Examination of Gram Positive Bacteria on Salt-Pack Cured Hides

by

Emel Aslan[1] And Meral Birbir[2*]

[1]*University of Waterloo, Department of Biology, 200 University Avenue West,*
Waterloo, Ontario, Canada N2L 3G1

[2]*Marmara University, Faculty of Science and Letters, Department of Biology,*
34722 Goztepe, Istanbul, Turkey

Abstract

Salt-pack curing method has been a widely used hide preservation method in worldwide. In order to evaluate the efficiency of the salt-pack curing process on the hides, Gram-positive bacteria on the hides were characterized and their proteolytic and lipolytic activities were examined. Salt-pack cured hides examined were collected from different tanneries in Leather Organized Tannery Region, Tuzla-Istanbul, Turkiye and 40% of the hides were imported from abroad. A total of 396 Gram-positive bacteria comprising from 12 different genera (*Aerococcus, Aneurinibacillus, Bacillus, Brevibacillus, Enterococcus, Geobacillus, Kocuira, Lactococcus, Paenibacillus, Streptococcus, Staphylococcus* and *Virgibacillus*) and 47 bacterial species were isolated and identified from the hides. The total numbers of proteolytic, lipolytic and both proteolytic and lipolytic isolates on the hides were found as 278, 274 and 226, respectively. The most common Gram-positive genera on the salted hides were *Staphylococcus* (115 isolates), *Bacillus* (111 isolates) and *Enterococcus* (75 isolates). *Bacillus* and *Staphylococcus* isolates showed both proteolytic and lipolytic activities in the highest number on the hides. The results verified that the salt-pack curing method was not efficient in preserving the raw cattle hides. As a conclusion, since the salt-pack curing method were not applied adequately, a wide variety of Gram- positive bacterial species were isolated from the salt-pack cured hides. Therefore, the salt-pack curing method should be modified to inactivate Gram-positive bacteria found on the salt-pack cured hide samples.

Resumen

La salazón en pilas de pieles ha sido el método más difundido en el mundo para la preservación de las mismas. En orden de evaluar la efectividad del proceso de salazón por pilas de pieles, las bacterias Gram positivas sobre las pieles fueron caracterizadas y sus actividades proteolíticas y lipolíticas examinadas. Pieles así curadas fueron recolectadas desde distintas curtiembres pertenecientes a la región curtidora organizada de Tuzla-Istanbul, Turquía, con un 40% de las pieles fueron importadas del exterior. Un total de 396 bacterias Gram positivas representando 12 diferentes géneros (*Aerococcus, Aneurinibacillus, Bacillus, Brevibacillus, Enterococcus, Geobacillus, Kocuira, Lactococcus, Paenibacillus, Streptococcus, Staphylococcus,* y *Virgibacillus*) y 47 especies bacterianas fueron aisladas e identificadas de las pieles. El número total de muestras proteolíticas, lípolíticas, combinado lipolítico/proteolítico, aisladas e identificadas fueron 278, 274 y 226 respectivamente. Los géneros más comunes de Gram positivo en las pieles saladas fueron los *Staphylococcus* (115 aisladas), *Bacillus* (111 aisladas) y *Enterococcus* (75 aisladas). Muestras de *Bacillus* y *Staphylococcus* de las pieles exhibieron actividades proteolítica tanto cómo lipolítica elevadas. Los resultados verificaron que la salazón en pilas no es un método efectivo para la preservación de pieles crudas. Cómo conclusión, ya que el método de salazón por apilado no fue adecuado, una gran variedad de especies de bacterias Gram-positivas fueron aisladas en las pieles curadas por salazón en pilas. El método de salazón en pilas entonces debe ser modificado tal que inactive las bacterias Gram-positivas encontradas en las muestras de piel.

*Corresponding author e-mail:mbirbir@marmara.edu.tr
Manuscript received March 30, 2011, accepted for publication August 12, 2011.

INTRODUCTION

Normal flora of animal skin harbor resident or transient populations of bacteria composed of Gram-positive and Gram-negative bacteria.[1] Differential-staining procedure called as Gram stain is commonly used to distinguish bacteria as Gram-positive and Gram-negative cells.[2] The difference between these organisms is the cell wall structure. The Gram-negative bacterial cell wall is a complex multilayered structure, whereas Gram-positive bacterial cell wall consists of almost a single type of molecule called as peptidoglycan.[3] Skin is a dry area compared to the other parts of animal body. Hence, normal flora of skin consists of Gram- positive *Bacteria* which is adapted to live in dry conditions. Gram-positive *Bacteria* such as *Staphylococcus* and *Streptococcus* are the most common and stable resident microorganisms on the skin. When the animal is alive, skin prevents the penetration of bacteria to inner layers.[1,2] After the animal is slaughtered, microorganisms may penetrate into animal tissues and multiply. Unchecked growth of these bacteria on the hides results in hide damage.[4-5] It is known that most of bacterial infection on skin are caused by Gram-positive *Bacteria* that produce hydrolytic enzymes.[1,2] Different species of microorganisms grown on the hides may cause microbiological defects such as hair slip, discoloration, serious grain peeling, fiber destruction, odor, looseness, weakness and holes in leather.[5-15] Bacteria on the raw hides may penetrate through the corium from the flesh surface in 8-12 hours and serious grain peeling and voids in hide may be formed by bacteria in 15 to 24 hours.[5]

