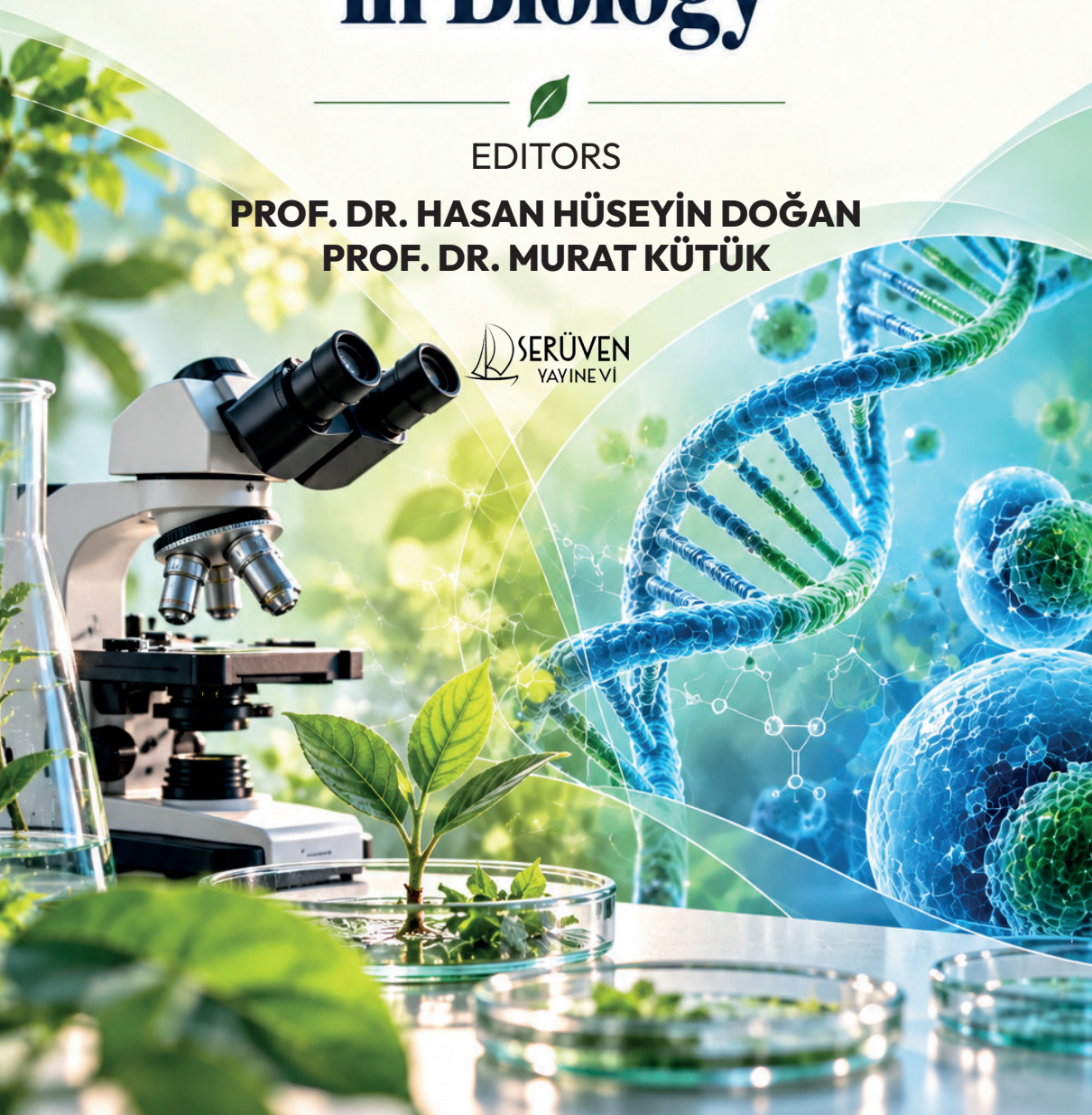
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PROF. DR. MURAT KÜTÜK

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Chapter 1

HALOVERSATILE MICROORGANISMS: A FASCINATING GROUP OF EXTREMOPHILES WITH UNIQUE PROPERTIES AND SURVIVAL STRATEGIES

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1.Introduction to Haloversatility

