Examination of Catabolic Activities of *Enterobacteriaceae* Isolated from Soaked Sheep Skins and Cattle Hides

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Summary

Detailed examination of the members of family *Enterobacteriaceae* found on soaked sheepskins and cattle hides offers important information about enteric species on the samples, the efficiency of antibacterial agents used in soaking process against these enteric species and their roles in deterioration of skins or hides during soaking process. Hence, the goal of this study was to examine members of the family *Enterobacteriaceae* on soaked sheepskins and cattle hides treated with antibacterial agent. In this study, bacterial species belonging to the family *Enterobacteriaceae* on five soaked sheepskins and ten cattle hides were identified using API® 20E test kits and the other biochemical tests. While *Citrobacter freundii, Citrobacter koseri, Cronobacter sakazakii, Enterobacter amnigenus, Enterobacter cloacae, Kluyvera intermedia, Morganella morganii, Proteus mirabilis* and *Providencia rettgeri* were isolated from the soaked sheepskins, *Citrobacter koseri, Cronobacter sakazakii, Ewingella americana, Kluyvera intermedia, Morganella morganii, Providencia rettgeri, Serratia marcescens, Serratia plymuthica* and *Serratia rubidae* were obtained from the soaked cattle hides. A fairly high percentage of soaked skin and hide samples contained *Enterobacteriaceae* members which have catabolic activities for deterioration of skins and hides. The presence of the members of *Enterobacteriaceae* on the soaked skin and hide samples was thought to be related to faecal contamination, the animal itself, environmental sources and inadequate preservation. As a conclusion, efficient antibacterial treatments should be applied in soaking process to kill *Enterobacteriaceae* members which may adversely affect leather quality.

INTRODUCTION

Soaked skins and hides may contain the family *Enterobacteriaceae* which are found in the gastrointestinal tract of animals, water, soil, insects and industrial processes. The *Enterobacteriaceae* containing 50 different genera have a great impact on human health, animal health, farming, food and hide industries.[1] Some species of these bacteria are pathogenic to human, animals and plants. *Citrobacter, Enterobacter, Erwinia, Escherichia, Klebsiella, Proteus, Salmonella, Serratia, Shigella* and *Yersinia*, which are pathogens, are among the important genera of the family *Enterobacteriaceae*.[2,3]

The bacterial species of the family *Enterobacteriaceae* called the enteric bacteria are facultatively aerobic Gram-negative rods, contain complex antigenic structures, produce different hydrolytic enzymes and toxins, and ferment a wide range of carbohydrates, producing various end products. While some species of enteric bacteria are non-motile, the others are motile by peritrichous flagella. The members of this family contain fimbria that help them to attach to the mucous membrane of animals. Moreover, virulence factors that enhance invasiveness are produced by enteric bacteria. Bacteriocins, produced by members of the family *Enterobacteriaceae*, may lyse closely related species of bacteria and help maintain the ecological balance of different enterics in the intestinal tract of animals.[2,3,4]

The members of the *Enterobacteriaceae* may contaminate skins and hides during the skinning operation on the slaughter line *via* direct hide or skin-to-faeces contact and hand/equipment contaminated by faeces.[5-6]

The presence of the *Enterobacteriaceae* on cattle hides and skins was detected in the previous studies.[5,7-14] *Enterobacter liquefaciens, Enterobacter cloacae, Enterobacter aerogenes, Klebsiella pneumoniae, Serratia sp.* and *Citrobacter sp.* were obtained from eighty-five hides.[5] *Citrobacter freundii* and *Proteus vulgaris* were isolated from both raw hides and soaked hides.[10] *Photorhabdus luminescens, Proteus mirabilis, Escherichia coli, Pantoea agglomerans* and *Shigella boydii* were obtained from raw buffalo hides.[11] In our previous study, the enteric species of genera *Edwardsiella, Enterobacter, Escherichia, Citrobacter, Klebsiella, Proteus, Salmonella, Serratia, Hafnia* and *Yersinia* were isolated from ten salted hides.[13] In another study, 16 enteric species belonging to genera *Enterobacter, Klebsiella, Citrobacter, Escherichia, Serratia, Proteus, Raoultella*