Although salt-packed curing method has been used commonly in preservation of the hides in most of countries, it may not control the bacterial growth adequately on the hides. Salt-tolerant bacteria and halophilic microorganisms may grow on the salt-pack cured hides, and salt tolerance of these bacteria may be related with compatible solutes. The bacteria synthesize compatible solutes in the medium with low water activity. Glycine betaine, proline (mainly Gram-positive), glutamate (mainly Gram- negative), sucrose, trehalose, a-glucosylglycerol, mannitol, various glycosides, dimetil sulfoniopropionate, KCl, ectoine, glycerol are several compatible solutes produced by different microorganisms.[1-2] It is known that some of the bacterial species are much more capable than the others growing in the salted foods and hides with low moisture or high osmotic pressures. Different microorganisms can tolerate different ranges of water potential. Especially, Gram-positive *Staphylococcus* and *Streptococcus* are fairly resistant to reduced water potential and tolerate drying and high salt.[1-2] It has been mentioned that genus *Staphylococcus* is relatively halotolerant and use the amino acid proline as a compatible solute.[1] Strains of *Staphylococcus* resistant to antimicrobial agents have become more common in hospitals and different industries.[2]

Researchers found that species belong to genera *Bacillus* and *Staphylococcus* were able to grow on the salted hides.[16-17] *Bacillus* are common in soil while *Staphylococcus* are found on the skin and mucous membranes of warm blooded animals.[2] In our previous study, *Bacillus brevis*, *Bacillus cereus*, *Bacillus firmus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus megaterium, Bacillus pumilis*, *Bacillus sphaericus*, *Kurthia variabilis*, *Micrococcus candidus*, *Micrococcus luteus*, *Micrococcus roseus*, *Micrococcus rubens*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were isolated and identified from 1 week old and 2 monhts old salted hides.[4] Orlita isolated 100 different isolates of Bacteria from the salted hides, 36 of which have been identified as halophilic cocci and the remaining 64 as either Gram- positive and negative rods.[18]

Our previous studies showed that the non-halophilic bacteria settled on the inadequately salt-pack cured hides produced proteases and lipases which may degrade hide proteins and lipids.[19] These bacteria may break down the tissue-supporting collagen network and lipids and diffuse into the hide. Polkade proved that *Bacillus cereus* shattered the corium layer completely.[20] Proteolytic and lipolytic Gram-positive bacteria on the salt-pack cured hides will damage to the hide by causing an important economic losses. To know the flora of Gram positive *Bacteria* on the salt-pack cured hides will help us to find the effective inactivation methods which increase the quality of the leather. Therefore, we wanted to know which species of Gram positive *Bacteria* are commonly found on the salt-pack cured hides, to examine their proteolytic and lipolytic activities and evaluate the efficiency of salt- pack curing methods applied in different countries in this study.

EXPERIMENTAL

In the present study, 10 salt-pack cured hides were collected from different tanneries in Leather Organized Tannery Region, Tuzla-İstanbul, Turkiye. Forty percent of the salted hides examined (Hide samples 1-4) were imported from different countries such as England and Australia. The rest of the hides (Hide Samples 5-10) were salt-pack cured hides in Turkiye. The hide samples were immediately placed into sterilized sample bags and containers and they were carried in ice boxes during the transportation. Before the experiments, all of the salt–pack cured hide samples were cleaned thoroughly of hair, fat and dirt. Then, the hide samples were cut into small pieces.

Isolation and Identification of *Bacillus* Species

A total of 10 gr of each salt-pack cured hides was separately placed in a flask containing 90 ml 0.85% sterilized physiological saline solution. The flask was placed in a shaking incubator (Edmund Bühler, Germany) for half an hour at 25°C in 100 rpm. 0.1 ml aliquots of direct and serial

dilutions (10^{-2}, 10^{-4}, 10^{-6}) of the bacterial suspensions were spreaded onto the agar plates containing nutrient agar. After the incubation at 37°C for 24-48 hours, characteristic colonies of the bacteria belonging to genus *Bacillus* were picked up and restreaked several times onto nutrient agar to obtain pure culture. A variety of tests including Gram stain, endospore staining (examination of sporangia and spore position), Voges-Proskauer test, citrate utilization test, catalase, lipase, gelatinase, amylase and caseinase activities, nitrate reduction and gas formation, carbohydrate fermentation tests (lactose, glucose, D-mannit, galactose, maltose and sucrose) were performed for identification of *Bacillus* species. All biochemical tests were accomplished according to earlier described procedures.[21-22] Furthermore, the API 50CH test kits (Biomèrieux, France) were used to characterize the isolates belonging to genus *Bacillus*. The isolates were grown on blood agar at 37°C for 24 hours and suspended in sterilized saline solutions (0.85 % NaCl) to adjust the density of the bacterial cultures to McFarland No. 2 as described in the manufacturers' instructions. The culture dilutions were then loaded to the test strips. These test strips were incubatedat 37°C for 24-48 hours. The results of all biochemical tests were read and evaluated after the incubation period.[23]

