Identification and Metabolic Activities of Bacterial Species Belonging to the *Enterobacteriaceae* on Salted Cattle Hides and Sheep Skins

by

K. Ulusoy[1] and M. Birbir*[2]

[1]*Marmara University, Institute of Pure and Applied Science,*
GOZTEPE, ISTANBUL 34722, TURKEY
[2]*Marmara University, Faculty of Science and Letters, Department of Biology,*
GOZTEPE, ISTANBUL 34722, TURKEY

Abstract

The detailed examination of the *Enterobacteriaceae* on salted hides and skins offers important information to assess faecal contamination of salted hides and skins, its roles in hide spoilage, and efficiency of hide preservation. Hence, salted cattle hide and skin samples were obtained from different countries and examined. Total counts of Gram-negative bacteria on hide and skin samples, respectively, were 10^4-10^6 and 10^5-10^6 CFU/g; of *Enterobacteriaceae* 10^4-10^5 and 10^5-10^6 CFU/g; of proteolytic *Enterobacteriaceae* 10^3-10^5 and 10^5-10^6 CFU/g; of lipolytic *Enterobacteriaceae* 10^2-10^5 and 10^4-10^5 CFU/g; and of each species belonging to *Enterobacteriaceae* 10^2-10^5 and 10^3-10^5 CFU/g. Moisture contents of the hide (between 17% - 29%) and skin samples (between 18% - 39%) were low, and salt saturations of most hide and skin samples were fairly high (100%), but these microorganisms were isolated from the samples in high numbers. Although 16 bacterial species belonging to genera of *Cedecea*, *Enterobacter*, *Escherichia*, *Ewingella*, *Klebsiella*, *Proteus*, *Raoultella*, *Serratia* and *Yersinia* were isolated from the hide samples, 16 bacterial species belonging to genera of *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus*, *Raoultella*, *Serratia* and *Yersinia* were isolated from the skin samples. These species were identified using API® 20E Test Kits. Although *Cedecea lapagei*, *Serratia rubidaea* and *Yersinia enterocolitica* were the most prevalent microorganisms on the hide samples, *Escherichia coli*, *Serratia rubidaea* and *Serratia plymuthica* were the most prevalent bacterial isolates on the skins. The most common bacterial species isolated from both hides and skins was *Serratia rubidaea*. The presence of the members of *Enterobacteriaceae* on the hides and skin samples in high numbers was evidence of faecal contamination and inadequate preservation. According to biochemical test results, isolates exhibited catabolic activity to break down carbohydrate, lipid and protein which may adversely affect leather quality. Hide and skin samples contain different species of *Enterobacteriaceae* which may cause deterioration of hides and skins; therefore, effective antibacterial applications should be applied to hides and skins to eradicate these microorganisms and prevent substantial economical losses in leather industry.

Introduction

Cattle hides and sheep skins may be contaminated by members of the family *Enterobacteriaceae*. The family is prevalent in nature, normally found in soil, human and animal intestines, water, decaying vegetation, fruits, grains, insects and eggs. These microorganisms may be transferred from faeces onto hide during the skinning operation on the slaughterline via direct hide-to-faeces contact and hand/equipment contaminated by faeces.[1-3] Moreover, contamination of hides and skins with these bacteria may be connected to the aforementioned sources.[2,3]

The *Enterobacteriaceae,* which belongs to the phylum *Proteobacteria* and subdivision gamma, contains facultatively aerobic, gram-negative, nonsporulating rod shaped bacteria. Although strains of some species are harmless commensals, others may cause diarrhea, abscesses, pneumonia, nosocomial infections, bacteremia, septicemia, meningitis, plant and insect diseases, infections of wounds, the urinary tract and the intestine.[3-6] Members of the *Enterobacteriaceae* contain endotoxin, exotoxin, cytotoxin, capsule, slime layer, adhesins, iron-binding compounds, hemolysins and type III secretion system that contribute to the pathogenity of these microorganisms in animals. Furthermore, these microorganisms establish their harmful presence by use of fimbriae that help them attach to hide surfaces, and sex pili which function in genetic exchange, especially antibiotic resistance and bacteriocins which may reinforce the ecological balance of these microorganisms. Therefore, these virulence factors lead to serious diseases in animals and humans.[3-6]

*Corresponding author e-mail: mbirbir@marmara.edu.tr.
Manuscript received January 16, 2015, accepted for publication March 13, 2015.

A very limited number of studies examine different species of the *Enterobacteriaceae* on the salted cattle hides and sheep skins.[2,7-13] In previous study, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Enterobacter liquefaciens*, *Citrobacter* and *Serratia* species were isolated from eighty-five hides.[2] *Proteus mirabilis*, *Escherichia coli, Shigella boydii*, *Pantoea agglomerans* and *Photorhabdus luminescens* have been isolated from raw buffalo hides.[7] In an earlier experiment, protease positive *Proteus vulgaris* and protease negative *Citrobacter freundii* have been isolated from both raw hides and soaked hides.[8] Researchers stated that the most common food-borne pathogens on cattle hides were *Escherichia coli* O157 (between 1.0% and 27.8% on cattle hides), *Salmonella* and *Campylobacter* species (5.5% and 5.0–53.0%, respectively) which are found in cattle intestine and shed in the faeces of the animals.[9-11] In another study, researchers examined the prevalence of *Escherichia coli* O157:H7 on 112 skin swabs collected from sheep in Ethiopia. *Escherichia coli* O157:H7 was isolated on 8% of the skin swabs.[12]

In our previous analysis of salted hide samples, the species of genera *Citrobacter*, *Edwardsiella, Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Proteus*, *Salmonella*, *Serratia* and *Yersinia*, which belong to the family *Enterobacteriaceae*, were isolated and identified, but their numbers and metabolic activities were not examined.[13] Identification of enteric bacterial species, determination of their numbers and examination of their metabolic activities may offer important information about 1) contamination of hides and skins with faeces, and 2) the efficiency of particular farming, slaughter and preservation methods. In addition, identification of the most frequently encountered *Enterobacteriaceae* on the hides and skins may provide valuable knowledge for eradicating these microorganisms or reducing bacterial contamination of hides and skins. Therefore, the aim of this research was to determine total counts of Gram-negative bacteria, total counts of *Enterobacteriaceae*, total counts of proteolytic *Enterobacteriaceae*, total counts of lipolytic *Enterobacteriaceae*, total counts of each species belonging to *Enterobacteriaceae* on the hides and sheep skins. Furthermore, *Enterobacteriaceae* isolates recovered from the samples were identified, and their prevalence on the hides and skins was determined. To examine degradation of protein, lipid, carbohydrate and metabolic activities of the members of *Enterobacteriaceae* isolated from the salted hide and sheep skin samples, various tests (oxidase, catalase, protease, lipase, β-galactosidase and urease tests, indol formation from L-tryptophan, H_2S production, Voges-Proskauer test, utilization of citrate as a sole carbon source, tryptophan deaminase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, production of acids from D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, and L-arabinose) were carried out in this study.

Experimental

Salted Cattle Hide and Sheep Skin Samples

In the present study, five salted hide and five salted skin samples were obtained from different tanneries in Leather Organized Tannery Region, Tuzla-Istanbul, Turkey. Salted cattle hides and sheep skins examined in this study originate in seven different countries. The cattle hides (HS1-HS5) were salt-cured in Dubai, Turkey and Israel; the sheep skin samples (SS1-SS5) were salt-cured in Australia, Lebanon, U.S.A. and South Africa. These samples were immediately placed into sterilized bags and containers and carried on ice during transportation. Before the experiments, all of the salted hide and skin samples were cleaned thoroughly of hair, fat and dirt.