Microorganisms inhabit every environment on Earth, including habitats characterized by extreme temperature, pH, pressure, radiation, desiccation, and salinity (Oren, 2002). Microorganisms capable of thriving under such harsh conditions are collectively referred to as *extremophiles*. High salt concentration is a burden for salt-adapted microorganisms (Ventosa et al., 1998). Salinity affects microorganisms through different mechanisms. High extracellular salt concentrations reduce water availability and lead to osmotic stress; furthermore, they adversely affect cellular ion balance, membrane properties, protein folding, and enzymatic activity. Nevertheless, diverse microorganisms have evolved sophisticated mechanisms that enable them to tolerate saline conditions and remain metabolically active under salinity (Rothschild and Mancinelli, 2001). Halophilic microorganisms are traditionally classified according to their salt requirements and tolerance. While extreme halophiles require very high salt concentrations, moderate halophiles grow optimally at intermediate salinities. In contrast, halotolerant microorganisms do not require salt to grow, but they can tolerate high NaCl concentrations. Among halophiles, haloversatile bacteria can grow across a wide range of salt concentrations (from 0 to >3 M NaCl, optimum 0.2-0.5 M NaCl) as well as in the absence of salt (James et al., 1990; Kristjansson et al., 2000). Evaporation, precipitation, freshwater input, tidal movements, seasonal changes, and geological processes can lead to fluctuations in salt concentration. Since saline environments are dynamic systems, microorganisms inhabiting such environments must be able to cope with high salinity and sudden osmotic changes. In addition, many studies have been focused on the ecological and biotechnological potentials of haloversatile microorganisms. Due to the adaptation mechanisms they have developed against harsh conditions, metabolic compounds such as enzymes, pigments, osmoprotective molecules, polymers, and lipids have been investigated by the researchers. This chapter summarizes the distinctive properties of haloversatile microorganisms.

2. Haloversatile Microorganisms and Their Differences from Other Salt-Adapted Microorganisms

Based on their relationship with salt, microorganisms can be classified as non-halophilic, halotolerant, moderately halophilic, or extremely halophilic organisms. *Halophiles* are microorganisms that require salt for optimal growth (Ventosa, 2006). The cellular structures and macromolecules of halophiles are adapted to saline conditions, and many exhibit reduced growth or complete loss of viability when salt concentrations decrease substantially. In contrast, *halotolerant microorganisms* do not require high salt concentrations but they can tolerate saline conditions (Caviccihioli and Thomas, 2004). *Haloversatile microorganisms* occupy a particularly interesting position due to their ability to

maintain functionality across a relatively wide salinity range. Their versatility may stem from the flexible regulation of intracellular ions, the synthesis or uptake of compatible solutes, the alteration of membrane composition, and rapid changes in gene expression. Therefore, a key feature of halo-versatility is physiological plasticity. Rather than permanently adapting to a single salinity level, these microorganisms can adjust their physiology in response to environmental conditions. Such flexibility confers a significant ecological advantage in habitats characterized by salinity fluctuations.

3. Ecological Distribution of Haloversatile Microorganisms

Haloversatile (or euryhaline) microorganisms can be found in a wide variety of environments where salt concentrations fluctuate or remain moderate to high levels (Ventosa et al., 1998; Margesin and Schinner, 2001; Litchfeld and Gillevet, 2002). For instance, haloversatile microorganisms are found in seawater (Kushner, 1978), saline environments (Grant et al., 1998), seawater samples in Antarctica (Ellis-Evans, 1985), Antarctic saline lakes (James et al., 1990), saline water samples (Vreeland and Huval, 1991), salt pan (Zafrilla et al., 2010), Safaga coast in Egypt (Ashour et al., 2011), hydrothermal vent (Kaye et al., 2011), benthic sponges (Velho-Pereira and Irene Furtado, 2014), Çamaltı Saltern salt samples used in leather industry (Caglayan, 2019a; Caglayan, 2019b), soak liquor samples in leather industry (Ozbay and Caglayan, 2022), salted hide (Caglayan, 2022), preservation salt in leather industry (Abdulhusein et al., 2023), estuarine and coastal waters (Xia et al., 2023), salted goatskin (Kose et al., 2024).