Isolation and Identification of *Staphylococcus* Species

0.1 ml aliquots of direct and serial dilutions (10^{-2}, 10^{-4}, 10^{-6}) of the bacterial suspensions were spreaded onto the agar plates containing Baird Parker RPF (Biomèrieux) agar. After the incubation at 37°C for 24 hours, characteristic colonies of the bacteria belonging to genus *Staphylococcus* were picked up and restreaked several times onto Baird Parker RPF (Biomèrieux) agar (R1+R2) to obtain pure culture. A variety of tests including Gram stain, Voges-Proskauer test, citrate utilization test, oxidase, DNase, urease, catalase, lipase and gelatinase activities, hemolysis patterns on blood agar, nitrate reduction and gas formation, carbohydrate fermentation tests (lactose, glucose, D-mannit, galactose, maltose and sucrose) were performed to identify *Staphylococcus* species. All biochemical tests were carried out according to earlier described procedures.[21-22] Moreover, the API Staph test kits (Biomèrieux, France) were used to characterize the isolates belonging to genus *Staphylococcus*. The isolates were grown on blood agar at 37°C for 24 hours and suspended in sterilized saline solutions (0.85 % NaCl) to adjust the density of the bacterial cultures to McFarland No. 0.5 as described in the manufacturers' instructions. The culture dilutions were then loaded to the test strips. These test strips were incubated at 37°C for 18-24 hours. The results of all biochemical tests were read and evaluated after the incubation period.[24]

Isolation and Identification of *Streptococcus* Species

0.1 ml aliquots of direct and serial dilutions (10^{-2}, 10^{-4}, 10^{-6}) of the bacterial suspensions were spreaded onto the agar plates containing D-coccosel agar (Biomèrieux). After the incubation at 37 °C for 24 hours, characteristic colonies of the bacteria belonging to genera *Streptococcus* and *Enterococcus (*group D- streptococci (enterococci)) were picked up and restreaked several times onto D-coccosel (Biomèrieux) agar to obtain pure cultures. A variety of tests including Gram stain, catalase activity, growth on 6.5% NaCl, hemolysis patterns on blood agar, esculin hydrolysis, Voges-Proskauer test, carbohydrate fermentation tests (lactose, glucose, D-mannit, galactose, maltose and sucrose), amylase, gelatinase and lipase activities, nitrate reduction and gas formation were performed for identification of *Streptococcus* and *Enterococcus* species. All biochemical tests were carried out according to earlier described procedures.[21-22] In addition to conventional biochemical tests, the API 20 Strep test kits (Biomèrieux, France) were used to characterize the isolates belonging to genera of *Streptococcus* and *Enterococcus*. The isolates were grown on D-coccosel agar at 37°C for 24 hours and suspended in 2 ml API GP medium to adjust the density of the bacterial cultures to McFarland No. 4 as described in the manufacturers' instructions. The culture dilutions were then loaded to the test strips. These test strips were incubated at 37°C for 4 hours and 24 hours. The results of all biochemical tests were read and evaluated after the incubation periods.[24]

Determination of protease activity

Proteolytic activity of the isolates was screened on gelatine agar medium containing triptone, 10 g; yeast extract, 2.5 g; D-mannit, 10 g; K_2P0_4, 5 g; gelatine, 30 g; ammonium sulfate 75 g; sodium hydroxide (10%), 6 cc; sodium chloride, 5 g; agar 20 g and distilled water, 1000 ml. After the incubation at 37°C for 7 days, clear zones around the colonies were taken as evidence of protease activity.[21]

Determination of lipase activity

Lipolytic activity of the isolates was examined on agar medium containing peptone, 10 g; sodium chloride, 5 g; $CaCl_2$, 0.1 g; 10 g, Tween 80; agar, 15 g and distilled water, 1000 ml. After the incubation at 37°C for 24-48 hours, clear zones around the colonies were taken as evidence of lipase activity.[21]

RESULTS AND DISCUSSION

Four hide samples were imported from England (HS1-2) and Australia (HS3-4). Six hide samples were obtained from different tanneries in Leather Organized Tannery Region, Tuzla-İstanbul, Turkiye (HS5-10). The temperatures of the tanneries where the hide samples were collected were between 14 and 30°C. It was found that all of the hides contained a wide variety of Gram-positive bacterial species in varying frequencies. Total numbers of different species of Gram-positive bacteria on the hides were between 17 and 31. While the hide sample 5 contained 31 different species of Gram-positive bacteria, the hide samples 2, 7 and 9 contained 17 different species of Gram-positive bacteria. In addition to

these different species, the hide sample 5 contained the highest number of the bacterial isolates (55) (Table I). In all, 12 genera and 47 species comprising 396 Gram positive bacteria were isolated and identified from the hide samples (Table I). *Staphylococcus* (115 isolates), *Bacillus* (111 isolates) and *Enterococcus* (75 isolates) were found as the most common genera of Gram positive bacteria on the hide samples examined.