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and Y*ersinia* were isolated from five salted skin samples. A total of 16 bacterial species belonging to genera *Enterobacter, Cedecea, Escherichia, Proteus, Klebsiella, Ewingella, Serratia, Raoultella* and *Yersinia* were isolated from five salted hide samples.[14] The presence of the *Enterobacteriaceae* such as *Escherichia coli, Enterobacter asburiae, Proteus* species and *Serratia* species in the tannery effluent collected from Indian tanneries was also stated by researchers.[15]

In addition to *Enterobacteriaceae* detected on salted cattle hide and sheepskin samples, *Enterobacteriaceae* members in soak liquor containing didecyl dimethyl ammonium chloride and benzyl dimethyl ammonium chloride were examined. *Enterobacter gergoviae, Enterobacter sakazakii, Enterobacter amnigenus* biogrup I and *Enterobacter cloacae* were isolated from this soak liquor.[16] Moreover, investigators isolated bacterial species belonging to genera *Bacillus, Chromobacter, Pseudomonas, Clostridium, Lactobacillus* and *Serratia* from soak liquor.[17] Among these micro-organisms, only *Serratia* species belonged to the family *Enterobacteriaceae*.

The presence of bacterial species belonging to genera *Bacillus, Chromobacter, Clostridium, Corynebacterium, Lactobacillus, Micrococcus, Pseudomonas, Sarcina, Staphylococcus, Proteus* and *Serratia* was stated in soak liquor in another study.[18] As seen, two enteric genera such as *Proteus* and *Serratia* were isolated from soak liquor.

Although there are several studies investigating the family *Enterobacteriaceae* on salted hides and skins,[5,7-14] the members of *Enterobacteriaceae* on soaked skins and hides treated with antibacterial agent and their metabolic activities have not yet been investigated. Examination of the family *Enterobacteriaceae* on soaked sheepskins and cattle hides treated with antibacterial agent and detailed investigation of their metabolic activities may present significant information about 1) faecal contamination of skins and hides, 2) the efficiency of antibacterial agent used in soaking liquor to kill enteric bacteria, and 3) catabolic activities of enteric bacteria on soaked skins and hides. Hence, we isolated the members of the *Enterobacteriaceae* from the soaked sheepskins and cattle hides treated with antibacterial agent, then API test kits and the other biochemical tests were used to identify these isolates and examine their metabolic activities.

EXPERIMENTAL PROCEDURES

Soaked sheepskin and cattle hide samples

Five soaked skin and ten soaked hide samples were collected from tanneries in the Leather Organized Tannery Region, Tuzla-Istanbul, Turkey.

Examination of pH values of the soaked sheepskin and cattle hide samples

Five grams of each sample were cut and added to a flask containing 100mL of sterile distilled water. After shaking the flasks at 200rpm for one hour, pH values of the samples were measured using a pH meter (Sartorius Professional Meter PT-10P, Goettingen, Germany.)[19]

Isolation of members of the family *Enterobacteriaceae* from the soaked hide and skin samples and their identification

Ten grams of soaked sheepskin and hide samples were separately put into 90ml sterile physiological saline solution. The flasks were placed into a shaking incubator (Edmund Bühler, Germany) and shaken at 100rpm for half an hour. Later, serial dilutions of skin and hide suspensions were prepared and 100μL of direct and serial dilutions were taken and separately spread on Eosin Methylene Blue (EMB) Agar media for isolation of members of the family *Enterobacteriaceae*. The inoculated plates were incubated at 37°C for 48 hours. Then, different colonies grown on the medium were selected and restreaked several times onto the test medium to obtain pure culture. Gram staining of each isolate was done.[20] When Gram-negative rods were detected, oxidase and catalase tests of these isolates were performed. Later, the API 20E test kits (Biomèrieux, France) were utilised. The isolates grown on EMB Agar at 37°C for 24 hours were suspended in sterilized saline solution (0.85% NaCl) to adjust the bacterial density to 10^8CFU/mL. The bacterial suspensions were separately placed into microtubes of API 20E test kits and incubated at 37°C for 24-48 hours. After incubation period, the test results were evaluated.[20] In the identification of enteric bacteria, Gram reactions, oxidase, catalase, protease and lipase tests of the isolates, biochemical test results obtained from API 20E test kits, and identification databases were used.

