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MICROBIOLOGY

Carbonate and silicate dissolving bacteria isolated from home-made yogurt samples

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Abstract: In the current study, twenty-eight bacterial strains were isolated from home-made yogurt samples from Ağrı Province, Turkey. The bacterial strains were identified by conventional and molecular techniques. Among the twenty- eight isolates, seventeen isolates were identified according to the 16 S rDNA region and determined to belong to five different genus including *Sphingomonas* (8 isolates), *Burkholderia* (5 isolates), *Lactobacillus* (2 isolates), *Lactococcus* (1 isolate), *Staphylococcus* (1 isolate). In this study, the presence of *Burkholderia* in home-made yogurt samples were reported for the first time, whereas *Sphingomonas* was detected for the second time. We also investigated the carbonate ($CaCO_3$ and $MgCO_3$) and silicate ($CaSiO_3$ and $MgSiO_3$) dissolving potential of seventeen bacterial isolates. Among these seventeen bacterial isolates, fifteen bacterial isolates have $CaCO_3$-dissolving and 10 bacterial isolates have $MgCO_3$-dissolving potential. The silicates dissolution ability was relatively less than that of carbonates dissolving. We observed that six bacterial isolates have $CaSiO_3$ and only two bacterial isolates have $MgSiO_3$ dissolution abilities. In conclusion, this work clearly shows the diversity of bacteria existing in fermented cow milk samples in Ağrı Province, Turkey, which could be considered as valuable sources for lactic acid bacteria (LAB) isolation and further probiotic potential.

Key words: Home-made yogurt, LAB, carbonate, silicate dissolving.

INTRODUCTION

Fermentation occurs primarily in an anaerobic process in which compounds such as sugars are converted to other compounds such as alcohols and results in energy production to be used by cells and microorganisms. The microorganisms involved in fermentation process can reduce the contamination risks of foods by producing compounds such as antimicrobials, ethanol and organic acids. Besides, fermentation process can result in an improved taste and texture of the starting material (milk, meat, vegetable etc.). Most of bacteria have enzymatic capacity for fermentation and produce lactic acid. Microorganisms can be found in fermented products either naturally or they can be added as starter culture and each of which are responsible for forming trademark tastes, textures and flavours of the fermented foods. As fermented products have nutritional value and variety of sensory attributes, they became increasingly popular as daily intake products in many countries. Popular fermented products consumed in various countries include kefir, sauerkraut, kimchi, cortido, sourdough, kvass, kombucha, pulque, kaffir beer, ogi, Igunaq, miso, tepa, dosa, cheddar and stilton cheeses, surströmming, crème fraîche, fermented sausage, wine and yogurt which are made of milk, vegetable and meat (Chilton et al. 2015). Among all foods in the marketplace yogurt was shown to be one of the most biologically active and highly nutritious protein-rich product produced

in the fermentation process. Yogurt contains higher levels of carbohydrate, calcium, protein and a variety of group B vitamins compared to milk (Deeth & Tamime 1981, Gurr 1987). Yogurt is a semisolid fermented milk product obtained in the presence of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, but, other bacteria such as *Lactobacillus jugurti*, *Lactobacillus helveticus*, *Lactobacillus casei*, and several species of *Bifidobacterium* are also commonly used. During the fermentation process these mentioned bacteria convert the carbohydrate into lactic acid and other metabolites, hydrolyse caseins into free amino acids and peptides and digest fat into free fatty acids of the milk. Worldwide, yogurt has increased its popularity over the past few decades due to its flavour, texture, aroma and many health benefits (Shah & Champagne 2015, Chandan 2015). The health benefits of yogurt include type 2 diabetes, obesity, gastrointestinal benefits, reduction in cardio-metabolic diseases and serum cholesterol, immuno-modulatory effects, control of infections, anti-carcinogenesis, production of vitamins and control of pathogenic organisms, weight management, skin health (Ley et al. 2006, Round & Mazmanian 2009, Astrup 2014, Marette & Picard-Deland 2014, Morelli 2014).

The aim of the present study was to isolate and identify the lactic acid bacteria in local yogurt samples from villages situated in the Ağri, Province, Turkey. For this purpose, eleven yogurt samples were collected from 5 different villages and the isolates obtained from the yogurt samples were tested for their characteristics. It is known that many lactic acid bacteria have carbonate dissolution potential due to their acid production. For this reason, we have also investigated seventeen bacterial isolates for their carbonate and silicate dissolution potential.

