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Isolation and characterization of Lactic acid Bacteria from fermented food product and milk product

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ABSTRACT

Conventional dairy products with health benefits such as improved nutrient absorption, toxin inactivation, and antipathogenic behaviours are used all over the world. Probiotic bacteria have been shown to improve the health of the human gastrointestinal tract. Lactic acid bacteria (LAB) are candidate probiotic bacteria that are widely distributed in nature and can be used in the food industry. This study is aimed at isolating, identifying, and in vitro characterizing LAB strains from traditional dairy products and fermented food products. Eleven suspected LAB were isolated from eight different types of raw and fermented milk products. The identification eight isolates using MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-Time of Flight). Three isolates were *Lactobacillus plantarum* (two from curd samples and one from Idli batter), two isolates were *Lactobacillus fermentum* (from curd and butter), one isolate was *Weissellaconfusa*(from Idli batter), one isolate was *Weissellaparamesenteroide* (from Sauerkraut), one isolate was *Sacchromycescerevisiae (from Curd)*. Probiotic characteristics like tolerance to acidic pH, resistance to bile were tested to establish their potential as probiotic.

Keywords: Probiotic, MALDI-TOF, Bile tolerance, acidic pH tolerance.

1.INTRODUCTION

Probiotic bacteria can confer health benefits to the human gastrointestinal tract[1-3]. Lactic acid bacteria (LAB) are candidate probiotic bacteria[4] that are widely distributed in nature and can be used in the food industry[5]. LAB, a group of microaerophilic or anaerobic Gram-positive bacteria that are unable to form spores or produce catalase, are characterized by the absence of the cytochrome system[5,6] and the ability to produce antimicrobials for bio preservation[7,8]. Certain foods, including dairy products, for example, yogurt, are considered good sources of probiotics[9,10]. The majority of microbiota in raw milk and fermented milk products include the genera *Lactobacillus*, *Enterococcus*,*Lactococcus*, *Leuconostoc*, *Pediococcus*, *Oenococcus*, *Carnobacterium*, *Streptococcus*, and *Weissella*[11] .The most obvious benefits of LAB fermentation include increased food palatability and improved shelf life[12]. LAB are generally recognized as safe (GRAS) because they are able to produce bacteriocins and their consumption confers several health benefits, such as controlling intestinal infections, improving lactose utilization, lowering blood ammonia levels, providing efficient resistance against gastric acid and bile[13,14], influencing the immune system, and lowering serum cholesterol levels[15]. LAB also adhere to the gastrointestinal tract and confer pathogen inhibition[16,17]. Interestingly, the presence of LAB resulted in no change or small changes in the abundance of other intestinal microbial groups[18].

The safety and functionality of probiotics are species and strain dependent and manufactures should clearly characterize the species and the concentrations of the

bacteria presented in their products. MALDI-TOF MS could be a useful tool for bacteria identification[19]

The isolation, identification, and characterization of novel LAB strains have two benefits. The first is to reveal the characteristic taxonomy of the LAB and the second is to obtain promising beneficial and functional probiotic LAB. There is a need for research regarding the isolation and characterization of LAB from dairy products. Therefore, this study is aimed at isolating, identifying, and in vitro characterizing LAB strains from traditional dairy products and fermented food products. These LAB need to be evaluated for their functional traits and probiotic properties. Ability of the LAB to inhibit the growth of pathogenic and food poisoning bacteria also requires further exploration.

2.MATERIALS AND METHODS

Sample collection:

A total of 10 non-commercial and commercial fermented product samples were collected and were kept in a refrigerator at 4 °C.Samples includedMeduVadabatter, Buffalo milk curd, two different Idli batter, five different Curd sample, Buttermilk sample.

Bacterial / yeast isolation:

One ml of the samples was added to 24 ml of Rogosa broth and incubated under microaerophilic conditions. Aloopful of the broth was then spread on Rogosa agar plates and incubated for 48 hours to obtain isolated colonies. The single colonies on the growth agar plate were selected and transferred to 15 ml of broth culture medium and incubated for 24 hours at 37°C. The isolates were stored in 30% (w/v) glycerol.

Primary Characterization of LAB Strains

Preliminary identification of the LAB isolates was based on their phenotypic and biochemical characteristics that included Gram's reaction and catalase test[20].

Identification of LAB.

The cultures were identified by MALDI-TOF as described by Angelakis et al [19].