Staphylococcus was found as the most common genus on the salt pack-cured hides and the genus also contained many different species. A total of 14 different species of genus *Staphylococcus* containing 115 isolates were isolated and identified from the hide samples. All of the hide samples contained *Staphylococcus intermedius* (32 isolates) whereas *Staphylococcus warneri, Staphylococcus epidermidis* and *Staphylococcus lentus were* isolated from the only 1 hide sample (Table I). The occurrence of *Staphylococcus* species in high numbers on the hides was thought to be related with their resistance to reduced water potential and drying. Most species of *Staphylococcus* grow in the presence of high NaCl concentrations. Skin, skin glands and mucous membranes of warm blooded animals harbor genus *Staphylococcus*. They are tolerant of desication, radiation and heat.[2] Staphylococci produce a number of enzymes that help to them survival and pathogenity. Hyaluranidase produced by 90% of *Staphylococcus aureus,* breaks down hyaluronic acid (intercellular matrix) and enables the bacteria to spread between tissue cells. Staphylococci may cause an infection of hair follicle and skin[1-2] Some species of this organism were also isolated from gastrointestinal canals of warm blooded animals, meat, milk, cheese, soil, sand, dust, air or natural waters.[2,23]

A total of 11 different species of genus *Bacillus* containing 111 isolates were isolated and identified from the hide samples. *Bacillus lentus* (15 isolates), *Bacillus licheniformis* (16 isolates) and *Bacillus pumilus* (20 isolates) were isolated from the 9 hide samples while *Bacillus laterosporus* (1 isolates), *Bacillus cereus* (3 isolates) and *Bacillus amyloliquefaciens* (3 isolates) were isolated from a few hide samples examined (Table I). Existence of *Bacillus* species in high numbers on the hides examined may be related with dispersal of these organisms or their endospores from soil to hides by wind, dust, water and feces. It was stated that most species of *Bacillus* are widely distributed in nature and some species are salt tolerant.[1,23] Endospores that are resistant to high concentration of salt, heat, drying, radiation and bactericide are found in *Bacillus* species. Endospor-forming *Bacillus* species are found most commonly in the soil. Also endospores enable to the organism to endure difficult times such as high temperature, drying, low oxygen and nutrient. Furthermore, endospores can live for long periods in a dormant stage in tanneries and can contaminate the hides continuously.

In addition, if salt pack curing process can not be applied homogenously, different parts of the hide contain different salt concentrations. *Bacillus* species containing peritrichous flagella may reach to new suitable parts of the salt-pack cured hides containing low salt and bactericide concentrations, and they can find suitable medium for growth. In our study, *Enterococcus* was found as the third common genus on the salt-pack cured hides. This result confirmed the previous study result. Researchers mentioned that *Enterococci* were salt tolerant.[23] Pathogenic strains of *Enterococcus* may be resistant to frequently used antimicrobials. A total of 6 different species of genus *Enterococcus* containing 75 isolates were isolated and identified from the hide samples. Although *Enterococcus faecium* (10 hide samples) and *Enterococcus avium* (9 hide samples) were the most common species isolated from the hides examined, *Enterococcus casseliflavus* (2 hides) was a less common isolated species. *Enterococcus gallinarum* was isolated from the hide samples 9 and 10 in high numbers (6 isolates). *Enterococcus faecalis* (12 isolates), *Enterococcus faecium* (14 isolates),*Enterococcus avium* (17 isolates) and *Enterococcus gallinarum* (23 isolates) which are residents of the intestinal tract of animals were isolated from the hides and presence of these species on the hides indicated fecal contamination (Table I).

Staphylococcus intermedius (32 isolates), *Aerococcus viridans* (24 isolates) *Enterococcus gallinarum* (23 isolates) and *Bacillus pumilus* (20 isolates) were the most commonly found Gram positive species on the hides examined (Table I).

