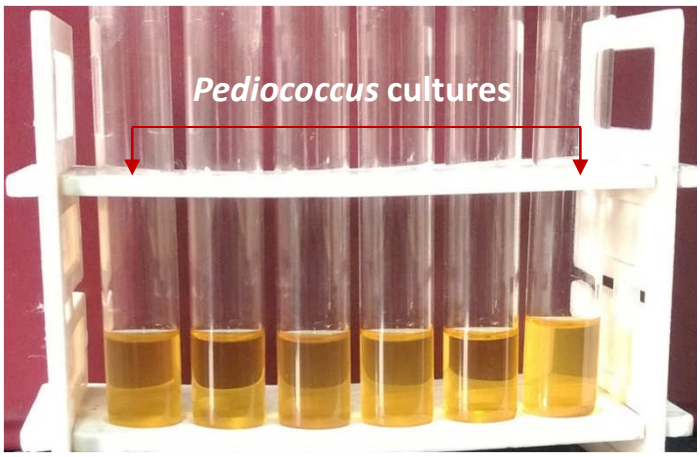
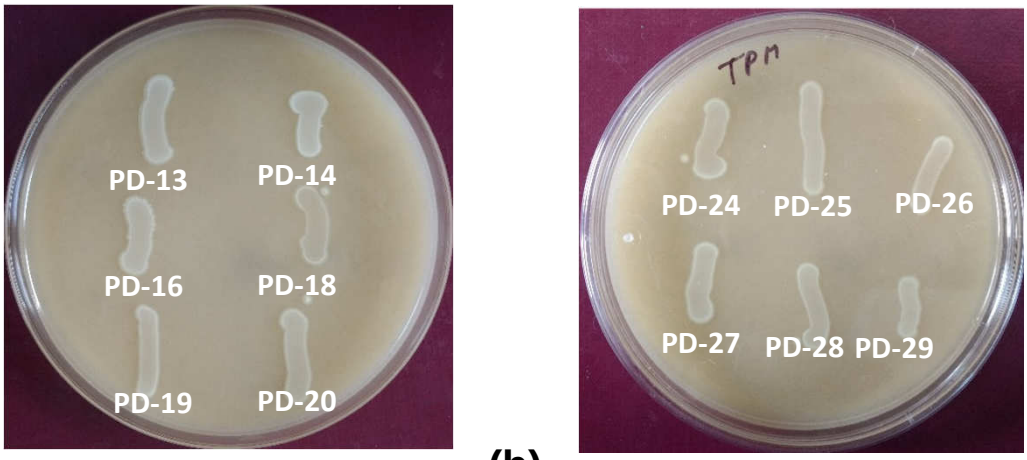


Fig. 4.21 Gelatinase activity of *Pediococcus* cultures



(a)



(b)

Fig. 4.22 Biogenic amine production by *Pediococcus* cultures

(a) Biogenic amine production by *Pediococcus* cultures in improved decarboxylase medium

(b) Biogenic amine production by *Pediococcus* cultures on tyrosine production medium

produce in very low amount which is under safe limit (Bover-Cid and Holzapfel, 1999; Tome *et al*., 2008; Albano *et al*., 2009; Ruiz-Moyano *et al*., 2010).

4.4.2 Antibiotic susceptibility test

Antibiotic susceptibility of the tested *Pediococcus* cultures was done using disc diffusion assay for 20 clinically important antibiotics according to Performance Standards for Antimicrobial Disk Susceptibility tests (CLSI, 2007). Based on the inhibition zone, varied range of responses by tested *Pediococcus* cultures against different antibiotics was observed (Fig. 4.23, Table 4.8). All the tested cultures were sensitive or intermediate sensitive to amoxycillin, erythromycin, ceftriaxone, cloxacillin, cefoperazone, penicillin, netillin, gentamicin, and chloramphenicol. Complete resistance towards vancomycin and nalidixic acid was exhibited by all the cultures except PD-39 which showed intermediate sensitivity towards vancomycin. Thirty eight cultures along with 3 NCDC cultures were resistant to co-trimoxazole. Nine cultures demonstrated sensitivity or moderate sensitivity towards 85% of the antibiotics while 2 cultures (PD-36 and PD-39) showed sensitivity or moderate sensitivity towards 90% of the studied antibiotics (Fig. 4.24).

Antibiotic susceptibility profiles of *Pediococcus* strains have been reported (Tankovic *et al*., 1993; Borges *et al*., 2013; Shukla and Goyal, 2014; Noohi *et al*., 2016). Penicillin G, chloramphenicol and erythromycin are usually active against *Pediococcus* spp. (Temmerman *et al*., 2003b; Danielsen *et al*., 2007; Cao *et al*., 2016). Ruiz-Moyano *et al.* (2010) also reported the susceptibility of *Pediococcus* cultures towards these antibiotics. Resistance to vancomycin among pediococci is very well reported and is considered as intrinsic resistance (Facklam *et al*., 1989; Barton *et al*., 2001; Sharma *et al*., 2014). Mandal *et al*. (2011) demonstrated the gene location of co-trimoxazole on chromosome. Yuksekdag and Aslim (2010) studied antibiotic resistance pattern of 5 pediococci strains and found that all were sensitive to amoxycillin and gentamycin, and resistant to vancomycin and amoxycillin. FAO/WHO guidelines have strongly recommended ascertaining antibiotic resistance/susceptibility pattern of every probiotic strain prior to consumption or their applications in food (FAO/WHO, 2002).

4.5 APPLICATION OF TECHNO-FUNCTIONALLY POTENT *PEDIOCOCCUS* CULTURES

On the basis of techno-functional attributes such as phytase activity, prebiotic utilization and probiotic properties, four *Pediococcus* cultures were selected for preparation of synbiotic fermented dairy beverage and yoghurt (Table 4.9).

4.5.1 Preparation of synbiotic fermented dairy beverage

Synbiotic fermented dairy beverage was prepared with selected *Pediococcus pentosaceus* cultures (PD-27, PD-32, PD-34 and 14-PD) in combination with fructo-oligosaccharide (FOS). Synbiotic product was prepared from skim milk. *Pediococcus* cultures were inoculated individually (@ 5%) and incubated at 37°C up-to 48 h. Prepared product was evaluated for physicochemical, microbiological and sensory quality (Table 4.10, Fig. 4.25). Phytase activity of synbiotic product was evaluated on sodium phytate MRS agar plate. pH was ranged from 3.83 to 4.08 at the end of 48 h. Maximum drop in pH was observed with *P. pentosaceus* 14-PD. Titratable acidity (% LA) was in the range of 0.61 to 0.71. Pediococci counts were >8 log CFU/mL of product. Coliform, and yeast and mold counts were <10 CFU/mL. Products prepared with *P. pentosaceus* PD-32 and PD-34 were of superior quality and had higher phytase activity. A synbiotic product named 'Synbiotic 2000' containing *P. pentosaceus* LMG P-20608 as probiotic has been prepared and reported to exert a myriad of beneficial effects on host (Olah *et al*., 2005; Rayes *et al*., 2005; Kotzampassi *et al*., 2006).

4.5.2 Yoghurt preparation

Yoghurt was also prepared with selected *Pediococcus* cultures in combination with low galactose utilizing *S. thermophilus* NCDC 428 and *Lb. delbrueckii* ssp. *bulgaricus* NCDC 277. Yoghurt was evaluated for physicochemical, microbiological and sensorial characteristics (Fig. 4.26, Table 4.11). pH of the yoghurt prepared with *P. pentosaceus* PD-27, PD-32, PD-34 and 14-PD was 4.51, 4.66, 4.86 and 4.92, respectively, while the pH of the control product was 4.62. Coliform, and yeast and mold counts were <10 CFU/mL. Overall quality of yoghurts prepared with PD-32 and PD-34 were comparable to control.

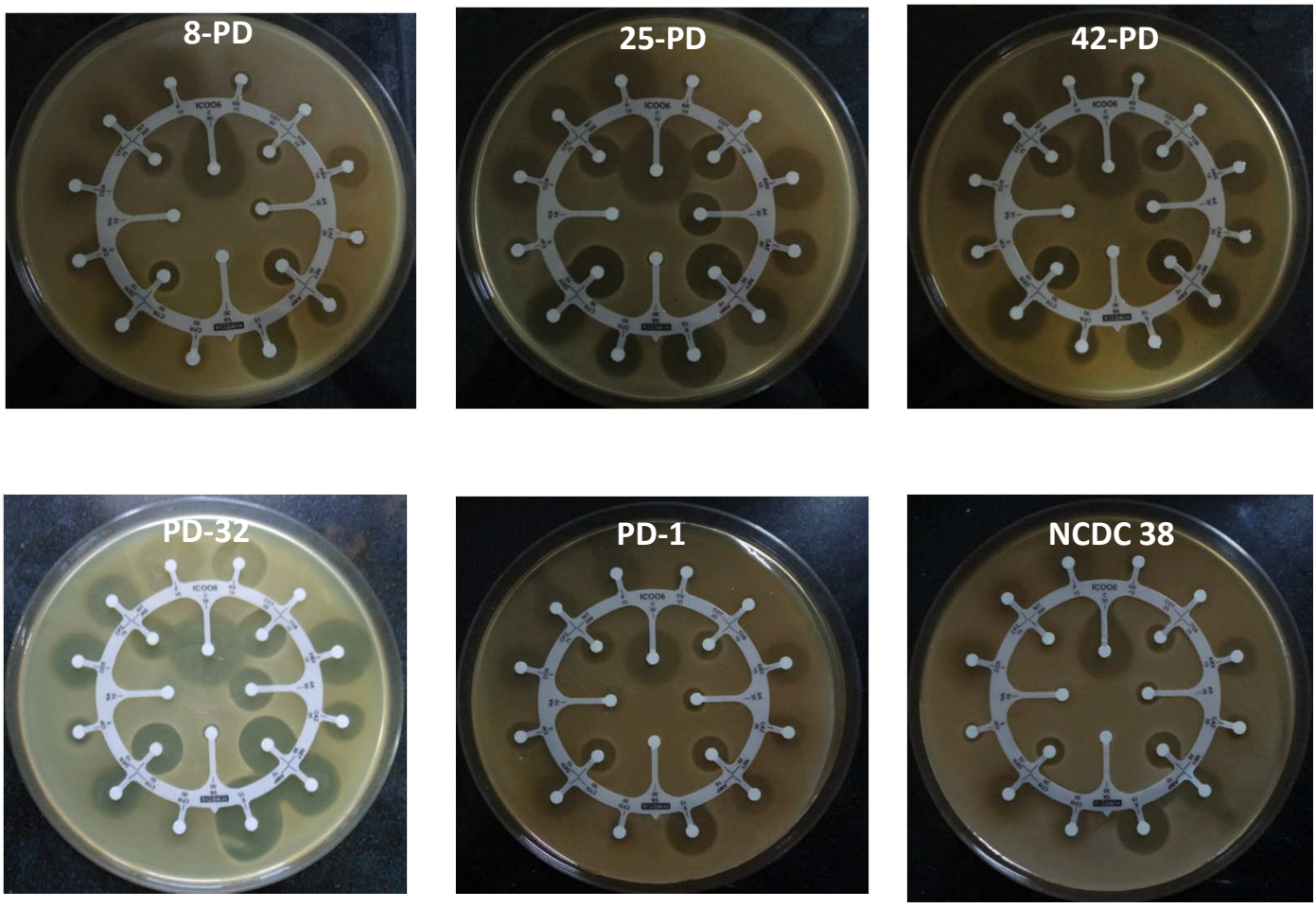


Fig. 4.23 Antibiotic susceptibility of *Pediococcus* cultures

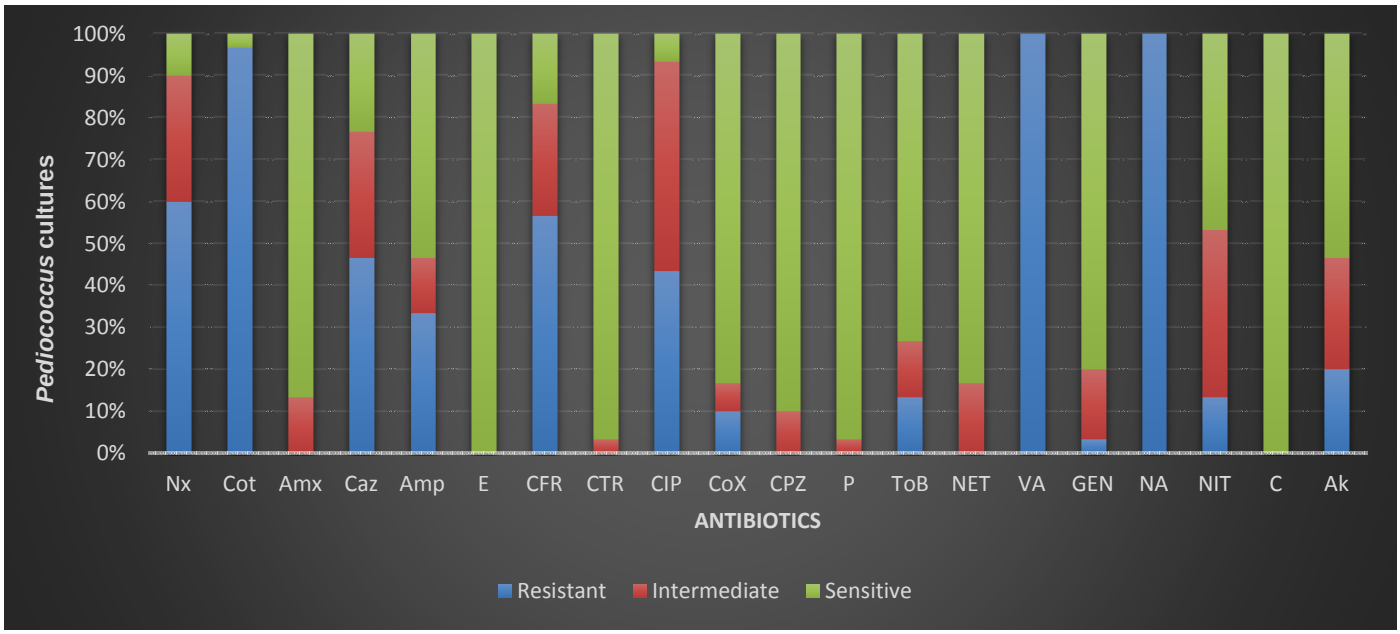


Fig. 4.24 Distribution (%) of *Pedicoccus* cultures based on antibiotic susceptibility