Oxidase activities of the enteric bacteria

To investigate catalase activities of the isolates, the pure culture of different colonies was inoculated onto the surface of EMB agar by the streak-plate technique and incubated at 37°C for 24-48 hours. First, 3 drops of the oxidase reagent were put onto filter paper, then colonies of the pure culture were transferred with a loop onto the filter paper. Formation of a dark purple colour within 20 seconds was considered as a positive test result.[20]

Catalase activities of the enteric bacteria

To examine catalase activities of the isolates, the pure culture of different colonies was inoculated onto the surface of EMB agar with the streak-plate technique. After incubation at 37°C for 24-48 hours, several drops of 3% H_2O_2 were added to the colonies. The appearance of gas bubbles was considered as a positive test result.[20]

Protease activities of the enteric bacteria

Protease activities of the enteric isolates were tested on gelatin agar medium containing tryptone, 15g; soytone, 5g; gelatin, 40g; sodium chloride, 5g; agar,

20g; and distilled water, 1000mL. Each isolate was streaked onto gelatin agar medium and incubated at 37°C for 72 hours. After incubation period, the gelatin agar medium was flooded with saturated ammonium sulphate solution. Clear zone around the colony was accepted as positive protease activity.[13,21]

Lipase activities of the enteric bacteria

Lipase activities of the enteric isolates were examined on Tween 80 agar medium containing peptone, 10g; sodium chloride, 5g; $CaCl_2$, 0.1g; Tween 80, 10g; agar, 20g; and distilled water, 1000mL. Each isolate was streaked onto Tween 80 agar medium and incubated at 37°C for 48 hours. Following the incubation period, an opaque zone around the bacterial colony was accepted as positive lipase activity.[13]

TABLE I
Information about the soaked skins and hides

Sample codes	Parts of the skin and hide used	pH values
SS1*	Butt	7.00
SS2	Belly	7.07
SS3	Flank	8.86
SS4	Butt	7.02
SS5	Hind flank	7.80
CH1**	Belly	7.60
CH2	Butt	7.77
CH3	Belly	7.39
CH4	Belly	7.15
CH5	Butt	9.50
CH6	Flank	10.00
CH7	Hind flank	7.50
CH8	Flank	9.00
CH9	Flank	8.50
CH10	Hind flank	8.00

*SS1: Skin sample 1, **CH1: Cattle hide 1

RESULTS AND DISCUSSION

The codes of the soaked sheepskins and cattle hides, the pH values and the parts of skins and hides used in this investigation are shown in Table I. The soaked skin and hide samples were collected from belly, butt and flank of the skins and hides. The pH values of the soaked hide samples (between 7.15-10.00) were higher than those of the skin samples (between 7.00-8.86). Since most bacterial species can grow best between pH4 and 9, these pH values were suitable for isolates' growth (Table II).

In the present study, salted sheepskin and hide samples were soaked in a soak liquor containing an antimicrobial agent (sodium dimethyl dithiocarbamate). Although an antimicrobial agent was used in the soaking process, all soaked sheepskins and cattle hides contained members of the family *Enterobacteriaceae*. While the number of enteric isolates on the cattle hides ranged from 1 to 2, the number of isolates on the sheepskins ranged from 1 to 6. In our previous study, conducted on salted cattle hides and salted sheepskins, the numbers of enteric isolates were detected as 4-8.[14] As seen, isolate numbers on soaked sheepskins and cattle hides were lower than that of salted cattle hides and salted sheepskins (Table II). This means that antimicrobial agent used in the soak liquors was effective in reducing enteric isolate numbers to low levels but were not able to kill all members of *Enterobacteriaceae*.