Although *Aerococcus viridans*, *Enterococcus faecium* and *Staphylococcus intermedius* were isolated from the 10 salt-pack cured hide samples, *Bacillus lentus, Bacillus licheniformis, Bacillus pumilus* and *Enterococcus avium* were found at the 9 hide samples (Table I). *Aerococcus viridans* was isolated from vegetation, outdoor air, dust and human clinical specimens.[25] Also, *Aerococcus viridans* is a common airborne organism in hospital environments and a marine organism causing fatal disease of lobsters.[24] A few species belong to genera of *Aneurinibacillus, Brevibacillus, Geobacillus, Kocuira, Paenibacillus* and *Virgibacillus* were isolated from the hides and their isolate numbers were found as very low (Table I).

Gram-positive bacterial species found on the salt-pack cured hides in the present study were similar to those reported by earlier investigators. In the previous studies, *Arthrobacter protophormiae, Bacillus cereus, Bacillus licheniformis, Bacillus brevis, Bacillus subtilis, Bacillus megaterium, Bacillus mycoides, Bacillus pumilis, Brevibacterium lutescens, Corynebacterium pyogenes, Enterococcus durans, Kurthia gibsonii, Micrococcus roseus, Micrococcus luteus, Micrococcus morrhuae,*

Staphylococcus aureus, Staphylococcus sciuri, Staphylococcus epidermidis, Staphylococcus xylosus and *Staphylococcus hominis* were isolated from the hides. [4, 8, 10,16,17, 20, 26-31]

If the bacteria found on the salt-pack cured hides can not be inactivated with an effective treatment, these bacteria will grow on soak liquor and damage to the hides. *Aerococcus viridans, Enterococcus avium, Enterococcus faecium, Kocuira varians, Lactococcus lactis, Staphylococcus capitis, Staphylococcus cohnii, Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus intermedius, Staphylococcus lugdunensis, Staphylococcus sciuri, Staphylococcus xylosus* and *Staphylococcus warneri* which were isolated from the salt-pack cured hides in the present study were also isolated from main soak liquor treated with bactericide in the our previous study.[32]

TABLE I
Frequencies of various species of Gram positive bacteria isolated from the salt-pack cured hides

	Hide samples										
Genera and species of Gram-positive bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Total**
Genus Aerococcus											**29**
Aerococcus urinae				1	2	1		1			5
Aerococcus viridans	2	1	3	3	3	2	3	3	2	2	24
Genus Aneurinibacillus											***4***
Aneurinibacillus aneurinilyticus	1						1	2			4
Genus Bacillus											**111**
Bacillus amyloliquefaciens		1		1	1						3
Bacillus cereus	1								2		3
Bacillus firmus		1		1	2	2		2			8
Bacillus laterosporus					1						1
Bacillus lentus	1	1	1	2	2	2		2	3	1	15
Bacillus licheniformis	1	1		1	1	2	2	3	3	2	16
Bacillus megaterium	1		1		1	3	2			2	10
Bacillus mycoides			1		1	1		1			4
Bacillus pumilus	1	3	1	1	3	2	1		5	3	20
Bacillus subtilis		1	1		3	1		1	4	3	14
Bacillus thuringiensis					2	2	5	3	2	3	17
Genus Brevibacillus											**2**
Brevibacillus laterosporus			1			1					2
Genus Enterococcus											**75**
Enterococcus avium	2	2	2	2	1	3	2		2	1	17
Enterococcus casseliflavus			2	1							3
Enterococcus durans	1	1					1		2	1	6
Enterococcus faecalis	2	3	1	1	2		2	1			12
Enterococcus faecium	1	1	1	1	1	2	1	3	2	1	14
Enterococcus gallinarum	1		3	4	2	1			6	6	23
Genus Geobacillus											**6**
Geobacillus stearothermophilus							2	1			3
Geobacillus thermoglucosidiasius						2		1			3

TABLE I
Frequencies of various species of Gram positive bacteria isolated from the salt-pack cured hides

	Hide samples										
Genera and species of Gram-positive bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Total**
Genus Kocuira											**8**
Kocuira kristanea				1	1	1				1	4
Kocuira varians				1	1	1				1	4
Genus Lactococcus											**13**
Lactococcus lactis	1	1			3	2		3	2	1	13
Genus Paenibacillus											**3**
Paenibacillus pabuli									2	1	3
Genus Staphylococcus											**115**
Staphylococcus aureus					1		4	2			7
Staphylococcus capitis	2		1	1	2	2		1	4	2	15
Staphylococcus caprae					1			1			2
Staphylococcus chromogenes			1					1			2
Staphylococcus cohnii	1	1		2	2	2	1	3		5	17
Staphylococcus epidermidis			1								1
Staphylococcus hominis			1		1	2			3	2	9
Staphylococcus hyicus			1		3	1				2	7
Staphylococcus intermedius	1	3	3	4	4	4	4	3	4	2	32
Staphylococcus lentus		1									1
Staphylococcus lugdunensis			1					1		1	3
Staphylococcus sciuri		1	1		1						3
Staphylococcus xylosus	1			3	2	3	2		3	1	15
Staphylococcus warneri			1								1
Genus Streptococcus											**27**
Streptococcus acidominimus				4	2	2	2	3			13
Streptococcus bovis				1		1					2
Streptococcus pluranimalium		1			1	1	1				4
Streptococcus thermophilus	1										1
Streptococcus uberis			3	2	2						7
Genus Virgibacillus											**3**
Virgibacillus panthothenticus	1									2	3
Total numbers of Gram-positive isolates	**23**	**24**	**32**	**38**	**55**	**49**	**36**	**42**	**51**	**46**	**396**
Total numbers of Gram-positive species	**19**	**17**	**22**	**21**	**31**	**27**	**17**	**22**	**17**	**23**	**216**