Table 4.8 Antibiotic susceptibility of *Pediococcus* cultures

S. No.	Cultures	Antibiotics																			
		Nx (10 mcg)	Cot (25 mcg)	Amx (10 mcg)	Caz (30 mcg)	Amp (10 mcg)	E (15 mcg)	CFR (30 mcg)	CTR (30 mcg)	CIP (5 mcg)	Cox (1 mcg)	CPZ (75 mcg)	P (10 units)	TOB (10 mcg)	NET (10 mcg)	VA (30 mcg)	GEN (10 mcg)	NA (10 mcg)	NIT (300 mcg)	C (30 mcg)	Ak (30 mcg)
1	*P. pentosaceus* PD-1	R	R	S	IS	S	S	R	S	R	IS	S	S	IS	IS	R	IS	R	IS	S	R
2	*P. pentosaceus* PD-2	R	R	S	IS	IS	S	R	S	S	S	S	S	S	S	R	S	R	S	S	S
3	*P. pentosaceus* PD-3	R	R	S	IS	S	S	S	S	IS	S	S	S	R	S	R	IS	R	IS	S	IS
4	*P. pentosaceus* PD-4	R	R	S	IS	S	S	R	S	R	S	S	S	IS	IS	R	IS	R	IS	S	R
5	*P. pentosaceus* PD-7	S	S	S	S	S	S	IS	S	R	S	S	S	R	S	R	S	R	S	S	S
6	*P. pentosaceus* PD-8	R	R	S	IS	S	S	IS	S	IS	S	S	S	S	S	R	S	R	S	S	IS
7	*P. pentosaceus* PD-10	IS	R	S	R	R	S	R	S	R	S	S	S	S	S	R	S	R	S	S	S
8	*P. pentosaceus* PD-13	R	R	S	S	S	S	R	S	IS	S	S	S	S	S	R	S	R	S	S	S
9	*P. pentosaceus* PD-14	IS	R	S	R	R	S	R	S	IS	S	S	S	S	S	R	S	R	IS	S	S
10	*P. pentosaceus* PD-16	R	R	S	R	IS	S	R	S	IS	S	S	S	S	S	R	S	R	S	S	S
11	*P. pentosaceus* PD-18	IS	R	S	R	R	S	R	S	IS	S	S	S	S	S	R	S	R	S	S	S
12	*P. pentosaceus* PD-19	IS	R	S	R	S	S	R	S	IS	IS	S	S	S	S	R	S	R	S	S	S
13	*P. pentosaceus* PD-20	S	R	S	S	S	S	R	S	IS	S	S	S	S	S	R	S	R	IS	S	S
14	*P. pentosaceus* PD-21	S	R	S	S	S	S	IS	S	IS	S	S	S	S	S	R	S	R	S	S	S
15	*P. pentosaceus* PD-22	IS	R	S	R	R	S	R	S	S	S	S	S	S	S	R	S	R	S	S	S
16	*P. pentosaceus* PD-24	R	R	S	R	R	S	S	S	R	S	S	S	S	S	R	S	R	S	S	IS
17	*P. pentosaceus* PD-25	IS	R	S	S	IS	S	R	S	IS	S	S	S	S	S	R	S	R	S	S	S
18	*P. pentosaceus* PD-26	S	R	IS	R	R	S	IS	S	IS	S	S	S	S	S	R	S	R	IS	S	S
19	*P. pentosaceus* PD-27	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
20	*P. pentosaceus* PD-28	S	R	S	S	S	S	R	S	R	S	S	S	S	S	R	S	R	IS	S	S
21	*P. pentosaceus* PD-29	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
22	*P. pentosaceus* PD-30	IS	R	S	S	S	S	IS	S	IS	S	S	S	S	S	R	S	R	IS	S	S
23	*P. pentosaceus* PD-31	S	R	S	S	S	S	R	S	IS	S	S	S	S	S	R	S	R	S	S	S

S. No.	Cultures	Antibiotics																			
		Nx (10 mcg)	Cot (25 mcg)	Amx (10 mcg)	Caz (30 mcg)	Amp (10 mcg)	E (15 mcg)	CFR (30 mcg)	CTR (30 mcg)	CIP (5 mcg)	Cox (1 mcg)	CPZ (75 mcg)	P (10 units)	TOB (10 mcg)	NET (10 mcg)	VA (30 mcg)	GEN (10 mcg)	NA (10 mcg)	NIT (300 mcg)	C (30 mcg)	Ak (30 mcg)
24	*P. pentosaceus* PD-32	S	R	S	S	S	S	R	S	R	S	S	S	S	S	R	S	R	S	S	S
25	*P. pentosaceus* PD-33	S	R	S	S	S	S	IS	S	IS	S	S	S	S	S	R	S	R	S	S	S
26	*P. pentosaceus* PD-34	S	R	S	S	S	S	R	S	IS	S	S	S	S	S	R	S	R	S	S	S
27	*P. pentosaceus* PD-35	S	R	S	S	S	S	IS	S	IS	S	S	S	S	S	R	S	R	S	S	S
28	*P. pentosaceus* PD-36	S	IS	S	S	S	S	S	S	IS	S	S	S	S	S	R	S	R	S	S	S
29	*P. pentosaceus* PD-37	S	R	S	S	S	S	S	S	IS	S	S	S	S	S	R	S	R	S	S	S
30	*P. pentosaceus* PD-38	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
31	*P. pentosaceus* PD-39	S	R	S	S	S	S	IS	S	IS	S	S	S	S	S	IS	S	R	S	S	S
32	*P. pentosaceus* PD-41	R	R	IS	R	R	S	R	S	R	S	S	S	S	S	R	S	R	IS	S	IS
33	*P. pentosaceus* PD-44	R	R	IS	IS	R	S	S	S	IS	IS	S	S	S	S	R	S	R	IS	S	IS
34	*P. acidilactici* PD-45	R	R	S	R	R	S	R	S	R	S	S	S	S	S	R	S	R	IS	S	S
35	*P. pentosaceus* 8-PD	R	R	S	R	S	S	R	S	IS	S	S	S	R	S	R	IS	R	IS	S	R
36	*P. pentosaceus* 14-PD	IS	R	S	IS	S	S	R	S	R	S	S	S	S	S	R	S	R	S	S	S
37	*P. pentosaceus* 25-PD	R	R	S	S	S	S	S	S	R	S	S	S	S	S	R	S	R	S	S	S
38	*P. pentosaceus* 42-PD	IS	R	S	S	S	S	IS	S	IS	S	S	S	S	S	R	S	R	S	S	IS
39	*P. pentosaceus* Sil-1	R	R	S	R	S	S	S	S	IS	S	IS	S	IS	IS	R	S	R	R	S	R
40	*P. pentosaceus* Sil-2	R	R	S	R	IS	S	R	IS	R	IS	IS	S	R	IS	R	IS	R	R	S	R
	P. pentosaceus NCDC 38	R	R	S	R	S	S	IS	S	R	IS	IS	S	IS	IS	R	IS	R	R	S	R
	P. pentosaceus NCDC 273	R	R	IS	IS	R	S	IS	S	R	S	S	IS	S	S	R	S	R	R	S	IS
	P. acidilactici NCDC 609	R	R	S	IS	S	S	IS	S	R	S	S	S	S	S	R	S	R	IS	S	S

AK: Amikacin, AMP: Ampicillin, AMX: Amoxycillin, CFR: Cefadroxil, CPZ: Cefoperazone, CAZ: Ceftazidime, CTR: Ceftriaxone, C: Chloramphenicol, CIP: Ciprofloxacin, COX: Cloxacillin, COT: Co-Trimoxazole, E: Erythromycin, GEN: Gentamicin, NA: Nalidixic acid, NET: Netillin, NIT: Nitrofurantoin, NX: Norfloxacin, P: Penicillin, TOB: Tobramycin, VA: Vancomycin

Table 4.9 Techno-functional attributes of selected *Pediococcus* cultures

Cultures	Phytase activity (Zone of clearance in mm)	Galactose utilization (%)	Acid tolerance (log CFU reduction)	Bile tolerance (log CFU reduction)	Prebiotic utilization (FOS)	Cell surface hydrophobicity (%)	Auto-aggregation (%)	Antimicrobial activity (Zone of inhibition in mm including well dia. of 8 mm)			
								S. aureus	*S. enterica* ssp. Typhi	*E. coli*	*L. monocytogenes*
P. pentosaceus PD-27	19	94.72	2.04	3.68	+	19.65	49.28	18.00	22.33	18.67	20.67
P. pentosaceus PD-32	20	91.65	2.60	4.17	+	26.36	45.99	17.67	17.00	17.67	19.67
P. pentosaceus PD-34	17	92.91	3.67	2.80	+	15.49	49.86	19.33	19.33	17.33	18.33
P. pentosaceus 14-PD	22	94.57	4.26	4.45	+	19.97	51.83	19.33	19.00	20.67	18.00

Table 4.10 Physico-chemical and microbiological characteristics of synbiotic fermented dairy beverage

Cultures	pH		Titratable acidity (% LA)		Total lactic count (log CFU/mL)	Phytase activity (Zone of clearance in mm)	Product quality
	24 h	48 h	24 h	48 h	48 h	48 h	
P. pentosaceus PD-27	4.61	4.04	0.46	0.61	9.38	-	+
P. pentosaceus PD-32	4.43	3.98	0.50	0.66	9.04	16	++
P. pentosaceus PD-34	4.38	3.98	0.54	0.68	8.48	14	++
P. pentosaceus 14-PD	4.11	3.83	0.61	0.71	8.56	12	+

*Coliform, and yeast and mold counts were <10 CFU/mL

Table 4.11 Physico-chemical and microbiological characteristics of yoghurt

Cultures	pH	Titratable acidity (% LA)	Total lactic count (log CFU/mL)		Product quality
			MRS	M-17	
S. thermophilus NCDC 428 (ST):*Lb. delbrueckii* ssp. *bulgaricus* NCDC 277 (LB) (1:1) (Control)	4.62	1.17	9.25	9.16	+ +
ST:LB:*P. pentosaceus* PD-27 (0.5:1:1)	4.51	1.01	8.85	8.56	+
ST:LB:*P. pentosaceus* PD-32 (0.5:1:1)	4.66	0.96	8.97	8.88	++
ST:LB:*P. pentosaceus* PD-34 (0.5:1:1)	4.86	0.93	8.75	8.63	++
ST:LB:*P. pentosaceus* 14-PD (0.5:1:1)	4.92	0.82	8.95	8.72	+

*Coliform, and yeast and mold counts were <10 CFU/mL



Fig. 4.25 Synbiotic fermented dairy beverage prepared using selected *Pediococcus* culture



Fig. 4.26 Yoghurt prepared using selected *Pediococcus* culture as an adjunct culture

CHAPTER –5

Summary and Conclusions

SUMMARY AND CONCLUSIONS

5.1 SUMMARY

An attempt was made to isolate *Pediococcus* spp. from dairy and non-dairy sources. The isolates were identified and characterized for techno-functional attributes for their application as probiotic and in dairy and food fermentations. The salient findings are as follows:

- A total 115 samples were collected from different places of India for isolation of *Pediococcus* spp.
- Three *Pediococcus* cultures such as *P. pentosaceus* NCDC 38 and NCDC 273, and *P. acidilactici* NCDC 609 were included in the study as reference cultures.
- Based on morphology (cocci in pairs and tetrads), Gram staining (Gram-positive), catalase activity (catalase negative) and physiological characteristics, 40 isolates (37 from non-dairy samples and 3 from dairy sources) were presumed as *Pediococcus* spp.
- Biochemical (APi 50CH sugar fermentation patterns) and genotypical characterization identified 39 isolates as *P. pentosaceus* and 1 as *P. acidilactici.*
- All the 40 isolated *Pediococcus* cultures along with 3 NCDC cultures were evaluated for technological (acidification profile, phytase activity, EPS production, diacetyl production and galactose utilization), functional (*In-vitro* acid and bile tolerance, cell surface hydrophobicity, auto-aggregation, antimicrobial activity, β-galactosidase activity, prebiotic utilization and bile salt hydrolase activity), and safety (*In-vitro* haemolysis, DNase, gelatinase, biogenic amine production, antibiotic susceptibility) aspects.
- Acidifying activity of all cultures was determined in CHL broth with lactose, skim milk, skim milk with protein hydrolysate and MRS (as control) at 37°C for 24 h. No considerable decrease in pH was observed in CHL broth with lactose and skim milk. However, supplementation of protein

hydrolysate in skim milk improved the curdling property. *Pediococcus acidilactici* NCDC 609 was able to ferment lactose sugar.

- Thirty four *Pediococcus* cultures along with *P. pentosaceus* NCDC 38 and NCDC 273 were positive for phytase activity and six cultures were potent phytase producers.
- Five *Pediococcus* cultures along with *P. pentosaceus* NCDC 38 showed clear mucoid colonies (EPS producers) on sucrose MRS agar and 18 cultures along with *P. acidilactici* NCDC 609 displayed viscous growth (weak EPS producers).
- Twelve cultures were found positive for diacetyl production.
- All the cultures were able to ferment galactose. Twenty eight *Pediococcus* cultures along with 3 NCDC cultures utilized >90% galactose in CHL media containing 1% galactose within 12 h.
- *In-vitro* acid tolerance test depicted that 27 *Pediococcus* cultures along with *P. pentosaceus* NCDC 273 and *P. acidilactici* NCDC 609 were able to survive up-to 1.5 h; while, after 3 h, only 19 of them could survive. *P. pentosaceus* 42-PD showed maximum survival followed by Sil-1 and PD-28.
- All the *Pediococcus* cultures tolerated 1% bile concentrations up-to 4 h. Viable counts were decreased with increase in bile concentration and exposure time. Most tolerant strain was Sil-2 followed by NCDC 609 and PD-21.
- A wide variation in cell surface hydrophobicity (7.95-59.35%) and auto-aggregation (39.28-83.28%) was demonstrated by tested *Pediococcus* cultures. Six cultures displayed >30% cell surface hydrophobicity towards n-hexadecane. Maximum cell auto-aggregation was exhibited by *P. pentosaceus* PD-4 followed by PD-7 and 42-PD.
- All the *Pediococcus* cultures demonstrated appreciable antimicrobial activity against *S. aureus*, *S. enterica* ssp. Typhi, *E. coli* and *L. monocytogenes*. *Pediococcus* cultures NCDC 38, PD-4, 25-PD and PD-8 were the most active cultures.

- Five *Pediococcus* cultures (PD-3, PD-10, PD-29, PD-30, PD-41) along with *P. pentosaceus* NCDC 273 and *P. acidilactici* NCDC 609 demonstrated intracellular β-galactosidase activity (0.035-0.597 IU/mg dry weight).
- All the cultures were able to ferment fructo-oligosaccharide (FOS) while only five cultures (PD-1, PD-7, PD-8, PD-10 and 8-PD) were positive for inulin fermentation.
- All the cultures showed growth on MRS agar with sodium taurocholate and sodium tauro-deoxycholate with negligible zone of precipitation.
- All the *Pediococcus* cultures and 3 NCDC cultures were negative for β-haemolysis, DNase, gelatinase activity and biogenic amine production. However, they exhibited greenish zones on blood agar which showed their α-haemolytic nature.
- The antibiogram of the tested cultures against 20 clinically relevant antibiotics demonstrated that most of the cultures were sensitive to the important antibiotics. Complete resistance towards vancomycin and nalidixic acid was exhibited by all the cultures except PD-39 which showed intermediate sensitivity towards vancomycin.
- Based on techno-functional attributes (phytase activity, prebiotic fermentation, galactose utilization and probiotic property), four *P. pentosaceus* cultures (PD-27, PD-32, PD-34 and 14-PD) were selected for product preparation.
- Synbiotic fermented dairy beverage was prepared using selected *Pediococcus* culture as single strain starter. Overall quality and phytase activity of the products prepared with PD-32 and PD-34 were found better.
- Yoghurt was prepared with selected *Pediococcus* culture as adjunct culture along with low galactose utilizing *S. thermophilus* NCDC 428 and *Lb. delbrueckii* ssp. *bulgaricus* NCDC 277. Yoghurts prepared with PD-32 and PD-34 were found better and comparable with control yoghurt.

5.2 CONCLUSIONS

Forty *Pediococcus* cultures were isolated, identified and characterized for their techno-functional and safety attributes from diverse dairy and non-dairy sources. Cultures endured with potent techno-functional attributes can be exploited for their applications as probiotic, synbiotic, and dairy and food fermentations.

