Research Article

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Detection of industrially potential enzymes of moderately halophilic bacteria on salted goat skins

Tuzlanmış Keçi Derileri Üzerindeki Ilımlı Halofil Bakterilerin Endüstriyel Potansiyele Sahip Enzimlerinin Belirlenmesi

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Abstract

Aim: This study aimed to isolate moderately halophilic bacteria from salted goat skins, to characterize these microorganisms and to determine their industrially important enzymes such as amylase, catalase, oxidase, caseinase, cellulase, DNase, lipase, lecithinase, protease, pullulanase, urease, phospholipase, xylanase and β-galactosidase.
Methods: Enzymes of these bacteria, isolated from skin samples belonging to eight countries and identified using phenotypic and genotypic methods, were examined in agar media.
Results: Thirty-nine isolates were fairly similar to species of genera *Staphylococcus*, *Bacillus*, *Salinicoccus*, *Gracilibacillus*, *Chromohalobacter* and *Halomonas*. Various carbon sources were utilized, and all isolates produced enzyme. Enzyme-producing species were *Staphylococcus saprophyticus* subsp. *saprophyticus*, *Staphylococcus arlettae*, *Bacillus pumilus*, *Gracilibacillus dipsosauri*, *Salinicoccus roseus*, *Bacillus licheniformis*, *Chromohalobacter beijerinckii*, *Staphylococcus xylosus*, *Halomonas eurihalina*, *Staphylococcus equorum* subsp. *equorum*, *Halomonas zhanjiangensis*, *Halomonas venusta* and *Chromohalobacter canadensis*. Fairly high percentage of isolates produced protease (87%) and catalase (100%). While more than 50% of isolates produced lipase (64%), β-galactosidase (59%) and oxidase (56%), less than 50% of isolates produced urease (46%), caseinase (28%), amylase (26%), lecithinase (8%) and cellulase (5%).
Conclusion: We detected that moderately halophilic bacteria on skins produced important enzymes, which may be used in diverse industrial applications in leather, feed, detergent, paper, food, chemical, medical, pharmaceutical, textile industries.

Keywords: Moderately halophilic bacteria; Industrially important enzymes; Biochemical characteristics; Leather industry; Molecular identification.

Özet

Amaç: Bu çalışma, ılımlı halofil bakterileri tuzlanmış keçi derilerinden izole etmeyi, bu mikroorganizmaları karakterize etmeyi ve bunların amilaz, katalaz, oksidaz, kaseinaz, selülaz, DNaz, lipaz, lesitinaz, proteaz, pullulanaz, üreaz, fosfolipaz, ksilinaz ve β-galaktosidaz gibi endüstriyel olarak önemli enzimlerini saptamayı amaçlamıştır.
Yöntemler: Sekiz ülkeye ait olan deri örneklerinden izole edilerek fenotipik ve genotipik metodlara göre tanımlanan bu bakterilerin enzimleri, agar besiyerlerinde incelendi.
Bulgular: *Staphylococcus*, *Bacillus*, *Salinicoccus*, *Gracilibacillus*, *Chromohalobacter* ve *Halomonas* cinslerine ait 39 izolat bu cinslere ait türlere oldukça benzerdi. Çeşitli karbon kaynakları kullanıldı ve izolatların tümü enzim

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üretti. Enzim üreten türler *Staphylococcus saprophyticus* subsp. *saprophyticus*, *Staphylococcus arlettae*, *Bacillus pumilus*, *Gracilibacillus dipsosauri*, *Salinicoccus roseus*, *Bacillus licheniformis*, *Chromohalobacter beijerinckii*, *Staphylococcus xylosus*, *Halomonas eurihalina*, *Staphylococcus equorum* subsp. *equorum*, *Halomonas zhanjiangensis*, *Halomonas venusta* ve *Chromohalobacter canadensis*'di. İzolatların oldukça yüksek yüzdesi proteaz (87%) ve katalaz (100%) enzimlerini üretti. İzolatların yarısından fazlası lipaz (64%), β-galaktosidaz (59%) ve oksidaz (56%) enzimlerini üretirken, yarısından azı üreaz (46%), kazeinaz (28%), amilaz (26%), lesitinaz (8%) ve selülaz (5%) üretti.

