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Molecular Identification and Characterization of Lactic Acid producing Bacterial Strains Isolated from Raw and Traditionally Processed Foods of Punjab, Pakistan

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SA conducted the experimental trials and wrote the manuscript. UH and NA helped in interpretation of results and finalizing the manuscript.

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D-Lactic acid, *Enterococcus, Lactobacillus, Leuconostoc, Pediococcus,* Sugars fermentation.

ABSTRACT

Seventeen (17) wild strains of lactic acid producing bacteria (LAB) were screened, out of one hundred and forty-four (144) bacterial strains isolated from different local food groups, on the basis of stress tolerance assays. Identification of the screened strains was further confirmed by 16S ribosomal DNA sequences and strain level differentiation was carried out by sugar fermentation tests. These strains were further analyzed for their ability to produce lactic acid. Overall, *Leuconostoc mesenteroides* was found as pre-dominant group (41.1% prevalence) among LAB strains in the isolated food samples. Some opportunistic pathogens were also isolated from these media. Among the tested strains maximum amount of lactic acid (26.457 mg/mL) was produced by the lactobacilli after 48 h growth in skim milk broth, while the least (12.131 mg/mL) was produced by pediococci. These LAB strains are being studied for their probiotic properties and good quality indigenous starter cultures from them are anticipated to be employed in food industry.

INTRODUCTION

Lactic acid producing bacteria (LAB) have always been a topic of interest for the researchers in the field of food science. They have a long history of being employed in various food fermentations as starter cultures. Various LAB strains can withstand harsh environmental conditions such as varying temperatures, pH and salt concentrations (Lee and Salminen, 2009; Rubio *et al*., 2014). Lactic acid produced by LAB helps in lowering the pH of foods and acts as antimicrobial agent against different pathogens and food poisoning microorganisms. These technological and biochemical properties enable LAB strains to survive under the gastrointestinal (GI) tract (Kocková *et al*., 2011; Tachedjian *et al*., 2017). Beneficial microorganisms that can thrive in the GI tract and exert positive health impacts on the host are technically known as probiotics and LAB are still known the best probiotic agents (Lee and Salminen, 2009; Scalfaro *et al*., 2017; Iqbal *et al*., 2018).

Well characterized probiotic LAB genera include *Lactobacillus, Leuconostoc, Enterococcus, Streptococcus, Pediococcus,* and *Lactococcus* (Merenstein and Salminen, 2017; Roskar *et al*., 2017). It is also a common concept that *Lactobacillus* is the predominant LAB group found in the fermented foods especially in dairy products and pickles (Stiles and Holzapfel, 1997; Heilig *et al*., 2002), but some recent studies showed the predominance of *Leuconostoc* group in the Korean fermented foods (Kaur *et al*., 2017; Sharma *et al*., 2018).

Studies on LAB strains from the indigenous foods of Pakistan with regard to probiotic characterization are limited. Ahmed and coworkers (2002) reported some strains of *Lactobacillus* and *Streptococcus* isolated from the camel milk. Later they employed these strains as starter culture in cheese manufacturing (Ahmad *et al*., 2002; Ahmed and Kanwal, 2004). Aslam and Qazi (2010) isolated some LAB from locally processed yogurt samples and analyzed their antimicrobial potential against fungal and bacterial pathogens. Riaz *et al*. (2010) isolated and characterized *Lactobacillus fermentum* and *Lactobacillus acidophillus* from fecal samples of birds and humans. Naeem *et al*. (2012) also isolated some *Lactobacillus* strains and studied their antibiotic properties. Isolation and identification of bacterial strains in above studies were based on cultivation on MRS/M-17 media and some

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of biochemical tests but lacked validated methods of identification.

