A REVIEW ON CATABOLIC ACTIVITY OF MICROORGANISMS IN LEATHER INDUSTRY

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A tremendously diverse group of microorganisms originated from animal skins/hides, animals' feces, preservation salt, dust, barn, water, air, soil, feed have been found on salted hides/skins. Growth and catabolic activities of these microorganisms have been supported by high organic and inorganic contents of salted hides/skins. As known, detail examination of catabolic activities of microorganisms offers an important information about their critical roles on hide/skin biodegradation. The goal of this review is to summarize experimental results of the previous studies to understand biodegradation capabilities of the microorganisms isolated from leather industry. Catabolic activities of microorganisms belonging to non-halophilic bacteria, moderately halophilic bacteria, extremely halophilic archaea and the members of family *Enterobacteriaceae* were summarized in the present study. The characterization of these microorganisms was performed according to molecular methods, conventional biochemical tests in the previous studies. Examination of research articles showed that aerobic microorganisms isolated from salted hides/skins produced protease, caseinase, lipase, β-galactosidase, amylase, cellulase, DNase, lecithinase and urease. Moreover, the isolates produced acid from different carbon sources, reduced nitrate to nitrite, produced NH_3 from peptone, decarboxylated different amino acids found in hides/skins. These studies demonstrated that salted hides/skins had a wide diversity of microorganisms which have different catabolic activities to breakdown carbon and energy sources for their growth.

Keywords: Bacteria, Archaea, Leather industry

INTRODUCTION

Skin is composed of water (64%), fats (2%), structural proteins (29% collagen, 2% keratin, 0.3% elastin), non-structural proteins (1% albumins and globulins, 0.7% mucins and mucoids), mineral salts (0.5%), other substances (0.5%) (Highberger, 1956; Hien, 2006). Freshly slaughtered skins are an ideal growth supporting organic medium for microbial cells which made up water, nucleic acids, proteins, polysaccharides, and fats. Presence of different of microorganisms on cattle hides, sheep and goat skins was detected by researchers and their high prevalence on hides and skins were related to high nutritional content of hides and skins. While some of these microorganisms are related to normal microbial flora of the animal hides and skins, the others may be contaminant microorganisms found in the air, water, soil, pasture, animal feeds, animal feces, barn, slaughterhouse, and tanneries (Sverre, 1956; Birbir and Ilgaz, 1996; Ulusoy and Birbir, 2015). When the animal is alive, growth of these contaminant microorganisms have been prevented by acidic pH of the hides/skins as well as normal microbial flora of the animals. After flaying process, these microorganisms release different enzymes to break down proteins, fats and carbohydrates into their building blocks for their carbon, nitrogen, hidrogen, and the other needs to grow. Hence, hides and skins are salted to prevent the growth and damage of these non-halophilic microorganisms but preservation salt contaminates hides and skins with extremely halophilic archaea, halotolerant, slightly halophilic and moderately halophilic bacteria.

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NON-HALOPHILIC BACTERIA ON HIDES AND SKINS

In the study of Venkatesan and colleagues (1970), non-halophilic protease positive *Achromobacter liquefaciens, Alcaligenes marshalli*, *Bacillus cereus*, *Bacillus circulans, Bacillus megatherium, Bacillus pantothenticus, Bacillus subtilis, Brevibacterium insectiphilum*, *Micrococcus roseus* and *Staphylococcus aureus* were isolated from three fresh goat skins obtained from India. After 24 hours curing process of the goat skins, non-halophilic protease positive *Alcaligenes marshalli*, *Bacillus cereus*, *Bacillus megaterium, Bacillus subtilis, Kurthia variabilis*, *Micrococcus rubens, Staphylococcus aureus* were isolated.

Twenty-one isolates of *Bacillus cereus* (4)*, Bacillus megaterium* (2)*, Bacillus sphaericus* (3)*, Bacillus subtilis* (7), *Kurthia variabilis* (1), *Micrococcus roseus* (2), *Staphylococcus aureus* (2) were isolated from newly slaughtered 10 fresh cattle hides collected from slaughterhouse in Istanbul, Turkey. All *Bacillus* species, two *Staphylococcus aureus* species (86% of the isolates) were able to digest gelatin. We detected that 81%, 62%, 81%, 10%, 52% of the isolates respectively produced urease, amylase, reduced nitrate to nitrite, produced indol from tryptophan, produced *acetoin* from glucose (Birbir and Ilgaz, 1996).