Sonuç: Derilerdeki ılımlı halofil bakterilerin, deri, yem, deterjan, kâğıt, gıda, kimya, medikal, ilaç, tekstil gibi çeşitli endüstriyel uygulamalarda kullanılabilecek önemli enzimleri ürettiklerini saptadık.

Anahtar Kelimeler: Ilımlı halofil bakteriler; Endüstriyel açıdan önemli enzimler; Biyokimyasal karakteristikler; Deri endüstrisi; Moleküler tanımlama.

Introduction

Enzymatic processes provide many advantages in terms of saving energy, water, raw materials and chemicals that cannot be achieved using conventional chemical processes. Due to enzymes' biodegradable structure, obtaining high quality product, low energy consumption, low-cost material and less environmental pollution, enzymes are more preferred as economically and ecologically alternatives to chemicals in industrial applications [1]. The use of microbial enzymes is widespread in biotechnological applications as metabolic catalysts for centuries. Industrial enzymes have been used in food, feed, chemical and pharmaceutical, textile, biofuel, paper and pulp, detergent and leather industries as well as production of fish sauce and soy sauce, bioremediation, saline waste water, and oil field waste treatment [2].

Annual spending on enzymes is quite high worlwide. According to report by BBC Research (2014), globally the enzyme market is approximately estimated at 7.1 billion dollars by 2018 [3]. Due to the high demand for industrial enzymes, researchers have been focused on finding new industrial microorganisms producing different enzymes that can withstand extreme conditions. Especially moderate halophiles are considered to be potential sources for industrial enzymes such as cellulases, lipases, xylanases, proteases, amylases, nucleases, and esterases which are able to perform their functions under a wide range of salinity (optimally 3–15% NaCl), pH, and temperatures [1, 2, 4–6]. Furthermore, moderate halophiles present opportunities owing to easily growing on low-cost substrate and low water content [7]. In the previous studies, different moderately halophilic bacterial genera (*Bacillus*, *Chromohalobacter*, *Halobacillus, Halomonas*, *Marinococcus*, *Oceanobacillus, Salibacillus*, *Salinicoccus*, *Salinivibrio* and *Thalassobacillus*), isolated from natural saline environments, produced DNase, lipase, xylanase, amylase, inulinase, protease, cellulase, pullulanase, pectinase and caseinase [5, 8, 9].

Moderately halophilic bacteria have been isolated from hypersaline environments such as saline lakes, salterns, solar salt evaporation ponds, saline soils, salt mine soil, marine sediments and other saline habitats such as salted fish, fermented anchovy sauce, meat and hides [10–16]. Although characterization of moderately halophilic bacteria, isolated from different saline environments, using conventional and molecular techniques and detail experiments on their hydrolytic enzymes have been carried out by several researchers [5, 8, 9], investigation of moderately halophilic bacteria found on salted goat skins, using both conventional and molecular techniques and examination of their industrial enzymes have not been reported previously. Screening of enzyme producing moderately halophilic bacteria on goat skins cured with different countries' salt will be important for determination of industrially potential microorganisms. Hence, the present study focused on the screening industrially important moderately halophilic bacteria producing amylase, catalase, oxidase, caseinase, cellulase, DNase, lipase, lecithinase, protease, pullulanase, urease, phospholipase, xylanase and β-galactosidase. In order to characterize moderately halophilic bacteria isolated from the skins and to understand their physiological and biochemical characteristics for enzyme production, phenotypic characteristics and comparative partial 16S rRNA gene sequence analysis of these microorganisms were also examined in this study.

Materials and methods

Sample collection

Twenty-three salted goat skin samples imported from Australia, Bulgaria, Israel, South Africa, Russia, China, France, and four salted goat skin samples preserved in Turkey were collected from different tanneries in the Leather Organized Tannery Region, Tuzla and Corlu, Turkey. The samples were then placed into sterile prelabeled translucent ziplock

bags and transported in a sterile and cold container. The samples were named according to its origin: Australia (AL, AVT), Turkey (E, KE, T, YE), Bulgaria (B, BLG, BT), Israel (IRL), South Africa (KT, K, NE, GK), Russia (RU, RA), China (CCN, CN, CC), France (F, FR, FN, FS) (Table 1).