MATERIALS AND METHODS

Yogurt samples: a total of eleven yogurt samples were collected from five different villages in Ağrı Province, Turkey. The samples were collected in sterile bottles and kept at 4 °C for further use. The isolates of 5 different villages [(Patnos-Akyemiş), (Ağrı center-Konuktepe), (Hamur-Karabal), (Eleşkirt-Yeşilova) and (Tutak-Güneşgören)] were (AD1-AD8), (AD9-AD17), (AD18-AD25), (AD26-AD30) and (AD31-AD32), respectively.

Isolation of bacterial strains: The collected yogurt samples were diluted 10-fold in sterile physiological saline solution (0.9 % NaCl). One mL of each diluted yogurt sample was mixed with MRS agar medium and then incubated at 30 °C for 48-72 h. The colonies with distinct morphological differences (based on color, shape and size, rough or smooth surface) were picked up with a loop and then spreaded on MRS agar medium for the purification of each isolate.

Characterization of Isolates

The isolates were screened for their Gram reaction as described previously (Wood & Krieg 1989) and followed by the determination of motility of the isolates with wet mounts. Additional tests were performed for the characterization of the isolates, which included determination of the oxidase and catalase, reduction of the nitrite and nitrate, utilization of arginine, hydrolysis of gelatine, urea, casein, starch and esculine, acid production from carbohydrates (galactose, mannose, inositol, sorbitol, sucrose, lactose, maltose and xylose) (Wayne et al. 1974, Barrow & Feltham 2004, Chuard & Reller 1998). The antibiotic susceptibility of the isolates was determined by using the disk diffusion method as described previously (Wayne 2002).

The genomic DNA isolation from the isolates was performed with a DNA extraction

kit (Qiagen). For the amplification of the 16S rDNA region, the universal primers (27F 5'-AGAGTTTGATCCTGGCTCAG-3'; 1492R 5'-GGTTACCTTGTTACGACTT-3') were used.

The amplification of 16 S rRNA region was performed as described previously (Orhan & Gulluce 2015). Afterwards, the PCR products were sent to Macrogen (http://www.macrogen.com) for sequence analysis. The sequence data of the seventeen isolates were analysed and the results were blasted at the NCBI sequence database.

Screening of Carbonates ($CaCO_3$ and $MgCO_3$) and Silicates ($CaSiO_3$ and $MgSiO_3$) dissolution potential

In order to determine the carbonates ($CaCO_3$ and $MgCO_3$) dissolution potential of bacterial isolates, the method previously described was used (Cacchio et al. 2004). The medium used for $CaCO_3$ dissolution potential was as follows (per liter): glucose, 20 g; NaCl, 10 g; $MgCl_2$, 3 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 0.4 g; $(NH_4)_2SO_4$, 0.2 g; agar, 15 g and $CaCO_3$, 5.0 g. Similar to the $CaCO_3$ dissolution medium, same amount of $MgCO_3$ was used instead $CaCO_3$ for the $MgCO_3$ dissolution medium. The zone of clearance around the colonies was considered as positive for $CaCO_3$ and $MgCO_3$ dissolution capabilities.

For the dissolution of silicates, soil extract was prepared as follows: 1 kg of garden land soil was added to 1000 mL of tap water and then autoclaved at 121 °C for 30 min. After then, the soil sample was filtered with a Whatman filter paper (No:1) and it was completed to a final volume of 1000 ml with tap water. For the dissolution of $CaSiO_3$, we used the method previously described (Bunt & Rovira 1955). The medium was prepared as follows (per liter): 10 g peptone, 20 g glucose, 0.1 g $MgCl_2$, 0.01 g $FeCl_3$, 1 g yeast extract, 0.5 g $(NH_4)_2SO_4$, 0.4 g Na_2HPO_4, 15 g agar and 2.5 g insoluble $CaSiO_3$ along with 500 ml of soil extract and the pH was adjusted to 7.20. Similar to the $CaSiO_3$ dissolution medium, same amount of $MgSiO_3$ was used instead $CaSiO_3$ for the $MgSiO_3$ dissolution medium. The bacterial isolates were incubated at 30 °C for 2 weeks on calcium silicate agar and magnesium silicate agar media for $CaSiO_3$ and $MgSiO_3$ dissolution potential, respectively. The zone of clearance around the colonies was considered as positive for $CaSiO_3$ and $MgSiO_3$ dissolution capabilities.