3.1. Saline and Hypersaline Lakes

Saline and hypersaline lakes, as inland aquatic ecosystems, constitute important reservoirs of salt-adapted microorganisms (Mcgenity and Oren, 2012; He et al., 2022). While evaporation can lead to significant increases in salt concentration, precipitation and freshwater inflow can cause rapid decreases (Kumar, 2012; Perera et al., 2024). Such fluctuations favor microorganisms capable of adjusting their physiology to changing osmotic conditions. Microbial communities in saline lakes may include members of the domains *Bacteria* and *Archaea* as well as diverse unicellular eukaryotes. Their metabolic activities contribute to carbon, nitrogen, sulfur, and other elemental cycles.

3.2. Solar Salterns

Javor (2026) considered solar salterns as outdoor chemostats. Solar salt production facilities are artificial hypersaline systems used for salt production. They contain a series of ponds with progressively increasing salinity, creating distinct microbial habitats. In solar salterns, cyanobacteria, phototrophic

bacteria, sulfur-oxidizing bacteria, aerobes, sulfate reducers, methanogenic archaea, algae, diamotes inhabit in the solar ponds (Javor, 2026). Haloversatile microorganisms may be particularly important in transition zones where salinity changes substantially over relatively short spatial distances.

3.3. Marine and Coastal Habitats

Although seawater is considerably less saline than hypersaline environments, coastal ecosystems can experience substantial salinity fluctuations. Tidal flats, estuaries, salt marshes, and coastal sediments cause periodic mixing of freshwater and seawater. Microorganisms inhabiting these environments must therefore tolerate both osmotic stress and rapid changes in ionic composition. Despite the Dead Sea's hypersaline water, filamentous fungi, bacteria, archaea, algae, protists, diatoms, purple bacteria, and cyanobacteria have been detected (Al-Daghistani et al., 2024).

3.4. Saline Soils

Soil salinization, the process of accumulating of soluble salts, poses a serious threat to global food security and agricultural development by reducing the quality of soil (Ammari et al., 2013; Otlewska et al., 2020; Liu et al., 2026). High concentrations of soluble salts can reduce plant water uptake and negatively affect soil microbial communities. Microorganisms perform various adaptation strategies in the interactions between plants, soil, and microorganisms within the rhizosphere (Acosta-Motos et al., 2017; Otlewska et al., 2020). However, salt-adapted microorganisms can survive in these environments and may contribute to nutrient cycling and plant growth. The plant growth promoting rhizosphere bacteria can influence plant developement by fixing nitrogen and producing cytokinin (CK), indole acetic acid (IAA), abscisic acid (ABA), and gibberellic acid (GA) to overcome salinity stress (Egamberdieva et al., 2019). Some haloversatile microorganisms associated with plant roots may improve plant responses to salinity through production of phytohormones, osmoprotectants, extracellular enzymes, and other metabolites. ACC-deaminase enzyme breaks down the precursor of plant stress hormone ethylene, which regulates the plant's response to drought and enables root developement despite environmental stress (Glick, 2012).

3.5. Saline Industrial Environments

The leather, food, chemical, oil and gas industries can generate saline wastewater (Lefebvre et al., 2006). Conventional microorganisms may show reduced activity under such conditions (Gonzalez and Aranda, 2023). Haloversatile microorganisms are potentially valuable in biological treatment systems because they can remain metabolically active when salt concentrations

inhibit many conventional microbial communities.

3.6. Salted and Fermented Foods

Salt is among the substances widely used as food additive, preservative, flavoring compound, and antioxidant (Sekar and Kim, 2020). Sodium salt is of critical importance in determining the quality of fermented vegetables due to its direct effects on physicochemical properties, textural characteristics, storage stability, and food safety (Liu et al., 2026). The haloversatile microorganisms can grow on fermented or salted food products. They may contribute to flavor formation, spoilage, fermentation. These microorganisms can contribute to fermentation, flavor formation, degradation of organic compounds, and, in some cases, spoilage. Long- and short-chain, highly unsaturated omega-3 fatty acids; produced through the fermentation of euryhaline *Thraustochytrium* and *Schizochytrium* microorganisms (Barclay, 2002). In that patent, the researcher stated that the lipids with highly unsaturated omega-3 fatty acids produced by euryhaline microorganisms can be used in food or pharmaceutical industries (Barclay, 2002). Halophilic *Desulfovermiculus*, *Salinibacter*, *Tepidimonas*, and *Halovibrio* positively influence chemical composition of the food during fermentation (Niu et al., 2024). However, yeasts capable of tolerating high salt concentrations such as *Saccharomyces* spp., *Candida* spp., *Zygosaccharomyces* spp., *Issatchenkia* spp., and *Pichia* spp. can lead to food spoilage by softening and degrading food structure (Zhao et al., 2021).