Evaluation of Probiotic Properties

Tolerance to Acids

The tolerance of the LAB isolates to both acidic pH value and bile salts was studied using the method described byHassanzadazar*et al* (). Rogosabroth were inoculated with overnight grown culture. The culture was then inoculated in Rogosa broth with pH adjusted to 6.5,5.5, 4.5, 3.5 and 2.5using 1N hydrochloric acid (HCl).The cultures were incubated under microaerophilic conditions at 37°C for 24hrs,thenloopful culture streaked on Rogosa agar to check the growth of the culture.

Tolerance Bile Salts

The Rogosa broth adjusted to an initial pH of 6.5 was considered as the control. MRS medium supplemented with 0.3% oxgall was used to test tolerance to bile salt. Rogosa broth without oxgall was considered as the control. The cultures were incubated under microaerophilic conditions at 37°C for time intervals 0, 2, and 4 h and a loopful was streaked on Rogosa agar to check the growth of the culture.

3.RESULTS AND DISCUSSION

Microorganisms have vital role in food preservation and human health. These bacteria can be raised for the production of various kinds of food and pharmaceutical products. They can also be used for the production of new functional foods. Therefore, increasing use of dairy products containing probiotics, identification and production of foods containing highest and most effective lactobacilli are recommended in daily diet.

Bacterial / yeast isolation:

We obtainedten isolates from different food / milk products[Table 1]. Two isolates were from idli batter, five from traditional curd samples, one from sauerkraut, one from meduvada batter and one from Buttermilk sample.

Table 1: Preliminary Characterization of LAB Strains.

Isolate number	Gram Staining	Catalase Test	Source
I1A	+ve short rod	-ve	**Idli batter**
I1B	+ve rod	-ve	**Idli batter**
C3	+ve rod	-ve	**Curd**
C4-1	+ve rod	-ve	**Curd**
C4-2	+ve rod	-ve	**Curd**
C5	+vecocci	-ve	**Curd**
C6	+ve	+ve	**Curd**
BTM-1	+ve rod	-ve	**Buttermilk**
S-1	+ve rod	-ve	**Sauerkraut**
MVB-1	+ve rod	-ve	**Meduvada batter**

(note:- +ve- positive; -ve- Negative)

Identification of isolates using MALDI-TOF

Identification of isolates was done using MALDI-TOF(Table.2).Three isolates were*Lactobacillus plantarum* (two from curd samples and one from Idli batter), two isolates were *Lactobacillus fermentum* (from curd and buttermilk), one isolate was *Weissellaconfusa*(from Idli batter), one isolate was *Weissellaparamesenteroide* (from Saurkraut), one isolate was *Sacchromycescerevisiae (from Curd)*.Isolate from Meduvada batter could not be identified.

During Idli batter fermentation given organisms are present. *Leuconostoclactis* strains came from in 6 and 9 h fermentation. *Pediococcuspentosaceus* strains were isolated from 6 h fermentation, *Lactococcuslactis* and *Micrococcus luteus* came from 12 h fermented samples, and *Weissellacibaria* came from 6 to 9 h of fermentation of idli batter. *Weissellaconfusa* and Bacillus subtilis strains were from throughout the fermentation. *Bacillus tequilensis* isolates came from 0, 3, 6, to 12 h of fermentation. *Bacillus amyloliquefaciens* present came from 0 to 12 hrs.during fermentation. *Bacillus cereus* and *Enterobacter cloacae* represented 6 h fermentation.The predominant microflora in the idli batter fermentation was *Leuconostocmesenteroides, Streptococcus, Pediococcuscerevisiae, Streptococcus faecalis* [21].We obtained two isolates from Idli batter that were identified as *Weisellaconfusa* and *Lactobacillus plantarum*.

Sauerkraut fermentation begins with the initial proliferation of *Leuconostocmesenteroides*, which rapidly produces carbon dioxide and acid. This quickly lowers the environmental pH, inhibiting the growth of undesirable microorganisms that might cause food spoilage while preserving the color of the cabbage. The action of *L. mesenteroides* changes the fermentation environment so that it favors the succession of other LAB, such as *Lactobacillus brevis* and *Lactobacillus plantarum*. In traditional Sauerkraut production, this process proceeds at 18 °C for roughly one month. Historically, the important species in Sauerkraut fermentation were considered to be *L. mesenteroides, L. plantarum, and L. brevis*, which is supported by recent studies. In the event of abnormally high heat or salinity, *Enterococcus faecalis* and *Pediococcuscerevisiae* are thought to play a role in the fermentation process. However, these observations were drawn from studies that used culture-based techniques to isolate bacteria, which are inherently biased due to their inability to capture the range of non-culturable bacteria. Recent studies have also identified the genus *Weissella* as important to early fermentative processes [22]. We obtained one isolate from Sauerkraut that was identified as*Weissellaparamesenteroide*.