RESULTS

In the current paper, the twenty-eight bacterial isolates (AD1- AD32) were characterized by conventional and molecular methods. In conventional methods, the morphology, motility and Gram of the twenty-eight bacterial isolates were investigated. As biochemical and physiological parameters catalase and oxidase tests, nitrate and nitrite reduction, arginine utilization, hydrolysis of gelatine, urea, casein, starch and esculin were investigated. The antibiotic susceptibility (neomycin and tetracycline) of the bacterial isolates was also investigated. In addition, the acid production from carbohydrates (glucose, galactose, mannose, inositol, sorbitol, sucrose, lactose, maltose and xylose) was tested. The DNA extraction of twenty-eight bacterial isolates were performed for molecular identification method. Afterwards, the amplification of 16S rRNA genes was performed and then the amplified 16S rRNA product was sequenced. However, only seventeen bacterial isolates could be characterized due to sequencing difficulties of *Burkholderia* and *Sphingomonas*.

As the bacterial isolates were isolated from yogurt samples, they were thought to have probiotic potential, so the isolates were tested for their probiotic potential as pH (2.0, 2.5, 3.0, 4.40 and 9.60), bile salt [cholic acid (0.05, 0.3 and 0.5) (w/v)], temperature (10 and 45°C) and salt (NaCl) (6.5, 12 and 18) tolerance.

The phenotypic characteristics of the isolates are given in Table I.

In respect to gelatine hydrolysis, only one isolate (AD16) was positive, seven isolates were not determined and twenty isolates were negative. None of the isolates were able to hydrolysis urea, casein or starch. Only one isolate (AD28) was sensitive to neomycin, while all the others were resistant to both neomycin and tetracycline (Table II).

Acid production from carbohydrates tested (glucose, galactose, mannose, inositol, sorbitol, sucrose, lactose, maltose and xylose) were recorded to be variable as summarized in Table III.

According to the blast analysis of seventeen bacterial isolates, five isolates (AD2, AD3, AD5, AD6 and AD13) were *Burkholderia sp*., eight isolates (AD4, AD8, AD10, AD11, AD12, AD14, AD20 and AD21) were *Sphingomonas* sp., one isolate (AD28) was *Staphylococcus epidermidis*, one isolate (AD30) was *Lactococcus lactis* and two isolates (AD31 and AD32) were *Lactobacillus* (*Lactobacillus paracasei* and *Lactobacillus casei*, respectively) (Table IV).

Majority of the isolates grew at a pH range from 2.0 to 9.60 (Table V). One isolate (AD9) did not grow at the pH 9.60 as shown in the Table V. Considering the bile salt tolerance, most of the isolates grew in the 0.005 and 0.3 % bile salt, while ten isolates grew in the 0.5 % bile salt.

Table I. Phenotypic characteristics of the isolates.

Isolate code	Gram (KOH)	Motility	Cell morphology	Catalase	Oxidase	Nitrate reduction	Nitrite reduction	Arginine utilization
AD1	-	-	Rod	+	-	-	-	-
AD2	-	-	Rod	+	-	-	-	-
AD3	-	-	Rod	+	-	-	+	-
AD4	-	-	Rod	+	-	-	+	-
AD5	-	-	Rod	+	-	-	+	-
AD6	-	-	Rod	+	-	-	+	-
AD7	-	-	Rod	+	-	-	+	-
AD8	-	-	Rod	+	-	-	+	-
AD9	-	-	Rod	+	-	-	+	-
AD10	-	-	Rod	+	-	-	+	-
AD11	-	-	Rod	+	-	-	-	-
AD12	-	-	Rod	+	-	-	+	-
AD13	-	-	Rod	+	-	-	-	-
AD14	-	-	Rod	-	-	-	-	-
AD15	-	-	Rod	+	-	-	+	-
AD16	-	-	Oval	-	-	-	-	-
AD17	-	-	Rod	+	-	-	-	-
AD18	-	-	Oval	+	-	-	+	-
AD19	-	-	Oval	+	-	-	-	-
AD20	-	-	Rod	+	-	-	-	-
AD21	-	-	Rod	+	-	-	-	+
AD22	-	-	Rod	+	-	-	-	-
AD23	-	-	Rod	-	-	-	-	-
AD24	-	-	Oval	+	-	-	-	-
AD28	+	-	Coccoid	+	-	-	+	+
AD30	+	-	Coccoid	+	-	-	+	+
AD31	+	-	Rod	-	-	-	-	-
AD32	+	-	Rod	-	-	-	-	+

-: Negative, +: Positive.