***Pediococcus* cultures**	**Specific property**	**Application**
P. pentosaceus PD-33, Sil-2, Sil-1 and 42-PD, and *P. acidilactici* NCDC 609	Good galactose fermenter and lactose fermenter	Thermophilic Starter blend
P. pentosaceus 25-PD, 14-PD and PD-25	Phytase active	Cereal based fermented products
P. pentosaceus PD-1, PD-44, 42-PD, Sil-1 and Sil-2	EPS producer	Bio-thickner in low fat dairy products
P. pentosaceus PD-18, PD-27, PD-28, PD-32, PD-34, 14-PD, 42-PD and Sil-2	Potent probiotics	Probiotic and synbiotic formulation
P. pentosaceus PD-2, PD-8 and 42-PD	Potent antimicrobial activity	Bio-protective culture

Bibliography

BIBLIOGRAPHY

Abbasiliasi, S., Tan, J. S., Ibrahim, T. A. T., Ramanan, R. N., Vakhshiteh, F., Mustafa, S., Ling, T.C., Rahim, R.A. and Ariff, A. B. (2012). Isolation of *Pediococcus acidilactici* Kp10 with ability to secrete bacteriocin-like inhibitory substance from milk products for applications in food industry. *BMC Microbiology*, *12*(1), 260.

Abriouel, H., Benomar, N., Cobo, A., Caballero, N., Fuentes, M. A. F., Perez-Pulido, R. and Galvez, A. (2012). Characterization of lactic acid bacteria from naturally-fermented Manzanilla Aloreña green table olives. *Food Microbiology*, *32*(2), 308-316.

Accolas, J. P. and Auclair, J. (1970). Determination of the acid-producing activity of concentrated frozen suspensions of lactic acid bacteria. *Lait*, *50*, 609-626.

Albano, H., Pinho, C., Leite, D., Barbosa, J., Silva, J., Carneiro, L., Magalhaes, R., Hogg, T. and Teixeira, P. (2009). Evaluation of a bacteriocin-producing strain of *Pediococcus acidilactici* as a biopreservative for “Alheira”, a fermented meat sausage. *Food Control*, *20*(8), 764-770.

Alonso-Calleja, C., Carballo, J., Capita, R., Bernardo, A. and Garcıca-Lopez, M. L. (2002). Changes in the microflora of Valdeteja raw goat's milk cheese throughout manufacturing and ripening. *LWT-Food Science and Technology*, *35*(3), 222-232.

Amado, I. R., Fuciños, C., Fajardo, P. and Pastrana, L. (2016). Pediocin SA-1: A selective bacteriocin for controlling *Listeria monocytogenes* in maize silages. *Journal of Dairy Science*, *99*(10), 8070-8080.

Ammor, M. S., Flórez, A. B. and Mayo, B. (2007). Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria. *Food Microbiology*, *24*(6), 559-570.

Aswathy, R. G., Ismail, B., John, R. P. and Nampoothiri, K. M. (2008). Evaluation of the probiotic characteristics of newly isolated lactic acid bacteria. *Applied Biochemistry and Biotechnology*, *151*(2-3), 244-255.

Attri, P., Jodha, D., Gandhi, D., Chanalia, P. and Dhanda, S. (2015). *In vitro* evaluation of *Pediococcus acidilactici* NCDC 252 for its probiotic attributes. *International Journal of Dairy Technology*, *68*(4), 533-542.

Badarinath, V., Raghavendra, P. and Halami, P. M. (2010). Characterization of lactic acid bacteria isolated from okara for probiotic properties. *International Journal of Probiotics and Prebiotics*, *5*(3), 149-156.

Badis, A., Guetarni, D., Boudjema, B. M., Henni, D. E. and Kihal, M. (2004). Identification and technological properties of lactic acid bacteria isolated from raw goat milk of four Algerian races. *Food Microbiology*, *21*(5), 579-588.

Bae, H. D., Yanke, L. J., Cheng, K. J. and Selinger, L. B. (1999). A novel staining method for detecting phytase activity. *Journal of Microbiological Methods*, *39*(1), 17-22.

Bajpai, V. K., Han, J. H., Rather, I. A., Majumder, R., Nam, G. J., ChanSeo, P., Lim, J., Paek, W. K. and Park, Y. H. (2016). Characterization of lactic acid bacterium *Pediococcus pentosaceus* 4I1 from fresh water fish *Zacco koreanus* and its antibacterial mode of action. *Peer Journal preprints*, *4*, e2121v1.

Bansal, S., Oberoi, H. S., Dhillon, G. S. and Patil, R. T. (2008). Production of β-galactosidase by *Kluyveromyces marxianus* MTCC 1388 using whey and effect of four different methods of enzyme extraction on β-galactosidase activity. *Indian Journal of Microbiology*, *48*(3), 337-341.

Banwo, K., Sanni, A. and Tan, H. (2013). Functional properties of *Pediococcus* species isolated from traditional fermented cereal gruel and milk in Nigeria. *Food Biotechnology*, *27*(1), 14-38.

Barney, M., Volgyi, A., Navarro, A. and Ryder, D. (2001). Riboprinting and 16S rRNA gene sequencing for identification of brewery *Pediococcus* isolates. *Applied and Environmental Microbiology*, *67*(2), 553-560.

Barouei, J., Karbassi, A., Ghoddusi, H. B., Mortazavi, A., Ramezani, R. and Moussavi, M. (2011). Impact of native *Lactobacillus paracasei* ssp. *paracasei* and *Pediococcus* spp. as adjunct cultures on sensory quality of Iranian white brined cheese. *International Journal of Dairy Technology*, *64*(4), 526-535.

Barros, R. R., Maria Da Glória, S. C., Peralta, J. M., Facklam, R. R. and Teixeira, L. M. (2001). Phenotypic and genotypic characterization of *Pediococcus* strains isolated from human clinical sources. *Journal of Clinical Microbiology*, *39*(4), 1241-1246.

Barton, L. L., Rider, E. D. and Coen, R. W. (2001). Bacteremic infection with Pediococcus: vancomycin-resistant opportunist. *Pediatrics*, *107*(4), 775-776.

Basu, S., Tomar, S. K. and Hati, S. (2015). Isolation and identification of phytate-degrading lactobacilli from Indian cereal-based fermented milk product "Raabadi". *International Journal of Fermented Foods*, *4*(2), 49-59.

Begley, M., Hill, C. and Gahan, C. G. (2006). Bile salt hydrolase activity in probiotics. *Applied and Environmental Microbiology*, *72*(3), 1729-1738.

Belhadj, H., Harzallah, D., Bouamra, D., Khennouf, S., Dahamna, S. and Ghadbane, M. (2014). Phenotypic and genotypic characterization of some lactic acid bacteria isolated from bee pollen: a preliminary study. *Bioscience of Microbiota, Food and Health*, *33*(1), 11-23.

Bennik, M., Smid, E. J. and Gorris, L. (1997). Vegetable-associated *Pediococcus parvulus* produces pediocin PA-1. *Applied and Environmental Microbiology*, *63*(5), 2074-2076.

Beshkova, D. M., Simova, E. D., Frengova, G. I., Simov, Z. I. and Dimitrov, Z. P. (2003). Production of volatile aroma compounds by kefir starter cultures. *International Dairy Journal*, *13*(7), 529-535.

Bhowmik, T. and Marth, E. H. (1990). β-Galactosidase of *Pediococcus* species: induction, purification and partial characterization. *Applied Microbiology and Biotechnology*, *33*(3), 317-323.

Boling, S. D., Douglas, M. W., Johnson, M. L., Wang, X., Parsons, C. M., Koelkebeck, K. W. and Zimmerman, R. A. (2000). The effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. *Poultry Science*, *79*(2), 224-230.

Borges, S., Barbosa, J., Silva, J. and Teixeira, P. (2013). Evaluation of characteristics of *Pediococcus* spp. to be used as a vaginal probiotic. *Journal of Applied Microbiology*, *115*(2), 527-538.

Bover-Cid, S. and Holzapfel, W. H. (1999). Improved screening procedure for biogenic amine production by lactic acid bacteria. *International Journal of Food Microbiology*, *53*(1), 33-41.

Broadbent, J. R. and Steele, J. L. (2005). Cheese flavor and the genomics of lactic acid bacteria. *ASM NEWS*, *71*(3), 121-128.

Bujnakova, D. and Kmet, V. (2002). Aggregation of animal lactobacilli with O157 enterohemorrhagic *Escherichia coli*. *Zoonoses and Public Health*, *49*(3), 152-154.

Cai, H., Rodriguez, B. T., Zhang, W., Broadbent, J. R. and Steele, J. L. (2007). Genotypic and phenotypic characterization of *Lactobacillus casei* strains isolated from different ecological niches suggests frequent recombination and niche specificity. *Microbiology*, *153*(8), 2655-2665.

Cai, Y., Kumai, S., Ogawa, M., Benno, Y. and Nakase, T. (1999). Characterization and Identification of *Pediococcus* species isolated from forage crops and their application for silage preparation. *Applied and Environmental Microbiology*, *65*(7), 2901-2906.

Caldwell, S. L., Hutkins, R. W., McMahon, D. J., Oberg, C. J. and Broadbent, J. R. (1998). Lactose and galactose uptake by genetically engineered *Pediococcus* species. *Applied Microbiology and Biotechnology*, *49*(3), 315-320.

Caldwell, S. L., McMahon, D. J., Oberg, C. J. and Broadbent, J. R. (1996). Development and characterization of lactose-positive *Pediococcus* species for milk fermentation. *Applied and Environmental Microbiology*, *62*(3), 936-941.

Calmin, G., Lefort, F. and Belbahri, L. (2008). Multi-Loci Sequence Typing (MLST) for Two Lacto-Acid Bacteria (LAB) Species: *Pediococcus parvulus* and *P. damnosus*. *Molecular Biotechnology*, *40*(2), 170-179.

Canzi, E., Guglielmetti, S., Mora, D., Tamagnini, I. and Parini, C. (2005). Conditions affecting cell surface properties of human intestinal bifidobacteria. *Antonie Van Leeuwenhoek*, *88*(3), 207-219.

Cao, Z., Pan, H., Tong, H., Gu, D., Li, S., Xu, Y., Ge, C. and Lin, Q. (2016). *In vitro* evaluation of probiotic potential of *Pediococcus pentosaceus* L1 isolated from paocai—a Chinese fermented vegetable. *Annals of Microbiology*, *66*(3), 963-971.

Caplice, E. and Fitzgerald, G. F. (1999). Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology*, *50*(1), 131-149.

Castex, M., Lemaire, P., Wabete, N. and Chim, L. (2009). Effect of dietary probiotic *Pediococcus acidilactici* on antioxidant defences and oxidative stress status of shrimp *Litopenaeus stylirostris*. *Aquaculture*, *294*(3), 306-313.

Clinical and Laboratory Standards Institute (CLSI) (2007). Performance standards 636 for Antimicrobial Disk Susceptibity tests, *27* (1).

Cizeikiene, D., Juodeikiene, G., Bartkiene, E., Damasius, J. and Paskevicius, A. (2015). Phytase activity of lactic acid bacteria and their impact on the solubility of minerals from wholemeal wheat bread. *International Journal of Food Sciences and Nutrition*, *66*(7), 736-742.

Cizeikiene, D., Juodeikiene, G., Paskevicius, A. and Bartkiene, E. (2013). Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread. *Food Control*, *31*(2), 539-545.

Clark, P. A., Cotton, L. N. and Martin, J. H. (1993). Selection of bifidobacteria for use as dietary adjuncts in cultured dairy foods. II. Tolerance to simulated pH of human stomachs. *Cultured Dairy Products Journal, 28,* 11-14.

Collado, M. C., Meriluoto, J. and Salminen, S. (2008). Adhesion and aggregation properties of probiotic and pathogen strains. *European Food Research and Technology*, *226*(5), 1065-1073.

Cosansu, S., Kuleasan, H., Ayhan, K. and Materon, L. (2007). Antimicrobial activity and protein profiles of *Pediococcus* spp. isolated from Turkish "Sucuk". *Journal of Food Processing and Preservation*, *31*(2), 190-200.

Coulon, J., Houlès, A., Dimopoulou, M., Maupeu, J. and Dols-Lafargue, M. (2012). Lysozyme resistance of the ropy strain *Pediococcus parvulus* IOEB 8801 is correlated with beta-glucan accumulation around the cell. *International Journal of Food Microbiology*, *159*(1), 25-29.

Crow, V., Curry, B. and Hayes, M. (2001). The ecology of non-starter lactic acid bacteria (NSLAB) and their use as adjuncts in New Zealand Cheddar. *International Dairy Journal*, *11*(4), 275-283.

Dalie, D. K. D., Deschamps, A. M. and Richard-Forget, F. (2010). Lactic acid bacteria–Potential for control of mould growth and mycotoxins: a review. *Food Control*, *21*(4), 370-380.

Damodharan, K., Lee, Y. S., Palaniyandi, S. A., Yang, S. H. and Suh, J. W. (2015). Preliminary probiotic and technological characterization of *Pediococcus pentosaceus* strain KID7 and *in vivo* assessment of its cholesterol-lowering activity. *Frontiers in Microbiology*, *6*, 768.

Danielsen, M. and Wind, A. (2003). Susceptibility of *Lactobacillus* spp. to antimicrobial agents. *International Journal of Food Microbiology*, *82*(1), 1-11.

Danielsen, M., Simpson, P. J., O'connor, E. B., Ross, R. P. and Stanton, C. (2007). Susceptibility of *Pediococcus* spp. to antimicrobial agents. *Journal of Applied Microbiology*, *102*(2), 384-389.

Davis, S. L., McKinnon, P. S., Hall, L. M., Delgado, G., Rose, W., Wilson, R. F. and Rybak, M. J. (2007). Daptomycin versus vancomycin for complicated skin and skin structure infections: clinical and economic outcomes. *Pharmacotherapy: The journal of Human Pharmacology and Drug Therapy*, *27*(12), 1611-1618.

De Angelis, M., Gallo, G., Corbo, M. R., McSweeney, P. L., Faccia, M., Giovine, M. and Gobbetti, M. (2003). Phytase activity in sourdough lactic acid bacteria: purification and characterization of a phytase from *Lactobacillus sanfranciscensis* CB1. *International Journal of Food Microbiology*, *87*(3), 259-270.

De Bruyne, K., Franz, C. M., Vancanneyt, M., Schillinger, U., Mozzi, F., de Valdez, G. F., De Vuyst, L. and Vandamme, P. (2008). *Pediococcus argentinicus* sp. nov. from Argentinean fermented wheat flour and identification of *Pediococcus* species by pheS, rpoA and atpA sequence analysis. *International Journal of Systematic and Evolutionary Microbiology*, *58*(12), 2909-2916.

de Man, J. C., Rogosa, D. and Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Microbiology*, *23*(1), 130-135.

de Palencia, P. F., Werning, M. L., Sierra-Filardi, E., Duenas, M. T., Irastorza, A., Corbí, A. L. and López, P. (2009). Probiotic properties of the 2-substituted (1, 3)-β-D-glucan-producing bacterium *Pediococcus parvulus* 2.6. *Applied and Environmental Microbiology*, *75*(14), 4887-4891.

De Vin, F., Radstrom, P., Herman, L. and De Vuyst, L. (2005). Molecular and biochemical analysis of the galactose phenotype of dairy *Streptococcus thermophilus* strains reveals four different fermentation profiles. *Applied and Environmental Microbiology*, *71*(7), 3659-3667.