16S ribosomal DNA typing or API identification systems are considered validated methods for bacterial identification but it is well understood that 16S ribosomal DNA typing is more superior (reliable) to any biochemical tests even API systems (Bosshard *et al*., 2004, 2006). A few studies from Pakistan reported the validated methods of LAB identification. Nawaz *et al*. (2011) reported lactobacilli species isolated from the fecal samples of breast-fed kids in Pakistan and studied some allergic responses induced by their screened isolates. Mahmood *et al*. (2013, 2014) isolated some strains of *Streptococcus thermophilus* and *Lactobacillus acidophilus* from yogurt and studied their antimicrobial and bacteriocin producing potential. Yousaf *et al*. (2016) isolated a strain of *Lactobacillus fermentum* and assessed its anti-diabetic potential in rat model. Asghar *et al*. (2016) and Rajoka *et al*. (2018) isolated strains of lactobacilli from poultry origin and assessed some of their probiotic potential. In these studies, isolations of LAB were done only from yogurt or non-food sources and *Lactobacillus* was reported to be the most prevalent LAB group. We could not find any report presenting the *Leuconostoc* or pediococci from the indigenous foods of Pakistan. Data on LAB strains from indigenous food environment (other than yogurt) of Pakistan is deficient especially in terms of probiotic potential. Any strain of Pakistani origin is not available commercially as starter culture or probiotic agent.

In search of potentially probiotic bacterial strains the current study was carried out to isolate and identify the wild strains of LAB from diversified local food groups (pickles, raw milks, yogurt, homemade cheese, sourdoughs *etc*.). Here we report the molecular identification, technological aspects and lactic acid production potential of our isolates.

MATERIALS AND METHODS

Reagents and chemicals

All the culture media, reagents and chemicals used in current study were of high purity and were purchased from Sigma Aldrich (Taufkirchen, Germany), Merck Millipore (Frankfurt, Germany), Difco (Detroit, USA) and Hi-Media (Mumbai, India). Kits for total genomic DNA extraction and PCR product purification were procured from GeneAll Biotechnology (Incheon, Korea). 2X Green Dye Mix PCR master mix and sugars for sugar fermentation tests were obtained from Merck Millipore (Frankfurt, Germany). Purified standard of D-Lactic acid was purchased from Tokyo Chemical Industries (TCI), Japan.

Isolation and screening of lactic acid bacteria

Thirty-two samples of traditionally processed and raw foods including homemade sweets and sour pickles, raw milks (from cow, buffalo and camel), locally processed yogurt, homemade cheese, sweetened milk (being sold in local markets) and sourdoughs (corn, rice and wheat) were collected from urban and rural areas of Lahore, Sheikhupura and Sargodha districts. Food sampling was done using prescribed methods of Lightfoot and Maier (1998). Food samples were collected from various locations. Samples from fermented foods were collected at the mature stage of fermentation to obtain the maximum number of live microflora. Optimal maturity of fermentation was decided on the basis of known shelf lives of various foods and was kept in care while sampling for any particular food. The samples were transported in the presence of ice and kept at 4°C until further analyses. Three replicates for each sample were processed. Each food sample (1g or 1mL) was used for inoculum preparation in 10 mL of broth media (MRS and M-17) and incubated anaerobically at 35°C for 24 h. Inoculum (100 µL) was spread on respective agar plates *i.e.,* on MRS and M-17 (Dallal *et al*., 2017; Downes and Ito, 2001). Bacterial strains were purified on the basis of Gram's staining, catalase, oxidase, motility and methyl red tests. Gram positive, non-motile, catalase and oxidase negative strains were subjected to screening through growth at varying temperature ranges (25, 30, 35, 40, 44°C), in the presence of various NaCl concentrations (0, 2, 4, 6, 8, 18%), growth at different pH (4, 5.5, 7, 8) and growth in the presence of methylene blue (0.1% and 0.3%). Strains showing viability at 25 to 40°C, 4% NaCl, and pH 4.0 to 8.0 were selected for further analyses (Ahmed and Kanwal, 2004; Dallal *et al*., 2017). All the experiments were performed in triplicates.

16s rDNA sequence analysis

Molecular identification of the selected strains was done by extraction of total genomic DNA using GeneAll Exgene™ genomic DNA purification kit and DNA was confirmed using 0.8% agarose gel electrophoresis (Green and Sambrook, 2012). Polymerase chain reaction (PCR) of each strain was carried out using 27f (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492r (5′-TACGGYTACCTTGTTACGACTT-3′) primers suggested by Frank *et al*. (2008). The optimized PCR conditions are: 50µl PCR cocktail was denatured at 94°C for 6 min followed by 30 cycles, each of denaturation at 94°C for 1 min, annealing at 55°C for 1 min. Primer elongation at 72°C for 1 min and extension at 72°C for 10 min. PCR products were purified using GeneAll Expin™ kit gel DNA purification kit. Purified PCR products were sequenced from Macrogen, Korea (commercial labs). Sequences were assembled using SeqMan tool of DNAStar, BLASTn analysis was performed to check their

homology at NCBI-GenBank database.