Isolation of the moderately halophilic bacteria

To isolate moderately halophilic bacteria, 20 g skin samples were separately soaked in flasks containing 180 mL of 10% NaCl. The flasks were placed into orbital shaker at 100 rpm for 4 h at 25°C. Sterile physiological saline solution containing 10% NaCl was used to dilute skin solutions. An aliquot of 0.1 mL of each direct and serial dilutions (from 10^{-1} to 10^{-6}) of skin solutions was spread onto the surface of the agar plates containing Complex Medium I (CMI) supplemented with 0.5% (w/v) yeast extract with 10% final salt concentration (SW10, saline water) consisting of (w/v): 8.1% NaCl, 0.2% KCl, 0.7% $MgCl_2$, 0.006% $NaHCO_3$, 0.96% $MgSO_4$, 0.0026% NaBr and 0.036% $CaCl_2$ [17]. Yeast extract and all chemicals used in SW10 were from the same company (Merck, Darmstadt, Germany). The pH of the media was adjusted to 7.5 prior to autoclaving. The plates were incubated at 37°C during 24 h. After incubation period, bacterial cultures were selected by their different colony morphologies and re-streaked on CMI agar to obtain pure isolates. Since the selections of isolates were made according to their different colony morphologies, a few different species having similar colony morphologies might have been missed. Then, the phenotypic and genotypic analysis of the pure cultures were performed.

Amplification and sequencing of 16S rRNA genes

The genomic DNA of the isolates was extracted by QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommended method. The 16S rRNA genes were amplified using the universal primers 16F27 (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 16R1488 (5′-CGG TTA CCT TGT TAG GAC TTC ACC-3′). A PCR cycler (Techne, Staffordshire, UK) was used for amplification. Amplification reactions contained each primer (2.5 µL), dNTPs (10 mM, 8 µL), PCR buffer (5 µL), $MgCl_2$ (25 mM, 2.5 µL), template DNA (1 µL), Taq DNA polymerase [(0.5 µL), (Thermo Fisher Scientific, Vilnius, Lithuania)], dH_2O (28 µL), in a final volume of 50 µL [18]. The following conditions were used in the amplification of 16S rRNA gene: 95°C for 5 min, followed by 25 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 2 min, with final 10 min extension at 72°C. The PCR products were then checked on agarose gel with ethidium bromide staining and purified using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). The 16S rRNA gene sequences were determined by IONTEK Laboratory (Turkey). 16S rRNA gene similarities between isolates and closely related species were determined further using ChromasPro (South Brisbane, Australia) and EzTaxon-e tool (Seoul, Korea) (Table 1) [19].

Phenotypic characterization of the isolates

The phenotypic characterization of bacterial cells was performed according to Gram reaction, pigmentation and cell morphology, growth at different salt concentrations (0, 0.5, 3, 5, 7.5, 10, 12.5, 15, 20 and 25%), growth at different pH (4, 5, 6, 7, 8, 9, 10, 11), growth at different temperatures (4, 28, 35, 37, 40, 45°C), citrate utilization, indole production, methyl red and Voges-Proskauer tests, H_2S production, reduction of nitrate to nitrite, production of N_2, production of NH_3 from peptone, the ability to produce acid from different carbon sources. Acid production from different carbon sources was separately examined using 1% (w/v) lactose, sucrose, D-glucose, D-galactose, D-trehalose, D-melibiose, D-mannose, D-xylose, D-cellobiose, L-arabinose, fructose, maltose, 0.5% (w/v) yeast extract, and 0.001% (w/v) phenol red [9, 20]. All carbon sources and phenol red used in this experiment were obtained from same company (Merck, Darmstadt, Germany).

Enzymatic activities of the moderately halophilic isolates

Catalase activity (hydrogen-peroxide oxidoreductase, EC 1.11.1.6) was determined by adding 3% H_2O_2 to colonies grown on CMI agar medium. The immediate appearance of bubbles was accepted as a positive test result. Oxidase activity (ferrocytochrome-c, EC 1.9.3.1) was examined by transfering colony of the isolate with a sterile loop onto filter paper moistened with oxidase reagent (Merck, Darmstadt, Germany). Occurrence of color change from pink to dark purple in a few seconds indicated positive oxidase activity [9, 20–22]. Amylase activity (4-α-D-glucan glucanohydrolase, EC 3.2.1.1) was detected using CMI agar medium supplemented with 0.5% (w/v) soluble starch. After incubation, the plate was flooded with 0.3% I_2–0.6% KI solution. Clear halos around the colonies indicated starch hydrolysis. The DNase test agar (Merck, Darmstadt, Germany) was used to determine DNase activity (deoxyribonuclease I, EC 3.1.21.1). After incubation, the plate

Table 1: Phenotypic characteristics of the moderately halophilic bacterial isolates.