While *Citrobacter freundii, Enterobacter amnigenus, Enterobacter cloacae* and *Proteus mirabilis* were isolated from only soaked sheepskins, *Ewingella americana, Serratia marcescens, Serratia plymuthica*

TABLE II
Prevalence of members of family *Enterobacteriaceae* on soaked sheepskins and soaked cattle hides

Enteric isolates	SS1	SS2	SS3	SS4	SS5	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10
Citrobacter freundii	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Citrobacter koseri	–	–	+	–	+	–	–	+	–	–	–	–	–	–	–
Cronobacter sakazakii	+	+	–	+	–	–	+	–	–	–	–	–	–	–	–
Enterobacter amnigenus	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Enterobacter cloacae	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ewingella americana	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Kluyvera intermedia	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–
Morganella morganii	+	–	+	–	–	–	–	–	–	+	–	–	–	–	–
Proteus mirabilis	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Providencia rettgeri	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–
Serratia marcescens	–	–	–	–	–	–	–	–	–	+	+	–	+	+	–
Serratia plymuthica	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Serratia rubidae	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
Total	6	1	3	2	2	2	1	1	1	2	1	1	1	1	1

and *Serratia rubidae* were isolated from only soaked cattle hides. However, *Citrobacter koseri, Cronobacter sakazakii, Kluyvera intermedia, Morganella morganii* and *Providencia rettgeri* were detected on both soaked sheepskins and cattle hides (Table II).

Among the samples examined, the highest isolate number was detected on the SS1. *Citrobacter freundii, Cronobacter sakazakii, Enterobacter cloacae, Morganella morganii, Proteus mirabilis* and *Providencia rettgeri* were isolated from the soaked skin sample 1 (Table II). Although *Cronobacter sakazakii* was the most common isolate on the soaked sheepskins, *Serratia marcescens* was the most prevalent enteric isolate on the soaked cattle hides (Table II). Enteric bacteria such as *Enterobacter gergoviae, Enterobacter sakazakii, Enterobacter amnigenus* biogroup I and *Enterobacter cloacae* in soak liquor containing quaternary ammonium compounds were detected in our previous study.[22]

All enteric bacteria isolated in this study showed Gram-negative reaction, and grew at pH7. In addition, a fairly high percentage of the isolates catabolized glucose, producing acidic end products as evidenced in the methyl red test. A high percentage of the isolates used citrate as a single carbon source for their energy needs. However, H_2S production by the isolates was fairly low. Lipase enzyme, which may cause deterioration of skin and hide samples, was produced by 27% of members of *Enterobacteriaceae*. The high percentage of the isolates produced β-galactosidase enzyme to hydrolyse lactose to galactose and glucose. Members of the family *Enterobacteriaceae* have been known as oxidase negative and catalase positive.[3] Test results of the present study confirmed this statement. All isolates reduced nitrate to nitrite and a high percentage of the isolates showed positive caseinase and ornithine decarboxylase activities. Almost half of the isolates hydrolysed urea. Some of the isolates showed positive protease and arginine dihydrolase activities, produced NH_3 from peptone and indol from tryptophan (Table III).

Skins and hides contain L-alanine, L-phenylalanine, L-proline, L-glycine, L-tyrosine, L-lysine and L-aspartic acid,[23] hence, amino acid utilization by the isolates was also examined in this study. Almost half of the isolates used L-alanine and L-phenylalanine. Some of the isolates utilised L-proline, L-glycine, L-tyrosine and L-lysine, but none of the isolates used L-aspartic acid. These biochemical test results proved that fairly high percentage of soaked sheepskins and cattle hides contained enteric bacteria which are active players in protein catabolism. Our study results indicated that the members of *Enterobacteriaceae* that use D-glucose and D-mannitol were common on the sheepskins and cattle hides. Almost half of the isolates used inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin and L-arabinose (Table III). These results showed that isolates of the family *Enterobacteriaceae* were metabolically active to use different carbon sources for their carbon and energy needs to grow on soaked sheepskins and cattle hides.