De Vrese, M. and Schrezenmeir, J. (2008). Probiotics, prebiotics, and synbiotics. In:*Food biotechnology* (pp. 1-66). Springer Berlin Heidelberg.

De Vuyst, L. (2000). Technology aspects related to the application of functional starter cultures. *Food Technology and Biotechnology*, *38*(2), 105-112.

Del Re, B., Sgorbati, B., Miglioli, M. and Palenzona, D. (2000). Adhesion, autoaggregation and hydrophobicity of 13 strains of *Bifidobacterium longum*. *Letters in Applied Microbiology*, *31*(6), 438-442.

Dobson, C. M., Deneer, H., Lee, S., Hemmingsen, S., Glaze, S. and Ziola, B. (2002). Phylogenetic analysis of the genus *Pediococcus*, including *Pediococcus claussenii* sp. nov., a novel lactic acid bacterium isolated from beer. *International Journal of Systematic and Evolutionary Microbiology*, *52*(6), 2003-2010.

Doi, K., Nishizaki, Y., Fujino, Y., Ohshima, T., Ohmomo, S. and Ogata, S. (2009). *Pediococcus lolii* sp. nov., isolated from ryegrass silage. *International Journal of Systematic and Evolutionary Microbiology*, *59*(5), 1007-1010.

Dols-Lafargue, M., Lee, H. Y., Le Marrec, C., Heyraud, A., Chambat, G. and Lonvaud-Funel, A. (2008). Characterization of gtf, a glucosyltransferase gene in the genomes of *Pediococcus parvulus* and *Oenococcus oeni*, two bacterial species commonly found in wine. *Applied and Environmental Microbiology*, *74*(13), 4079-4090.

EFSA (2012). Scientific Opinion on the safety and efficacy of *Pediococcus acidilactici* (CNCM I-3237, CNCM MA 18/5M—DSM 11673) and *Pediococcus pentosaceus* (DSM 23376, NCIMB 12455, NCIMB 30237 and NCIMB 30168) as silage additives for all species. *EFSA Journal*, *10*(6), 2733.

EFSA (2016). Scientific Panel on Additives and Products or Substances Used in Animal Feed (Parma, PR, Italy, April 19–21th, 2016).

Ehrmann, M. A. and Vogel, R. F. (2005). Molecular taxonomy and genetics of sourdough lactic acid bacteria. *Trends in Food Science and Technology*, *16*(1), 31-42.

El-Katcha, M. I., Soltan, M. A. and Essi, M. S. (2016). Effect of *Pediococcus* spp. supplementation on growth performance, nutrient digestibility and some blood serum biochemical changes of fattening lambs. *Alexandria Journal of Veterinary Sciences*, *49*, 44-54.

Erkkila, S. and Petaja, E. (2000). Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use. *Meat Science*, *55*(3), 297-300.

Facklam, R., Hollis, D. and Collins, M. D. (1989). Identification of gram-positive coccal and coccobacillary vancomycin-resistant bacteria. *Journal of Clinical Microbiology*, *27*(4), 724-730.

FAO/WHO (2002). Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. ftp://ftp.fao.org/es/esn/food/wgreport2.pdf.

Federici, S., Ciarrocchi, F., Campana, R., Ciandrini, E., Blasi, G. and Baffone, W. (2014). Identification and functional traits of lactic acid bacteria isolated from Ciauscolo salami produced in Central Italy. *Meat Science*, *98*(4), 575-584.

Felis, G. E., Torriani, S. and Dellaglio, F. (2005). Reclassification of *Pediococcus urinaeequi* (ex Mees 1934) Garvie 1988 as *Aerococcus urinaeequi* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, *55*(3), 1325-1327.

Felton, E. A., Evans, J. B. and Niven Jr, C. F. (1953). Production of catalase by the pediococci. *Journal of Bacteriology*, *65*(4), 481.

Fernandez, K., Dueñas, M., Irastorza, A., Bilbao, A. and Campo, G. D. (1996). Characterization and DNA plasmid analysis of ropy *Pediococcus* spp. strains isolated from Basque Country ciders. *Journal of Food Protection*, *59*(1), 35-40.

Fernandez, M. F., Boris, S. and Barbes, C. (2003). Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. *Journal of Applied Microbiology*, *94*(3), 449-455.

Filya, I., Muck, R. E. and Contreras-Govea, F. E. (2007). Inoculant effects on alfalfa silage: fermentation products and nutritive value. *Journal of Dairy Science*, *90*(11), 5108-5114.

Fontana, C., Li, S., Yang, Z. and Widmalm, G. (2015). Structural studies of the exopolysaccharide from *Lactobacillus plantarum* C88 using NMR spectroscopy and the program CASPER. *Carbohydrate Research*, *402*, 87-94.

Franciosi, E., Settanni, L., Carlin, S., Cavazza, A. and Poznanski, E. (2008). A factory-scale application of secondary adjunct cultures selected from lactic acid bacteria during Puzzone di Moena cheese ripening. *Journal of Dairy Science*, *91*(8), 2981-2991.

Franz, C. M., Vancanneyt, M., Vandemeulebroecke, K., De Wachter, M., Cleenwerck, I., Hoste, B., Schillinger, U., Holzapfel, W.H. and Swings, J. (2006). *Pediococcus stilesii* sp. nov., isolated from maize grains. *International Journal of Systematic and Evolutionary Microbiology*, *56*(2), 329-333.

Gallego, C. G. and Salminen, S. (2016). Novel probiotics and prebiotics: How can they help in human gut microbiota dysbiosis? *Applied Food Biotechnology*, *3*(2), 72-81.

Galvez, A., Abriouel, H., López, R. L. and Omar, N. B. (2007). Bacteriocin-based strategies for food biopreservation. *International Journal of Food Microbiology*, *120*(1), 51-70.

Gao, Y., Li, D., Liu, S. and Liu, Y. (2012). Probiotic potential of *L. sake* C2 isolated from traditional Chinese fermented cabbage. *European Food Research and Technology*, *234*(1), 45-51.

Garg, N. and Kaur, B. (2015). Biopreservative potential and stability assessment of *Pediococcus acidilactici* BA28. *International Journal of Advanced Research*, *3*(9), 980-989.

Garsa, A. K., Kumariya, R., Kumar, A., Lather, P., Kapila, S., Sood, S. K. and Kapasiya, M. (2014). *In vitro* evaluation of the probiotic attributes of two pediococci strains producing pediocin PA-1 with selective potency as compared to nisin. *European Food Research and Technology*, *239*(3), 491-499.

Garvie, E. I. (1986). Genus Pediococcus. In: Sneath PHA, Mair NS, Sharp ME, Holt JG (eds) *Bergey's Manual of Systematic Bacteriology*. The Williams and Wilkins Co, Baltimore, vol. 2, pp 1075-1079.

Ghany, K. A. E., Hamouda, R. A., Mahrous, H., Abd Elhafez, E., Ahmed, F. A. H. and Hamza, H. A. (2016). Description of isolated LAB producing beta-glucan from Egyptian sources and evaluation of its therapeutic effect. *International Journal of Pharmacology*, *12*(8), 801-811.

Ghareeb, K., Awad, W. A., Mohnl, M., Porta, R., Biarnes, M., Bohm, J. and Schatzmayr, G. (2012). Evaluating the efficacy of an avian-specific probiotic to reduce the colonization of *Campylobacter jejuni* in broiler chickens. *Poultry Science*, *91*(8), 1825-1832.

Gilliland, S. E., Staley, T. E. and Bush, L. J. (1984). Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. *Journal of Dairy Science*, *67*(12), 3045-3051.

Giraffa, G., Paris, A., Valcavi, L., Gatti, M. and Neviani, E. (2001). Genotypic and phenotypic heterogeneity of *Streptococcus thermophilus* strains isolated from dairy products. *Journal of Applied Microbiology*, *91*(5), 937-943.

Goldin, B. R. (2011). Probiotics and health: from history to future. In W. Kneifel and S. Salminen (Eds.), *Probiotics and health claims*, West Sussex, UK: John Wiley and Sons Ltd., 1-16.

Gomez Zavaglia, A., Kociubinski, G., Perez, P., Disalvo, E. and De Antoni, G. (2002). Effect of bile on the lipid composition and surface properties of bifidobacteria. *Journal of Applied Microbiology*, *93*(5), 794-799.

Goodwins, J., Mornet, A. and Manoury, E. (2008). Aromatization of a milk product using at least one bacteria producing a bacteriocin and belonging to the *Pediococcus* genus. Patent 10/577,351 (US 2008/0292749 A1) Jonathan Goodwins, Annie Mornet Elise Manoury, Denmark.

Gupta, A. and Sharma, N. (2017). Characterization of potential probiotic lactic acid bacteria-*Pediococcus acidilactici* Ch-2 isolated from Chuli-A traditional apricot product of Himalayan region for the production of novel bioactive compounds with special therapeutic properties. *Journal of Food: Microbiology, Safety and Hygiene*, *2*(1), 119.

Gupta, H. and Malik, R. K. (2007). Incidence of virulence in bacteriocin-producing enterococcal isolates. *Le Lait*, *87*(6), 587-601.

Haakensen, M., Dobson, C. M., Hill, J. E. and Ziola, B. (2009). Reclassification of *Pediococcus dextrinicus* (Coster and White 1964) Back 1978 (Approved Lists 1980) as *Lactobacillus dextrinicus* comb. nov., and emended description of the genus Lactobacillus. *International Journal of Systematic and Evolutionary Microbiology*, *59*(3), 615-621.

Halami, P. M. and Chandrashekar, A. (2005). Enhanced production of pediocin C20 by a native strain of *Pediococcus acidilactici* C20 in an optimized food-grade medium. *Process Biochemistry*, *40*(5), 1835-1840.

Herreros, M. A., Sandoval, H., González, L., Castro, J. M., Fresno, J. M. and Tornadijo, M. E. (2005). Antimicrobial activity and antibiotic resistance of lactic acid bacteria isolated from Armada cheese (a Spanish goats' milk cheese). *Food Microbiology*, *22*(5), 455-459.

Higashikawa, F., Noda, M., Awaya, T., Danshiitsoodol, N., Matoba, Y., Kumagai, T. and Sugiyama, M. (2016). Antiobesity effect of *Pediococcus*

pentosaceus LP28 on overweight subjects: a randomized, double-blind, placebo-controlled clinical trial. *European Journal of Clinical Nutrition*, *70*(5), 582-588.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Gananj, R. B., Flint, H. J., Salminen, S. and Calder, P. C. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology*, *11*(8), 506-514.

Hitchener, B. J., Egan, A. F. and Rogers, P. J. (1982). Characteristics of lactic acid bacteria isolated from vacuum-packaged beef. *Journal of Applied Microbiology*, *52*(1), 31-37.

Hladikova, Z., Smetanková, J., Greif, G. and Greifová, M. (2012). Antimicrobial activity of selected lactic acid cocci and production of organic acids. *Acta Chimica Slovaca*, *5*(1), 80-85.

Holzapfel, W. H. and Schillinger, U. (2002). Introduction to pre-and probiotics. *Food Research International*, *35*(2), 109-116.

Holzapfel, W. H., Franz, C. M., Ludwig, W., Back, W. and Dicks, L. M. (2006). The genera *Pediococcus* and *Tetragenococcus*. In *The Prokaryotes* (pp. 229-266). Springer US.

Huang, Y., Luo, Y., Zhai, Z., Zhang, H., Yang, C., Tian, H., Li, Z., Feng, J., Liu, H. and Hao, Y. (2009). Characterization and application of an anti-Listeria bacteriocin produced by *Pediococcus pentosaceus* 05-10 isolated from Sichuan Pickle, a traditionally fermented vegetable product from China. *Food Control*, *20*(11), 1030-1035.

Hummel, A. S., Hertel, C., Holzapfel, W. H. and Franz, C. M. (2007). Antibiotic resistances of starter and probiotic strains of lactic acid bacteria. *Applied and Environmental Microbiology*, *73*(3), 730-739.

Huys, G., Vancanneyt, M., D'Haene, K., Vankerckhoven, V., Goossens, H. and Swings, J. (2006). Accuracy of species identity of commercial bacterial cultures intended for probiotic or nutritional use. *Research in Microbiology*, *157*(9), 803-810.

ICMR-DBT (2011). Guidelines for evaluation of probiotics in food. New Delhi, India: Indian Council of Medical Research.

Ilavenil, S., Vijayakumar, M., Kim, D. H., Valan Arasu, M., Park, H. S., Ravikumar, S. and Choi, K. C. (2016). Assessment of probiotic, antifungal and cholesterol lowering properties of *Pediococcus pentosaceus* KCC-23 isolated from Italian ryegrass. *Journal of the Science of Food and Agriculture*, *96*(2), 593-601.

INRA (2005). A Lactic Acid Bacteria collection of importance to the dairy industry. INRA Press.

Irmler, S., Bavan, T., Oberli, A., Roetschi, A., Badertscher, R., Guggenbühl, B. and Berthoud, H. (2013). Catabolism of Serine by *Pediococcus acidilactici* and *Pediococcus pentosaceus*. *Applied and Environmental Microbiology*, *79*(4), 1309-1315.

Jain, J. and Singh, B. (2016). Characteristics and biotechnological applications of bacterial phytases. *Process Biochemistry*, *51*(2), 159-169.

Jang, S., Lee, D. K., jin Yang, H., An, H. M., Baek, E. H., Kim, M. J., Lee, K. O., Chung, M. J., Kim, J. E. and Ha, N. J. (2010). Anti-stress effect of lactic acid bacteria (*Lactobacillus acidophilus*, *Pediococcus pentosaseus*, *Bifidobacterium longum* SPM1205). *Environmental Health and Toxicology*, *25*(1), 79-84.

Jang, S., Lee, D., Jang, I. S., Choi, H. S. and Suh, H. J. (2015). The culture of *Pediococcus pentosaceus* T1 inhibits *Listeria* proliferation in salmon fillets and controls maturation of kimchi. *Food Technology and Biotechnology*, *53*(1), 29-37.

Jang, S., Lee, J., Jung, U., Choi, H. S. and Suh, H. J. (2014). Identification of an anti-listerial domain from *Pediococcus pentosaceus* T1 derived from Kimchi, a traditional fermented vegetable. *Food Control*, *43*, 42-48.

Jankovic, I., Ventura, M., Meylan, V., Rouvet, M., Elli, M. and Zink, R. (2003). Contribution of aggregation-promoting factor to maintenance of cell shape in *Lactobacillus gasseri* 4B2. *Journal of Bacteriology*, *185*(11), 3288-3296.

Jensen, H., Grimmer, S., Naterstad, K. and Axelsson, L. (2012). *In vitro* testing of commercial and potential probiotic lactic acid bacteria. *International Journal of Food Microbiology*, *153*(1), 216-222.

Jonganurakkun, B., Wang, Q., Xu, S. H., Tada, Y., Minamida, K., Yasokawa, D., Sugi, M., Hara., H. and Asano, K. (2008). *Pediococcus pentosaceus* NB-17 for probiotic use. *Journal of Bioscience and Bioengineering*, *106*(1), 69-73.

Juskiewicz, J., Semaskaite, A., Zduńczyk, Z., Wróblewska, M., Gružauskas, R. and Juśkiewicz, M. (2007). Minor effect of the dietary combination of probiotic *Pediococcus acidilactici* with fructooligosaccharides or polysaccharidases on beneficial changes in the cecum of rats. *Nutrition Research*, *27*(3), 133-139.