The total numbers and percentages of proteolytic, lipolytic and both proteolytic and lipolytic Gram-positive bacteria isolated from the hides were found as 278 (70.20%), 274 (69.19%) and 226 (57.07%), respectively (Table II). All isolates of *Staphylococcus intermedius, Staphylococcus cohnii, Staphylococcus aureus*, *Staphylococcus hyicus*, *Staphylococcus epidermidis* and *Staphylococcus lentus* showed both protease and lipase activities. The number of lipolytic isolates of genus *Staphylococcus* was higher than that of the proteolytic isolates (Table II). Lipases produced by most

of these microorganisms allow staphylococci grow on the surface of the hide and cutaneous oil gland.[2]

All isolates of *Bacillus pumilus, Bacillus subtilis, Bacillus mycoides* and *Bacillus amyloliquefaciens* showed both protease and lipase activities. A total of 14 isolates of *Bacillus thuringiensis* isolated from the 6 hide samples showed both protease and lipase activities. The number of proteolytic isolates of *Bacillus* was higher than that of the lipolytic isolates. Among the isolates, the highest proteolytic activity was detected at *Bacillus* species (Table II). Our results were consisted with the results of Polkade.[20] Researcher explained that *Bacillus* species isolated from raw buffulo hides showed highest protease and lipase activities.[20] In the present study, the percentages of proteolytic, lipolytic and both proteolytic and lipolytic *Bacillus* isolates were found as 92%, 62% and 60%, respectively (Table III).

We detected both lipase and protease activities in all isolates of *Enterococcus faecalis* and *Enterococcus durans* and 12 isolates of *Enterococcus faecium*. Protease and both protease and lipase activities were detected in 30 isolates of genus *Enterococcus,* whereas 32 isolates of this genus were lipase positive (Table II). All isolates of *Staphylococcus intermedius* isolated from the 10 salt-pack cured hides were protease and lipase positive while 12 isolates of *Enterococcus faecium* were found to be protease and lipase positive. It was found that all isolates of *Bacillus pumilus* isolated from the 9 hide samples were protease and lipase positive.

Several investigators emphasized that efficient decontamination treatments or effective antimicrobials may be used as decontamination processes to reduce microbial populations on cattle hides before removal from carcasses.[33, 34]

Nou and his co-workers proposed that cleaning cattle hides by removing extraneous matter before hide removal will decrease number of bacteria and improve the quality of carcasses in commercial beef processing plants.[33] Small and his colleagues searched the efficacy of different decontamination treatments in reducing microbial populations on cattle hides.[34] Researchers found that treatment of hide with a food industry sanitizer solution (10% Betane Plus) resulted in significant reductions of 1.80 and 1.98 $\log_{10}$ cfu/cm^2 without and with subsequent drying, respectively. They also suggested that treatment of hide with a food industry disinfectant (P3-Topactive DES) significantly reduced total viable counts of bacteria to 0.97 and 1.18 $\log_{10}$ cfu/cm^2 without and with subsequent drying, respectively.[34]

CONCLUSION

The presence of a wide variety of proteolytic and lipolytic Gram-positive bacterial species on the salt-pack cured hides was thought to be associated with contamination of the hide by external sources, and this was a sign that decomposition of the hides might occur. Therefore, hides should be cleaned very well with water and effective antiseptics just before

TABLE II
Total numbers of proteolytic, lipolytic, both proteolytic and lipolytic genera of Gram-positive bacteria isolated from the salt-pack cured hides

Genera of Gram-positive bacteria	Total numbers of isolates	Total numbers of proteolytic isolates	Total numbers of lipolytic isolates	Total numbers of both proteolytic and lipolytic isolates
Aerococcus	29	24	24	24
Aneurinibacillus	4	1	0	0
Bacillus	111	102	69	67
Brevibacillus	2	2	0	0
Enterococcus	75	30	32	30
Geobacillus	6	6	6	6
Kocuira	8	4	4	4
Lactococcus	13	13	11	11
Paenibacillus	3	2	2	1
Staphylococcus	115	71	106	65
Streptococcus	27	20	20	18
Virgibacillus	3	3	0	0
Total	**396**	**278**	**274**	**226**

TABLE III
The percentages of proteolytic, lipolytic, both proteolytic and lipolytic genera of Gram-positive bacteria isolated from the salt-pack cured hides