Determination of pH Values of the Hide and Skin Samples

Five grams each of salted hide and skin samples were separately placed into the flasks containing 100 ml of sterile distilled water at 20°C, and then these flasks were shaken in a shaking incubator (Edmund Bühler, Germany) for one hour at 200 rpm. The pH values of the hide and skin samples were measured by using a pH meter (Sartorius Professional Meter PT- 10P, Goettingen, Germany).[14]

Determination of Salt Saturation, Moisture and Ash Contents of the Hide and Skin Samples

Three grams of the salted hide and skin samples were cut into small pieces. The moisture content of the samples was determined by drying the samples in an oven at 102 ºC for 6 hours. The samples were placed into a dessicator for 30 minutes to cool. After cooling, the samples were weighed, then placed into an oven for one hour before being weighed again. The drying procedure was repeated until first dry weight was equal to second dry weight, and finally moisture contents of the samples were calculated.[14,15] Then, the dry samples were placed in ceramic crucibles and ashed in a muffle furnace at 600°C for 8 hours. After cooling, the samples were weighed to determine ash content.[15,16] Salt saturations of the samples were calculated according to the following formula:

Per cent saturation of salt = [(total ash % x 100/ moisture % x 100) / 0.359] x 100

Determination of Total Counts of Gram-negative Bacteria and the *Enterobacteriaceae* on the Hide and Skin Samples

Spread plate technique was used to determine the total counts of both Gram-negative bacteria and the *Enterobacteriaceae* on the hide and skin samples.[17] Ten grams each of salted hide and skin samples were separately placed into a flask containing 90 ml sterile physiological saline solution. The flasks were shaken in a shaking incubator (Edmund Bühler, Germany) for half an hour at 25°C and 100 rpm. After shaking procedure, serial dilutions (from 10^{-2} to 10^{-6}) of the hide and skin sample suspensions were prepared. Then 100μL of direct and serial dilutions (from 10^{-2} to 10^{-6}) of the suspensions were spread plated on Eosin Methylene Blue (EMB) Agar media to determine total counts of Gram-negative bacteria and the *Enterobacteriaceae*. Later, the plates were incubated for 48 hours at 37°C. After incubation period, the colonies on the agar plates were counted to determine total counts of Gram-negative bacteria. To determine total counts of the *Enterobacteriaceae*, different colonies on the agar plates were counted after Gram staining procedures, oxidase and catalase tests were performed.[6] In this study, all experiments were conducted in duplicate.

Isolation and Identification of the Members of *Enterobacteriaceae* from the Hide and Skin Samples

Different colonies on EMB Agar were picked up and restreaked several times onto EMB Agar to obtain pure culture. Gram staining of different pure cultures was performed.[17] When Gram-negative rods were detected, we applied oxidase and catalase tests to these pure cultures. The API 20E test kits (Biomèrieux, France) were used to identify the isolates belonging to the family *Enterobacteriaceae*. The isolates were grown on EMB Agar at 37°C for 24 hours and suspended in sterilized saline solution (0.85% NaCl) to adjust the density of the bacterial cultures to McFarland No. 0.5 (10^8 CFU/mL) as described in the manufacturers' instructions. The bacterial dilutions were then loaded into the test strips. These test strips were incubated at 37°C for 24-48 hours. The results of all biochemical tests were read and evaluated after incubation period.[17]

Determination of Protease Activity of the Isolates

The isolates were streaked out over the surface of gelatin agar medium containing tryptone, 15 g; soytone, 5 g; gelatin, 40 g; sodium chloride, 5 g; agar, 20 g; and distilled water, 1000 ml. Then, the gelatin agar plates were incubated at 37°C for three days, and the plates were flooded with saturated ammonium sulphate solution. Clear zone around the colony was taken as evidence of protease activity.[13,18]

Determination of Lipase Activity of the Isolates

The isolates were streaked out over the surface of Tween 80 agar medium containing peptone, 10 g; sodium chloride, 5 g; $CaCl_2$, 0.1 g; Tween 80, 10 g; agar, 20 g; and distilled water, 1000 ml. After incubation of the plates at 37°C for 48 hours, lipid hydrolysis was evidenced by opaque zone around the bacterial colony.[13,18]

Determination of Oxidase Activities of the Isolates

The isolates were streaked out over the surface of EMB agar and incubated at 37°C for 24-48 hours. After incubation period, colonies of isolates were transferred with a loop onto filter paper moistened with oxidase reagent. Occurrence of a color change from pink to dark purple within 20 to 30 seconds was accepted as positive oxidase activity.[17]

Determination of Catalase Activity of the Isolates

The isolates were streaked out over the surface of EMB agar and incubated at 37°C for 24-48 hours. After incubation period, catalase activity was detected by adding 3% H_2O_2 to colonies grown on EMB agar. The appearance of gas bubbles was accepted as a positive test result.[17]

Methylene Blue Agar, Gram reactions, oxidase, catalase, protease and lipase tests of the isolates, API 20E test kits, API test results of the isolates and API & 32 Identification databases were used for grouping of the bacteria isolated from the salted hides and skins.[3,17-18]

Results and Discussion

The origins of the hide and skin samples, the parts of hides and skins used in the present study are presented in Table I. The salted hide and skin samples were usually taken from belly and buttocks sections of the hides and skins. These samples were examined for pH value, moisture content, ash content, salt saturation and for determining aforementioned bacterial counts. In addition, metabolic activities of isolates were examined according to biochemical tests used in their identification (API 20E test kit, protease, lipase, catalase, oxidase activity tests).

TABLE I
Information about the salted hides and skins.

Sample No	Origins of the hides and skins	Animal	The parts of the hides and skins used
HS*-1	Dubai	Cattle	Belly
HS-2	Turkey	Cattle	Buttocks
HS-3	Turkey	Cattle	Belly
HS-4	Turkey	Cattle	Belly
HS-5	Israel	Cattle	Buttocks
SS-1**	Australia	Sheep	Buttocks
SS-2	Lebanon	Sheep	Belly
SS-3	U.S.A.	Sheep	Flank
SS-4	South Africa	Sheep	Buttocks
SS-5	South Africa	Sheep	Buttocks

*** HS:Hide Sample, **SS:Skin Sample**

The pH values of the hide samples (between 7.02-7.77) were higher than those of the skin samples (between 5.99-7.07). As known, most bacteria can grow best around pH 4-9. Therefore, these values were suitable for bacterial growth (Table II). Although moisture contents of the hide samples were found between 17% and 29%, moisture contents of the skin samples were between 18% and 39%. These values were lower than the standard values (40-48%) stated by Bailey (2003).[19] Ash contents of the hide (between 20%-32%) and skin samples (between 19%-26%) were found nearly equivalent. Salt saturations of all hide and skin samples were fairly high (100%) except HS-3. The ash content and salt saturation values of the samples were consistent, respectively, with the standard values (14-48% and 100%)[19] (Table II).