Characteristics	*Staphylococcus saprophyticus* subsp. *saprophyticus*	*Staphylococcus arlettae*	*Bacillus pumilus*	*Gracilibacillus dipsosauri*	*Salinicoccus roseus*	*Bacillus licheniformis*	*Chromohalobacter beijerinckii*
Isolate numbers	7	6	6	5	3	2	2
Pigmentation	Yellow	Cream	White	White	Pink	White	Cream
Gram staining	+	+	+	+	+	+	–
Cell morphology	Cocci	Cocci	Rod	Rod	Cocci	Rod	Rod
NaCl range (%)	3–12.5	0.5–15	3–15	0.5–20	0.5–25	3–12.5	3–25
Optimum NaCl (%)	10	10	10	10	10	10	7.5–10
Temperature range (°C)	20–40	20–40	20–40	20–45	20–40	20–45	4–40
Optimum temperature (°C)	37	37	37	37	37	37	30–37
pH range	6–8	6–8	6–8	5–9	5–9	6–10	5–9
Optimum pH	7.5	7.5	7.5	7.5	7.5	7	7.5
Endospore formation	–	–	+	+	–	+	–
Flagella	–	–	+	+	–	+	+
Production of indole	–	–	–	–	–	–	–
Citrate utilization	–	–	+	–	–	+	+
Methyl-red test	+	+	–	+	–	+	+
Voges-Proskauer test	–	–	+	–	–	+	–
Production of H_2S	–	–	–	–	–	–	–
Nitrate reduction	–	–	–	+	+	+	+
Production of N_2	–	–	–	–	+	–	–
Production of NH_3	+	–	+	+	+	+	+
Production of acid from different carbon energy sources							
Sucrose	+	+	+	+	–	+	–
D-glucose	+	+	+	+	–	+	+
D-galactose	+	–	+	–	–	+	+
Fructose	+	+	+	+	–	+	–
D-trehalose	+	+	+	+	–	+	+
D-melibiose	–	+	–	+	–	+	+
D-mannose	–	+	+	+	–	+	+
D-xylose	–	+	+	+	–	+	+
Lactose	+	–	+	+	+	–	–
Maltose	+	+	–	+	–	–	–
L-arabinose	–	+	+	+	–	+	+
D-cellobiose	–	–	+	–	–	+	–
Hydrolytic activities							
Catalase	+	+	+	+	+	+	+
Protease	+	+	+	+	+	+	–
Lipase	+	–	+	+	+	+	–
B-galactosidase	+	–	+	+	–	+	–

Table 1 (continued)

Characteristics	*Staphylococcus saprophyticus* subsp. *saprophyticus*	*Staphylococcus arlettae*	*Bacillus pumilus*	*Gracilibacillus dipsosauri*	*Salinicoccus roseus*	*Bacillus licheniformis*	*Chromohalobacter beijerinckii*
Oxidase	–	–	+	+	+	+	+
Urease	+	+	–	–	–	–	–
Caseinase	–	–	+	–	+	+	–
Amylase	–	–	–	+	+	+	–
Lecithinase	–	–	–	–	–	–	–
Cellulase	–	–	–	–	–	+	–
Pullulanase	–	–	–	–	–	–	–
Xylanase	–	–	–	–	–	–	–
Phospholipase	–	–	–	–	–	–	–
DNase	–	–	–	–	–	–	–
	Staphylococcus xylosus	*Halomonas eurihalina*	*Staphylococcus equorum* subsp. *equorum*	*Halomonas zhanjiangensis*	*Halomonas venusta*	*Chromohalobacter canadensis*	
Isolate numbers	2	2	1	1	1	1	Positive isolates (%)
Pigmentation	Yellow	Cream	Cream	Yellow	Cream	White	
Gram staining	+	–	+	–	–	–	
Cell morphology	Cocci	Rod	Cocci	Rod	Rod	Rod	
NaCl range (%)	3–12.5	0.5–25	0.5–15	3–20	3–15	3–20	
Optimum NaCl (%)	10	10	10	7.5–10	10	7.5–10	
Temperature range (°C)	20–40	4–45	20–40	4–40	20–40	20–45	
Optimum temperature (°C)	37	37	37	30	37	30–37	
pH range	6–8	5–9	6–8	6–10	6–10	5–9	
Optimum pH	7	7	7.5	7.5	7.5	7.5	
Endospore formation	–	–	–	–	–	–	33
Flagella	–	+	–	+	+	+	51
Production of indole	–	–	–	–	–	+	3
Citrate utilization	–	–	–	+	+	–	31
Methyl-red test	+	+	+	–	+	+	74
Voges-Proskauer test	–	–	–	–	–	–	21
Production of H_2S	–	+	–	–	–	–	5
Nitrate reduction	+	+	+	+	+	+	51
Production of N_2	–	–	–	–	–	–	8
Production of NH_3	+	+	+	+	+	+	85
Production of acid from different carbon energy sources							
Sucrose	+	+	+	+	+	–	85
D-glucose	+	+	+	+	+	+	92
D-galactose	+	+	–	–	–	+	56