Sugars fermentation tests

In order to differentiate various strains, sugar fermentation tests were performed using the method of Harrigan (1998). Eleven different sugars including lactose, mannose, cellobiose, raffinose, dextrose, arabinose, maltose, fructose, sucrose, xylose, and mannitol were used. Results were compared with Bergey's manual of systematics of archaea and bacteria (Whitman, 2015).

Determination of lactic acid production

D-lactic acid (CAS#10326-41-7) was used to construct the standard curve using concentrations of 2, 4, 6, 8, 10 mg/mL of D-lactic acid. Bacterial strains were inoculated on skimmed milk broth (10% w/v); 1 mL sample from each strain was taken at intervals of 12, 24 and 48 h; tested for production of lactic acid by the method of Borshchevskaya *et al*. (2016). Briefly, cells were harvested by centrifugation (13000 rpm, 8 min, 4°C) and supernatant was procured. 50 µL from each supernatant was mixed with 2 mL $FeCl_3.6H_2O$ (0.2%) till the yellow color development (by stirring up to 8 min) and absorbance was taken at 390 nm (instantly after color development) by autozeroing spectrophotometer (HOLO DB 20, Dynamica) with $FeCl_3.6H_2O$ (0.2%). Lactic acid concentration (mg/mL) was calculated using linear regression equation constructed from standard curve.

Statistical analysis

The lactic acid data was subjected to two-way analysis of variance (ANOVA) and mean values were compared by Tuckey HSD test, using Statistix 10.1.

RESULTS AND DISCUSSION

Food samples were subjected to inoculum preparation using M-17 and MRS broth for enrichment of lactic acid producing strains (Dallal *et al*., 2017; Ahmed and Kanwal, 2004). Total 144 discrete bacterial colonies were purified using standard protocols and subjected to morphological and biochemical characterization. Eighty-four strains indicating Gram positive, catalase negative, oxidase negative, and non-motile characters, were screened as LAB strains and preserved in glycerol (20%) as well as in agar slants (Adesulu-Dahunsi *et al*., 2017; Khalid, 2011; Ahmed *et al*., 2002).

Table I.- Screening and identification details of isolated LAB strains.

Organism	Strain code	Isolation source	Isolation media	GenBank accession No.	FCBP accession No.	Growth at 44°C	Methyl red test	Growth in the presence of NaCl (%)			Methylene blue (%)	
								6	8	18	0.1	0.3
Ln. mesenteroides	BSM-41	Cardamom Milk	MRS	MH155203	FCBP-706	+	—	+	+^{w}	—	+	+
Ln. mesenteroides	BSM-43	Raw Milk	MRS	MH155204	FCBP-707	+	—	+	+^{w}	—	+	—
Ln. mesenteroides	WFD-111	Wheat Dough	MRS	MH155205	FCBP-708	+	+	+	+^{w}	—	+	—
E. durans	RFD-112	Rice Dough	MRS	MH220789	FCBP-709	+	—	+	+	—	+	—
Ln. mesenteroides	WFD-113	Wheat Dough	MRS	MH248364	FCBP-710	+^{w}	+	+	+^{w}	—	+	+
P. acidilacticci	CFD-121	Corn Dough	MRS	MH220780	FCBP-711	+	—	+	+	—	+	+
E. faecium	CFD-122	Corn Dough	MRS	MH220781	FCBP-712	+	—	+	+	+^{w}	+	+
E. faecium	WFD-128	Wheat Dough	MRS	MH220793	FCBP-714	+	—	+	+	+^{w}	+	+
Ln. mesenteroides	WFD-131	Wheat Dough	MRS	MH220794	FCBP-715	+^{w}	+	+	+^{w}	—	+	+
Ln. mesenteroides	WFD-132	Wheat Dough	MRS	MH220795	FCBP-716	+^{w}	—	+^{w}	—	—	+	—
E. faecium	RFD-154	Rice Dough	MRS	MH220790	FCBP-718	+	—	+	+	—	+	+
E. faecium	CFD-174	Corn Dough	MRS	MH220784	FCBP-719	+	—	+	+	—	+	+
Lb. plantarum	TRNP-181	Turnip Pickle	M-17	MH220791	FCBP-720	+	+	+	+	+	—	—
Lb. plantarum	COTG-331	Homemade Cheese	MRS	MH220785	FCBP-723	+	+	+	+	+^{w}	—	—
Lb. brevis	COTG-332	Homemade Cheese	MRS	MH220786	FCBP-724	+	+	+	+^{w}	—	—	—
E. faecium	COTG-352	Homemade Cheese	M-17	MH220788	FCBP-726	+	—	+	+	—	+	—
Ln. mesenteroides	CYG-362	Homemade Yogurt	MRS	MH570186	FCBP-729	+^{w}	+	+	+^{w}	—	+	—