Although moisture contents of the hide (between 17%-29%) and skin samples (between 18%-39%) were found low, and salt saturations of most hide and skin samples were fairly high (100%), total counts of Gram-negative bacteria, total counts of the *Enterobacteriaceae*, total counts of proteolytic *Enterobacteriaceae*, total counts of lipolytic *Enterobacteriaceae*, and total counts of each species belonging to *Enterobacteriaceae* on the hide and skin samples were found to be high (Tables III and IV). These bacterial counts on the hide and skin samples varied slightly. Counts of these bacteria on the skin samples were found higher than those on the hide samples. Total counts of Gram-negative bacteria on the salted cattle hide samples and salted sheep skin samples were between $2.3x10^4$ CFU/g-$1.4x10^6$ CFU/g and between $2.9x10^5$ CFU/g-$4.1x10^6$ CFU/g, respectively. Total *Enterobacteriaceae* counts on the hide samples and the skin samples were between $1.7x10^4$ CFU/g-$4.5x10^5$ CFU/g and between $1.7x10^5$ CFU/g-$1.5x10^6$ CFU/g, respectively (Table III).

TABLE II
pH, moisture content, ash content and salt saturation values of the salted hide and skin samples.

Sample No	pH	Moisture content (%)	Ash content (%)	Salt saturation (%)
HS-1	7.60	22	24	100
HS-2	7.77	23	21	100
HS-3	7.39	17	32	52
HS-4	7.15	20	24	100
HS-5	7.02	29	20	100
SS-1	5.99	39	19	100
SS-2	6.95	27	22	100
SS-3	7.07	23	22	100
SS-4	6.86	20	25	100
SS-5	6.65	18	26	100

It was observed that the sheep skins were dirty and contaminated by faeces. Presence of the *Enterobacteriaceae* on the hide and skin samples was probably related to contamination of the hide and skin samples with faeces. In the previous research, the *Enterobacteriaceae* count on 40 randomly selected bovine hides (35 dairy cows and 5 beef cattle; Simmental and Holstein-Friesian breeds) originating from three geographical regions in Serbia was found as $2x10^4$ CFU/cm^2. Researchers stated that *Enterobacteriaceae* counts were found as approximately $3.5x10^4$ and $2x10^4$ CFU/cm^2 on distal leg (metacarpus) and brisket areas, respectively.[1]

Total counts of proteolytic *Enterobacteriaceae* on the hides and skin samples were between $9.1x10^3$ CFU/g-$3.9x10^5$ CFU/g and between $1.2x10^5$ CFU/g-$1.1x10^6$ CFU/g, respectively. Total counts of lipolytic *Enterobacteriaceae* on the hide and skin samples were respectively between $6.0x10^2$ CFU/g-$3.7x10^5$ CFU/g and between $1.7x10^4$ CFU/g-$5.0x10^5$ CFU/g (Table III).

In the present study, totally, 10 different genera and 20 distinct species of the *Enterobacteriaceae* were isolated from the hide and skin samples. While 9 genera and 16 different species of the *Enterobacteriaceae* were isolated from the hide, 8 genera and 16 different species of the family were isolated from the skin samples (Table IV). Total numbers of different species of the *Enterobacteriaceae* on both hide and skin samples were between four and eight. HS-1 and SS-3 contained the highest number (8) of different bacterial species (Table IV). The presence and prevalence of different genera and different species of this family on the samples examined is likely a consequence of the animal faeces.[3,6]

While genera of *Cedecea*, *Enterobacter, Escherichia, Ewingella, Klebsiella, Proteus, Raoultella, Serratia* and *Yersinia* were isolated from the hide samples, genera of *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus*, *Raoultella*, *Serratia* and *Yersinia* were isolated from the skin samples (Table IV). These results were consistent with the results of our other study. Genera of *Citrobacter*, *Edwardsiella*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Proteus*, *Salmonella*, *Serratia* and *Yersinia* were isolated from 10 salted hides.[13]

It has been known that *Enterobacter* spp., *Citrobacter* spp., *Klebsiella* spp., and especially *Escherichia coli* are mostly used as faecal indicator organisms by the food and water industry due to their presence in the gastrointestinal tract of

TABLE III
Total counts of Gram-negative bacteria, total counts of *Enterobacteriaceae*, total counts of proteolytic *Enterobacteriaceae* and total counts of lipolytic *Enterobacteriaceae* on the salted hide and skin samples.

Sample No	Total counts of Gram-negative bacteria (CFU/g)	Total counts of *Enterobacteriaceae* (CFU/g)	Total counts of proteolytic *Enterobacteriaceae* (CFU/g)	Total counts of lipolytic *Enterobacteriaceae* (CFU/g)
HS-1	$6.8x10^5$	$1.0x10^5$	$9.1x10^3$	$7.1x10^3$
HS-2	$2.3x10^4$	$1.7x10^4$	$1.0x10^4$	$1.5x10^4$
HS-3	$6.3x10^5$	$4.5x10^5$	$3.9x10^5$	$3.7x10^5$
HS-4	$7.6x10^5$	$4.0x10^5$	$6.5x10^4$	$6.0x10^2$
HS-5	$1.4x10^6$	$3.1x10^5$	$3.4x10^4$	$3.4x10^4$
SS-1	$2.9x10^5$	$1.7x10^5$	$1.7x10^5$	$1.2x10^5$
SS-2	$1.6x10^6$	$4.1x10^5$	$1.8x10^5$	$1.5x10^5$
SS-3	$3.4x10^5$	$2.4x10^5$	$1.2x10^5$	$1.7x10^4$
SS-4	$4.1x10^6$	$1.5x10^6$	$1.1x10^6$	$5.0x10^5$
SS-5	$3.0x10^6$	$4.7x10^5$	$3.7x10^5$	$1.6x10^5$

mammals.[6] Our results showed that hide and skin samples contained these faecel indicator organisms (*Enterobacter spp., Escherichia coli* and *Klebsiella spp.*).

It is well known that hide and skin containing proteins, lipids and carbohydrates offer an ideal environment for bacterial growth. In addition, blood, manure, urine, milk, dirt, dust, soil, animal feed, contaminated water are the other nutrients that support the growth of microorganisms. Degradation of these macromolecules by members of the *Enterobacteriaceae* may cause deterioration of hide and skin. Therefore, metabolic activities of the *Enterobacteriaceae* on the samples were evaluated.

In this investigation, all isolates obtained from the salted hides and skins were Gram-negative, and grew at pH 7. Our test results related to protein catabolism showed that some isolates had the ability to hydrolyse protein to amino acids and utilize these amino acids as a carbon, nitrogen and energy source for growth. Forty-five per cent of the isolates showed positive protease activity. Twenty-five per cent of the isolates deaminated L-tryptophan amino acid, which is found in meat and milk, and showed positive tryptophan deaminase activity. Thirty-five per cent of our isolates formed indol. Decarboxylation of amino acid such as L-ornithine by some isolates was also detected, and thirty-five per cent of the isolates produced ornithine decarboxylase enzyme. Decarboxylase enzyme produced by bacteria breaks down amino acids. Thirty-five per cent of the isolates showed positive arginine dihydrolase activity. Furthermore, decarboxylation of L-lysine amino acid by some isolates was also observed and forty-five per cent of the isolates showed positive lysine decarboxylase test. Thirty-five per cent of the isolates exhibited positive urease activity. The enzyme urease liberates ammonia from urea, which is a waste product of protein digestion in mammals, causing a shift in pH to alkaline.[17,20] None of the isolates produced H_2S from the sulfur containing sodium thiosulfate (Table V). These test results confirm that ammonia odor released from the hide and skin samples in our study was related to protein catabolism. Oxidase reactions of all isolates were negative. In aerobic respiration, oxidase enzymes have a significant role in the electron transport system.[17] Oxidase test is utilized to detect the presence of cyctochrome *c*. *Enterobacteriaceae* generally

TABLE IV
Total counts of each species of the *Enterobacteriaceae* isolated from the hide and skin samples.