Curd (fermented milk), freshly made by inoculation with starter curd, is a good source of bioavailable vitamin, minerals and folate, and has less lactose and galactose than milk. Consumption of curd may enhance the immune response in the elderly [23].Out of the ten isolates we could identify 8 isolates using MALDI-TOF [Table 2]. One of the isolate from curd was an yeast

Saccharomyces cereviciae. Two more isolates from curd were identified as *Lactobacillus fermentum* and *Lactobacillus plantarum*.

Isolate from butter milk was *Lactobacillus fermentum* whereas isolate from Meduvada batter could not be identified. We are performing 16S rRNA PCR for the identification of the isolates using sequencing of the amplicon obtained [Data not shown].

Table 2: Identification of isolates using MALDI-TOF

I1A	***Weissellaconfusa***
I1B	***Lactobacillus plantarum***
C3	***Lactobacillus Fermentum***
C4-1	***Not yet indentified***
C4-2	***Lactobacillus plantarum***
C5	***Lactobacillus plantarum***
C6	***Saccharomyces cereviciae***
BTM(Buttermilk sample)	***Lactobacillus fermentum***
S-1	***Weissellaparamesenteroide***
MVB-1	**Not yet indentified**

Curd (fermented milk), freshly made by inoculation with starter curd, is a good source of bioavailable vitamin, minerals and folate, and has less lactose and galactose than milk. Consumption of curd may enhance the immune response in the elderly [23].Out of the ten isolates we could identify 8 isolates using MALDI-TOF [Table 2]. One of the isolate from curd was an yeast *Saccharomyces cereviciae*. Two more isolates from curdwere identified as *Lactobacillus fermentum* and *Lactobacillus plantarum*.

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Evaluation of Probiotic Properties

Tolerance to Acids and Bile Salts:

Tolerance to Acids

Isolated *Lactobacillus* were taken from 24 hours cultured MRS broth (exponential growth phase) and directly subjected to each of the two stress factors such as low pH, bile salts.

The results of our study show that isolate I1Ai.e*Weissellaconfusa*survive at pH 6.5 buton decrease in pH the organism was unable to tolerate the acidic pH. This needs to be confirmed further as the isolate was from a fermented food product.*Lactobacillus plantarum, Lactobacillus fermentum, Saccharomyces cereviciae,Weissellaparamesenteroide* ,MVB-1 are able to tolerate the pH up to 2.5 [Table: 3]

Isolate	**Tolerance to acidic pH**				
	pH 6.5	**pH 5.5**	**pH 4.5**	**pH 3.5**	**pH 2.5**
I1A	+ve	-ve	-ve	-ve	-ve
I1B	+ve	+ve	+ve	+ve	+ve
C3	+ve	+ve	+ve	+ve	+ve
C6	+ve	+ve	+ve	+ve	+ve
S-1	+ve	+ve	+ve	+ve	+ve
MVB-1	+ve	+ve	+ve	+ve	+ve

Table 3:Tolerance to acidic pH

[note:- +ve- growth; -ve- no growth]

Tolerance to Bile Salts:

All of the 4 LAB isolates (I1B, C6, S-1, MVB-1) i.e*Lactobacillus plantarum, Saccharomyces cereviciae,Weissellaparamesenteroide*andMVB-1(not yet identified)are able to survive in the presence of 0.3% of bile salt at 3 hrs of intervals whereas Isolate I1A and i.e*Weissellaconfusa*was unable to tolerant bile stress [Table:4]. In case of *Lactobacillus plantarum.*we need to retest its ability to tolerate exposure to bile salts as it has been reported earlier that it can sustain 0.3 % [20].

Table:4Tolerance to Bile Salts

Isolate	Tolerance to bile salt			
	0hr	1hr	2hrs	3hr
I1A	-ve	-ve	-ve	-ve
I1B	+ve	+ve	+ve	+ve
C3	-ve	-ve	-ve	-ve
C6	+ve	+ve	+ve	+ve
S-1	+ve	+ve	+ve	+ve
MVB-1	+ve	+ve	+ve	+ve

(note:- +ve- growth; -ve- no growth)

4.CONCLUSION

The present study,three isolates were identified as*Lactobacillus plantarum* (two from curd samples and one from Idli batter), two isolates were *Lactobacillus fermentum* (one each from curd and buttermilk), one isolate was *Weissellaconfusa*(from Idli batter), one isolate was *Weissellaparamesenteroide* (from Saurkraut), one isolate was *Sacchromycescerevisiae (from Curd)*.Probiotic characteristics like tolerance to acidic pH, resistance to bile were tested to establish their probioticcharacheristic. Potential of the organisms to be an effective probiotic needs to be tested further.

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