Ln., Leuconostoc; E., Enterococcus; Lb., Lactobacillus; P., Pediococcus; W, weak growth. Growth of bacterial isolates was monitored spectrophotometrically by observing absorbance at 600 nm. Positive symbol indicates an absorbance of more than 1.2 after 24 h of incubation. 'W' shows a weak growth that means an absorbance of less than 0.5 after 24 h of incubation.

Out of these eighty-four bacterial strains, twenty-six strains showing viability at 25 to 40°C, 4% NaCl, and pH 4.0 to 8.0 were further selected whose viability at 44°C, 6%, 8% and 18% NaCl was also evaluated (Table I). These twenty-six strains indicating the biochemical characters of LAB were subjected to 16s rDNA analysis, and seventeen strains were identified as LAB including *Leuconostoc mesenteroides* (seven strains), *Enterococcus facecium* (five strains), *Lactobacillus plantarum* (two strains), *Enterococcus durans* (one strain), *Lactobacillus brevis* (one strain), and *Pediococcus acidilactici* (one strain). *Leuconostoc mesenteroides* was found to be the most prevalent group (41.1%) among total LAB microflora (Fig. 1).

Previous studies conducted in Pakistan revealed that *Lactobacillus* was the most prevalent group in Pakistani yogurt samples while some strains of *Streptococcus* have also been reported (Mahmood *et al*., 2013, 2014; Yousaf *et al*., 2016). Some strains of *Enterococcus* and *Weissela* were isolated and identified by Shahid *et al*. (2017). Information is lacking about the prevalence of *Leuconostoc* and *Pediococcus* in Pakistani foods however some studies conducted in Korea indicate that *Ln. mesenteroides* was the major microflora prevailing in locally fermented Korean foods (Kaur *et al*., 2017; Sharma *et al*., 2018). The DNA sequences were submitted to NCBI GenBank and bacterial cultures were submitted to first fungal culture bank of Pakistan (FCBP). Accession numbers obtained from GenBank and FCBP are listed in Table I.

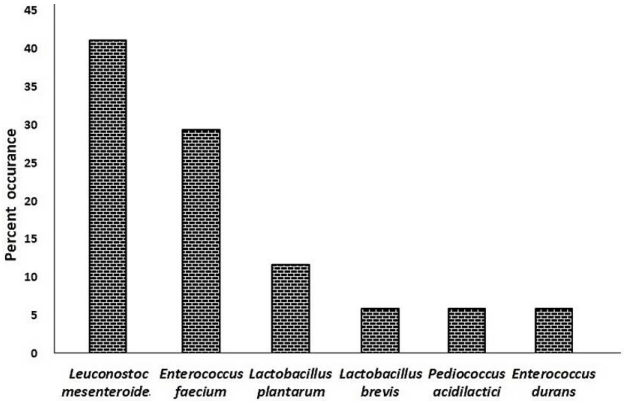

Fig. 1. Percent occurrence of lactic acid bacterial species in the food samples analyzed during current study.

Sugar fermentations (Table II) and growth in the presence of methylene blue (Table I) further confirmed the strain level differentiation. Results of sugar fermentations were in accordance with Bergey's manual of systematics of archaea and bacteria. Sugars fermentation showed that some of the strains of *Leuconostoc mesenteroides* were fermenting mannose, mannitol and xylose while some strains did not ferment these sugars (Holzapfel *et al*., 2015). Both strains of *Lactobacillus plantarum* also showed the variable behavior in fermenting the maltose.

Table II.- Sugar fermentation behavior of LAB strains. LAC, Lactose; MNS, Mannose; CEL, Celliobiose; RAF, Raffinose; DEX, Dextrose; ARB, Arabinose; MAL, Maltose; FRT; Fructose; SUR, Sucrose; XYL, Xylose; MNL, Mannitol.