Sixty-six bacterial isolates of *Bacillus brevis* (2), *Bacillus cereus* (8)*, Bacillus licheniformis* (3), *Bacillus megaterium* (2)*, Bacillus pumilus* (3), *Bacillus sphaericus*(5), *Bacillus subtilis* (10), *Kurthia variabilis* (3), *Micrococcus candidus* (1), *Micrococcus luteus* (10), *Micrococcus roseus* (*3*)*, Micrococcus rubens* (6), *Staphylococcus aureus*(7) and *Staphylococcus epidermidis* (3) were obtained from one week old 15 salted cattle hides collected from tanneries of Turkey. High catabolic activities and different bacterial species were detected at salted hides compare to the fresh cattle hides. Proteolytic activities were observed in all *Bacillus* species, all isolates of *Micrococcus* roseus, *Staphylococcus aureus* and *Staphylococcus epidermidis*. While 56% of the isolates reduced nitrate to nitrite and produced urease enzyme, 46%, 32% and 5% of the isolates produced amylase, the neutral product acetoin from glucose, and indol from tryptophan, respectively (Birbir and Ilgaz, 1996).

Furthermore, 71 isolates of *Bacillus cereus* (3), *Bacillus firmus* (1), *Bacillus licheniformis* (4)*, Bacillus megaterium* (7)*, Bacillus pumilus* (4), *Bacillus sphaericus*(8), *Bacillus subtilis* (24)*, Kurthia variabilis* (1), *Micrococcus candidus* (1), *Micrococcus luteus* (5), *Micrococcus roseus* (6), *Micrococcus rubens* (3), *Staphylococcus aureus*(3) and *Staphylococcus epidermidis* (1) were isolated from two months old 25 salted cattle hides. Proteolytic activities (96 % of the isolates) were observed in all *Bacillus* species, all isolates of *Micrococcus luteus, Micrococcus roseus* and *Staphylococcus aureus* and all isolates of *Staphylococcus epidermidis, Kurthia variabilis,Micrococcus candidus.* While 79% and 90 of the isolates respectively reduced nitrate to nitrite and produced urease enzyme, 65%, 49%, 8% of the isolates produced amylase, acetoin from glucose, and indol from tryptophan, respectively. Epidermis, dermis and hipodermis layers and collagen fibers of the hides were damaged by bacterial attack (Birbir and Ilgaz, 1996).

In another study, 256 non-halophilic Gram-negative bacterial isolates of *Acinetobacter baumannii* (3), *Acinetobacter calcoaceticus* (2), *Acinetobacter haemolyticus* (1), *Acinetobacter junii ssp. johnsonii* (5), *Acinetobacter lwoffii* (5), *Aeromonas caviae* (1), *Aeromonas hydrophila* (1), *Alcaligenes faecalis* (3), *Burkholderia gladioli* (7), *Citrobacter amalonaticus* (1), *Citrobacter ferundii* (3), *Comamonas testesteroni* (2), *Edwardsiella tarda* (1), *Enterobacter aerogenes* (4), *Enterobacter agglomerans* (4),*Enterobacter amnigenus* (12), *Enterobacter cloacae* (22), *Enterobacter gergoviae* (9), *Enterobacter intermedius* (4),

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Enterobacter liquefaciens(3), *Enterobacter sakazakii* (8), *Escherichia coli*(9), *Hafnia alvei*(14), *Klebsiella oxytoca* (1), *Klebsiella pneumoniae ssp. ozanae* (1), *Mannheimia haemolytica* (3), *Pasteurella multocida* (4), *Pasteurella pneumotropica* (5), *Proteus mirabilis* (1), *Pseudomonas aeruginosa* (1), *Pseudomonas fluorescens* (9), *Pseudomonas luteola* (29), *Pseudomonas maltophila* (2), *Pseudomonas paucimobilis* (1), *Pseudomonas pseudoalcaligenes* (1), *Pseudomonas putida* (16), *Salmonella choleraesuis ssp. arizonae* (1), *Salmonella paratyphi A* (1), *Salmonella typhimurium* (1), *Serratia marcescens* (1), *Sphingomonas paucimobilis* (5), *Stenotrophomonas maltophila* (12), *Vibrio fluvialis* (27), *Vibrio vulnificus* (5), *Yersinia pseudotuberculosis* (1), *Yersinia ruckeri* (4) were isolated from ten salted hides cured in England, Australia and Turkey (Aslan and Birbir, 2012).