Genera of Gram-positive bacteria	Proteolytic activity (%)	Lipolytic activity (%)	Both proteolytic and lipolytic activities (%)
Aerococcus	82.76	82.75	82.75
Aneurinibacillus	25.00	0	0
Bacillus	92.00	62.00	60.00
Brevibacillus	100	0	0
Enterococcus	40.00	42.70	40.00
Geobacillus	100	100	100
Kocuira	50.00	50.00	50.00
Lactococcus	100	84.62	84.62
Paenibacillus	66.66	66.66	33.30
Staphylococcus	61.74	92.17	56.52
Streptococcus	74.10	74.10	66.66
Virgibacillus	100	0	0

slaughtering. Furthermore, traditional salt-pack curing method should be modified to inactivate all Gram-positive bacteria on the salt-pack cured hides. Otherwise, the proteolytic and lipolytic Gram- positive bacteria on the salt-pack cured hides can reach considerably high numbers and may cause serious damages on the soaked hides during the soaking process by affecting the hide quality adversely. Antimicrobials containing potassium dimethyl-dithiocarbamate or didecyldimethyl ammonium chloride and low level electric curent treatment may be used Gram-positive bacteria on the salt-pack cured hides.[32,35-36]

ACKNOWLEDGEMENTS

This work was supported by the Scientific Research Project Commission of Marmara University, Project No. FEN-C-YLP-030408-0080. We thank Dr.Didem Berber, Miss Sema Anık and the tanneries in Leather Organized Tannery Region, Tuzla-Istanbul for technical assistance and providing salt-pack cured hide samples for testing.

REFERENCES

1. Madigan, M.T., Martinko, J.M., Dunlop, P.V. and Clarck, D.P.; Brock Biology of Microorganisms, 12th Edition, Pearson Education, Benjamin Cummings, 2009.
2. Bauman, R.W.; Microbiology with Diseases by Taxonomy, 3th Edition, Pearson Education, Benjamin Cummings, 2011.
3. Tortora, G.J.; Funke, B.R. and Case, C.L.; Microbiology an Introduction, 10th Edition, Pearson Education, Benjamin Cummings, 2010.
4. Birbir, M. and Ilgaz, A.; Isolation and Identification of Bacteria Adversely Affecting Hide and Leather Quality. *Journal of the Society of Leather Technologists and Chemists* **80,** 147-153, 1996.
5. Haines, B.M.; Quality Rawstock. *JALCA* **56**, 164-173, 1984.
6. Tancous, J.J., Roddy, T.W. and O'Flaherty, F.; Skin, Hide and Leather Defects. *Tanners Council Laboratory University of Cincinnati*, The Western Hills Publishing Company, 1986.
7. Cordon, T.C., Everett, A.L. and Windus, W.; The Influence of Bacteria on Depilation of Hides by Enzymes. *JALCA* **56**, 164-173, 1961.
8. Roddy, W.T.; Relationship Between Hide Deterioration and Leather Defects Proceedings of the Fifteenth Research Conference, 45-52, 1963.
9. Hendry, M.F., Cooper, D.R. and Woods, D.R.; The Microbiology Curing and Tanning Process, Part I, II, III, VI. *JALCA* **66,** 1970.
10. Venkatesan, R.A., Nandy, S.C. and Sen, S.N.; Effect of Storage and Pretanning Operation on the Bacterial Flora and Its Population on Goat Skin. *Leather Science* **17** (12), 395-404, 1970.
11. Mitchell, W.; Prevention of Bacterial Damage on Brine Cured and Fresh Cattle Hides. *JALCA* **82**, 372, 1987.
12. Bailey, D.G. and Birbir, M.; A Study of the Extremely Halophilic Microorganisms Found on Commercially Brine-Cured Cattle Hides. *JALCA* **88**, 285-293, 1993.