Juven, B. J., Meinersmann, R. J. and Stern, N. J. (1991). A review: antagonistic effects of lactobacilli and pediococci to control intestinal colonization by human enteropathogens in live poultry. *Journal of Applied Microbiology*, *70*(2), 95-103.

Kamran, A., Bibi, Z., Aman, A. and Qader, S. A. U. (2016). Lactose hydrolysis approach: isolation and production of β-galactosidase from newly isolated *Bacillus* strain B-2. *Biocatalysis and Agricultural Biotechnology*, *5*, 99-103.

Kaur, B., Garg, N., Sachdev, A., Kumar, B., Mittu, B. and Chauhan, A. (2012). Isolation and molecular characterization of anti-*Helicobacter pylori* bacteriocin producing *Pediococcus acidilactici* BA28. *Open Access Scientific Reports*, *1*, 1-6.

Kaur, N. and Gupta, A. K. (2002). Applications of inulin and oligofructose in health and nutrition. *Journal of Biosciences*, *27*(7), 703-714.

Kaushik, J. K., Kumar, A., Duary, R. K., Mohanty, A. K., Grover, S. and Batish, V. K. (2009). Functional and probiotic attributes of an indigenous isolate of *Lactobacillus plantarum*. *PloS One*, *4*(12), e8099.

Kekkonen, R. A., Vasankari, T. J., Vuorimaa, T., Haahtela, T., Julkunen, I. and Korpela, R. (2007). The effect of probiotics on respiratory infections and gastrointestinal symptoms during training in marathon runners. *International Journal of Sport Nutrition and Exercise Metabolism*, *17*(4), 352-363.

Klaenhammer, T. R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiology Reviews*, *12*(1-3), 39-85.

Kleinschmit, D. H. and Kung, L. (2006). The effects of *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus* R1094 on the fermentation of corn silage. *Journal of Dairy Science*, *89*(10), 3999-4004.

Kokkiligadda, A., Beniwal, A., Saini, P. and Vij, S. (2016). Utilization of cheese whey using synergistic immobilization of β-galactosidase and *Saccharomyces cerevisiae* cells in dual matrices. *Applied Biochemistry and Biotechnology*, *179*(8), 1469-1484.

Korasapati, N. R. (1998). Genetic and biochemical characterization of bacteriocin produced by a selected *Pediococcus* sp. Ph.D. Thesis, Dairy Microbiology Division, National Dairy Research Institute, Karnal-132001, Haryana (India).

Kotzampassi, K., Giamarellos-Bourboulis, E. J., Voudouris, A., Kazamias, P. and Eleftheriadis, E. (2006). Benefits of a synbiotic formula (Synbiotic 2000Forte®) in critically ill trauma patients: early results of a randomized controlled trial. *World Journal of Surgery*, *30*(10), 1848-1855.

Kuda, T., Kawahara, M., Nemoto, M., Takahashi, H. and Kimura, B. (2014). *In vitro* antioxidant and anti-inflammation properties of lactic acid bacteria isolated from fish intestines and fermented fish from the Sanriku Satoumi region in Japan. *Food Research International*, *64*, 248-255.

Kumar, V., Kumari, A., Angmo, K. and Bhalla, T. C. (2017). Isolation and characterization of lactic acid bacteria from traditional pickles of Himachal Pradesh, India. *Journal of Food Science and Technology*, *54*(7), 1945-1952.

Kurzak, P., Ehrmann, M. A. and Vogel, R. F. (1998). Diversity of lactic acid bacteria associated with ducks. *Systematic and Applied Microbiology*, *21*(4), 588-592.

Lactose Intolerance- Scientific status report (2011). http://www.nationaldairycouncil.org/SiteCollectionDocuments/research/research summaries/ScientificStatusReportLactoseIntolerance.pdf.

Laitila, A., Sweins, H., Vilpola, A., Kotaviita, E., Olkku, J., Home, S. and Haikara, A. (2006). *Lactobacillus plantarum* and *Pediococcus pentosaceus* starter cultures as a tool for microflora management in malting and for enhancement of malt processability. *Journal of Agricultural and Food Chemistry*, *54*(11), 3840-3851.

Leroy, F. and De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science and Technology*, *15*(2), 67-78.

Lima, A. F., Cavalcante, K. F., de Freitas, M. D. F. M., Rodrigues, T. H. S., Rocha, M. V. P. and Gonçalves, L. R. B. (2013). Comparative biochemical characterization of soluble and chitosan immobilized β-galactosidase from *Kluyveromyces lactis* NRRL Y1564. *Process Biochemistry*, *48*(3), 443-452.

Litopoulou-Tzanetaki, E., Vafopoulou-Mastrojiannaki, A. and Tzanetakis, N. (1989). Biotechnologically important metabolic activities of *Pediococcus* isolates from milk and cheese. *MAN Microbiologie, Aliments, Nutrition*, *7*(2), 113-122.

Liu, S. Q. (2003). Practical implications of lactate and pyruvate metabolism by lactic acid bacteria in food and beverage fermentations. *International Journal of Food Microbiology*, *83*(2), 115-131.

Lv, L. X., Li, Y. D., Hu, X. J., Shi, H. Y. and Li, L. J. (2014). Whole-genome sequence assembly of *Pediococcus pentosaceus* LI05 (CGMCC 7049) from the human gastrointestinal tract and comparative analysis with representative sequences from three food-borne strains. *Gut Pathogens*, *6*(1), 36.

Mandal, V., Sen, S. K. and Mandal, N. C. (2011). Isolation and characterization of pediocin NV 5 producing *Pediococcus acidilactici* LAB 5 from vacuum-packed fermented meat product. *Indian Journal of Microbiology*, *51*(1), 22-29.

Martino, M. E., Maifreni, M., Marino, M., Bartolomeoli, I., Carraro, L., Fasolato, L. and Cardazzo, B. (2013). Genotypic and phenotypic diversity of *Pediococcus pentosaceus* strains isolated from food matrices and characterisation of the penocin operon. *Antonie Van Leeuwenhoek*, *103*(5), 1149-1163.

Mathara, J. M., Schillinger, U., Kutima, P. M., Mbugua, S. K., Guigas, C., Franz, C. and Holzapfel, W. H. (2008). Functional properties of *Lactobacillus plantarum* strains isolated from Maasai traditional fermented milk products in Kenya. *Current Microbiology*, *56*(4), 315-321.

McSweeney, P. L. H. and Fox, P. F. (2004). Metabolism of residual lactose and of lactate and citrate. *Cheese: Chemistry, Physics and Microbiology*, *1*, 361-371.

Mercenier, A., Wijnkoop, I. L. and Sanders, M. E. (2008). Physiological and functional properties of probiotics. *Bulletin of the International Dairy Federation*, *429*, 1–7.

Monnet, C. and Corrieu, G. (2007). Selection and properties of α-acetolactate decarboxylase-deficient spontaneous mutants of *Streptococcus thermophilus*. *Food Microbiology*, *24*(6), 601-606.

Mora, D., Fortina, M. G., Parini, C., Daffonchio, D. and Manachini, P. L. (2000). Genomic subpopulations within the species *Pediococcus acidilactici*

detected by multilocus typing analysis: relationships between pediocin AcH/PA-1 producing and non-producing strains. *Microbiology*, *146*(8), 2027-2038.

Mora, D., Fortina, M. G., Parini, C., Ricci, G., Gatti, M., Giraffa, G. and Manachini, P. L. (2002). Genetic diversity and technological properties of *Streptococcus thermophilus* strains isolated from dairy products. *Journal of Applied Microbiology*, *93*(2), 278-287.

Morita, H., Miyamoto, T., Kataoka, K. and Nabeshima, S. (1991). Intergeneric transfer of lactose-fermenting ability to *Pediococcus acidilactici* by conjugation. *Agricultural and Biological Chemistry*, *55*(11), 2871-2873.

Moser, S. A. and Savage, D. C. (2001). Bile salt hydrolase activity and resistance to toxicity of conjugated bile salts are unrelated properties in lactobacilli. *Applied and Environmental Microbiology*, *67*(8), 3476-3480.

Mostefaoui, A., Hakem, A., Yabrir, B., Boutaiba, S. and Badis, A. (2014). Screening for exopolysaccharide-producing strains of thermophilic lactic acid bacteria isolated from Algerian raw camel milk. *African Journal of Microbiology Research*, *8*(22), 2208-2214.

Mukherjee, K. K. and Hutkins, R. W. (1994). Isolation of Galactose-Fermenting Thermophilic Cultures and Their Use in the Manufacture of Low Browning Mozzarella Cheese. *Journal of Dairy Science*, *77*(10), 2839-2849.

Mukherjee, S., Singh, A. K., Adhikari, M. D. and Ramesh, A. (2013). Quantitative Appraisal of the Probiotic Attributes and *In Vitro* Adhesion Potential of Anti-listerial Bacteriocin-producing Lactic Acid Bacteria. *Probiotics and Antimicrobial Proteins*, *5*(2), 99-109.

Muller, T. (1990). Comparison of methods for differentiation between homofermentative and heterofermentative lactic acid bacteria. *Zentralblatt Für Mikrobiologie*, *145*(5), 363-366.

Naqvi, S. W. A., Jayakumar, D. A., Narvekar, P. V. and Nalk, H. (2000). Increased marine production of N_2O due to intensifying anoxia on the Indian continental shelf. *Nature*, *408*(6810), 346-349.

Narayanan, R., Sumanth, G. K., Chowdary, R. and Jyothi, C. P. (2017). *In vitro* study of potential probiotic *Pediococcus pentosaceus* isolated from idli batter and biomass production using whey. *International Journal of Food and Nutritional Science*, *6*(2), 34-45.

Nielsen, J., Nikolajsen, K. and Villadsen, J. (1989). FIA for on-line monitoring of important lactic acid fermentation variables. *Biotechnology and Bioengineering*, *33*(9), 1127-1134.

Noohi, N., Ebrahimipour, G., Rohani, M., Talebi, M. and Pourshafie, M. R. (2016). Evaluation of potential probiotic characteristics and antibacterial effects of strains of *Pediococcus* species isolated from broiler chickens. *British Poultry Science*, *57*(3), 317-323.

Nout, M. R., Sarkar, P. K. and Beuchat, L. R. (2007). Indigenous fermented foods. In *Food Microbiology: Fundamentals and Frontiers, Third Edition* (pp. 817-835). American Society of Microbiology.

Novelli, G. and Reichardt, J. K. (2000). Molecular basis of disorders of human galactose metabolism: past, present, and future. *Molecular Genetics and Metabolism*, *71*(1), 62-65.

Nuobariene, L., Cizeikiene, D., Gradzeviciute, E., Hansen, A. S., Rasmussen, S. K., Juodeikiene, G. and Vogensen, F. K. (2015). Phytase-active lactic acid bacteria from sourdoughs: Isolation and identification. *LWT-Food Science and Technology*, *63*(1), 766-772.

Oh, Y. J. and Jung, D. S. (2015). Evaluation of probiotic properties of *Lactobacillus* and *Pediococcus* strains isolated from Omegisool, a traditionally fermented millet alcoholic beverage in Korea. *LWT-Food Science and Technology*, *63*(1), 437-444.

Olah, A., Belagyi, T., Issekutz, A. and Olgyai, G. (2005). Combination of early nasojejunal feeding with modern synbiotic therapy in the treatment of severe acute pancreatitis (prospective, randomized, double-blind study). *Magyar Sebeszet*, *58*(3), 173-178.

Osmanagaoglu, O., Kiran, F. and Ataoglu, H. (2010). Evaluation of *in vitro* probiotic potential of *Pediococcus pentosaceus* OZF isolated from human breast milk. *Probiotics and Antimicrobial Proteins*, *2*(3), 162-174.

Osmanagaoglu, O., Kiran, F. and Nes, I. F. (2011). A probiotic bacterium, *Pediococcus pentosaceus* OZF, isolated from human breast milk produces pediocin AcH/PA-1. *African Journal of Biotechnology*, *10*(11), 2070-2079.

Osmanagaoglu, O., Kiran, F., Yagci, F. C. and Gursel, I. (2013). Immunomodulatory function and in vivo properties of *Pediococcus*

pentosaceus OZF, a promising probiotic strain. *Annals of Microbiology*, *63*(4), 1311-1318.

Pal, A., Lobo, M. and Khanum, F. (2013). Extraction, Purification and Thermodynamic Characterization of Almond (*Amygdalus communis*) [Beta]-Galactosidase for the Preparation of Delactosed Milk. *Food Technology and Biotechnology*, *51*(1), 53.

Palacios, M. C., Haros, M., Sanz, Y. and Rosell, C. M. (2008). Selection of lactic acid bacteria with high phytate degrading activity for application in whole wheat breadmaking. *LWT-Food Science and Technology*, *41*(1), 82-92.

Pandey, K. R., Naik, S. R. and Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics-a review. *Journal of Food Science and Technology*, *52*(12), 7577-7587.

Papagianni, M. and Anastasiadou, S. (2009). Pediocins: The bacteriocins of Pediococci. Sources, production, properties and applications. *Microbial Cell Factories*, *8*(1), 3.

Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E. and Kotzekidou, P. (2003). Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Science*, *65*(2), 859-867.

Pasteris, S. E. and de Saad, A. S. (2005). Aerobic glycerol catabolism by *Pediococcus pentosaceus* isolated from wine. *Food Microbiology*, *22*(5), 399-407.

Patel, A. K., Singhania, R. R., Pandey, A. and Chincholkar, S. B. (2010). Probiotic bile salt hydrolase: current developments and perspectives. *Applied Biochemistry and Biotechnology*, *162*(1), 166-180.

Patel, A. and Prajapati, J. B. (2013). Food and health applications of exopolysaccharides produced by lactic acid bacteria. *Advances in Dairy Research*, *1*, 107.

Pederson, C. S. (1949). The genus Pediococcus. *Bacteriological Reviews*, *13*(4), 225-232.

Pfannebecker, J. and Frohlich, J. (2008). Use of a species-specific multiplex PCR for the identification of pediococci. *International Journal of Food Microbiology*, *128*(2), 288-296.

Piasecka-Jozwiak, K., Rozmierska, J., Chabłowska, B., Stecka, K. M., Skąpska, S., Kliszcz, M. and Szkudzińska-Rzeszowiak, E. (2013). Starter cultures for lactic acid fermentation of sweet pepper, pattypan squash and tomatoes. *Polish Journal of Food and Nutrition Sciences*, *63*(2), 95-102.

Pinto, M. G. V., Franz, C. M., Schillinger, U. and Holzapfel, W. H. (2006). *Lactobacillus* spp. with *in vitro* probiotic properties from human faeces and traditional fermented products. *International Journal of Food Microbiology*, *109*(3), 205-214.

Porto, M. C. W., Kuniyoshi, T. M., de Azevedo, P. O. D. S., Vitolo, M. and de Souza Oliveira, R. P. (2017). *Pediococcus* spp.: An important genus of lactic acid bacteria and pediocin producers. *Biotechnology Advances*, *35*(3), 361-374.

Pospiech, A. and Neumann, B. (1995). A versatile quick-prep of genomic DNA from gram-positive bacteria. *Trends in Genetics*, *11*(6), 217-218.

Prakash, R., Sinha, P. R., Sinha, R. N. and Singh, B. (1997). Adherence of lactobacilli to epithelial cells and hexadecane for use as probiotics. *Indian Journal of Dairy Science*, *10*, 43-47.