It has been known that hides and skins containing lipids, proteins, carbohydrates, blood, urine, faeces, milk, soil and animal feed are excellent media for bacterial growth. The present study results demonstrated that enteric bacteria found on sheepskins and cattle hides during soaking process may degrade these organic molecules and cause hide and skin deterioration.

In this study, although protease and lipase producer isolates were commonly found on the soaked cattle hides (CH4, CH5, CH6, CH7, CH8 and CH9), enteric micro-organisms producing these enzymes were not common on the soaked sheepskins. Urease producer isolates were detected at three sheepskin samples (SS1, SS3 and SS5) and three cattle hide samples (CH1, CH3 and CH5). Members of the family *Enterobacteriaceae* using different amino acids were detected on all soaked sheepskin and cattle hide samples except CH10. Moreover, all soaked sheepskin and cattle hide samples contained enteric isolates producing caseinase, β-galactosidase and utilising different sugars. An unpleasant odour detected in soaked sheepskins and cattle hides may be related to decomposition of macromolecules in sheepskins and cattle hides by these enteric isolates.

Although *Citrobacter freundii* was not detected on cattle hides, *Citrobacter koseri* strains were found at both sheepskins and cattle hide. *Citrobacter freundii* and *Citrobacter koseri* are known as opportunistic pathogens.[4] *Citrobacter* species have been commonly found in the intestinal tract of humans and animals. Moreover, soil, water, sewage and food may contain these micro-organisms due to faecal contamination.[24] The presence of *Citrobacter freundii* and *Citrobacter koseri* on the samples was thought to be related to faecal contamination of skins and hides.

Cronobacter sakazakii strains were detected on both sheepskins and cattle hide. *Cronobacter* species may be found in different plants such as wheat, soy, corn, rice, herbs, spices and vegetables. Moreover, meats, milk powder, powdered infant formula, salads and cheese may contain *Cronobacter* species.[25] Detection of *Cronobacter sakazakii* strains at both sheepskins and cattle hide may be related to animal feed.

Enterobacter amnigenus and *Enterobacter cloacae* strains were found on only soaked sheepskins. *Enterobacter* species are seldomly known as human pathogens. Sewage, water, soil and vegetables may contain *Enterobacter* species. While *Enterobacter cloacae* was isolated from meat, hospital environments, the human skin, and from the intestinal tracts of humans and animals, *Enterobacter amnigenus* was mostly isolated from water.[26,27] In addition, some species of this genus were detected in clinic samples of respiratory tract, wound or faeces.[26,27] Presence of *Enterobacter amnigenus* and *Enterobacter cloacae* on soaked sheepskins was thought to be related to faecal contamination and the animal itself.

Ewingella americana, which was found on only one cattle hide, has been known as rare human pathogen. Although this species was isolated from human blood,

TABLE III
Biochemical tests results of the enteric bacteria isolated from the soaked skin and hide samples