Moreover, 396 Gram-positive bacteria such as *Aerococcus urinae* (5), *Aerococcus viridans* (24), *Aneurinibacillus aneurinilyticus* (4), *Bacillus amyloliquefaciens* (3), *Bacillus cereus* (3), *Bacillus firmus* (8), *Bacillus laterosporus* (1), *Bacillus lentus* (15), *Bacillus licheniformis* (16), *Bacillus megaterium* (10), *Bacillus mycoides* (4), *Bacillus pumilus* (20), *Bacillus subtilis* (14), *Bacillus thuringiensis* (17), *Brevibacillus laterosporus* (2), *Enterococcus avium* (17), *Enterococcus casseliflavus* (3), *Enterococcus durans* (6), *Enterococcus faecalis* (12), *Enterococcus faecium* (14), *Enterococcus gallinarum* (23), *Geobacillus stearothermophilus* (3), *Geobacillus thermoglucosidiasius* (3), *Kocuira kristanea* (4), *Kocuira varians* (4), *Lactococcus lactis* (13), *Paenibacillus pabuli* (3), *Streptococcus acidominimus* (13), *Streptococcus bovis* (2), *Streptococcus pluranimalium* (4), *Streptococcus thermophilus* (1), *Streptococcus uberis* (7), *Staphylococcus aureus* (7), *Staphylococcus capitis* (15), *Staphylococcus caprae*(2), *Staphylococcus chromogenes*(2),*Staphylococcus cohnii* (17), *Staphylococcus epidermidis* (1), *Staphylococcus hominis* (9), *Staphylococcus hyicus* (7), *Staphylococcus intermedius* (32), *Staphylococcus lentus* (1), *Staphylococcus lugdunensis* (3), *Staphylococcus sciuri* (3), *Staphylococcus xylosus* (15), *Staphylococcus warneri* (1), *Virgibacillus panthothenticus* (3) were isolated (Aslan and Birbir, 2011). While 68%, 52%, 43% of Gram-negative bacterial isolates and 70%, 69% and 57% of the Gram-positive bacterial isolates demonstrated respectively proteolytic, lipolytic and both proteolytic and lipolytic activities (Aslan and Birbir, 2012;Aslan and Birbir, 2011).

Extremely Halophilic Archaea and Moderately Halophilic Bacteria on Salted Hides and Skins

Kallenberger (1985) stated that halophilic microorganisms found in curing salt contaminate hides and skins. Examination of 131 brine-cured cattle hides collected from USA showed that 94% of salted hide samples were contaminated with extremely halophilic archaea (Bailey and Birbir, 1993). Fifty three percent of these extremely halophilic archaea showed protease activities. When 35 salted hides cured in France and Russia were examined, it was observed that 29%, 37%, 86%, 91% of the samples respectively contained halotolerant, slightly halophilic, moderately halophilic bacteria, and extremely halophilic archaea (Birbir, 1997).

In the study of Akpolat *et al*. (2015), 101 extremely halophilic archaea such as *Halorubrum tebenquichense* (54), *Halorubrum saccharovorum* (24), *Halorubrum lipolyticum* (1), *Halorubrum kocurii* (1), *Halorubrum terrestre* (1), *Halococcus morrhuae* (2), *Halococcus dombrowskii* (9), *Halococcus qingdaonensis* (3), *Natrinema versiforme* (1), *Natrinema pellirubrum* (3), *Halostagnicola larsenii* (1), *Haloterrigena saccharevitans* (1) were isolated from salted sheep skins collected from Spain. Proteolytic and lipolytic activities of these isolates were detected as 15% and 5%.

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In a study carried out with salted cattle hides imported from Australia and England, 13 moderately halophilic bacteria such as *Alkalibacillus salilacus, Salimicrobium album*, *Salimicrobium halophilum*, *Salimicrobium luteum*, *Marinococcus halophilus*, *Halomonas koreensis*, *Halomonas alimentaria*, *Halomonas elongata*, *Halomonas halmophila*, *Halomonas eurihalina*, *Thalassobacillus devorans*, *Chromohalobacter salexigens*, *Oceanobacillus picturae* and five extremely halophilic archaeal species such as *Halorubrum saccharovorum*, *Halorubrum tebenquichense*, *Halorubrum lacusprofundi*, *Natrinema pallidum* and *Natrinema gari* were isolated from these hides. Amylase, protease, lipase, caseinase, urease, pullulanase and DNase activities of the moderately halophilic isolates were respectively detected as 15%, 31%, 15%, 15%, 31%, 8%, 38%. Amylase, protease, urease, DNase activities of the extremely halophilic isolates were detected as 20%. In addition, 54%, 77%, 54, 38, 15 of the moderately halophilic isolates and 40%, 60%, 40%, 20%, 20% of the extremely halophilic archaeal isolates respectively used citrate as a sole carbon source, reduced nitrate to nitrite, produced acid from glucose, sucrose and lactose (Caglayan *et al*., 2015).