Table 1 (continued)

	Staphylococcus xylosus	*Halomonas eurihalina*	*Staphylococcus equorum* subsp. *equorum*	*Halomonas zhanjiangensis*	*Halomonas venusta*	*Chromohalobacter canadensis*	
Fructose	+	–	–	+	–	–	74
D-trehalose	+	+	+	+	+	–	90
D-melibiose	–	–	+	–	–	+	44
D-mannose	+	+	+	+	+	+	74
D-xylose	+	–	+	+	+	+	69
Lactose	+	+	+	+	–	+	72
Maltose	+	+	–	+	–	+	62
L-arabinose	+	+	+	+	–	+	72
D-cellobiose	–	+	–	–	–	+	28
Hydrolytic activities							
Catalase	+	+	+	+	+	+	100
Protease	+	+	+	–	–	–	87
Lipase	–	+	–	–	–	–	64
B-Galactosidase	+	–	+	–	–	–	59
Oxidase	–	+	–	+	+	–	56
Urease	+	+	+	–	–	–	46
Caseinase	–	–	–	–	–	–	28
Amylase	–	–	–	–	–	–	26
Lecithinase	+	–	+	–	–	–	8
Cellulase	–	–	–	–	–	–	5
Pullulanase	–	–	–	–	–	–	–
Xylanase	–	–	–	–	–	–	–
Phospholipase	–	–	–	–	–	–	–
DNase	–	–	–	–	–	–	–

was flooded with 1N HCl. Clear zones around the colonies showed hydrolysis of DNA [8, 21, 22]. The cellulose medium agar plate containing 0.2% (w/v) carboxymethyl cellulose was used to detect production of cellulase (4-β-D-glucan cellobiohydrolase, EC 3.2.1.91). After incubation, 0.1% congo red test reagent (Merck, Darmstadt, Germany) was flooded on the colonies and left for 30 min. Then, the colonies were washed with 1 M NaCl solution. Clear zones around the colonies showed cellulase activity [21–23]. Hydrolysis of casein was tested with the Plate Count Agar medium containing 2% skim milk. After incubation, clear zones around the colonies were interpreted as caseinase production (caseinolytic protease, EC 3.4.21.92) [9, 21, 22]. Lipase activity (triacylglycerol acylhydrolase, EC 3.1.1.3) was screened on Tween 80 agar medium containing 1% (w/v) Tween 80 (Merck, Darmstadt, Germany). After incubation, opaque zones around the colonies were accepted as evidence of lipase activity [21–23]. Due to the presence of fats on the skins, lipase activity of the isolates was also tested in agar medium containing 5% (w/v) butter. After incubation, 20% cupper-sulfate solution was flooded onto the plates. Positive bluish green colonies were interpreted as phospholipase activity (phosphatidylcholine 1-acylhydrolase, EC 3.1.1.32) [21, 22, 24, 25]. Protease activity (gelatinase A, EC 3.4.24.24) was screened on gelatin agar medium containing 2% gelatin (w/v). After incubation, the plate was flooded with Frazier solution. Clear zones around the colonies were interpreted as positive protease activities [8, 21, 22]. Urease activity (urea amidohydrolase, EC 3.5.1.5) was detected on Christensen Urea Agar (Difco, Detroit, USA). After growth was obtained, the tube was examined for pink or red color changes [21, 22, 26]. To detect pullulolytic (pullulan 6-α-glucanohydrolase, EC 3.2.1.41) and xylanolytic (4-β-D-xylan xylanohydrolase, EC 3.2.1.8) activities of the isolates, plates containing the chromogenic substrates such as azurine-cross-linked (AZCL)-pullulan and AZCL-xylan (Megazyme, Wicklow, Finland) were used. Clear zones around the colonies were accepted as positive pullulolytic and xylanolytic activities [8, 21, 22]. Lecithinase activity (phosphatidylcholine 2-acylhydrolase, EC 3.1.1.4) was investigated on lecithine agar plate containing 5% egg yolk (w/v). Opaque zones around the colonies showed lecithinase activity [21, 22, 24, 25]. Beta-galactosidase activity (β-D-galactoside galactohydrolase, EC 3.2.1.23) was detected in test tubes containing 1 mL of 10% NaCl (w/v) sterile distilled water and ONPG (*ortho*-nitrophenyl-β-galactoside) discs (Sigma-Aldrich, Buchs, Switzerland). The formation of yellow color was accepted as positive β-galactosidase activity [21–23]. The pH of all media was adjusted to 7.5. The salt mixture (SW10) was used in all biochemical test media.