LAB Strains	LAC	MNS	CEL	RAF	DEX	ARB	MAL	FRT	SUR	XYL	MNL
Ln. mesenteroides strain BSM-41	+	—	+	—	+	+	+	+	+	+	+
Ln. mesenteroides strain BSM-43	+	+	+	—	—	+	+	+	+	—	—
Ln. mesenteroides strain WFD-111	+	+	+	—	—	+	+	+	+	+	+
E. durans strain RFD-112	+	+	+	—	+	—	+	+	—	—	—
Ln. mesenteroides strain WFD-113	—	+	—	+	—	+	+	+	+	—	—
P. acidilacticci strain CFD-121	+	—	—	—	+	+	—	—	—	—	—
E. faecium strain CFD-122	+	+	+	—	+	—	+	+	—	+	—
E. faecium strain WFD-128	+	+	+	—	+	—	+	+	—	—	—
Ln. mesenteroides strain WFD-131	+	—	—	—	+	+	+	+	+	—	—
Ln. mesenteroides strain WFD-132	—	—	—	—	—	+	+	+	+	—	+
E. faecium strain RFD-154	+	+	+	—	+	—	+	+	—	—	+
E. faecium strain CFD-174	+	+	+	—	+	—	+	+	—	—	—
Lb. plantarum strain TRNP-181	+	—	+	+	+	—	—	—	+	+	+
Lb. plantarum strain COTG-331	+	—	+	+	+	—	+	—	+	+	+
Lb. brevis strain COTG-332	+	—	—	—	+	+	+	—	+	+	—
E. faecium strain COTG-352	+	+	+	—	+	—	+	+	+	—	—
Ln. mesenteroides strain CYG-362	+	—	+	—	+	+	+	+	+	—	—

L. plantarum isolated from pickle fermented maltose, while that isolated from cheese did not ferment it (Hammes and Hertel, 2015). This variable metabolism was also observed in the strains of *Enterococcus* (Svec and Devriese, 2015) as well as *Pediococcus* (Holzapfel *et al*., 2015). During this study, two strains of *Pediococcus acidilactici* were isolated from the corn dough, but metabolic typing (stress tolerance assays and sugar fermentation tests) showed the same results for both of strains, so it was concluded that it was single strain cultured twice.

Lactic acid is a major end-metabolite of LAB. Although it is not considered as importance indicator for probiotic potential of these bacteria but plays an important role in pathogens inhibition by lowering the pH up to 2.0. In these highly acidic conditions, most of the pathogenic/poisoning microbes could not grow (Porto *et al*., 2017). Colorimetric method for the determination of lactic was used which was initially developed by Steinsholt and Calbert (1960) being considered as rapid and effective method. Toksoy (1996), Yaman *et al*. (1998), Sabir *et al*. (2010) and many other researchers have efficiently used this method. Borshchevskaya *et al*. (2016) improved this method and compared Spectrophotometric method with the enzymatic assay kits. This method was proved as rapid and efficient method for determination of lactic acid production by bacterial strains rather than the enzymatic assay kits, in which instability of NAD^+ and NADH is serious issue (Borshchevskaya *et al*., 2016).

Strains were cultured on skimmed milk broth and after incubation for 48 h, highest level of lactic acid (26.457 mg/mL) was produced by the *Lactobacillus planatraum* strain COTG-331, while minimum lactic acid (12.131 mg/mL) was produced by *Leuconostoc mesenteroides* strain WFD-111. A remarkable difference was observed in the levels of lactic acid produced after 12 and 24 h, but a slight difference was observed between 24 and 48 h except the strains isolated from homemade cheese (Fig. 2). After 12 and 24 h growth the minimum lactic acid (0.427 and 4.713 mg/mL, respectively) was produced by *Pediococcus acidilactici* strain CFD-121. Sabir *et al*. (2010) also reported that lactic acid production capacity in lactobacilli species was the highest (17.4 mg/mL); while