In a study of Bilgi *et al*. (2015), 186 extremely halophilic archaea were isolated from eight salted hides and skins cured in Turkey, Iraq, Turkmenistan, Kazakhstan and Armenia. Isolation of extremely halophilic archaeal isolates of *Natronococcus* sp., *Natronococcus jeotgali*, *Natrialba aegyptia*, *Halovivax* sp. E107, *Halovivax asiaticus*, *Halococcus morrhuae*, *Halococcus thailandensis*, *Halococcus dombrowskii*, *Halorubrum* sp. CH3, *Natrinema pallidum*, *Natrinema versiforme*, *Haloterrigena thermotolerans*, *Halobacterium noricense* was stated by researchers. While all isolates had DNase activities, 12%, 57%, 11%, 35%, 18% of the isolates showed positive reactions for caseinase, protease, amylase, esterase, lipase activities. Moreover, it was detected that 8% and 62% of the isolates produced indole from tryptophan and reduced nitrate to nitrite.

In addition, 39 bacterial isolates of moderately halophilic bacteria such as *Staphylococcus saprophyticus* subsp. *saprophyticus* (7), *Staphylococcus xylosus* (2), *Staphylococcus equorum* subsp. *equorum* (1), *Staphylococcus arlettae* (6), *Bacillus pumilus* (6), *Bacillus licheniformis* (2), *Salinicoccus roseus* (3), *Gracilibacillus dipsosauri* (5), *Chromohalobacter beijerinckii* (2), *Chromohalobacter canadensis* (1), *Halomonas eurihalina* (2), *Halomonas zhanjiangensis* (1), *Halomonas venusta* (1) were isolated from 25 goat skins cured in Bulgaria, Israel, China, Australia, South Africa, Russia, France and Turkey. Protease, lipase, β-galactosidase, urease, caseinase, amylase, lecithinase, cellulase activities of the isolates were detected as 87%, 64%, 59%, 46%, 28%, 26%, 8% and 5% (Birbir *et al*., 2015; Caglayan *et al*., 2017). Indole production from tryptophan, citrate utilization, hydrogen sulfide (H_2S), reduction of nitrate to nitrite, ammonia production from peptone by the isolates were respectively detected as 3%, 31%, 5%, 51%, 85%. Moreover, acid production from glucose, sucrose, galactose, mannose by the isolates were found as 92%, 85%, 56%, 74%, respectively.

In a study of Caglayan *et al.* (2017), 77 bacterial isolates of moderately halophilic bacteria such as *Staphylococcus equorum* subsp. *equorum* (4), *Staphylococcus cohnii* subsp. *cohnii* (3), *Staphylococcus xylosus* (2), *Staphylococcus lentus* (2), *Staphylococcus saprophyticus* subsp. *saprophyticus* (1), *Salimicrobium salexigens*(4), *Bacillus pumilus* (2), *Bacillus licheniformis* (7), *Bacillus safensis* (2), *Bacillus siamensis* (1), *Bacillus tequilensis* (2), *Salinicoccus roseus* (2), *Planococcus rifietoensis* (1), *Alkalibacillus halophilus* (1), *Gracilibacillus dipsosauri* (4), *Marinococcus luteus* (1), *Marinococcus tarijensis* (1), *Oceanobacillus picturae* (1), *Halomonas halmophila* (5), *Halomonas eurihalina* (1), *Halomonas zhanjiangensis* (4), *Halomonas venusta* (2), *Halomonas alkaliphila* (3),

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Salinivibrio costicola subsp. *alkaliphilus* (4), *Chromohalobacter canadensis* (6), *Chromohalobacter beijerinckii* (6), *Chromohalobacter japonicus* (3), *Chromohalobacter israelensis* (1), *Idiomarina loihiensis* (1) were isolated from 23 salted sheep skins cured in Australia, Bulgaria, Dubai, Greece, Israel, Kuwait, South Africa, Turkey and U.S.A. Protease, lipase, β-galactosidase, amylase, caseinase, DNase, urease, cellulase, lecithinase activities of these isolates were respectively found as 60%, 43%, 39%, 26%, 23%, 17%, 12%, 12%, 10%. Indole production from tryptophan, citrate utilization, production of H_2S, reduction of nitrate to nitrite, production of ammonia from peptone by the isolates were detected as 12%, 47%, 8%, 78%, 87%, respectively. Acid production from glucose, sucrose, galactose, mannose by the isolates were found as 95%, 71%, 78%, 90%.