Results

The 16S rRNA the pairwise sequence similarities of the isolates were found as 98.9–100% for the isolates belonging to *Firmicutes* and 99–99.9% for the isolates belonging to *Proteobacteria* [25]. While four different genera [*Staphylococcus* (16 isolates), *Bacillus* (8 isolates), *Gracilibacillus* (5 isolates) and *Salinicoccus* (3 isolates)] were determined in *Firmicutes*, two different genera [*Halomonas* (4 isolates) and *Chromohalobacter* (3 isolates)] were found in *Proteobacteria* (Table 1).

Goat skin samples used in this study were cured with each country's preservation salt. Different species of moderately halophilic bacteria may be found in these salts. Hence, every country's goat skin samples may have different moderately halophilic bacterial species. To verify this, we collected salted goat skin samples belonging to eight different countries. Our study demonstrated that the isolate numbers, presence and prevalence of different moderately halophilic bacterial species on the goat skin samples showed differences according to countries. While the goat skins belonging to Africa contained five different species, goat skin samples belonging to Israel and Russia contained only one species. The other skin samples contained a few species (Table 2).

While all isolates were catalase positive, more than half of the isolates were oxidase positive. It has been known that catalase and oxidase enzymes are related with aerobic microorganisms. A few bacteria produced indole from tryptophan; fermented glucose and produced acetoin and 2,3 butanediol in Voges-Proskauer test; formed H_2S and N_2 gases. More than half of the isolates catabolized glucose and produced acidic end products; used nitrate as a terminal electron acceptor and reduced nitrate to nitrite; produced NH_3 from peptone broth. Ammonia odor released from the goat skin samples was related to protein catabolism. While 85%, 92%, 56%, 74%, 90%, 74%, 69%, 72%, 62% and 72% of the isolates respectively produced acid from sucrose, D-glucose, D-galactose, fructose, D-trehalose, D-mannose, D-xylose, lactose, maltose and L-arabinose, acid productions from D-melibiose and D-cellobiose were detected as 44% and 28%, respectively. Thirty one percent of the isolates utilized citrate as a sole carbon source for their energy needs (Table 1).

While all isolates produced catalase, 87%, 64%, 59%, 46%, 28%, 26%, 8%, 5% of the isolates produced protease, lipase, β-galactosidase, urease, caseinase, amylase, lecithinase and cellulase, respectively (Table 1).

Some of the isolates exhibited combined enzymatic activities. While 5%, 36% and 31% of isolates produced eight, six and five different enzymes, respectively, 15%

Table 2: The presence and prevalence of different moderately halophilic bacterial species on the goat skin samples.