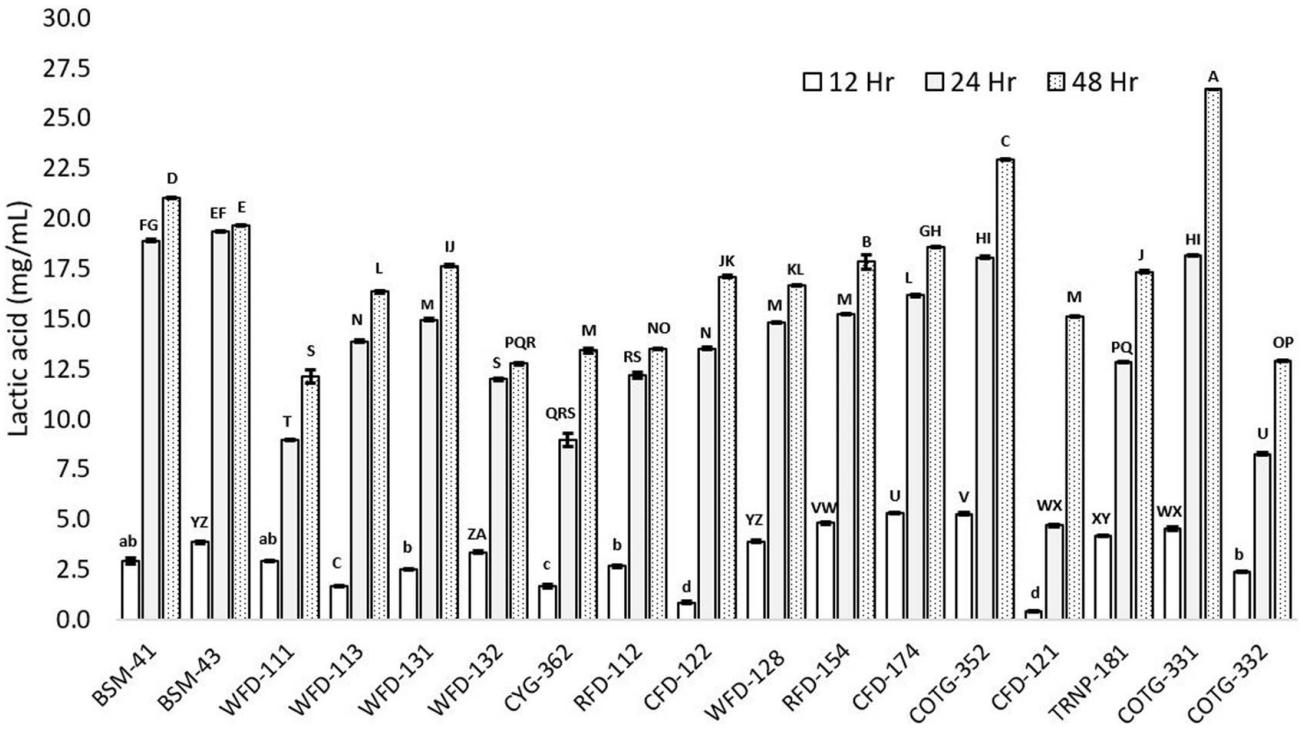

Fig. 2. Comparison of the lactic acid producing potential among various strains of isolated LAB species. Codes of strains are presented on X-axis while lactic acid concentration (mg/mL) is shown on Y-axis. Samples were taken at various intervals (12 h, 24 h, and 48 h) during growth and analyzed for lactic acid concentration (mg/mL) in the cell free culture broth. Codes used for the lactic acid producing bacterial strains were; *Leuconostoc mesenteroides* (BSM-41, BSM-43, WFD-111, WFD-113, WFD-131, WFD-132, CYG-362); *Enterococcus durans* (RFD-112); *Enterococcus faecium* (CFD-122, WFD-128, RFD-154, COTG-352, CFD-174); *Pediococcus acidilacticci* (CFD-121); *Lactobacillus plantarum* (TRNP-181, COTG-331); *Lactobacillus brevis* (COTG-332).

Pediococcus acidilactici produced the lowest quantity of lactic acid (8.1 mg/mL), after incubation of 24 h, among the tested strains in their study. Other studies reported that after 24 h incubation, various species of *Pediococcus* produced lactic acid in range of 5.0 to 7.5 mg/mL (Toksoy, 1996) and 3.2 to 7.75 mg/mL (Yaman *et al*. 1998). During our study, *Pediococcus acidilactici* produced 4.713 mg/mL lactic acid after 24 h incubation, while its production remarkably increased after 48 h incubation up to 15.128 mg/mL. Probable reason for this variation may be the use of different basal media and origin of strains. Other reason may also be that we used the amended method (Borshchevskaya *et al*., 2016) while they used old method of lactic acid determination (Stensholt and Calbert, 1960).