Prasirtsak, B., Tanasupawat, S., Boonsombat, R., Kodama, K. and Thongchul, N. (2013). Characterization of lactic acid producing bacteria from Thai sources. *Journal of Applied Pharmaceutical Science*, *03*(1), 33-38.

Priyodip, P., Prakash, P. Y. and Balaji, S. (2017). Phytases of Probiotic Bacteria: Characteristics and Beneficial Aspects. *Indian Journal of Microbiology*, *57*(2), 148-154.

Raboy, V. (2003). myo-Inositol-1, 2, 3, 4, 5, 6-hexakisphosphate. *Phytochemistry*, *64*(6), 1033-1043.

Raghavendra, P. and Halami, P. M. (2009). Screening, selection and characterization of phytic acid degrading lactic acid bacteria from chicken intestine. *International Journal of Food Microbiology*, *133*(1), 129-134.

Raghavendra, P., Rao, T. and Halami, P. (2010). Evaluation of beneficial attributes for phytate-degrading *Pediococcus pentosaceus* CFR R123. *Beneficial Microbes*, *1*(3), 259-264.

Raghavendra, P., Ushakumari, S. and Halami, P. (2011). Phytate-degrading *Pediococcus pentosaceus* CFR R123 for application in functional foods. *Beneficial Microbes*, *2*(1), 57-61.

Rahman, M. M., Kim, W. S., Kumura, H. and Shimazaki, K. I. (2008). Autoaggregation and surface hydrophobicity of bifidobacteria. *World Journal of Microbiology and Biotechnology*, *24*(8), 1593-1598.

Ramirez-Chavarin, M. L., Wacher, C., Eslava-Campos, C. A. and Perez-Chabela, M. L. (2013). Probiotic potential of thermotolerant lactic acid bacteria strains isolated from cooked meat products. *International Food Research Journal*, *20*(2), 991-1000.

Rayes, N., Seehofer, D., Theruvath, T., Schiller, R. A., Langrehr, J. M., Jonas, S., Bengmark, S. and Neuhaus, P. (2005). Supply of Pre-and Probiotics Reduces Bacterial Infection Rates After Liver Transplantation—A Randomized, Double-Blind Trial. *American Journal of Transplantation*, *5*(1), 125-130.

Ribeiro, M. C. D. O., Vandenberghe, L. P. D. S., Spier, M. R., Paludo, K. S., Soccol, C. R. and Soccol, V. T. (2014). Evaluation of probiotic properties of *Pediococcus acidilactici* B14 in association with *Lactobacillus acidophilus* ATCC 4356 for application in a soy based aerated symbiotic dessert. *Brazilian Archives of Biology and Technology*, *57*(5), 755-765.

Riebel, W. J. and Washington, J. A. (1990). Clinical and microbiologic characteristics of pediococci. *Journal of Clinical Microbiology*, *28*(6), 1348-1355.

Rodas, A. M., Ferrer, S. and Pardo, I. (2003). 16S-ARDRA, a tool for identification of lactic acid bacteria isolated from grape must and wine. *Systematic and Applied Microbiology*, *26*(3), 412-422.

Rodríguez, J. M., Martínez, M. I. and Kok, J. (2002). Pediocin PA-1, a wide-spectrum bacteriocin from lactic acid bacteria. *Critical Reviews in Food Science and Nutrition*, *42*(2), 91-121.

Rosenberg, M., Gutnick, D. and Rosenberg, E. (1980). Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiology Letters*, *9*(1), 29-33.

Ruiz-Moyano, S., Martín, A., Benito, M. J., Hernández, A., Casquete, R. and Córdoba, M. D. G. (2010). Safety and functional aspects of pre-selected pediococci for probiotic use in Iberian dry-fermented

sausages. *International Journal of Food Science and Technology*, *45*(6), 1138-1145.

Ruiz-Moyano, S., Martín, A., Benito, M. J., Nevado, F. P. and de Guia Cordoba, M. (2008). Screening of lactic acid bacteria and bifidobacteria for potential probiotic use in Iberian dry fermented sausages. *Meat Science*, *80*(3), 715-721.

Ryu, E. H. and Chang, H. C. (2013). *In vitro* study of potentially probiotic lactic acid bacteria strains isolated from kimchi. *Annals of Microbiology*, *63*(4), 1387-1395.

Sabir, F., Beyatli, Y., Cokmus, C. and Onal-Darilmaz, D. (2010). Assessment of potential probiotic properties of *Lactobacillus* spp., *Lactococcus* spp., and *Pediococcus* spp. strains isolated from kefir. *Journal of Food Science*, *75*(9), M568-M573.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual 1989 2nd edn Cold Spring Harbor. *NY Cold Spring Harbor Laboratory*.

Sanalibaba, P. and Çakmak, G. A. (2016). Exopolysaccharides production by lactic acid bacteria. *Applied Microbiology: Open Access*, *2*(2), Article ID 1000115.

Santos, E. M., Jaime, I., Rovira, J., Lyhs, U., Korkeala, H. and Bjorkroth, J. (2005). Characterization and identification of lactic acid bacteria in "morcilla de Burgos". *International Journal of Food Microbiology*, *97*(3), 285-296.

Saravanan, C., Gopu, V. and Shetty, P. H. (2015). Diversity and functional characterization of microflora isolated from traditional fermented food idli. *Journal of Food Science and Technology*, *52*(11), 7425-7432.

Schillinger, U., Guigas, C. and Holzapfel, W. H. (2005). *In vitro* adherence and other properties of lactobacilli used in probiotic yoghurt-like products. *International Dairy Journal*, *15*(12), 1289-1297.

Sellamani, M., Kalagatur, N. K., Siddaiah, C., Mudili, V., Krishna, K., Natarajan, G. and Putcha, V. L. R. (2016). Antifungal and zearalenone inhibitory activity of *Pediococcus pentosaceus* isolated from dairy products on *Fusarium graminearum*. *Frontiers in Microbiology*, *7*.

Shah, N. P. (2001). Functional foods from probiotics and prebiotics. *Food Technology*, 55, 46-53.

Sharma, P., Tomar, S. K., Goswami, P., Sangwan, V. and Singh, R. (2014). Antibiotic resistance among commercially available probiotics. *Food Research International*, *57*, 176-195.

Shivanna, G. B. and Venkateswaran, G. (2015). Dephytinization of Cereals and Pulses by Phytase Producing Lactic Acid Bacteria. *International Journal of Current Research and Academic Review*, *3*(12), 61-69.

Shobharani, P. and Agrawal, R. (2011). A potent probiotic strain from cheddar cheese. *Indian Journal of Microbiology*, *51*(3), 251-258.

Shukla, R. and Goyal, A. (2013). Novel dextran from *Pediococcus pentosaceus* CRAG3 isolated from fermented cucumber with anti-cancer properties. *International Journal of Biological Macromolecules*, *62*, 352-357.

Shukla, R. and Goyal, A. (2014). Probiotic potential of *Pediococcus pentosaceus* CRAG3: a new isolate from fermented cucumber. *Probiotics and Antimicrobial Proteins*, *6*(1), 11-21.

Simpson, P. J., Stanton, C., Fitzgerald, G. F. and Ross, R. P. (2002). Genomic diversity within the genus Pediococcus as revealed by randomly amplified polymorphic DNA PCR and pulsed-field gel electrophoresis. *Applied and Environmental Microbiology*, *68*(2), 765-771.

Simpson, W. J. and Taguchi, H. (1995). The genus Pediococcus, with notes on the genera Tetratogenococcus and Aerococcus. In *The genera of lactic acid bacteria* (pp. 125-172). Springer US.

Singh, B. and Satyanarayana, T. (2015). Fungal phytases: characteristics and amelioration of nutritional quality and growth of non-ruminants. *Journal of Animal Physiology and Animal Nutrition*, *99*(4), 646-660.

Singh, S., Goswami, P., Singh, R. and Heller, K. J. (2009). Application of molecular identification tools for Lactobacillus, with a focus on discrimination between closely related species: a review. *LWT-Food Science and Technology*, *42*(2), 448-457.

Singh, T. P., Kaur, G., Malik, R. K., Schillinger, U., Guigas, C. and Kapila, S. (2012). Characterization of intestinal *Lactobacillus reuteri* strains as potential probiotics. *Probiotics and Antimicrobial Proteins*, *4*(1), 47-58.

Singla, V., Mandal, S., Anand, S. and Tomar, S. K. (2017). Techno-functional attributes of *Pediococcus* spp.: A Review. *Indian Journal of Dairy Science*, *70*(4), 381-390.

Siripornadulsil, W., Tasaku, S., Buahorm, J. and Siripornadulsil, S. (2014). Probiotic properties of lactic acid bacteria isolated from fermented food. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, *8*, 364-366.

Somkuti, G. A. and Steinberg, D. H. (2010). Pediocin production in milk by *Pediococcus acidilactici* in co-culture with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Journal of Industrial Microbiology and Biotechnology*, *37*(1), 65-69.

Song, Y. R., Cha, Y. S. and Baik, S. H. (2013). Exopolysaccharide produced by *Pediococcus acidilactici* M76 isolated from the Korean traditional rice wine, Makgeolli. *Journal of Microbiology and Biotechnology*, *23*(5), 681-688.

Songre-Ouattara, L. T., Mouquet-Rivier, C., Icard-Vernière, C., Humblot, C., Diawara, B. and Guyot, J. P. (2008). Enzyme activities of lactic acid bacteria from a pearl millet fermented gruel (ben-saalga) of functional interest in nutrition. *International Journal of Food Microbiology*, *128*(2), 395-400.

Suganthi, V. and Mohanasrinivasan, V. (2015). Optimization studies for enhanced bacteriocin production by *Pediococcus pentosaceus* KC692718 using response surface methodology. *Journal of Food Science and Technology*, *52*(6), 3773-3783.

Sukumar, G. and Ghosh, A. R. (2010). *Pediococcus* spp. A potential probiotic isolated from Khadi (an Indian fermented food) and identified by 16S rDNA sequence analysis. *African Journal of Food Science*, *4*(9), 597-602.

Swain, M. R., Anandharaj, M., Ray, R. C. and Parveen Rani, R. (2014). Fermented fruits and vegetables of Asia: a potential source of probiotics. *Biotechnology Research International*, Article ID 250424.

Tadesse, G., Ephraim, E. and Ashenafi, M. (2005). Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamita, traditional Ethiopian fermented beverages, on some food-borne pathogens and effect of growth medium on the inhibitory activity. *Internet Journal of Food Safety*, *5*(5), 13-20.

Taheri, H. R., Moravej, H., Malakzadegan, A., Tabandeh, F., Zaghari, M., Shivazad, M. and Adibmoradi, M. (2010). Efficacy of *Pediococcus acidilactici*-based probiotic on intestinal Coliforms and villus height,

serum cholesterol level and performance of broiler chickens. *African Journal of Biotechnology*, *9*(44), 7564-7567.

Tamang, J. P., Tamang, B., Schillinger, U., Guigas, C. and Holzapfel, W. H. (2009). Functional properties of lactic acid bacteria isolated from ethnic fermented vegetables of the Himalayas. *International Journal of Food Microbiology*, *135*(1), 28-33.

Tanasupawat, S., Pakdeeto, A., Thawai, C., Yukphan, P. and Okada, S. (2007). Identification of lactic acid bacteria from fermented tea leaves (miang) in Thailand and proposals of *Lactobacillus thailandensis* sp. nov., *Lactobacillus camelliae* sp. nov., and *Pediococcus siamensis* sp. nov. *The Journal of General and Applied Microbiology*, *53*(1), 7-15.

Tankovic, J., Leclercq, R. and Duval, J. (1993). Antimicrobial susceptibility of *Pediococcus* spp. and genetic basis of macrolide resistance in *Pediococcus acidilactici* HM3020. *Antimicrobial Agents and Chemotherapy*, *37*(4), 789-792.

Tejero-Sarinena, S., Barlow, J., Costabile, A., Gibson, G. R. and Rowland, I. (2012). *In vitro* evaluation of the antimicrobial activity of a range of probiotics against pathogens: evidence for the effects of organic acids. *Anaerobe*, *18*(5), 530-538.

Temmerman, R., Pot, B., Huys, G. and Swings, J. (2003b). Identification and antibiotic susceptibility of bacterial isolates from probiotic products. *International Journal of Food Microbiology*, *81*(1), 1-10.

Temmerman, R., Scheirlinck, I., Huys, G. and Swings, J. (2003a). Culture-independent analysis of probiotic products by denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology*, *69*(1), 220-226.

Tiwari, G., Tiwari, R., Pandey, S. and Pandey, P. (2012). Promising future of probiotics for human health: Current scenario. *Chronicles of Young Scientists*, *3*(1), 17.

Todorov, S. D. and Dicks, L. M. (2009). Bacteriocin production by *Pediococcus pentosaceus* isolated from marula (*Scerocarya birrea*). *International Journal of Food Microbiology*, *132*(2), 117-126.

Tome, E., Pereira, V. L., Lopes, C. I., Gibbs, P. A. and Teixeira, P. C. (2008). *In vitro* tests of suitability of bacteriocin-producing lactic acid bacteria, as potential biopreservation cultures in vacuum-packaged cold-smoked salmon. *Food Control*, *19*(5), 535-543.

Torino, M. I., de Valdez, G. F. and Mozzi, F. (2015). Biopolymers from lactic acid bacteria. Novel applications in foods and beverages. *Frontiers in Microbiology*, *6*, 834.

Turgis, M., Vu, K. D., Millette, M., Dupont, C. and Lacroix, M. (2016). Influence of environmental factors on bacteriocin production by human isolates of *Lactococcus lactis* MM19 and *Pediococcus acidilactici* MM33. *Probiotics and Antimicrobial Proteins*, *8*(1), 53-59.

Uymaz, B., Şimşek, O., Akkoç, N., Ataoğlu, H. and Akçelik, M. (2009). *In vitro* characterization of probiotic properties of *Pediococcus pentosaceus* BH105 isolated from human faeces. *Annals of Microbiology*, *59*(3), 485-491.

Vanbelle, M., Teller, E. and Focant, M. (1990). Probiotics in animal nutrition: a review. *Archives of Animal Nutrition*, *40*(7), 543-567.

Vandevoorde, L., Christiaens, H. and Verstraete, W. (1992). Prevalence of coaggregation reactions among chicken lactobacilli. *Journal of Applied Microbiology*, *72*(3), 214-219.

Vankerckhoven, V. V., Autgaerden, T. V., Huys, G., Vancanneyt, M., Swings, J. and Goossens, H. (2004). Establishment of the PROSAFE collection of probiotic and human lactic acid bacteria. *Microbial Ecology in Health and Disease*, *16*(2-3), 131-136.

Venkateshwari, S., Halami, P. and Vijayendra, S. (2010). Characterisation of the heat-stable bacteriocin-producing and vancomycin-sensitive *Pediococcus pentosaceus* CFR B19 isolated from beans. *Beneficial Microbes*, *1*(2), 159-164.

Vidhyasagar, V. and Jeevaratnam, K. (2012). Isolation and characterization of *Pediococcus pentosaceus* from idly batter: a traditional South Indian fermented food source. *Biosciences Biotechnology Research Asia*, *9*, 427-431.

Vidhyasagar, V. and Jeevaratnam, K. (2013). Evaluation of *Pediococcus pentosaceus* strains isolated from Idly batter for probiotic properties *in vitro*. *Journal of Functional Foods*, *5*(1), 235-243.