The temperature tolerance test showed that one isolate (AD32) did not grow at 10 °C and one isolate (AD23) did not grow at 45 °C, while all the others grew at both 10 and 45 °C. Twenty isolates grew in 6.5 % NaCl, nineteen isolates grew in 12 % NaCl, and fifteen isolates slightly grew in 18 % NaCl (Table V).

Among seventeen bacterial isolates, fifteen bacterial isolates were able to dissolve $CaCO_3$ and ten bacterial isolates were able to dissolve $MgCO_3$.While, six bacterial isolates (AD13, AD14, AD20, AD30, AD31 and AD32) were able to dissolve $CaSiO_3$, only two isolates (AD31 and AD32) were able to dissolve $MgSiO_3$ (Table VI and Figure 1).

The results of bacterial isolates producing yogurt/yogurt-like product are shown in Figure 2.

DISCUSSION

The word 'yoghurt' was derived from the Turkish word 'jugurt' or 'yoğurt' and in different countries it has various names as 'Tiaourti' in Greece, 'Cieddu' in Italy, 'Kissel Mleka' in Balkans, 'Mezzoradu' in Sicily, 'Leben/Laben' in Scandinavian, 'Zabady' in Egytp and Sudan, 'Tarho' in Hungary, 'Mast/Dough' in Iran and

Table II. Phenotypic characteristics (hydrolysis of various substrates and antibiotic susceptibility) of the isolates.

Isolate code	Hydrolysis of					Antibiotic Susceptibility (mm)	
	Gelatin	Urea	Casein	Starch	Esculin	Neomycin	Tetracycline
AD1	-	-	-	-	+	R	R
AD2	-	-	-	-	+	R	R
AD3	-	-	-	-	+	R	R
AD4	-	-	-	-	+	R	R
AD5	-	-	-	-	-	R	R
AD6	-	-	-	-	-	R	R
AD7	ND	-	-	-	+	R	R
AD8	-	-	-	-	+	R	R
AD9	ND	-	-	-	+	R	R
AD10	-	-	-	-	+	R	R
AD11	-	-	-	-	+	R	R
AD12	-	-	-	-	+	R	R
AD13	-	-	-	-	+	R	R
AD14	-	-	-	-	+	R	R
AD15	-	-	-	-	-	R	R
AD16	+	-	-	-	+	R	R
AD17	ND	-	-	-	+	R	R
AD18	-	-	-	-	+	R	R
AD19	-	-	-	-	+	R	R
AD20	ND	-	-	-	-	R	R
AD21	-	-	-	-	-	R	R
AD22	ND	-	-	-	-	R	R
AD23	-	-	-	-	-	R	R
AD24	-	-	-	-	+	R	R
AD28	-	-	-	-	-	18	R
AD30	ND	-	+	-	+	R	R
AD31	ND	-	-	-	+	R	R
AD32	-	-	-	-	+	R	R

-: Negative, +: Positive, ND: Not determined, R: Resistant, mm: millimeter.

Table III. Phenotypic characteristics (acid production from various sugars) of the isolates.