Isolate numbers	Phylogenetically similar to	Country origins and codes of the goat skin samples								Prevalence of different bacterial species on the skins
		Turkey * E * KE *T *YE	Australia * AL * AVT	Africa * KT * K *NE *GK	Bulgaria * B * BLG *BT	China *CCN *CN *CC	Israel * IRL	Russia *RU *RA	France *F *FR *FN *FS	
7	*S. saprophyticus* subsp. *saprophyticus*	+	–	–	–	–	–	–	+	2
6	*S. arlettae*	–	–	+	+	+	–	–	–	3
6	*B. pumilus*	+	–	+	–	+	–	–	–	3
5	*G. dipsosauri*	–	–	+	–	–	–	+	–	2
3	*S. roseus*	–	+	–	–	–	–	–	+	2
2	*B. licheniformis*	–	–	–	–	+	+	–	–	2
2	*S. xylosus*	–	–	–	+	–	–	–	–	1
2	*C. beijerinckii*	–	–	–	+	–	–	–	–	1
2	*H. eurihalina*	–	+	–	–	–	–	–	–	1
1	*S. equorum* subsp. *equorum*	–	–	+	–	–	–	–	–	1
1	*H. zhanjiangensis*	–	–	+	–	–	–	–	–	1
1	*H. venusta*	+	–	–	–	–	–	–	–	1
1	*C. canadensis*	–	–	–	+	–	–	–	–	1
Total numbers of different species for each country		**3**	**2**	**5**	**4**	**3**	**1**	**1**	**2**	

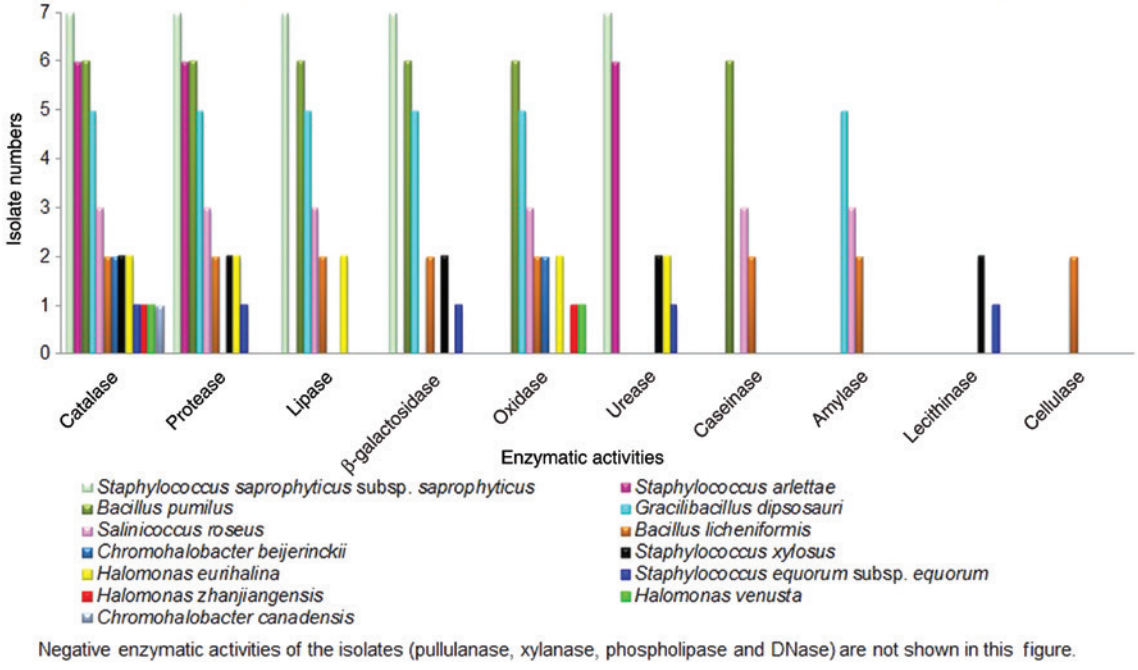

Figure 1: Comparison of enzymatic activities of moderately halophilic bacterial isolates.

of isolates produced three different enzymes. Furthermore, 10% and 3% of the isolates produced two and one enzymes (Figure 1). In the present study, isolates exhibiting most combined activities were belong to the genera *Bacillus*, *Staphylococcus*, *Gracilibacillus*, *Salinicoccus* and *Halomonas*. Among the isolates *B. licheniformis* produced the highest number of enzymes (Figure 1). In order to characterize these enzymes and determine their biochemical properties, more detailed investigation is currently under way.

Discussion

Production of lipase, pullulanase, amylase, protease, xylanase, inulinases, pectinase, cellulases and DNase enzymes by moderately halophilic bacteria (*Salicola, Salinicoccus, Marinococcus, Salinivibrio, Virgibacillus, Oceanobacillus, Thalassobacillus, Halovibrio, Halobacillus, Piscibacillus, Halomonas, Gracilibacillus, Bacillus, Chromohalobacter*) which were isolated from various saline environments in Spain and Howz Soltan playa (a hypersaline lake) were stated in the previous studies [5, 8]. The enzymatic activities of our test isolates were similar to the enzymatic activities of the same species previously identified [27–44].