Genera and species of *Enterobacteriaceae*	**HS-1**	**HS-2**	**HS-3**	**HS-4**	**HS-5**	**SS-1**	**SS-2**	**SS-3**	**SS-4**	**SS-5**
Citrobacter										
Citrobacter koseri										$1.5x10^{4}$
Cedecea										
Cedecea lapagei		$5.0x10^{3}$	$4.0x10^{4}$	$6.0x10^{2}$						
Enterobacter										
Enterobacter cloacae	$6.0x10^{3}$	$8.0x10^{2}$					$1.8x10^{4}$	$1.0x10^{5}$		
Enterobacter sakazakii			$1.9x10^{4}$							
Escherichia										
Escherichia coli	$6.0x10^{3}$				$6.3x10^{3}$		$2.0x10^{5}$	$1.4x10^{4}$	$4.0x10^{5}$	$2.5x10^{4}$
Escherichia vulneris	$2.8x10^{4}$						$1.3x10^{4}$			
Ewingella										
Ewingella americana	$2.8x10^{4}$			$2.0x10^{5}$						
Klebsiella										
Klebsiella pneumoniaea ssp. ozaenae	$2.4x10^{4}$									$7.6x10^{3}$
Klebsiella oxytoca				$2.5x10^{4}$	$1.9x10^{3}$			$4.0x10^{3}$		
Proteus										
Proteus vulgaris		$8.0x10^{2}$		$6.5x10^{4}$				$1.6x10^{3}$		$8.0x10^{4}$
Proteus penneri								$1.0x10^{5}$		
Raoultella										
Raoultella planticola					$3.4x10^{4}$	$2.0x10^{4}$				
Raoultella ornithinolytica					$2.7x10^{5}$					
Serratia										
Serratia odorifera	$2.0x10^{3}$					$5.0x10^{4}$				
Serratia liquefacicens			$2.2x10^{5}$			$1.0x10^{5}$				
Serratia plymuthica			$6.0x10^{4}$				$3.0x10^{4}$		$6.0x10^{5}$	$1.3x10^{5}$

Table IV continues on following page.

Table IV continued.

Serratia ficaria						$4.0x10^3$				
Serratia marcescens								$1.5x10^4$	$3.7x10^3$	
Serratia rubidaea	$7.1x10^3$	$1.0x10^4$	$1.1x10^5$				$1.5x10^5$	$2.0x10^3$	$5.0x10^5$	$1.6x10^5$
Yersinia										
Yersinia enterocolitica	$7.1x10^3$	$6.0x10^2$		$1.1x10^5$				$2.3x10^3$		$5.0x10^4$
Total numbers of different species on the samples	8	5	5	5	4	4	5	8	4	7

do not have cytochrome *c* oxidase. Catalase reactions of all isolates were positive. Ninety per cent of isolates produced β-galactosidase and hydrolysed lactose to galactose and glucose. Thirty per cent of the isolates hydrolysed Tween 80, producing lipase. All isolates grew fermentatively on D-glucose, producing acid. Sixty, seventy-five, fifty-five, eighty-five and seventy per cent of the isolates grew fermentatively on L-rhamnose, D-sucrose, D-melibiose, amygdalin, and L-arabinose, respectively, producing acid. Ninety and seventy per cent of isolates grew fermentatively on, respectively, D-mannitol and D-sorbitol, which are sugar alcohols, producing acid. Forty-five per cent of the isolates grew fermentatively on inositol, producing acid in our study. Seventy per cent of our isolates used citrate as a sole carbon source. Sixty-five per cent of isolates produced neutral products, acetoin, from glucose by fermentation process. That majority tested positive in Voges-Proskauer tests. These experimental data showed that the isolates have catabolic activity to break down proteins, lipids and carbohydrates which may be related to hide and skin deterioration (Table V).

Although the most prevalent bacterial isolates on the hides were found as *Cedecea lapagei* (10^2-10^4 CFU/g), *Serratia rubidaea* (10^3-10^5 CFU/g) and *Yersinia enterocolitica* (10^2-10^5 CFU/g), *Escherichia coli* (10^4-10^5 CFU/g), *Serratia rubidaea* (10^3-10^5 CFU/g) and *Serratia plymuthica* (10^4-10^5 CFU/g) were the most prevalent bacterial isolates on the skins (Table IV).

In the present study, only one species of genus *Cedecea, Cedecea lapagei* ($6x10^2$-$4x10^4$ CFU/g)*,* was isolated from three hide samples (Table IV). The common presence of *Cedecea lapagei* on the hide samples in our study may originate with the animal itself or proceed from environmental sources.

Cedecea species were isolated from vegetables, human clinical specimens and especially from the respiratory tract.[3,21] *Cedecea lapagei* has been isolated from agricultural dusts, environmental reservoirs, the peritoneal fluid, throat, sputum and lung of animals.[4, 22,23] All strains of *Cedecea lapagei* are protease, indol, H_2S, urease, lysine decarboxylase and ornithine decarboxylase negative; but lipase positive. Most strains are Voges-Proskauer, citrate, arginine dihydrolase and β-galactosidase positive. *Cedecea lapagei* can ferment glucose and some other carbohydrates, producing acid.[3,24]

In the present study, *Cedecea lapagei* showed positive catalase, β-galactosidase, lipase, arginine dihydrolase activities; used citrate as a sole carbon source; produced neutral products (acetoin) from glucose by fermentation process; and tested positive in Voges-Proskauer test. This microorganism used D-glucose, D-mannitol and amygdalin as carbon sources. The other tests carried out in this study showed negative results for *Cedecea lapagei* (Table V).

Especially the genus Serratia was found as the most common genus of *Enterobacteriaceae* on both hide and skin samples. Six different species of genus Serratia were isolated from the samples. Although four different species of genus Serratia were isolated from three hide samples ($2.0x10^3$ CFU/g-$2.2x10^5$ CFU/g), six different species of this genus were recovered from five skin samples ($2.0x10^3$ CFU/g-$6.0x10^5$ CFU/g). *Serratia odorifera* (1 hide and 1 skin samples), *Serratia liquefacicens* (1 hide and 1 skin samples), *Serratia plymuthica* (1 hide and 3 skin samples), *Serratia rubidaea* (3 hide and 4 skin samples*)* species were isolated from both hide and skin samples, while *Serratia ficaria* (1 skin sample) and *Serratia marcescens* (2 skin samples) were found only on the sheep skins (Table IV).

Serratia species occur in natural environments, soil, water, the digestive tracts of rodents, in insects and on plants. These species are also found in human patients and may be opportunistic human pathogens.[3] Among *Serratia* species, *Serratia marcescens* is the most important.[3,6] Contaminated soil and plants with *Serratia* species may cause community-acquired infections.[6] *Serratia* species can cause mastitis in cows and other infections in animals.[3] *Serratia* species can grow in the media containing 4% NaCl. Most strains are

TABLE V
Biochemical reactions of the isolates obtained from the hide and skin samples.