Isolate code	Acid production from								
	Glucose	Galactose	Mannose	Inositol	Sorbitol	Sucrose	Lactose	Maltose	Xylose
AD1	+	+	+	-	-	+	+	-	+
AD2	+	+	+	-	-	+	+	-	+
AD3	+	+	+	-	-	+	+	-	+
AD4	+	+	+	-	-	+	+	-	+
AD5	+	+	+	-	-	+	+	-	+
AD6	-	-	-	-	-	-	-	-	-
AD7	+	+	+	-	+	+	+	-	+
AD8	-	+	Slight	-	-	+	+	-	+
AD9	+	+	+	-	-	+	+	-	+
AD10	-	+	+	-	-	+	+	-	+
AD11	+	+	+	-	+	+	+	-	+
AD12	+	+	+	-	-	+	+	-	+
AD13	+	+	+	-	-	+	+	-	+
AD14	+	+	+	-	-	+	+	-	+
AD15	+	+	+	-	-	+	+	-	+
AD16	+	+	+	-	+	+	+	-	+
AD17	-	+	+	-	-	+	+	-	+
AD18	+	+	+	-	-	+	+	-	+
AD19	+	+	+	-	-	+	+	-	+
AD20	+	+	+	-	-	+	+	-	+
AD21	+	+	+	-	-	+	+	-	+
AD22	+	+	+	-	-	+	+	-	+
AD23	-	+	+	-	-	+	-	-	+
AD24	-	+	+	-	-	+	+	-	+
AD28	-	+	+	-	-	+	+	-	+
AD30	-	+	+	-	-	-	-	+	-
AD31	-	+	+	-	+	+	+	-	+
AD32	-	-	-	-	+	Slight	+	-	+

-: Negative, +: Positive.

Afghanistan, 'Villi' in Finland, 'Roba' in Iraq, 'Skyr' in Iceland, 'Dahi/Dadhi/Dahee' in India, Bangladesh and Nepal, 'Mazun' in Armenia and 'Yoghurt/Yogurt/Yaourt' in the rest of the world (Tamime & Deeth 1980). Yogurt is generally prepared from cow's milk, however, milk from sheep, water buffaloes, yaks, horses, and camels can also be used (Donovan & Shamir 2014). The texture, taste and aroma of the yogurt vary according to the type of milk used, the method used for milk processing and the starter cultures used in the yogurt production (Chandan & Kilara 2013). It has been documented that the use of defined starter cultures led to a product with better aroma, acceptability, taste and appearance than spontaneously fermented beverages (Peyer et al. 2016). For this reason, researchers are attempting to obtain better result in producing yogurt by improving the methods for the yogurt production and isolation of new strains capable in yogurt fermentation. In this regard, members of *Lactobacillus*, *Streptococcus*, *Weissella*, *Pediococcus*, *Enterococcus* and *Leuconostoc* from various yogurt, cheese and milk samples have been documented (Nakhdjavani et al. 1996, Azadnia & Khan Nazer 2009, Ebrahimi et al. 2011, RoushanZadeh et al. 2014, Velikova et al. 2018).

In home-made yogurt and raw milk samples collected in Pakistan, several unexpected bacteria including *Sphingomonas*, *Pseudomonas* and

Table IV. Identification of the isolates.

Isolate code	Accession no.	Nearest type strain	Sequence length	Sequence identity (%)
AD2	MK454715	*Burkholderia* sp.	1397	99
AD3	MK454716	*Burkholderia* sp.	1380	99
AD4	MK454717	*Sphingomonas* sp.	1167	99
AD5	MK454718	*Burkholderia* sp.	1382	99
AD6	MK454719	*Burkholderia* sp.	1407	99
AD8	MK454720	*Sphingomonas* sp.	1050	97
AD10	MK454721	*Sphingomonas* sp.	1153	99
AD11	MK454722	*Sphingomonas* sp.	1126	99
AD12	MK454723	*Sphingomonas* sp.	1173	99
AD13	MK454724	*Burkholderia* sp.	1184	100
AD14	MK454725	*Sphingomonas* sp.	1077	99
AD20	MK454726	*Sphingomonas* sp.	1080	98
AD21	MK454727	*Sphingomonas* sp.	1051	99
AD28	MK454728	*Staphylococcus epidermidis*	1372	99
AD30	MK454729	*Lactococcus lactis*	1383	99
AD31	MK454730	*Lactobacillus paracasei*	1373	99
AD32	MK454731	*Lactobacillus casei*	1366	99

Bacillus have been reported (Asma & Qazi 2014). In accordance with the data in above line, the genus *Sphingomonas* has also been detected in our study. Indeed, this genus have been isolated from a variety of sources such as drinking water system, hospital water equipment's, seawater, river, waste water, sea ice, soil, mineral water, milk of bovine and human (Ferreira et al. 1996, Geldreich 1996, Bowman et al. 1997, Vachée et al. 1997, Oie et al. 1998, Gauthier et al. 1999, Tabata et al. 1999, Hunt et al. 2011, Kuehn et al. 2013, Zhang et al. 2015). According to the available literature, this is the second paper documenting the presence of the genus *Sphingomonas* in home-made yogurt samples.