Catalase enzymes are used in textile industry as a bleaching agent and elimination of hydrogen peroxide in dairy industry [45, 46]. Our all isolates produced this enzyme. Proteases are used in removing hair from hides, leather processing, laundry, detergent production, cheese production, softening meat, improving wool quality [47]. The species of *Bacillus licheniformis*, *Bacillus pumilus*, *Gracilibacillus dipsosauri*, *Halomonas eurihalina*, *Staphylococcus arlettae*, *Staphylococcus equorum* subsp. *equorum*, *Staphylococcus saprophyticus* subsp. *saprophyticus*, *Salinicoccus roseus* and *Staphylococcus xylosus* were able to produce protease in this study. Lipases are used in dairy industry for hydrolysis of milk fat, removal of subcutaneous fat in leather industry, biosysnthesis of drugs in pharmaceutical industry [47]. *Bacillus licheniformis, Bacillus pumilus*, *Gracilibacillus dipsosauri*, *Halomonas eurihalina, Salinicoccus roseus* and *Staphylococcus saprophyticus* subsp. *saprophyticus* were able to produce lipase (Table 1). Proteases and lipases are notably important because they are mainly used in bating, soaking, degrasing, tanning and final stages of leather product. Hence, salt-tolerant enzymes produced by moderately halophilic bacteria are good candidates for leather industry [48]. The enzyme β-galactosidase, produced by *Bacillus licheniformis*, *Bacillus pumilus*, *Gracilibacillus dipsosauri*, *Staphylococcus equorum* subsp. *equorum*, *Staphylococcus saprophyticus* subsp. *saprophyticus* and *Staphylococcus xylosus*, in this study, may be used in the synthesis of galacto-oligosaccharides from lactose [49]. In the present study, moderately halophilic *Halomonas eurihalina*, *Staphylococcus arlettae*, *Staphylococcus equorum* subsp. *equorum*, *Staphylococcus saprophyticus* subsp. *saprophyticus* and *Staphylococcus xylosus* showed positive urease activity. Microbial ureases are used for wine production to remove urea [50]. Caseinase plays an essential role in degrading casein found in milk [26]. Caseinase activity was seen in *Bacillus licheniformis, Bacillus pumilus* and *Salinicoccus roseus*. Amylases play a vital role in starch hydrolysis, food, textile, paper and pulp industry, bread and baking, detergent, pharmaceutical [47, 51]. In the present study, *Bacillus licheniformis*, *Gracilibacillus dipsosauri* and *Salinicoccus roseus* produced amylase. *Staphylococcus equorum* subsp. *equorum* and *Staphylococcus xylosus* in the present study were capable of producing lecithinase enzyme that hydrolysis lecithine. In our study, only moderately halophilic *Bacillus licheniformis* produced cellulase enzyme which may be used in food, chemical, textile, feed, paper and detergent industries, biomedical science and agriculture [47]. In accordance with data from previous studies that investigated enzymatic studies, members of genera *Bacillus, Gracilibacillus*, *Halomonas, Salinicoccus*, *Salinivibrio* and *Staphylococcus* were known to secrete extracellular enzymes such as protease, lipase, amylase, urease [5, 8, 51]. None of our moderately halophilic isolates produced pullulanase, xylanase, phospholipase and DNase in the present study (Table 1).

In the present study, moderately halophilic bacteria were especially isolated from goat skin samples belonging to different countries. Presence of moderately halophilic bacteria on all salted goat skins was closely related with the preservation salt used in the curing of goat skin. It has been known that salted goat skin containing fats, proteins, carbohydrates and blood offers an ideal saline environment for growth of moderately halophilic bacteria and production of hydrolytic enzymes. Our biochemical test results proved that all isolates were able to utilize a wide variety of organic compounds and carbon sources. These isolates produced different enzymes such as protease, catalase, lipase, β-galactosidase, urease, caseinase, amylase, lecithinase and cellulase. These enzymes may have a wide range of potential applications in different industries such as baking, beverage, dairy, dry cleaning, feed, food, laundry, meat, paper, pharmaceutical, starch and textile.

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