Isolate name	*Cronobacter sakazakii*	*Ewingella americana*	*Citrobacter freundii*	*Citrobacter koseri*	*Proteus mirabilis*	*Morganella morganii*	*Serratia marcescens*	*Enterobacter cloacae*	*Enterobacter amnigenus*	*Kluyvera intermedia*	*Providencia rettgeri*	*Serratia plymuthica*	*Serratia rubidaea*	Percentage of positive isolates
Codes of skin and hide samples	SS1 SS2 SS4 CH2	CH10	SS1	SS3 SS5 CH3	SS1	SS1 SS3 CH5	CH5 CH6 CH8 CH9	SS1	SS4	SS3 CH1	SS1 SS5 CH1	CH7	CH4	
Gram-negative reaction	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Growth at pH7.0	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Methyl red reaction	+	–	+	+	+	+	+	+	+	+	+	–	+	92
Citrate utilisation	+	+	+	+	+	–	+	+	–	–	+	+	+	77
H_2S production	–	–	+	–	+	–	–	–	–	–	–	–	–	8
Enzymatic activities														
Lipase	–	–	–	–	+	–	+	–	–	–	–	+	+	27
β–galactosidase	+	+	+	+	–	–	+	+	+	+	–	+	+	73
Oxidase	–	–	–	–	–	–	–	–	–	–	–	–	–	0
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Biochemical tests related to protein catabolism														
Protease	–	–	–	–	+	–	+	–	–	–	–	+	+	27
Urease	–	–	+	+	+	+	–	–	–	–	+	–	–	42
Ornithine decarboxylase	+	–	–	+	+	+	+	+	+	+	–	–	–	73
Arginine dihydrolase	+	–	–	+	–	–	–	+	–	–	–	–	–	31
Indol production	–	–	–	+	–	+	–	–	–	–	+	–	–	35
NH_3 production	–	–	–	–	–	–	+	–	–	+	–	+	+	31
Caseinase	+	+	–	+	–	+	+	+	+	+	+	+	+	92
Nitrate reduction to nitrite	+	+	+	+	+	+	+	+	+	+	+	+	+	100
L-alanine	–	–	–	+	–	–	+	–	+	+	–	–	+	42
L-phenylalanine	–	–	–	+	–	–	+	+	–	+	–	+	–	42
L-proline	+	–	–	–	–	–	+	–	–	+	–	–	–	38
L-glycine	–	–	+	+	–	–	–	–	–	+	–	–	–	23
L-tyrosine	–	–	–	–	–	–	–	–	+	+	–	–	–	12
L-lysine	–	–	–	–	–	–	+	–	–	–	–	–	–	15
L-aspartic acid	–	–	–	–	–	–	–	–	–	–	–	–	–	0
Production of acid from different carbon sources														
D-glucose	+	+	+	+	+	+	+	+	+	+	+	+	–	96
D-mannitol	+	+	+	+	–	–	+	+	+	+	+	–	+	81
Inositol	+	–	+	–	–	–	+	–	–	–	+	+	–	50
D-sorbitol	–	–	+	+	–	–	+	+	–	+	–	+	–	46
L-rhamnose	+	–	+	+	–	–	–	+	+	+	–	–	–	46
D-sucrose	+	–	+	+	–	–	+	+	+	–	–	+	+	62
D-melibiose	+	–	+	+	–	–	–	+	+	+	–	+	–	50
Amygdalin	+	–	–	+	–	–	–	+	+	+	+	+	+	62
L-arabinose	+	–	+	+	–	–	–	+	+	+	–	–	+	50

conjunctiva, respiratory tract, throat, sputum, wounds and stool, the intestinal contents of snails and slugs, vacuum packaged meat and mushrooms, the original source of *Ewingella americana* was believed to be through the water.[28] The presence of *Ewingella americana* on soaked cattle hide was thought to be related to the water in our study.

Kluyvera intermedia, which was isolated from only one soaked sheepskin and only one cattle hide, has been known as a potential pathogen. *Kluyvera* species can be found in milk, water, sewage and soil samples. This micro-organism was also isolated from fresh vegetables and cattle.[28] The presence of *Kluyvera intermedia* on the samples was thought to be related to environmental contamination and the animal itself.

Morganella morganii has been found in intestinal tract of humans and animals. This species was isolated from stools, blood, wound, sputum, eyes, bile and gastric ulcers.[29] The presence of *Morganella morganii* on both sheepskin and cattle hide may be as a consequence of faecal contamination and the animal itself.

Proteus mirabilis, which was found on only one soaked sheepskin, was considered as an opportunistic pathogen. This micro-organism is a member of the normal bacterial flora of the mammals' intestine.[29] *Proteus mirabilis* has been isolated from cattle, sheep, dog, human, monkey, pig, cat and rat. Moreover, *Proteus mirabilis* is commonly found in water, soil, faeces and sewage.[29] The presence of this microorganism on the sheepskin may be related to water, soil or faeces.