Other unexpected bacteria isolated from the yogurt samples in this study was *Burkholderia*. The members of this genus are able to inhabit diverse sources including soil, water, fungus, plant rhizosphere, infected human and animals (Burkholder 1950, Vial et al. 2008, Morelli 2014, Eberl & Vandamme 2016). Interestingly, they have both pathogenic and beneficial members. For example, *Burkholderia mallei*, *B. pseudomallei* and *B. solanacearum* are human, animal and plant pathogens, respectively (Burkholder 1950, Eberl & Vandamme 2016). The beneficial member of this genus is *B. tropica* which can inhibit the growth of pathogenic fungi such as *Colletotrichum, Fusarium* and *Sclerotium* (Tenorio-Salgado et al. 2013). The other beneficial member of this genus is *B. vietnamiensis* which has plant (rice) growth promoting activities (Van et al. 2000). The data obtained from the pH and bile salt (cholic acid) tolerances in this study showed that the bacterial isolates may have probiotic potential as most of the isolates (except for the known pathogen, *Staphylococcus epidermis*) have both pH and bile salt tolerances.

Among the twenty-eight bacterial isolates, seventeen isolates have been identified in terms of their 16S rRNA gene sequences region. The data of the 16S rRNA gene sequences region shows that among these seventeen isolates, five isolates (AD2, AD3, AD5, AD6 and AD13) belong to the genus *Burkholderia*, eight isolates (AD4, AD8, AD10, AD11, AD12, AD14, AD20 and AD21) belong to the genus *Sphingomonas*, two isolates (AD31

Table V. Phenotypic characteristics (tolerance of pH, bile salt, temperature and salt) of the isolates.

Isolate code	pH tolerance					Bile salt (cholic acid) tolerance (%)			Temperature tolerance (°C)		Salt tolerance (% NaCl)		
	2.0	2.5	3.0	4.40	9.60	0.05	0.3	0.5	10	45	6.5	12	18
AD1	+	+	+	+	+	+	+	+	+	+	+	+	-
AD2	+	+	+	+	+	+	+	+	+	+	+	+	-
AD3	+	+	+	+	+	+	+	+	+	+	+	+	-
AD4	+	+	+	+	+	+	+	+	+	+	+	-	-
AD5	+	+	+	+	+	+	+	+	+	+	+	+	-
AD6	+	+	+	+	+	+	+	+	+	+	+	+	+
AD7	+	+	+	+	+	+	+	+	+	+	+	+	+
AD8	Slight	+	+	+	+	+	+	+	+	+	+	+	-
AD9	+	+	+	+	-	+	+	+	+	+	+	-	-
AD10	+	+	+	+	+	+	+	+	+	+	+	+	-
AD11	+	+	+	+	+	+	+	+	+	+	+	+	-
AD12	+	+	+	+	+	+	+	+	+	+	+	+	-
AD13	+	+	+	+	+	+	+	+	+	+	+	+	+
AD14	+	+	+	+	+	+	+	+	+	+	+	+	+
AD15	+	+	+	+	+	+	+	+	+	+	+	+	+
AD16	+	+	+	+	+	+	+	+	+	+	+	+	+
AD17	+	+	+	+	+	+	+	+	+	+	+	+	-
AD18	+	+	+	+	+	+	+	+	+	+	+	+	+
AD19	+	+	+	+	+	+	+	+	+	+	+	+	+
AD20	+	+	+	+	+	+	+	+	+	+	+	+	+
AD21	+	+	+	+	+	+	+	+	+	+	+	+	-
AD22	+	+	+	+	+	+	+	+	+	+	+	+	+
AD23	+	+	+	+	+	+	+	+	+	-	+	+	-
AD24	+	+	+	+	+	+	+	+	+	+	+	+	+
AD28	+	+	+	+	+	+	+	+	+	+	+	+	+
AD30	+	+	+	+	+	+	+	+	+	+	+	+	+
AD31	+	+	+	+	+	+	+	+	+	+	+	+	+
AD32	+	+	+	+	+	+	+	+	-	+	+	+	+

-: Negative, +: Positive.

and AD32) belong to the genus *Lactobacillus*, one isolate (AD30) belong to the genus *Lactococcus* and one isolate (AD28) belong to the genus *Staphylococcus*.