Characteristics of isolates	**Cedecea lapagei (H*)**	**Enterobacter sakazakii (H)**	**Ewingella americana (H)**	**Raoultella ornithinolytica (H)**	**Citrobacter koseri (S**)**	**Proteus penneri (S)**	**Serratia ficaria (S)**	**Serratia marcescens (S)**	**Enterobacter cloacae (HS***)**	**Escherichia coli (HS)**	**Escherichia vulneris (HS)**	**Klebsiella pneumoniaea ssp. ozaenae (HS)**	**Klebsiella oxytoca (HS)**	**Proteus vulgaris (HS)**	**Raoultella planticola (HS)**	**Serratia odorifera (HS)**	**Serratia liquefaciens (HS)**	**Serratia plymuthica (HS)**	**Serratia rubidaea (HS)**	**Yersinia enterocolitica (HS)**	**Per centage of positive isolates (tests)**
Gram-negative reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100
Growth at pH 7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Biochemical tests related to protein catabolism																					
Protease activity	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	+	+	+	-	45
Deamination of tryptophan	-	-	-	+	+	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	25
Indol production	-	-	-	+	+	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	35
Ornithine decarboxylase	-	+	-	+	+	-	-	+	+	-	-	-	-	-	-	-	+	-	-	+	35
Arginine dihydrolase	+	+	-	+	+	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	35
Lysine decarboxylase	-	-	-	+	-	-	-	+	-	+	+	+	+	-	+	+	+	-	-	-	45
Urease activity	-	-	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	35
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Enzymatic activities																					
Oxidase activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Catalase activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
β-galactosidase activity	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	90
Lipase activity	+	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	+	-	+	-	30

Table V continues on following page.

Table V continued.

Fermentation tests acid from:																					
D-glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
L-rhamnose	-	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	60
D-sucrose	-	+	-	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	75
D-melibiose	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	+	-	+	+	-	55
Amygdalin	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	-	85
L-arabinose	-	-	-	+	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	70
D-mannitol	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	90
D-sorbitol	-	-	-	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	+	70
Inositol	-	+	-	+	-	-	-	+	-	-	-	+	+	-	-	+	+	+	+	-	45
Citrate utilization	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	+	+	+	+	-	70
Voges–Proskauer	+	+	+	+	-	-	+	+	+	-	-	-	+	-	+	+	+	+	+	-	65

* H:Hide sample, **S:Skin sample, ***HS: Hide and Skin sample

Voges-Proskauer and citrate positive. All species are H_2S negative. Most species are urease and arginine dihydrolase negative. Some species are lysine decarboxylase positive. Most *Serratia* species are protease and β-galactosidase positive. Some species produce lipase. Some strains may use different sugars and amino acids as a carbon source.[3,24]

In our study, all *Serratia* species were catalase and protease positive. None of *Serratia* species formed indol from L-tryptophan except *Serratia odorifera*. All *Serratia* species showed negative arginine dihydrolase and tryptophane deaminase. All *Serratia* species did not form H_2S and urease enzyme but used citrate as a sole carbon source. All *Serratia* species used D-glucose, D-mannitol and amygdalin as carbon sources. The other carbon sources were used by some *Serratia* species, and half the *Serratia* species used D-melibiose. Most *Serratia* species used inositol, D-sorbitol, D-sucrose and L-arabinose; a few of *Serratia* species used L-rhamnose. Fifty and thirty-three per cent of *Serratia* species respectively tested positive for Lysine decarboxylase and Ornithine decarboxylase tests. Most *Serratia* species showed positive lipase activity. All *Serratia* species showed positive Voges-Proskauer and β-galactosidase activities (Table V).

Serratia rubidaea was found as the most prevalent microorganism on both hide and skin samples. This isolate showed positive catalase, β-galactosidase, lipase activities; used citrate as a sole carbon source; showed positive Voges-Proskauer test; used D-glucose, D-mannitol, inositol, D-sucrose, D-melibiose, amygdalin and L-arabinose as carbon sources. The other tests on this microorganism were negative (Table V). Our results are similar to those of other experiments. Researchers explained that most strains of *Serratia rubidaea* are citrate, protease, lipase and β-galactosidase positive. All strains are indol, H_2S, arginine dihydrolase and ornithine decarboxylase negative; but Voges-Proskauer positive. Most strains are urease negative, and some strains are lysine decarboxylase negative. *Serratia rubidaea* can ferment glucose and most of the other carbohydrates, producing acid.[3,24]

Serratia plymuthica was the other *Serratia* species which commonly found on the skin samples in our study. Researchers mentioned that most strains are citrate, Voges-Proskauer and β-galactosidase positive. All strains are indol, H_2S, urease, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase negative. Some strains are protease positive. A few strains are lipase negative. Most strains can ferment glucose and carbohydrates, producing acid.[3,24]

In the present study, *Serratia plymuthica* showed positive protease and β-galactosidase activities and used citrate as a sole carbon source. But lipase, indol, H_2S, urease, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase tests were negative. This species used D-glucose, D-mannitol, inositol, D-sorbitol, D-sucrose, D-melibiose, amygdalin and L-arabinose, producing acid (Table V).

Serratia strains produce a moldy or potato-like odor.[3] Bad odor was detected in some of our samples, and this bad odor may be a sign of the common presence of *Serratia* species on the samples. The frequency of *Serratia* species on both cattle hides and sheep skins was thought to be related to the animal itself and natural environments such as soil and water.

In another research, *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Serratia marcensces, Escherichia coli, Streptococcus pyogenes* and *Klebsiella pneumoniae* were isolated from soil contaminated by tannery effluents. The presence of these microorganisms in the soil was attributed to tannery activities and discharge of tannery waste (effluents) into the surrounding soil.[25] In our previous study, *Serratia marcescens* was isolated from only one salted hide sample.[13]

Yersinia enterocolitica was isolated from three hide ($6x10^2$-$1.1x10^5$ CFU/g) and two skin samples ($2.3x10^3$-$5.0x10^4$ CFU/g) in our study. Researchers classify *Yersinia enterocolitica* as a global pathogen.[26-27] This species has been isolated in various countries on all five continents.[3] In our study, isolates of this species were also recovered from the hide samples procedent from different continents. Some strains of *Yersinia enterocolitica* are indol and lipase negative. Most strains are Voges-Proskauer negative. All strains are H_2S, citrate, protease, arginine dihydrolase and lysine decarboxylase negative; but most strains are urease, ornithine decarboxylase and β-galactosidase positive. *Yersinia enterocolitica* may produce acid from glucose and other carbohydrates.[3]

In the present study, *Yersinia enterocolitica* showed positive catalase, β-galactosidase, ornithine decarboxylase, urease. Our results also showed that *Yersinia enterocolitica* used D-glucose, D-mannitol, D-sorbitol, D-sucrose and L-arabinose, producing acid (Table V). Otherwise this microorganism tested negative.

Researchers have determined that *Yersinia enterocolitica* is widely distributed in nature and may be found in an ample variety of animal species, human, water, soil, milk, faeces, meat products, vegetables, eggs and cheese.[6,26] *Yersinia enterocolitica* may also transmit through the faecal-oral route. The organism may be ingested with contaminated food.[26] This microorganism was isolated from sheep, cow, and buffalo.[26,27] *Yersinia enterocolitica* may be pathogenic and cause arthritis, septicemia, acute enteritis and diarrhea in animals and humans.[6,26] The existence of this isolate on the samples in this study may originate primarily with the animal itself.