Utilization of different amino acids found in the skin by 137 moderately halophilic bacterial species belonging to genera *Halomonas*, *Planococcus*, *Salimicrobium*, *Alkalibacillus*,*Salinicoccus*,*Staphylococcus,Bacillus*,*Chromohalobacter*,*Gracilibacillus*, *Idiomarina,Marinococcus*, *Oceanobacillus, Salinivibrio* was investigated. Arginine was used by all moderately halophilic isolates obtained from the salted goat and sheep skins. L-hydroxyproline, L-proline, L-tyrosine, L-alanine, L-glycine amino acids were respectively utilized by 66%, 64%, 85%, 66%, 86% of the isolates but L-cysteine was used only 4% of the isolates. These results showed that salted sheep and goat skin isolates were able to utilize L-hydroxyproline, L-proline, L-tyrosine, L-alanine, L-glycine and L-cysteine amino acids found in the skin structure Caglayan *et al*. (2018).

Bacterial Species of the Family *Enterobacteriaceae* on the Salted Hides and Skins

In the other study, Ulusoy and Birbir (2015) examined members of the family *Enterobacteriaceae* on the salted hides and skins. While 27 isolates of *Enterobacter cloacae* (2), *Enterobacter sakazakii* (1), *Raoultella planticola* (1), *Raoultella ornithinolytica* (1), *Serratia odorifera* (1), *Serratia liquefacicens* (1), *Serratia plymuthica* (1), *Serratia rubidaea* (3), *Escherichia coli* (2), *Escherichia vulneris* (1), *Cedecea lapagei* (3), *Ewingella americana* (2), *Klebsiella pneumoniaea ssp. ozaenae (1), Klebsiella oxytoca* (2), *Proteus vulgaris* (2), *Yersinia enterocolitica* (3) were obtained from the hides salt cured in Dubai, Turkey and Israel and 28 isolates of *Citrobacter koseri* (1), *Enterobacter cloacae* (2), *Escherichia coli* (4), *Escherichia vulneris* (1), *Klebsiella pneumoniaea ssp. ozaenae* (1), *Klebsiella oxytoca* (1), *Proteus vulgaris* (2), *Proteus penneri* (1), *Raoultella planticola* (1), *Serratia odorifera* (1), *Serratia liquefacicens* (1), *Serratia plymuthica* (3), *Serratia ficaria* (1), *Serratia marcescens* (2), *Serratia rubidaea* (4), *Yersinia enterocolitica* (2) were obtained from the skin samples salt cured in Australia, Lebanon, U.S.A., South Africa. Protease, urease, β-galactosidase, lipase activities of the isolates were respectively found as 45%, 35%, 90%, 30%. Moreover, 25%, 35%, 35%, 45%, 35%, 70% of the isolates respectively were found to be positive for tryptophan deaminase, arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase, indol production, utilization of citrate. While all isolates produced acid from glucose, 75%, 70%, 90% of the enteric isolates produced acid from sucrose, arabinose, mannitol, respectively.

CONCLUSIONS

All of these studies carried out fresh, salted hides and skins clearly showed that non-halophilic bacteria, moderately halophilic bacteria, extremely halophilic archaea and bacterial species of the family *Enterobacteriaceae* were metabolically active to degrade

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proteins, fats, carbohydrates and use their building blocks for their nutritional and structural needs, growth and energy. While most of the examined hides and skin samples had bad odor and red, yellow, cream discolorations, some of them had hair slip which were related to microbial activities. These experimental study results clearly demonstrated that most of the organisms found on the skins and hides were belong to contaminant microorganisms found in the air, water, soil, curing salt, pasture, animal feeds, animal feces, barn, slaughterhouse, and tanneries. These studies also showed that traditional salt curing process does not prevent the growth of these different species of microorganisms causing huge economic losses in leather industry. Hence, we suggest using effective antimicrobial applications to exterminate these hide and skin degrading microorganisms in the leather industry.

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