Providencia rettgeri, which was found on both sheepskins and cattle hide, is a clinically important nosocomial pathogen.[29,30] *Providencia* species are commonly found in water, sewage and soil. They are also found in animals such as cats, penguins, dogs and birds.[29,30] The presence of *Providencia rettgeri* on skins and hide was thought to be related to the natural environments such as soil and water.

Serratia species, isolated from six soaked cattle hides, have been known as opportunistic human pathogens.[4] These species were isolated from urine, faeces, wound exudates, human blood, the intestinal canals of rodents and insects, water, soil, plants and animal territories.[31] Detection of *Serratia marcescens, Serratia plymuthica* and *Serratia rubidae* on soaked cattle hides may be related to water, soil, animal feed, insects, animal territories and animal itself.

The presence of the family *Enterobacteriaceae* was also detected in our previous study conducted with 10 salted hides from England, Australia and Turkey. Ninety-eight enteric species belonging to genera *Edwardsiella, Escherichia, Enterobacter, Salmonella, Serratia, Citrobacter, Yersinia, Hafnia, Klebsiella* and *Proteus* were isolated from the salted hides. *Enterobacter* species (66 isolates) were detected as the most common bacteria on these hide samples.[13] Furthermore, 32 enteric isolates belonging to the family *Enterobacteriaceae* were isolated from five salted cattle hides (Dubai, Turkey, Israel) and five salted sheepskins (Australia, Lebanon, USA, South Africa). While enteric species belonging to genera *Cedecea, Enterobacter, Escherichia, Ewingella, Klebsiella, Proteus, Raoultella, Serratia* and *Yersinia* were isolated from the cattle hide samples, enteric species belonging to genera *Citrobacter, Enterobacter, Escherichia, Klebsiella, Proteus, Raoultella, Serratia* and *Yersinia* were isolated from the sheepskin samples. Although *Serratia rubidaea, Yersinia enterocolitica* and *Cedecea lapagei* were found as the most common isolates on the hides, *Serratia plymuthica, Escherichia coli* and *Serratia rubidaea* were detected as the most common isolates on the skin samples. Among these isolates, the most common isolate on both hides and skins was *Serratia rubidaea*.[14]

The genera *Citrobacter, Enterobacter, Ewingella, Proteus* and *Serratia* detected in the present study were also detected in the salted hide and skin samples examined in the previous studies.[13,14] While some members of genera *Citrobacter, Serratia* and *Enterobacter* detected in the present study were stated as opportunistic pathogens, *Proteus* species were stated as human intestinal bacteria, which may be occasional pathogen.[2]

CONCLUSIONS

In the present study a total of 26 isolates and 13 different enteric species belonging to nine genera of the family *Enterobacteriaceae* were obtained from soaked skin and hide samples. 14 enteric isolates of the soaked sheepskins belonged to genera *Citrobacter, Cronobacter, Enterobacter, Kluyvera, Morganella, Proteus* and *Providencia*, 12 enteric isolates of the soaked cattle hides belonged to genera *Citrobacter, Cronobacter, Ewingella, Kluyvera, Morganella, Providencia* and *Serratia.* The prevalence of members of the family *Enterobacteriaceae* on all soaked sheepskins and cattle hides has been thought to be related to animal faeces, water, animal feed, soil and the animal itself. The prevalence of members of the family *Enterobacteriaceae* on the soaked sheepskins and cattle hides detected in the present study was found to be lower than the salted cattle hides and salted sheepskins examined in a previous study.[14] However, these enteric bacteria found on the soaked sheepskins and cattle hides could not be completely killed with commonly used antimicrobial agent in soaking solutions. Biochemical test results belonging to protein, lipid and carbohydrate catabolism demonstrated that a fairly high percentage of the soaked samples contained enteric isolates, which may break down sheepskins and cattle hides. Since some of these isolates may be opportunistic pathogens in humans, effective antibacterial treatments should be applied in salt curing and soaking processes. These treatments will help to kill pathogenic enteric bacteria, and to improve the leather's commercial value and the industry's financial viability.

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