We also have investigated yogurt making potential of these twenty-eight bacterial isolates. Among the twenty-eight isolates, except for *Lactococcus* and *Lactobacillus*, four identified isolates [*Burkholderia* sp. (AD3), *Burkholderia* sp. (AD5), *Burkholderia* sp. (AD13) and *Sphingomonas* sp. AD20)] and one unidentified isolate (AD17) were able to produce yogurt/yogurt-like product.

The obtained yogurts/yogurt-like products by the genus *Burkholderia* could be due to their acid tolerance as this genus has a high acid tolerance and their distribution is strongly affected by pH (Tago et al. 2014, Stopnisek et al. 2014, Sermswan et al. 2015, Hall et al. 2015), which is in compliance with our results. On the other hand, there is no data available with the acid tolerance of the genus *Sphingomonas*.

It is known that bacteria and their metabolites can be used in many industrial mineral processing applications (Johnson & Hallberg 2005, Olson et al. 2003). There are several bacterial isolates whose utilization have started long time ago in different bioleaching processes including *Acidithiobacillus* sp. (sulphides), *Micobacteria phlei* (haematite), *Bacillus circulans* (bauxite) and *Burkholdaria* sp. (iron) (Rawlings 2002, Rao & Subramanian 2007, Groudev 1987, Delvasto et al. 2009). Recently investigates regarding utilization of microorganisms in ore enrichment processes gained importance due to increase in demand and decrease in high grade materials. Recently, the use of bacterial isolates including *Pseudomonas oryzihabitans* and *Lactobacillus* sp. in magnesite ores enrichment has been reported (Karaoglu et al. 2016, Yanmis et al. 2015). More recently, *Enterobacter* sp., *Klebsiella* sp., *Leclercia* sp. and *Leclercia adecarboxylata* have been shown to dissolve silisium in boron clay (Efe et al. 2019). According to the literature regarding the determination of bioleaching mechanism performed with alumina-silicate and calcium carbonate, organic acids were mainly responsible for the dissolution of the tested materials (Rezza et al. 2001, Efe et al. 2020). Considering the fact that yogurt/yogurt-like product producing bacterial isolates may also secrete organic acids, we investigated carbonate and silicate dissolution potential of the seventeen bacterial isolates.

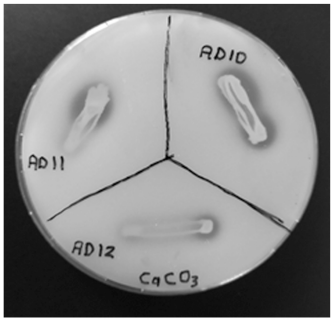
The results of carbonate dissolution experiments showed that many of the bacterial isolates were able to dissolve carbonates. On the other hand, the silicate dissolving ability of the same bacterial isolates was less than that of carbonates dissolving ability. In fact, the dissolution of carbonates by lactic acid bacteria has previously been reported (Yanmis et al. 2015). Similarly, the carbonate dissolving phenomenon continuously occurs in nature via microbial activity (Ehrlich 1998, Friis et al. 2003, Tang et al. 2012). However, the dissolution capabilities of insoluble compounds (carbonates and silicates) via *Burkholderia* and *Sphingomonas* has not been published elsewhere.

Considering the previous reports and the data obtained in the current work, it can be concluded that the presence of genus *Burkholderia* and *Sphingomonas* is not expected in home-made yogurt samples. On the other hand, the presence of pathogenic bacteria, *Staphylococcus*, in the yogurt samples indicates contamination which could be due to milking with either dirty hands, collecting in dirty milking pails or un-cleaned udders. In this study, the partial bacterial diversity of home-made yogurt samples in Agri Province has been shown for the first time. Moreover, the preliminary tests in making yogurts showed that the five bacterial isolates (except for known LAB) have potential in making yogurt/yogurt-like products. However, further fermentation applications and pathogenic traits of these bacterial isolates should be investigated in order to illustrate the potential of these bacterial isolates as a new yogurt producer. Therefore, the determination of the organic acids and volatile compound profiles of the yogurt/yogurt-like products should be investigated. Furthermore, the mechanism(s) underlying the carbonates and silicates dissolving capabilities of the bacterial isolates should be investigated.