Vinderola, C. G., Medici, M. and Perdigon, G. (2004). Relationship between interaction sites in the gut, hydrophobicity, mucosal immunomodulating capacities and cell wall protein profiles in indigenous and exogenous bacteria. *Journal of Applied Microbiology*, *96*(2), 230-243.

Walter, J., Hertel, C., Tannock, G. W., Lis, C. M., Munro, K. and Hammes, W. P. (2001). Detection of Lactobacillus, Pediococcus, Leuconostoc, and Weissella species in human feces by using group-specific PCR primers and denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology*, *67*(6), 2578-2585.

Welman, A. D. and Maddox, I. S. (2003). Exopolysaccharides from lactic acid bacteria: perspectives and challenges. *Trends in Biotechnology*, *21*(6), 269-274.

Werning, M. L., Ibarburu, I., Duenas, M. T., Irastorza, A., Navas, J. and Lopez, P. (2006). *Pediococcus parvulus* gtf gene encoding the GTF glycosyltransferase and its application for specific PCR detection of β-D-glucan–producing bacteria in foods and beverages. *Journal of Food Protection*, *69*(1), 161-169.

Westerfeld, W. W. (1945). A colorimetric determination of blood acetoin. *Journal of Biological Chemistry*, *161*(2), 495-502.

Whittenbury, R. (1965). A study of some pediococci and their relationship to *Aerococcus viridans* and the enterococci. *Microbiology*, *40*(1), 97-106.

Wieme, A., Cleenwerck, I., Van Landschoot, A. and Vandamme, P. (2012). *Pediococcus lolii* DSM 19927T and JCM 15055T are strains of *Pediococcus acidilactici*. *International Journal of Systematic and Evolutionary Microbiology*, *62*(12), 3105-3108.

Yasutake, T., Kumagai, T., Inoue, A., Kobayashi, K., Noda, M., Orikawa, A., Matoba, Y. and Sugiyama, M. (2016). Characterization of the LP28 strain-specific exopolysaccharide biosynthetic gene cluster found in the whole circular genome of *Pediococcus pentosaceus*. *Biochemistry and Biophysics Reports*, *5*, 266-271.

Yuksekdag, Z. N. and Aslim, B. (2010). Assessment of potential probiotic-and starter properties of *Pediococcus* spp. isolated from Turkish-type fermented sausages (sucuk). *Journal of Microbiology and Biotechnology*, *20*(1), 161-168.

Zajsek, K., Gorsek, A. and Kolar, M. (2013). Cultivating conditions effects on kefiran production by the mixed culture of lactic acid bacteria imbedded within kefir grains. *Food Chemistry*, *139*(1), 970-977.

Zavaglia, A. G., Kociubinski, G., Perez, P. and de Antoni, G. (1998). Isolation and characterization of *Bifidobacterium* strains for probiotic formulation. *Journal of Food Protection*, *61*(7), 865-873.

Appendix

APPENDIX I

Composition of different Media and Buffers used in the study are given below. Sterilization of media and buffers were performed at 121ºC for 15 min. at 15 psi pressure**.**

Composition of Different Culture Media and Buffers

1. De Man Rogosa Sharpe Broth or MRS Broth

Ingredients	**Quantity (g/L)**
Peptone	10.0
Beef extract	10.0
Yeast extract	5.0
Dextrose	20.0
Dipotassium hydrogen phosphate	2.0
Sodium acetate	5.0
Ammonium citrate	2.0
Magnesium sulfate	0.1
Manganese sulfate	0.05
Tween 80	1.0
Distilled water	1000 mL
pH 6.5	

For preparation of readymade media supplied by HiMedia, powder was reconstituted in 900 mL of distilled water. pH of the medium was adjusted to 6.5 using 1N HCl and then volume was make up-to 1000 mL. Media was dispensed in tubes or as required and autoclaved. For preparation of MRS agar media, agar powder was added @ 1.5% in MRS broth.

2. Low Glucose Medium

Ingredients	**Quantity (g/L)**
Tryptone	10.0
Yeast extract	5.0
Glucose	0.5
Sodium chloride	5.0

Dipotassium hydrogen phosphate	2.0
Distilled water	1000 mL
Agar	15
pH 6.8	

3. Arginine Broth

Ingredients	**Quantity (g/L)**
Tryptone	5.0
Yeast extract	5.0
Dipotassium hydrogen phosphate	2.0
L- Arginine monohydrochloride	3.0
Dextrose	0.5
Distilled Water	1000 mL
pH 7.0	

4. Tryptone Glucose Extract Agar (**TGE**)

Ingredients	**Quantity (g/L)**
Casein enzymic hydrolysate	5.0
Yeast extract	3.0
Glucose	1.0
Distilled Water	1000 mL
Agar	15.0
pH 7.0	

5. CHL Broth

Ingredients	**Quantity (g/L)**
Peptone	10.0
Yeast extract	5.0
Dipotassium phosphate	2.0
Sodium acetate	5.0

Ammonium citrate	2.0
Magnesium sulfate	0.05
Manganese sulfate	0.12
Bromocresol purple	0.17
Tween 80	1.0
Distilled water	1000 mL
pH 6.5	
10% filter sterilized sugar added aseptically to the final concentration of 1%	

6. Citrate Broth

Ingredients	**Quantity (g/L)**
Tryptone	10.0
Yeast extract	5.0
Dextrose	10.0
Dipotassium phosphate	1.0
Tri-sodium citrate	20.0
Magnesium sulfate	1.0
Distilled water	1000 mL
pH 6.8	

7. Gelatin Agar Medium

Ingredients	**Quantity (g/L)**
Peptone	5.0
Yeast extract	3.0
Gelatin	30.0
Agar	17.0
Distilled water	1000 mL
pH 7.0	

8. Improved Decarboxylase Medium

Ingredients	Quantity (g/L)
Tryptone	5.0
Yeast extract	5.0
Meat extract	5.0
Sodium chloride	2.5
Glucose	0.5
Tween 80	1.0
Magnesium sulfate	0.2
Manganese sulfate	0.05
Ferrous sulfate	0.04
Ammonium citrate	2.0
Thiamine	0.01
Dipotassium phosphate	2.0
Calcium carbonate	0.1
Pyridoxal-5-phosphate	0.05
Bromocresol purple	0.06
Distilled water	1000 mL
pH 5.3	

9. Tyrosine Plate Media

Ingredients	Quantity (g/L)
Tryptone	5.0
Yeast extract	5.0
Meat extract	5.0
Sodium chloride	2.5
Glucose	0.5
Fructose	1.0
Tween 80	1.0
Magnesium sulfate	0.2

Manganese sulfate	0.05
Ferrous sulfate	0.04
Ammonium citrate	2.0
Thiamine	0.01
Dipotassium phosphate	2.0
Calcium carbonate	0.1
Pyridoxal-5-phosphate	0.05
Tyrosine	10.0
Agar	18.0
Distilled water	1000 mL
pH 5.3	

10. Phosphate Urea Magnesium Sulfate (PUM) Buffer

Ingredients	**Quantity (g/L)**
Dipotassium hydrogen phosphate	22.2
Potassium dihydrogen phosphate	7.26
Urea	1.8
Magnesium sulfate	0.2
Distilled water	1000 mL
pH 7.1	

11. Phosphate Buffer Saline (PBS)

Ingredients	**Quantity (g/L)**
Sodium chloride	8.00
Potassium dihydrogen phosphate	0.34
Dipotassium hydrogen phosphate	1.21
Distilled water	1000 mL
pH 6.0	

12. Glycerol Stock (30%)

Ingredients	Quantity (mL)
Glycerol	60.0
Distilled water	100

Glycerol stock was sterilized twice by autoclaving. Glycerol solution added in equal amount to the active culture in each cryovial at the time of preservation and stored at -20°C.

13. Normal Saline

Dissolved 0.85 g NaCl in 100 mL distilled water. Adjusted the pH to the solution 7.0. Autoclaved the solution at 121°C, 15 psi for 15 min.

APPENDIX II

1. Reagents for Isolation of Genomic DNA

1.1 SET Buffer

Sucrose	6.70 g	Final conc. 6.7%
Tris	0.606 g	50 mM
EDTA (Di-Sodium EDTA)	0.0372 g	1 mM
Deionized water	100 mL	

All the components were dissolved in 80 mL of water and pH was adjusted to 8.0 with 6N NaOH and volume was made to 100 mL. Buffer was autoclaved (121ºC/ 15 min.) after preparation.

1.2 SDS Solution (10%)

SDS	10 g
Deionized water	100 mL

Solution was autoclaved before use.

1.3 5M NaCl

Sodium chloride	29.22 g
Deionized water	100 mL

1.4 Chloroform:Isoamyl alcohol (24:1)

Isoamyl alcohol	4.0 mL
Chloroform	96.0 mL

1.5 TE Buffer

Tris	0.1212 g	Final conc. 10 mM
Di-Sodium EDTA	0.0372 g	Final conc. 1 mM
Deionized water	100 mL	

All the components were dissolved in 80 mL of water and pH was adjusted to 8.0 with 6N NaOH and volume was made to 100 mL. Buffer was autoclaved (121ºC/ 15 min) after preparation.

2. Reagents for Agarose Gel Electrophoresis of DNA

2.1 TAE Buffer (50X) (Stock Solution)

Tris (10 mM)	24.20 g
0.5 mM Di-Sodium EDTA (pH 8.0)	10 mL
Glacial Acetic Acid	5.71 mL
Deionized water	100 mL

The solution was autoclaved at 121ºC/ 15 min.

2.1.1 Working Solution (1X)

Working solution was prepared by diluting 1 mL of stock solution to 50 mL with distilled water.

2.2 Gel Loading Solution

Bromophenol Blue	0.05 g
Sucrose	40.00 g
Di-Sodium EDTA	3.72 g
SDS	0.50 g
Deionized water	100 mL

2.3 Staining Solution

2.3.1 Stock Solution (10 mg/mL)

Ethidium bromide (EtBr) was prepared as a stock solution of 10 mg/mL in water, stored at room temperature in screw cap tubes wrapped in aluminium foil.

2.3.2 Working Solution (0.5 µg/mL)

The working solution was prepared by adding 50 µL of stock solution to 1L of distilled water.

Appendix III

Sugar fermentation pattern of *Pediococcus* isolates by APi 50CH

	Sugar	*Pediococcus* isolates											
		PD-1	PD-2	PD-3	PD-4	PD-7	PD-8	PD-10	PD-13	PD-14	PD-16	PD-18	PD-19
0	CONTROL	+	+	+	+	+	+	+	+	+	+	+	+
1	GLYcerol	-	-	-	-	-	-	-	-	-	-	-	-
2	ERYthrytol	-	-	-	-	-	-	-	-	-	-	-	-
3	D ARAbinose	-	-	-	-	-	-	-	-	-	-	-	-
4	L ARAbinose	+	-	-	+	+	+	+	+	+	+	+	+
5	RIBose	+	+	+	+	+	+	+	+	+	+	+	+
6	D XYLose	-	-	-	-	+	-	-	+	+	+	+	+
7	L XYLose	-	-	-	-	-	-	-	-	-	-	-	-
8	ADOnitol	-	-	-	-	-	-	-	-	-	-	-	-
9	β-Methyl-D-Xyloside	-	-	-	-	-	-	-	-	-	-	-	-
10	GALactose	+	+	+	+	+	+	+	+	+	+	+	+
11	GLUcose	+	+	+	+	+	+	+	+	+	+	+	+
12	FRUctose	+	+	+	+	+	+	+	+	+	+	+	+
13	MaNnose	+	+	+	+	+	+	+	+	+	+	+	+
14	SorBose	-	-	-	-	-	-	-	-	-	-	-	-
15	RHAmnose	?	-	-	?	-	?	-	-	-	-	-	-
16	DULcitol	-	-	-	-	-	-	-	-	-	-	-	-
17	INOsitol	-	-	-	-	-	-	-	-	-	-	-	-
18	MANnitol	-	-	-	-	-	-	-	-	-	-	-	-
19	SORbitol	-	-	-	-	-	-	-	-	-	-	-	-
20	α-Methyl-D-Mannoside	-	-	-	-	-	-	-	-	-	-	-	-
21	α-Methyl-D-Glucoside	-	-	-	-	-	-	-	-	-	-	-	-
22	N-Acetyl-Glucosamine	+	+	+	+	+	+	+	+	+	+	+	+
23	AMY gdalin	?	+	+	+	?	+	+	?	+	+	?	+
24	ARButin	+	+	+	+	+	+	+	+	+	+	+	+

	Sugar	*Pediococcus* isolates											
		PD-1	PD-2	PD-3	PD-4	PD-7	PD-8	PD-10	PD-13	PD-14	PD-16	PD-18	PD-19
25	ESCulin	+	+	+	+	+	+	+	+	+	+	+	+
26	SALicin	?	+	+	+	+	+	+	?	+	+	+	+
27	CELlobiose	+	+	+	+	+	+	+	+	+	+	+	+
28	MALtose	+	+	+	+	+	+	+	+	+	+	+	+
29	LACtose	?	-	-	-	-	?	-	-	-	-	-	-
30	MELibiose	-	-	-	-	-	-	-	-	-	-	+	-
31	Sucrose	-	-	-	-	-	-	+	-	-	-	+	-
32	TREhalose	+	+	+	+	-	+	+	+	+	+	+	+
33	INUlin	?	-	-	-	?	+	+	-	-	-	-	-
34	MeLeZitose	-	-	-	-	-	-	-	-	-	-	-	-
35	RAFfinose	-	-	-	-	-	-	-	-	-	-	?	-
36	Starch	-	-	-	-	-	-	-	-	-	-	-	-
37	GLYcoGen	-	-	-	-	-	-	-	-	-	-	-	-
38	XYLiTol	-	-	-	-	-	-	-	-	-	-	-	-
39	GENtobiose	+	?	-	+	?	+	-	?	+	+	?	+
40	D TURanose	-	-	-	-	-	-	-	-	-	-	-	-
41	D LYXose	-	-	-	-	-	-	-	-	-	-	-	-
42	D TAGatose	+	+	+	+	+	+	+	-	-	-	-	?
43	D FUCose	-	-	-	-	-	-	-	-	-	-	-	-
44	L FUCose	-	-	-	-	-	-	-	-	-	-	-	-
45	D Arabitol	-	-	-	-	-	-	-	-	-	-	-	-
46	L ARabitol	-	-	-	-	-	-	-	-	-	-	-	-
47	GlucoNaTe	?	-	-	?	-	?	-	-	-	-	-	-
48	2-Keto-Gluconate	-	-	-	-	-	-	-	-	-	-	-	-
49	5-Keto-Gluconate	-	-	-	-	-	-	-	-	-	-	-	-