The other *Yersinia* species such as *Yersinia pseudotuberculosis* and *Yersinia ruckeri* were isolated from one and two salted hide samples in the previous study, respectively.[13]

Genus *Escherichia* was the other common bacteria on the skin samples. Two different species of genus *Escherichia* were isolated from both salted hides and skin samples. *Escherichia* species were isolated from two hide samples (between $6.0x10^3$ CFU/g-$2.8x10^4$ CFU/g) and four sheep samples (between $1.3x10^4$ CFU/g-$4.0x10^5$ CFU/g). *Escherichia coli* was more prevalent than *Escherichia vulneris* on the samples. Although *Escherichia coli* was isolated from two hide samples, four sheep samples contained this species. *Escherichia vulneris* was isolated from only one salted hide and one skin sample. The common presence of *Escherichia coli* on the samples was thought to be related to faecal contamination of hides and skins.

In our previous study, *Escherichia* coli was isolated from four of ten salted hides.[13] *Escherichia* coli strains were also isolated from all 40 randomly selected bovine hides in Serbia.[1] In another investigation, *Escherichia coli* counts on the exterior hide samples ranged from $3.2x10^5$ to $3.2x10^7$ CFU/100 cm^2.[28]

Escherichia coli, which causes intestinal and extraintestinal diseases, is found in the large intestine of warm-blooded animals. This microorganism is a mostly opportunistic pathogen, and some *Escherichia coli* strains may cause diarrhea in animals and humans, neonatal meningitis, and dysentery-like disease.[3,29] Enterotoxigenic *Escherichia coli* strains were isolated from newborn calves. This species was also isolated from soil and water contaminated with faeces.[3,29] *Escherichia vulneris* strains were isolated from humans, animals and environment. Researchers also isolated this species from human wounds, blood, urine and respiratory tract of patients.[24]

Most *Escherichia coli* strains are citrate, H_2S, and urease negative. Most strains of *Escherichia coli* are lysine decarboxylase and β-galactosidase positive. Most strains are arginine dihydrolase negative, but some strains are ornithine decarboxylase negative. All strains are protease, lipase and Voges-Proskauer negative; but indol positive. *Escherichia coli* can ferment D-glucose and other carbohydrates, producing acid.[3,24] In our research, *Escherichia coli* showed positive catalase, β-galactosidase and lysine decarboxylase activities; produced indol from L-tryptophan; used D-glucose, D-mannitol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose and L-arabinose, producing acid. The other tests of *Escherichia coli* were found to be negative (Table V).

In an earlier study, investigators collected bovine rectal swabs and samples of meat and surface swabs from beef carcasses to examine *Escherichia coli* O157 in South Yorkshire. This microorganism was isolated from 84 of 2103 bovine rectal swabs; 78 of these 84 were found as verocytotoxin-producing *Escherichia coli* O157. This microorganism was also isolated from 7 of 23 carcasses of rectal swab-positive cattle and from 2 of 25 carcasses of rectal swab-negative cattle. The study proved that verocytotoxin-producing *Escherichia coli* O157 may be found in cattle intestine, and that contamination of carcasses during slaughter and processing may contaminate beef and beef carcasses, and transmit verocytotoxin-producing *Escherichia coli* O157 to humans.[30]

Scientists examined *Escherichia coli* O157 and indicator microorganisms on cattle hides at two commercial beef processing plants. *Escherichia coli* O157 was recovered from 76% of animal hides coming into the plants. Aerobic bacterial counts and *Enterobacteriaceae* counts on hides were found as $6.35x10^7$ and $1.60x10^6$ CFU/100 cm^2, respectively.[31]

Although *Enterobacter sakazakii* ($1.9x10^4$ CFU/g) was isolated from only one hide sample, *Enterobacter cloacae* were isolated from two hide samples ($8.0x10^2$ CFU/g-$6.0x10^3$ CFU/g) and two sheep samples ($1.8x10^4$ CFU/g-$1.0x10^5$ CFU/g) in the present study (Table IV). In our earlier experiment, *Enterobacter aerogenes* (3 hides), *Enterobacter agglomerans* (4 hides), *Enterobacter amnigenus* (8 hides), *Enterobacter cloacae* (10 hides), *Enterobacter gergoviae* (7 hides), *Enterobacter intermedius* (3 hides), *Enterobacter liquefaciens* (3 hides), *Enterobacter sakazakii* (6 hides) were isolated from the salted hides. In that study *Enterobacter cloacae* was the commonly found *Enterobacteriaceae* member on the salted hides.[13]

Investigators stated that *Enterobacter cloacae* was isolated from food, meat, soil, animal and human faeces, skin, sewage, spinal fluid, blood, urine, wounds, respiratory tract, intestinal tracts of animals and humans.[3,24] *Enterobacter cloacae* has been found in different infections such as ventriculitis, endocarditis, meningitis, arthritis or osteomyelitis, urinary tract infections and pneumonia.[24,32]

All strains of *Enterobacter cloacae* are indol, H_2S, lysine decarboxylase, protease and lipase negative; but citrate and Voges-Proskauer positive. Most strains are arginine dihydrolase, ornithine decarboxylase and β-galactosidase positive; but some strains are urease negative. *Enterobacter cloacae* can ferment glucose and other carbohydrates, producing acid.[3,24] In our investigation, *Enterobacter cloacae* showed positive catalase, β-galactosidase, arginine dihydrolase and ornithine decarboxylase activities; used citrate as a sole carbon; tested positive in Voges-Proskauer test; used D-glucose, D-mannitol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin and L-arabinose as carbon sources, producing acid. The other tests of this species were negative (Table V).

Enterobacter sakazakii may be found in the environment and in foods. *Enterobacter sakazakii* strains were isolated from sputum, blood, urine, feaces, spinal fluid, nose, bone marrow, bronchial washing, and milk powder. This microorganism may cause meningitis and sepsis in newborn babies; urosepsis and bacteremia in elderly patients. *Enterobacter sakazakii* was also isolated from an ulcer in the foot of a diabetic patient.[3,33]

Although *Klebsiella pneumoniae* ssp. *ozaenae* was isolated from one hide sample ($2.4x10^4$ CFU/g) and one skin sample ($7.0x10^3$ CFU/g), *Klebsiella oxytoca* was isolated from two hide ($1.9x10^3$-$2.5x10^4$ CFU/g) and one skin samples ($4.0x10^3$ CFU/g) (Table IV). Comparable research results also were recorded in the other study. *Klebsiella oxytoca* and *Klebsiella pneumoniae* ssp. *ozaenae* were isolated from only one hide sample. Investigators stated that these species were not common microorganisms on the hides.[13]

Most *Klebsiella* species may use citrate. *Klebsiella* species show mostly positive Vogeus-Proskauer reaction and β-galactosidase activity. They cannot produce H_2S, protease, lipase and ornithine decarboxylase. Some species are indol and urease positive. Most species can ferment glucose and other carbohydrates, producing acid.[3,24] *Klebsiella* species are normally found in the animal intestines, clinical samples, soil, water, and grain. These microorganisms are opportunistic pathogens and may cause bacteremia, urinary tract, wound or surgical site infections, pneumonia and meningitis.[3,24] In our study, neither *Klebsiella pneumoniae* ssp. *ozaenae* nor *Klebsiella oxytoca* produced protease, lipase, arginine dihydrolase, ornithine decarboxylase and H_2S. Both produced lysine decarboxylase and β-galactosidase. These species showed positive catalase activity and negative oxidase test results. Although *Klebsiella oxytoca* used citrate as a sole carbon source, tested positive in Vogeus-Proskauer test, and produced indol, tryptophan deaminase and urease; *Klebsiella pneumoniae* ssp. *ozaenae* showed negative results for these tests. Both species used D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-melibiose, amygdalin, and L-arabinose as carbon sources; but D-sucrose was used only by *Klebsiella oxytoca* (Table V).