13. Bailey, D.G. and Birbir, M.; The Impact of Halophilic Organisms on the Grain Quality of Brine Cured Hides. *JALCA* **91**, 47-51, 1996.
14. Birbir, M. and Bailey, D.G.; Controlling the Growth of Extremely Halophilic Bacteria on Brine Cured Cattle Hides. *JALCA* **84,** 201-204**,** 2000.
15. Rangarajan, R., Didato, T.D. and Bryant, S.; Measurement of Bacterial Populations in Typical Tannery Soak Solutions by Traditional and New Approaches. *JALCA* **98**, 477-485, 2003.
16. Anderson, H.; The Bacteriology of the Hide Preservation. *Journal of the Society of Leather Trades Chemists* **33,** 251-256 **,** 1949.
17. Woods, D.R., Atkinson, P., Cooper, D.R. and Galloway, A.C.; The Microbiology of Curing And Tanning Processes. Part II. Analysis of Aerobic Bacteria in Static Hide Brining, *JALCA* **65**, 164 **,**1970.
18. Orlita, A.; Microbial Biodeterioration of Leather and Its Control: A Review. *International Biodeterioration & Biodegradation* **53**, 157-163**,** 2004.
19. Berber, D. and Birbir M.; Examination of Bacterial Populations in Salt, Salted Hides, Soaked Hides and Soak Liquors. *JALCA* **105** (10), 320-326, 2010.
20. Polkade, A.V.; Molecular and Phylogenetic Studies on Microbial Diversity of Raw Buffalo Hide to be Used in Leather Manufacturing. *Doctorate Thesis*, University of Pune, Faculty of Science, India, Pune 104, 2007.
21. Harley, J.P. and Prescott, L.M.; Laboratory Exercises in Microbiology, 5th ed., The McGraw-Hill Companies, 2002.
22. Gonzales, C., Gutierrez, C. and Ramirez, C.; *Halobacterium vallismortis* sp. nov. An Amylolytic and Carbohydrate-Metabolizing Extremely Halophilic Bacterium. *Canadian Microbiology* **24,** 710-715 , 1978.
23. Sneath, P.H.A.; Endospore-forming Gram-Positive Rods and Cocci, Section 13, in: Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holt, J.G., *Bergey's Manual of Systematic Bacteriology* **2**, Williams & Wilkins, Baltimore MD 1104-1139, 1986.
24. Schleifer, K.H.; Gram Positive Cocci. Section 12, in: Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holt, J.G., Bergey's Manual of Systematic Bacteriology **2**, Williams&Wilkins, Baltimore, MD 999-1103, 1013-1034,1043-1071,1986.
25. Holzapfel, W.H., Franz, C.M.A.P., Ludwig, W., Back, W. and Dick, L. M.T.; The Genera *Pediococcus* and *Tetragenococcus*. Prokaryotes Chapter 1.2.8. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., Stackebranat, E.; The Prokaryotes-A Handbook on the Biology of Bacteria, 3rd Edition, **4**, Bacteria: *Firmicutes*, *Cyanobacteria*, 229-266, 2006.
26. Dempsey, M.; The Penetration of Bacteria into Hides and Skins. *Journal of the Society of Leather Technologists and Chemists* **53**, 32-35, 1969.
27. Champion, R.H., Gilman, T., Rook, A.J. and Sims, R.T.; An Introduction to the Biology of the Skin. *Blackwell Scientific Publications*, 197-205**,** 1970.
28. Antychowicz, J. and Rogulska, A.; Preliminary Investigations on The Bacterial Flora of the Skin and Erythrodermatitis Ulsers of Cap. *Bulletin of The Veterinary Institute in Pulawy*, 1-45,1985.
29. Kuroczkin, J. and Strzelczyk, B.A.; Studies on the Microbial Degradation of Ancient Leather Bookbindings. *International Biodeterioration* **25** (2), 39-47**,** 1989.
30. Hanlin, M.B., Field, R.A. and Ray, B.; Characterization of Predominant Bacteria in Cattle Hides and Their Control by a Bacteriocin Based Preservative. *JALCA* **90**, 380-320**,** 1995.
31. Shede, P.N., Kanekar, P.P., Polkade, A.V., Dhakephalkar, P.K. and Sarnaik, S.S.; Bacterial Succession On Raw Buffalo Hide and Their Degradative Activities During Ambient Storage. *International Biodeterioration & Biodegradation* **62**, 65-74, 2008.
32. Berber,D.,Birbir,M.andHacioglu,H.;EfficacyAssessment of Bactericide Containing Didecyldimethylammonium Chloride on Bacteria Found in Soak Liquor at Different Exposure Times. *JALCA* **105**, 354-387, 2010.
33. Nou, X.W., Rivera-Betancourt, M., Bosilevac, J.M., Wheeler, T.L., Shackelford, S.D., Gwartney, B.L., Reagan, J.O. and Koohmaraie, M.; Effect of Chemical Dehairing on the Prevalence of *Escherichia coli* 0157:H7 and The Levels of Aerobic Bacteria and *Enterobacteriaceae* on Carcasses in a Commercial Beef Processing Plant. *Journal of Food Protection* **66** (11), 2005-2009, 2003.
34. Small, A., Wells-Burr, B. And Buncic, S.; An Evaluation of Selected Methods for the Decontamination of Cattle Hides Prior to Skinning. *Meat Science* **69** (2), 263-268, 2005.
35. Birbir, M. and Kose, N., The Control of Microorganisms Isolated from the Soak Liquor with Antimicrobial Containing Potassium Dimethyl-Dithiocarbamate, M.U. Scientific Research Project, Project Number, FEN-C-YLP-090409-0077, 2011.
36. Birbir,Y., Uğur, G. and Birbir, M. Inactivation of Bacterial Population in Hide Soak Liquors Via Direct Electric Current, *Journal of Electrostatics*, **6**6 355-360, 2008.