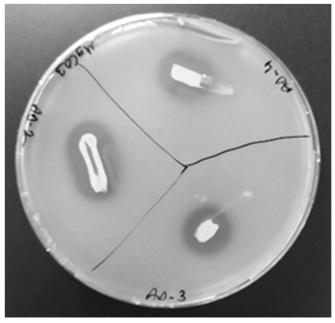
Table VI. Carbonates and Silicates dissolution potential of the isolates.

Isolate code	Nearest type strain	$CaCO_3$	$MgCO_3$	$CaSiO_3$	$MgSiO_3$
AD2	*Burkholderia* sp.	+	+	-	-
AD3	*Burkholderia* sp.	+	+	-	-
AD4	*Sphingomonas* sp.	+	+	-	-
AD5	*Burkholderia* sp.	+	+	-	-
AD6	*Burkholderia* sp.	-	-	-	-
AD8	*Sphingomonas* sp.	+	+	-	-
AD10	*Sphingomonas* sp.	+	+	-	-
AD11	*Sphingomonas* sp.	+	+	-	-
AD12	*Sphingomonas* sp.	+	+	-	-
AD13	*Burkholderia* sp.	+	Slight	+	-
AD14	*Sphingomonas* sp.	+	-	+	-
AD20	*Sphingomonas* sp.	+	-	+	-
AD21	*Sphingomonas* sp.	+	-	Slight	-
AD28	*Staphylococcus epidermidis*	-	+	-	-
AD30	*Lactococcus lactis*	+	-	+	-
AD31	*Lactobacillus paracasei*	+	+	+	+
AD32	*Lactobacillus casei*	+	-	+	+

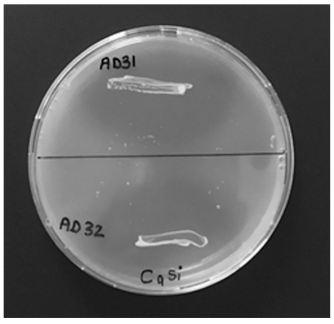
-: Negative, +: Positive.



a: $CaCO_3$



b: $MgCO_3$



c: $CaSiO_3$

d: $MgSiO_3$

Figure 1. Carbonate and Silicate dissolving potential of the isolates.
a: $CaCO_3$, b: $MgCO_3$ c: $CaSiO_3$ d: $MgSiO_3$ containing media.

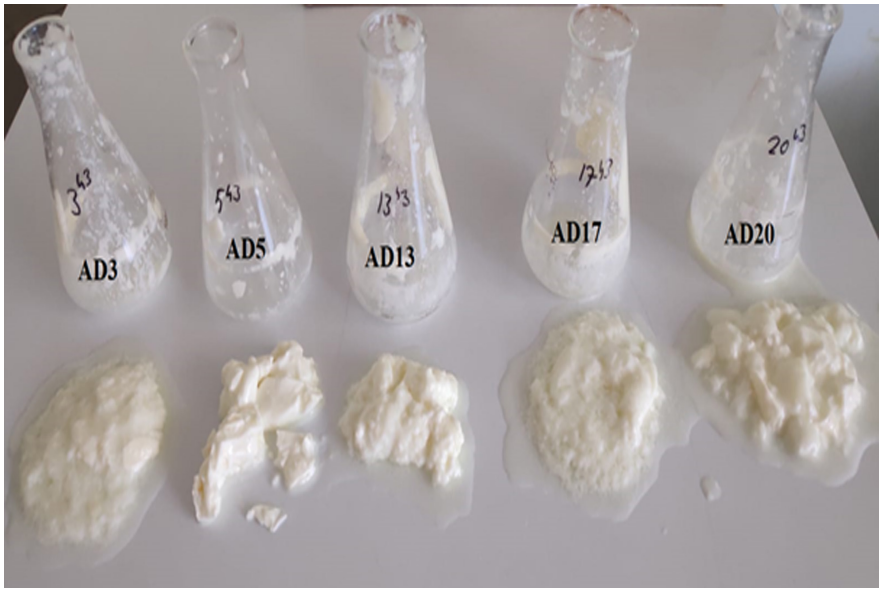


Figure 2. The bacterial isolates producing yogurt/yogurt-like product.

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F.O. and A.D: performed the growth of bacterial isolates, biochemical and molecular tests; F.O: coordinated the study and wrote the manuscript; A.G: wrote the methods; all authors commented on and improved the manuscript.