Proteus vulgaris was isolated from two hides ($8.0x10^2$-$6.5x10^4$ CFU/g) and two skin samples ($1.6x10^3$- $8.0x10^4$ CFU/g), while *Proteus penneri* was isolated from only one skin sample ($1.0x10^5$ CFU/g) (Table IV). Researchers stated that *Proteus vulgaris* and *Proteus penneri* are important human pathogens. *Proteus vulgaris* is commonly found in the environment. This species has been isolated from the intestinal tract of humans and animals, as well as contaminated soil and water.[3] *Proteus penneri* strains have been isolated from human blood, urine, wounds, respiratory tract, and stool.[24] The presence of *Proteus* species on the salted hide was also observed in our previous report, but only one salted hide sample contained *Proteus mirabilis*.[13]

Although *Citrobacter koseri* ($1.5x10^4$ CFU/g), *Enterobacter sakazakii* ($1.9x10^4$ CFU/g), *Ewingella americana* ($2.8x10^4$-$2.0x10^5$ CFU/g), *Raoultella planticola* ($2.0x10^4$-$3.4x10^4$ CFU/g), *Raoultella ornithinolytica* ($2.7x10^5$ CFU/g) were isolated from the samples, these species were not common isolates on the hide and skin samples (Table IV). *Citrobacter* species are commonly found in the environment, in the intestinal tract of humans and other animals. Some strains of this genus may be found in soil, water, sewage, and food. *Citrobacter* species are opportunistic pathogens.[3] In our previous study, *Citrobacter amalonaticus* (1 hide) and *Citrobacter ferundii* (3 hides) were isolated from salted hides samples. In that research, we concluded that *Citrobacter* species were not common bacteria on the salted hides.[13] In another study, it was mentioned that *Citrobacter koseri* was not common in animal, food and soil. Researchers usually isolated *Citrobacter koseri* strains from humans. Although this species was most often isolated from urine and cerebrospinal fluid, its presence in human stools, blood, wounds and sputum was also mentioned. *Citrobacter koseri* is known as an opportunistic pathogen.[34] *Ewingella americana* strains were isolated from human blood, respiratory tract, throat, sputum, wounds and stool.[24] *Raoultella planticola* has been isolated from environmental sources such as soil, water, plants, mammal mucosae, human tissues, sputum, stools, wounds and urine. This species has been accepted as an environmental isolate.[35]

Conclusion

These study results showed that all hide and skin samples contained a variety of bacteria belonging to the *Enterobacteriaceae,* and their common presence on the samples may be mostly a result of faecal contamination. There is also a possibility that these microorganisms were introduced onto the hides and skins from soil, water, feed and the environment. Different species belonging to genera of *Cedecea*, *Citrobacter, Enterobacter, Escherichia, Ewingella*, *Klebsiella, Proteus, Raoultella, Serratia* and *Yersinia,* which may cause health risks for humans and deterioration of hides, were isolated from salted cattle hides and sheep skins. Most of these microorganisms have potential to degrade macromolecules on the hides and skins and use monomers of these macromolecules. Each of the hide and skin samples contained proteolytic and lipolytic *Enterobacteriaceae*. Therefore, effective treatments should be applied during preservation of hides and skins to kill these microorganisms. In addition, the results obtained from this study emphasize that cattle hide and sheep skin should be cleaned with effective applications to remove these bacteria before the slaughter. The animals should be washed and brushed regularly with sanitizers, organic acid solutions such as acetic and lactic acids, chlorinated water or warm water.[36,37] Ozonated and electrolyzed oxidizing waters can be used to reduce *Enterobacteriaceae* counts on the hides.[38] These strategies are necessary to improve the leather's commercial value and, consequently, the industry's financial viability.

Acknowledgement

This work was supported by the Scientific Research Project Commission of Marmara University, Project No. FEN-C-YLP-030912-0303. We thank the Scientific Research Project Commission, the tanneries in Leather Organized Tannery Region, Tuzla-Istanbul for valuable support, technical assistance and providing salted hide and skin samples for testing. We thank also Mrs.Pinar Cağlayan and Mrs.Yasemin Ozturk for valuable help, and Dr. Martin Louis Duncan for proofreading our manuscript.

References

1. Antic, D., Blagojevic, B., Ducic, M., Nastasijevic, I., Mitrovic, R and Buncic, S.; Distribution of Microflora on Cattle Hides and Its Transmission to Meat Via Direct Contact. *Food Control*. **21**,1025-1029, 2010.
2. Newton, K. G., Harrision, J. C. L and Smith, K. M.; Coliforms from Hides and Meat. *Appl. Environ. Microbiol*. **33**,199-200, 1977.
3. Brenner, D. J and Farmer, J. J. III; Order XIII *Enterobacteriales,* Family I. *Enterobacteriaceae,* In: Brenner, D. J., Krieg, N. R., Staley, J. T and Garrity, G. M. (Eds.), Bergey's Manual of Systematic Bacteriology. 2nd ed., Vol. 2, The Proteobacteria, Part B The Gammaproteobacteria, Springer, pp. 587-850, 2005.
4. Janda, J. M.; New Members of the Family *Enterobacteriaceae*, In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K. H and Stackebrandt, E. (Eds.), The Prokaryotes, A Handbook on the Biology of Bacteria: Proteobacteria: Gamma Subclass, 3rd ed., Vol. 6, Springer, New York, pp. 5-40, 2006.
5. Bauman, R.W.; Microbiology with Diseases by Taxonomy, 3rd ed., Pearson Education, Benjamin Cummings, 2011.
6. Baylis, C., Uyttendaele, M., Joosten, H and Davies, A.; The *Enterobacteriaceae* and Their Significance to the Food Industry Report. ILSI Europe Report Series, Commissioned by the ILSI Europe Emerging Microbiological Issues Task Force, *ILSI* **47**, 2011.
7. 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. *Int. Biodeter. Biodegr.* **62**, 65-74, 2008.
8. Oppong, D., Bryant, S., Rangarajan, R., Steele, S., Radwell, D and Hyllengren, L.; Application of Molecular Techniques to Identify Bacteria Isolated from the Leather Industry. *JALCA* **101**, 140-145, 2006.
9. Reid, C. A., Small, A., Avery, S. M and Buncic, S.; Presence of Food-borne Pathogens on Cattle Hides. *Food Control* **13**, 411-415, 2002.