		***Pediococcus* isolates**											
	Sugar	**PD-20**	**PD-21**	**PD-22**	**PD -24**	**PD-25**	**PD-26**	**PD-27**	**PD-28**	**PD-29**	**PD-30**	**PD-31**	**PD-32**
0	CONTROL	+	+	+	+	+	+	+	+	+	+	+	+
1	GLYcerol	-	-	-	-	-	-	-	-	-	-	-	-
2	ERYthrytol	-	-	-	-	-	-	-	-	-	-	-	-
3	D ARAbinose	-	-	-	-	-	-	-	-	-	-	-	-
4	L ARAbinose	+	+	+	+	+	+	+	+	+	+	+	+
5	RIBose	+	+	+	+	+	+	+	+	+	+	+	+
6	D XYLose	+	+	+	+	+	+	+	+	+	+	+	+
7	L XYLose	-	-	-	-	-	-	-	-	-	-	-	-
8	ADOnitol	-	-	-	-	-	-	-	-	-	-	-	-
9	β-Methyl-D-Xyloside	-	-	-	-	-	-	-	-	-	-	-	-
10	GALactose	+	+	+	+	+	+	+	+	+	+	+	+
11	GLUcose	+	+	+	+	+	+	+	+	+	+	+	+
12	FRUctose	+	+	+	+	+	+	+	+	+	+	+	+
13	MaNnose	+	+	+	+	+	+	+	+	+	+	+	+
14	SorBose	-	-	-	-	-	-	-	-	-	-	-	-
15	RHAmnose	-	-	-	-	-	-	-	+	-	+	-	-
16	DULcitol	-	-	-	-	-	-	-	-	-	-	-	-
17	INOsitol	-	-	-	-	-	-	-	-	-	-	-	-
18	MANnitol	-	-	-	-	-	-	-	-	-	-	-	-
19	SORbitol	-	-	-	-	-	-	-	-	-	-	-	-
20	α-Methyl-D-Mannoside	-	-	-	-	-	-	-	-	-	-	-	-
21	α-Methyl-D-Glucoside	-	-	-	-	-	-	-	-	-	-	-	-
22	N-Acetyl-Glucosamine	+	+	+	+	+	+	+	+	+	+	+	+
23	AMY gdalin	+	+	+	+	+	+	+	+	+	+	+	+
24	ARButin	+	+	+	+	+	+	+	+	+	+	+	+

		***Pediococcus* isolates**											
	Sugar	**PD-20**	**PD-21**	**PD-22**	**PD -24**	**PD-25**	**PD-26**	**PD-27**	**PD-28**	**PD-29**	**PD-30**	**PD-31**	**PD-32**
25	ESCulin	+	+	+	+	+	+	+	+	+	+	+	+
26	SALicin	+	+	+	+	+	+	+	+	+	+	+	+
27	CELlobiose	+	+	+	+	+	+	+	+	+	+	+	+
28	MALtose	+	+	+	+	+	+	+	+	+	+	+	+
29	LACtose	-	-	-	-	-	-	-	-	-	-	-	-
30	MELibiose	-	+	-	-	-	-	-	-	-	-	-	-
31	Sucrose	-	+	-	-	-	-	-	-	-	-	-	-
32	TREhalose	+	+	+	+	+	+	+	+	+	+	+	+
33	INUlin	-	-	-	-	-	-	-	-	-	-	-	-
34	MeLeZitose	-	-	-	-	-	-	-	-	-	-	-	-
35	RAFfinose	-	+	-	-	-	-	-	-	-	-	-	-
36	Starch	-	-	-	-	-	-	-	-	-	-	-	-
37	GLYcoGen	-	-	-	-	-	-	-	-	-	-	-	-
38	XYLiTol	-	-	-	-	-	-	-	-	-	-	-	-
39	GENtobiose	?	?	+	?	-	?	?	-	+	-	+	+
40	D TURanose	-	-	-	-	-	-	-	-	-	-	-	-
41	D LYXose	-	-	-	-	-	-	-	-	-	-	-	-
42	D TAGatose	-	-	-	-	-	-	-	+	-	+	-	-
43	D FUCose	-	-	-	-	-	-	-	-	-	-	-	-
44	L FUCose	-	-	-	-	-	-	-	-	-	-	-	-
45	D Arabitol	-	-	-	-	-	-	-	-	-	-	-	-
46	L ARabitol	-	-	-	-	-	-	-	-	-	-	-	-
47	GlucoNaTe	-	-	-	-	-	-	-	-	-	-	-	-
48	2-Keto-Gluconate	-	-	-	-	-	-	-	-	-	-	-	-
49	5-Keto-Gluconate	-	-	-	-	-	-	-	-	-	-	-	-

		***Pediococcus* isolates**											
	Sugar	**PD-33**	**PD-34**	**PD-35**	**PD-36**	**PD-37**	**PD-38**	**PD-39**	**PD-41**	**PD-44**	**PD-45**	**8-PD**	**14-PD**
0	CONTROL	+	+	+	+	+	+	+	+	+	+	+	+
1	GLYcerol	-	-	-	-	-	-	-	-	-	-	-	-
2	ERYthrytol	-	-	-	-	-	-	-	-	-	-	-	-
3	D ARAbinose	-	-	-	-	-	-	-	-	-	-	-	-
4	L ARAbinose	+	+	+	+	+	+	+	+	+	+	+	+
5	RIBose	+	+	+	+	+	+	+	+	+	+	+	+
6	D XYLose	+	+	+	+	+	+	+	+	+	+	-	+
7	L XYLose	-	-	-	-	-	-	-	-	-	-	-	-
8	ADOnitol	-	-	-	-	-	-	-	-	-	-	-	-
9	β-Methyl-D-Xyloside	-	-	-	-	-	-	-	-	-	-	-	-
10	GALactose	+	+	+	+	+	+	+	+	+	+	+	+
11	GLUcose	+	+	+	+	+	+	+	+	+	+	+	+
12	FRUctose	+	+	+	+	+	+	+	+	+	+	+	+
13	MaNnose	+	+	+	+	+	+	+	+	+	+	+	+
14	SorBose	-	-	-	-	-	-	-	-	-	-	-	-
15	RHAmnose	-	-	-	-	-	-	-	-	-	-	-	-
16	DULcitol	-	-	-	-	-	-	-	-	-	-	-	-
17	INOsitol	-	-	-	-	-	-	-	-	-	-	-	-
18	MANnitol	-	-	-	-	-	-	-	-	-	-	-	-
19	SORbitol	-	-	-	-	-	-	-	-	-	-	-	-
20	α-Methyl-D-Mannoside	-	-	-	-	-	-	-	-	-	-	-	-
21	α-Methyl-D-Glucoside	-	-	-	-	-	-	-	-	-	-	-	-
22	N-Acetyl-Glucosamine	-	+	+	+	+	+	+	+	+	-	+	+
23	AMY gdalin	-	+	?	+	+	?	+	+	+	-	+	+
24	ARButin	+	+	+	+	+	+	+	+	+	-	+	+

		***Pediococcus* isolates**											
	Sugar	**PD-33**	**PD-34**	**PD-35**	**PD-36**	**PD-37**	**PD-38**	**PD-39**	**PD-41**	**PD-44**	**PD-45**	**8-PD**	**14-PD**
25	ESCulin	+	+	+	+	+	+	+	+	+	+	+	+
26	SALicin	+	+	+	+	+	?	+	+	+	-	+	+
27	CELlobiose	+	+	+	+	+	+	+	+	+	+	+	+
28	MALtose	+	+	+	+	+	+	+	+	+	-	+	+
29	LACtose	-	-	-	-	-	-	-	-	-	-	-	-
30	MELibiose	-	-	-	-	+	-	-	-	+	-	-	-
31	Sucrose	-	-	-	-	+	-	-	-	+	-	-	-
32	TREhalose	+	+	+	+	+	+	+	+	+	+	+	+
33	INUlin	-	-	-	-	-	-	-	-	-	-	+	-
34	MeLeZitose	-	-	-	-	-	-	-	-	-	-	-	-
35	RAFfinose	-	-	-	-	+	-	-	-	-	-	-	-
36	Starch	-	-	-	-	-	-	-	-	-	-	-	-
37	GLYcoGen	-	-	-	-	-	-	-	-	-	-	-	-
38	XYLiTol	-	-	-	-	-	-	-	-	-	-	-	-
39	GENtobiose	-	-	+	-	?	-	+	-	-	-	+	+
40	D TURanose	-	-	-	-	-	-	-	-	-	-	-	-
41	D LYXose	-	-	-	-	-	-	-	-	-	-	-	-
42	D TAGatose	+	+	-	-	-	-	-	-	+	+	+	-
43	D FUCose	-	-	-	-	-	-	-	-	-	-	-	-
44	L FUCose	-	-	-	-	-	-	-	-	-	-	-	-
45	D Arabitol	-	-	-	-	-	-	-	-	-	-	-	-
46	L ARabitol	-	-	-	-	-	-	-	-	-	-	-	-
47	GlucoNaTe	-	-	-	-	-	-	-	-	-	-	-	-
48	2-Keto-Gluconate	-	-	-	-	-	-	-	-	-	-	-	-
49	5-Keto-Gluconate	-	-	-	-	-	-	-	-	-	-	-	-

		***Pediococcus* isolates**						
	Sugar	**25-PD**	**42-PD**	**Sil-1**	**Sil-2**	***P. pentosaceus* NCDC 38**	***P. pentosaceus* NCDC 273**	***P. acidilactici* NCDC 609**
0	CONTROL	+	+	+	+	+	+	+
1	GLYcerol	-	-	-	-	-	-	-
2	ERYthrytol	-	-	-	-	-	-	-
3	D ARAbinose	-	-	-	-	-	-	-
4	L ARAbinose	+	+	+	+	+	+	+
5	RIBose	+	+	+	+	+	+	+
6	D XYLose	+	+	+	+	+	+	+
7	L XYLose	-	-	-	-	-	-	-
8	ADOnitol	-	-	-	-	-	-	-
9	β-Methyl-D-Xyloside	-	-	-	-	-	-	-
10	GALactose	+	+	+	+	+	+	+
11	GLUcose	+	+	+	+	+	+	+
12	FRUctose	+	+	+	+	+	+	+
13	MaNnose	+	+	+	+	+	+	+
14	SorBose	-	-	-	-	-	-	-
15	RHAmnose	?	?	-	-	-	-	-
16	DULcitol	-	-	-	-	-	-	-
17	INOsitol	-	-	-	-	-	-	-
18	MANnitol	-	-	-	-	-	-	-
19	SORbitol	-	-	-	-	-	-	-
20	α-Methyl-D-Mannoside	-	-	-	-	-	-	-
21	α-Methyl-D-Glucoside	-	-	-	-	-	-	-
22	N-Acetyl-Glucosamine	+	+	+	+	+	+	+
23	AMY gdalin	+	+	+	+	+	+	-
24	ARButin	+	+	+	+	+	+	-

		***Pediococcus* isolates**						
	Sugar	**25-PD**	**42-PD**	**Sil-1**	**Sil-2**	***P. pentosaceus* NCDC 38**	***P. pentosaceus* NCDC 273**	***P. acidilactici* NCDC 609**
25	ESCulin	+	+	+	+	+	+	+
26	SALicin	+	+	+	?	+	+	-
27	CELlobiose	+	+	+	+	+	+	+
28	MALtose	+	+	+	+	+	+	-
29	LACtose	?	?	?	-	-	-	+
30	MELibiose	-	-	-	+	+	-	-
31	Sucrose	-	-	+	+	+	-	-
32	TREhalose	+	+	+	+	+	-	+
33	INUlin	-	-	-	-	-	-	-
34	MeLeZitose	-	-	-	-	-	-	-
35	RAFfinose	-	-	-	-	+	-	-
36	Starch	-	-	-	-	-	-	-
37	GLYcoGen	-	-	-	-	-	-	-
38	XYLiTol	-	-	-	-	-	-	-
39	GENtobiose	+	?	?	-	-	+	+
40	D TURanose	-	-	-	-	-	-	-
41	D LYXose	-	-	-	-	-	-	-
42	D TAGatose	+	-	+	+	+	+	+
43	D FUCose	-	-	-	-	-	-	-
44	L FUCose	-	-	-	-	-	-	-
45	D Arabitol	-	-	-	-	-	-	-
46	L ARabitol	-	-	-	-	-	-	-
47	GlucoNaTe	?	-	-	-	-	-	-
48	2-Keto-Gluconate	-	-	-	-	-	-	-
49	5-Keto-Gluconate	-	-	-	-	-	-	-

Curriculum Vitae

VARSHA



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Chopra Chowk, Narwana-126116

Distt. Jind, State- Haryana, India

Contact No. +91-8901242126

E-mail: varshagarg9@gmail.com

Personal Profile:

Name	:	Varsha
Husband's Name	:	Dr. Ankit Singla
Date of Birth	:	20-05-1987
Permanent Address	:	House No. 103, Ward No. 14, Chopra Chowk, Narwana- 126116
		Distt. Jind, State- Haryana, India
Gender	:	Female
Marital Status	:	Married
Nationality	:	Indian
Languages known	:	English, Hindi
Contact No.	:	+91-8901242126

Career Objective:

Seeking a platform to utilize my skills that offer professional and personal growth and prove myself as a valuable asset for the organization.

Educational Qualification:

Degree	***Area of Specialization***	***Name of university/ Board***	***Passing Year***	***OGPA/ Percentage***
PhD	Dairy Microbiology	National Dairy Research Institute, Karnal, India	Pursuing	8.10/10.00 (equivalent to 81 %)
M.Sc.	Microbiology	CCS Haryana Agricultural University, Hisar, India	2011	7.81/10.00 (equivalent to 78.10 %)
B.Sc.	Biotechnology	Kurukshetra University, Kurukshetra, India	2008	79.65 %
Senior Secondary	Medical	B.S.E.H., India	2005	65.00 %
Matriculation	General	B.S.E.H., India	2003	89.50 %

- **Qualified ICAR-NET (2014-II) in Agricultural Microbiology**

PhD thesis title: "Functional characterization of *Pediococcus* spp. for their application in dairy foods"

Advisor: Dr. Surajit Mandal

M.Sc. thesis title: "Isolation, Characterization and Molecular Diversity of Berseem (*Trifolium alexandrinum* L.) rhizobia from Haryana soils"

Advisor: Prof. Kamlesh Kukreja

Area of Specialization:

Microbiology, Biotechnology

Publications:

Original articles:

1. **Varsha Garg**, Kamlesh Kukreja and Ankit Singla. 2016. Efficacy of native rhizobial strains for nodulation and plant biomass production in berseem (*Trifolium alexandrinum* L). *AgricINTERNATIONAL*. 3(1-2): 89-95.
2. **Varsha Garg,** Kamlesh Kukreja, Rajesh Gera and Ankit Singla. 2015. Production of indole-3-acetic acid by Berseem (*Trifolium alexandrinum* L.) rhizobia isolated from Haryana, India. *Agricultural Science Digest.* 35(3): 229-232.
3. **Varsha Garg,** Kamlesh Kukreja and Rajesh Gera. 2016. Molecular Diversity of Berseem (*Trifolium alexandrinum* L.) rhizobia isolated from Haryana soil. *Legume Research.* 39(5): 729-733.

Review articles:

4. **Varsha Singla,** Surajit Mandal, Santosh Anand and Sudhir Kumar Tomar. 2017. Techno-functional attributes of *Pediococcus* spp.: A Review. *Indian Journal of Dairy Science*. 70(4): 381-390.
5. Prasad Patil, Akanksha Wadehra, **Varsha Garg**, Kanchan Munjal, Sudhir Kumar Tomar and Surajit Mandal. 2015. Biofunctional properties of milk protein derived bioactive peptides-A review. *Asian Journal of Dairy & Food Research.* 34(4): 253-258.

Book Chapters:

1. Ankit Singla, **Varsha Singla** and Kazuyuki Inubushi. 2016. Agricultural waste to biochar: a new eco-friendly approach in Indian Scenario. In: Trends in Food Technology (accepted).

Techniques Known:

Spectrophotometer, PCR, HPLC, Microscopy, Basic microbiological techniques (Staining, Plating, Most probable number count), Gel Documentation system.

Declaration:

I hereby declare that the above facts given by me are true to the best of my knowledge and belief.

Date: 4th Dec, 2017
Place: Karnal **(VARSHA)**