4. Stress in Saline Conditions

Salinity represents a complex environmental stress rather than a single physiological challenge. Like halophiles, haloversatile microorganisms possess distinctive characteristics, such as cell memrane structures and funtional proteins that enable them to adapt to high external osmotic stress (Foophow et al., 2022).

4.1. Osmotic Stress

One of the immediate consequences of high external salt concentration is a reduction in water activity (Yang et al., 2017). When the extracellular environment becomes more concentrated than the cytoplasm, water tends to move out of the cell. Intracellular water loss can lead to cell shrinkage, disruption of macromolecular interactions, reduced enzymatic activity, and impairment of cellular metabolism. Haloversatile microorganisms neutralize this effect by increasing intracellular osmotic pressure through the controlled accumulation of inorganic ions or compatible solutes.

4.2. Membrane Stress

Osmolarity fluctuations shape microbial diversity in addition to profoundly affecting cellular physiological processes such as nutrient uptake,

phenotypic traits, cell size, cell shape, and antibiotic susceptibility (Greenwood and Grady, 1972; Rojas et al., 2014; He et al., 2022; Hunter et al., 2026).

Sodium ions can disrupt fundamental cellular processes when they accumulate in excessive amounts in the cytoplasm (Oren, 2013). High intracellular sodium concentrations can disrupt enzyme activity, protein structure, protein folding, membrane structure, membrane permeability, and potassium homeostasis (Stautz et al., 2021). Hence, maintaining an appropriate intracellular Na^+/K^+ balance is of fundamental importance for the adaptation process (Suhail, 2010). The proteins of salt-adapted microorganisms are generally remain soluble and functional under high osmotic conentrations. Researchers reported that the euryhaline alga *Chlorella autotrophica*, cultivated under high-salinity conditions, exhibited an increase in cell volume and a slight decrease in cellular water content (Ahmad and Hellebust, 1984).

4.3. Oxidative Stress

Salinity stress can indirectly increase the formation of reactive oxygen species (ROS). Excessive ROS can damage proteins, nucleic acids, lipids, and other cellular components. Consequently, antioxidant defense systems constitute a key component of adaptation to salt stress (Kesawat et al., 2023).

4.4. Solar Radiation

Solar radiation is another stress factor for halophilic microorganisms (Andrei et al., 2012). Mattimore and Battista (1995) noted that astobiological studies revelaed a strong link between UV resistance and resistance to dessication. Furthermore, the researchers stated that halophilic archaea accumulate trehalose and glycine betaine as compatible solutes to overcome the effects of UV radiation (Goh et al., 2011; Leuko et al., 2015).

5. Survival Strategies of Haloversatile Microorganisms

The extraordinary adaptability of haloversatile microorganisms stems from the integration of various complementary mechanisms. When bacteria are exposed to high osmotic conditions, specific solutes such as K^+, glutamate, trehalose, proline, or glycine betaine are accumulated in the cytoplasm to maintain osmolarity (Csonka, 1989).

5.1. The 'Salt-In' Strategy

The *salt-in* strategy is characterized by the accumulation of inorganic ions, such as K^+ ions, within the cell. Microorganisms can reduce the osmotic difference between the cytoplasm and the external environment. Intracellular solute concentrations can be rapidly adjusted in response to external salinity; consequently, microorganisms employing this *low intracellular salt* strategy

can generally adapt to life across a wide range of salinity levels (Galinski, 1995; Oren, 2013).

5.2. Compatible Solutes

An alternative and widespread mechanism involves accumulation of organic compounds known as compatible solutes or osmolytes. Compatible solutes such as glycine betaine, glycerol, ectoine, hydroxyectoine, proline, trehalose, glutamate, sugars and polyols can accumulate at high concentrations without significantly disrupting cellular biochemical processes (Goszcz et al., 2025).