10. Chapman, P. A., Siddons, C. A., Cerdan Malo, A. T and Harkin, M. A.; A 1 Year Study of *Escherichia coli* O157 in Cattle, Sheep, Pigs and Poultry. *Epidemiol. Infect.*, Cambridge University Press, Vol. 119, No. 2, pp. 245-250, 1997.
11. Fedorka-Cray, P. J., Dargatz, D. A., Thomas, L. A and Gray, J. T.; Survey of *Salmonella* Serotypes in Feedlot Cattle. *J. Food Protect.* **5**, 513-648, 1998.
12. Mersha, G., Asrat, D., Zewde, B. M and Kyule, M.; Occurrence of *Escherichia coli* O157:H7 in Faeces, Skin and Carcasses from Sheep and Goats in Ethiopia. *Lett. Appl. Microbiol.* **50**, 71-76, 2010.
13. Aslan, E and Birbir, M.; Examination of Gram Negative Bacteria on Salt Pack Cured Hides. *JALCA* **107**, 106-115, 2012.
14. Birbir, M and Ilgaz, A.; Isolation and Identification of Bacteria Adversely Affecting Hide and Leather Quality. *JSLTC* **80**, 147-153, 1996.
15. O'Flaherty, F and Roddy, W. T.; The Chemistry and Technology of Leather, Vol. 4, Evaluation of Leather, Reinhold Publishing Corporation, The Science Pres Inc. Ephrata, Pennsylvania, 1965.
16. Didato, D., Bowen, J and Hurlow, E.; Microorganism Control During Leather Manufacture. Leather Technologist Pocket Book Chapter 20, Leafe, M. K. (Editor), The Society of Leather Technologist and Chemists, pp. 339-352, 1999.
17. Harley, J. P and Prescott, L. M.; Laboratory Exercises in Microbiology, 5th ed., The McGraw-Hill Companies, New York, 2002.
18. Barnett, M. E and Venghaus, J. D.; Microbiology Laboratory Exercises, Wm.C. Brown Publishers, Dubuque, Iowa, 1988.
19. Bailey, D. G.; The Preservation of Hides and Skins. *JALCA* **98**, 308-319, 2003.
20. Ted, R and Case, J. C.; Laboratory Experiments in Microbiology. 9th ed., Pearson Benjamin Cummings, 2010.
21. Österblad, M., Pensala, O. M., Peterzéns, M., Heleniusc, H and Huovinen, P.; Antimicrobial Susceptibility of *Enterobacteriaceae* Isolated from Vegetables. *J. Antimicrob. Chemoth.* **43**, 503-509, 1999.
22. Pande, B. N., Traczyk, E.W., Prazmo, Z., Skorska, C., Sitkowska, J and Dutkiewicz, J.; Occupational Biohazards in Agricultural Dusts from India. *Ann. Agric. Environ. Med.* **7**, 133-139, 2000.
23. Grimont, P. A. D., Grimont, F., Farmer III, J. J and Asbury, M. A.; *Cedecea davisae* gen. nov., sp. nov. and *Cedecea lapagei* sp. nov., New *Enterobacteriaceae* from Clinical Specimens. *Int. J. Syst. Bacteriol.* **31**, 317-326, 1981.
24. Farmer III, J.J., Davis, B. R., Hickman-Brenner, F.W., McWhorter, A., Huntley-Carter, G. P., Asbury, M. A., Wathen-Grady, H. G., Elias, C and Fanning, G. R.; Biochemical Identification of New Species and Biogroups of *Enterobacteriaceae* Isolated from Clinical Specimen. *J. Clin. Microbiol.* **21**, 46-76, 1985.
25. Rabah, A. B and Ibrahim, M. L.; Physico-chemical and Microbiological Characterization of Soils Laden with Tannery Effluents in Sokoto, Nigeria. *Niger. J. Basic Appl. Sci.* **18**, 65-71, 2010.
26. Carniel, E., Autenrieth, I., Cornelis, G., Fukushima, H., Guinet, F., Isberg, R., Pham, J., Prentice, M., Simonet, M., Skurnik, M and Wauters, G.; *Y. enterocolitica* and *Y. pseudotuberculosis,* In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H and Stackebrandt, E. (Eds.), The Prokaryotes, A Handbook on the Biology of Bacteria: Proteobacteria: Gamma Subclass, 3rd ed., Vol. 6, Springer, New York, pp. 270-398, 2006.
27. Slee, K. J and Button, C.; Enteritis in Sheep and Goats due to *Yersinia enterocolitica*. *Aust. Vet. J.* **67**, 396-398, 1990.
28. Bacon, R. T., Belk, K. E., Sofos, J. N., Clayton, R. P., Reagan, J. O and Smith, G. C.; Microbial Populations on Animal Hides and Beef Carcasses at Different Stages of Slaughter in Plants Employing Multiple-Sequential Interventions for Decontamination. *J. Food Protect.* **63**, 1080-1086, 2000.
29. Nataro, J. P and Kaper, J. B.; Diarrheagenic *Escherichia coli*. *Clin. Microb. Rev.* **11**, 142-201, 1998.
30. Chapman, P. A., Siddons, C. A., Wright, D. J., Norman, P., Fox, J and Crick, E.; Cattle as a Possible Source of Verocytotoxin-producing *Escherichia coli* O157 Infections in Man. *Epidemiol. Infect.* **111**, 439-448, 1993.
31. Arthur, T. M., Bosilevac, J. M., Nou, X., Shackelford S. D., Wheeler, T. L., Kent, M. P., Jaroni, D., Pauling, B., Allen, D. M and Koohmaraie, M.; *Escherichia coli* O157 Prevalance and Enumeration of Aerobic Bacteria *Enterobacteriaceae* and *Escherichia coli* O157 at Various Steps in Commercial Beef Processing Plants. *J. Food Protect.* **67**, 658-65, 2004.
32. Grimont, F., Patrick, A. D and Grimont, P. A. D.; Chapter 3.3.9, The Genus *Enterobacter*, In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K. H and Stackebrandt, E. (Eds.), The Prokaryotes, A Handbook on the Biology of Bacteria: Proteobacteria: Gamma Subclass, 3rd ed., Vol. 6, Springer, New York, pp. 197-214, 2006.
33. Farmer III, J. J., Asbury, M. A., Hickman, F. W and Brenner, D. J.; The *Enterobacteriaceae* Study Group, *Enterobacter sakazakii*: A New Species of "*Enterobacteriaceae*" Isolated from Clinical Specimens. *Int. J. Syst. Bacteriol.* **30**, 569-584, 1980.

34. Brenner, D. J., Patrick, A. Grimont, D., Arnold, G., Steigerwalt, G. R., Ageron, E and Riddle, C. F.; Classification of *Citrobacteria* by DNA Hybridization: Designation of *Citrobacter famzeri* sp. Nov., *Citrobacter youngae* sp. Nov., *Citrobacter braakii* sp. Nov., *Citrobacter werkmanii sp*. Nov., *Citrobacter sedlakii sp*. Nov., and Three Unnamed *Citrobacter* Genomospecies. *Int. J. Syst. Evol. Micr.* **43**, 645-658, 1993.
35. Drancourt, M., Bollet, C., Carta, A and Rousselier, P.; Phylogenetic Analyses of *Klebsiella* Species Delineate *Klebsiella* and *Raoultella* gen. nov.,with Description of *Raoultella ornithinolytica* comb. nov., *Raoultella terrigena* comb. nov. and *Raoultella planticola* comb. nov. *Int. J. Syst. Evol. Micr.* **51**, 925-932, 2001.
36. Dickson, J. E and Anderson, M. E.; Microbiological Decontamination of Food Carcasses by Washing and Sanitizing Systems; a Review. *J. Food Protect*. **55**, 133-140, 1992.
37. Bell, R. G., Distribution and Sources of Microbial Contamination on Beef Carcasses, *J. Appl. Microbiol*. **82**, 292-300, 1997.
38. Bosilevac, J. M., Shackelford, S. D., Brichta, D. M and Koohmaraie, M.; Efficacy of Ozonated and Electrolyzed Oxidative Waters to Decontaminate Hides of Cattle Before Slaughter. *J. Food Protect*. **68**, 1393-1398, 2005.