Among *Leuconostoc mesenteroides* strains after incubation of 48 h (Fig. 2), maximum lactic acid (21.059 mg/mL) was produced by the strain BSM-41 isolated from cardamom milk and minimum (12.131 mg/mL) was produced by WFD-111 isolated from wheat flour dough. In case of *Enterococcus*, after 48 h incubation, maximum level (22.952 mg/mL) was produced by *Enterococcus faecium* strain COTG-352 isolated from homemade cheese while minimum (13.504 mg/mL) lactic acid was produced by *Enterococcus durans* strain RFD-112 (Fig. 2). Findings of this study showed that lactic acid production may vary even at strain level rather than at genera or specie level. To the best of our efforts, we were unable to find any study in which lactic acid productivity of *Leuconostoc* and *Enterococcus* strains was analyzed spectrophotometrically.

MRS medium was formulated by de Man *et al*. (1960) in a way that it inhibits the growth of cocci (Marshall, 1992; Downes and Ito, 2001) while Terzaghi and Sandine (1975) formulated the M-17 media and it was claimed that M-17 media contains β-glycerophosphate which suppresses the growth of bacilli therefore inhibits the lactobacilli (Shanker and Davies, 1977). MRS and M-17 media are still known as selective for lactic acid producing bacilli and cocci, respectively (Downs and Ito, 2001). But during our study *Lactobacillus brevis* was isolated on M17, while pediococci and enterococci were isolated on MRS. Total 144 strains were isolated on the selective media for LAB. Among those only 17 strains were confirmed through DNA sequences as LAB indicating only 11.8% turnout of selective media.

Furthermore, an opportunistic pathogen; *Stenotrophomonas maltophillia* (GenBank # MH119141; FCBP-705), Species of *Lysinbacillus, Bacillus,* and *Serratia* have also been isolated on MRS during this study on MRS/M-17 media. Results were verified by multiple sub-culturing (several times) on these media and by changing the source/manufacturers of media *i.e.,* using media from Merck, Difco and Hi-Media Labs. These findings were further confirmed by repeating the 16S ribosomal DNA sequencing. Our findings were supported by the studies of Lee and Salminen (2009) who also revealed that MRS and M-17 media cannot be used as selective media for lactobacilli and Cocci.

Ravula and Shah (1998) devised some modifications in MRS media *i.e.,* addition of HCI until pH 5.1, Bromocresol green and ribose to make is selective for *Lactobacillus* spp. Yuki *et al*. (1999) suggested the use of some monoclonal antibodies for enrichment of MRS culture media to make it selective for *Lactobacillus* spp. Fujiwara *et al*. (2001) and Oozeer *et al*. (2006) suggested the use of antibiotics in MRS media for selection of probiotic LAB strains. Aritonang *et al*. (2017) and Shahid *et al*. (2017) supplemented MRS media with various concentrations of calcium carbonate and for selection of LAB strain and they found up to some extent better results. Keeping in view our results and findings of other researchers, we postulate that MRS and M-17 media can also support various organisms other than lactic acid producing bacilli and Cocci.

Hence, it is recommended that during enumeration and identification of lactic acid producing probiotic strains one should not merely rely on selective media but should also use some advanced methods including PCR-based tools (Ben Amor *et al*., 2007).

CONCLUSION

Seventeen LAB strains resistant to various environmental and stress conditions have been characterized from raw and traditionally processed foods of Pakistan. On the basis of metabolic finger printing and 16S ribosomal DNA sequencing it is concluded that *Leuoconostoc mesenteroides* is the predominant LAB group (41.1%) in the indigenous foods analyzed during this study. Among the tested strains *Lactobacillus plantarum* had the highest potential to produce lactic acid, while *Pediococcus acidilactici* produced the lowest amount of lactic acid, however lactic acid production level varies from strain to strain. This study also revealed that commonly known selective media for LAB group *e.g.,* MRS and M-17 are no more selective for lactic acid producing Bacilli and Cocci, respectively. These identified seventeen LAB strains are being characterized for their probiotic potential.

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Statement of conflict of interest

There is no conflict of interest in this study.

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