Osmotic balance is mainted by these compounds; proteins, membranes, and other cellular structures are also protected. Sucrose and proline have been detected in *Chlorella autotrophica*, an euryhaline alga, to balance external salt concentrations in the cells (Ahmad and Hellebust, 1984).

5.3. Ion Transport and Homeostasis

Transport proteins play a crucial role in maintaining intracellular ion concentrations. Potassium transport systems, Na /H antiporters, ion channels, and ATP-dependent transporters contribute to the regulation of cytoplasmic ionic composition. Efficient ion homeostasis enables haloversatile microorganisms to rapidly respond to changes in external salinity.

5.4. Membrane Adaptation

Membrane properties, characteristics, structure, and composition are dynamically regulated in response to environmental stress. Fatty acid chain length, degree of saturation, branching, and other membrane lipid modifications can help maintain appropriate membrane fluidity.

5.5. Protein Adaptation

Proteins and enzymes adapted to high salinity possess structural features that enhance their stability under high ionic strength conditions, owing to their high content of acidic amino acids (Saker and Kim, 2020). These proteins can maintain catalytic activity under environmental stress. Heat shock stress proteins (HSPs), universal stress proteins, thermoprotectants are among the elements of haloarchaeal stress response (Matarredona et al., 2020).

6. Metabolic Versatility and Ecological Roles of Haloversatile Microorganisms under Saline Conditions

The ecological success of haloversatile microorganisms is not solely linked to their ability to tolerate salt. Many species exhibit broad metabolic capabilities that enable them to utilize diverse carbon and energy sources. Depending on the organism and the habitat, haloversatile microorganisms

can play roles in processes such as anaerobic respiration, aerobic respiration, fermentation, sulfur transformation, carbon fixation, the breakdown of organic polymers, the nitrogen cycle, the degradation of hydrocarbons, and the utilization of complex carbohydrates. Their metabolic flexibility can provide a competitive advantage in environments where nutrient availability varies in conjunction with salinity.

6.1. Carbon Cycling

Calvin-Benson-Bassham, reductive tricarxylic acid, Wood-Ljungdahl pathways are of vital importance for the carbon cycle in saline environments (Sorokin et al., 2014; Zhao et al., 2019). Haloversatile microorganisms can contribute to decomposition, fermentation, carbon fixation, and the transformation of organic matter. In saline ecosystems, the activities of these microorganisms can influence the accumulation and cycling of dissolved and particulate organic carbon.

6.2. Nitrogen Cycling

There is a significant correlation beween saline enviroments and nitrogen cycle. Salt-adapted microorganisms can participate in nitrogen fixation, ammonification, nitrification, denitrification, and other nitrogen transformations (Gao et al., 2024).

6.3. Sulfur Cycling

Sulfur metabolism is particularly important in saline sediments and hypersaline ecosystems. Microorganisms involved in sulfur oxidation and reduction can influence sulfur availability and ecosystem chemistry. While sulfur-oxidizing bacteria use H_2S, S^0, $S_2O_3^{-2}$, sulfur-reducing bacteria use SO_4^{-2} (Bryanskaya et al., 2022). Haloversatile microorganisms interact with their environment through competition, syntrophy, predation, signaling, metabolite exchange, and biofilm formation. These interactions may be particularly important in hypersaline environments.

6.4. Phospate and Lead Cycling

Phosphate-solubilizing bacteria convert phosphorus into a form usable by plants and form lead precipitates to reduce heavy metal pollution (Zhang et al., 2023).

7. Enzymes from Haloversatile Microorganisms

One of the most important biotechnological characteristics of haloversatile microorganisms is their capacity to produce salt-tolerant enzymes. Salt-tolerant enzymes remain catalytically active in the presence of high concentrations of NaCl and other salts. These valuable enzymes—such as lipases, proteases, xylanases, pullulanases, esterases, amylases, cellulases—are widely used in

industrial processes where high ionic strength is unavoidable. Thanks to their stability under organic solvents, pH, temperature, and salt conditions, more efficient biocatalytic processes can be carried out. Since conventional culture-dependent methods can reveal only limited microbial diversity, enzymes from haloversatile microorganisms hold promise. Proteomic, metagenomic, metatranscriptomic, and genome mining approaches can uncover novel enzymes from microorganisms that have not previously been cultured or whose characteristics have not been fully defined.

8. Biotechnological Applications

8.1. Bioremediation

Halophilic microorganisms can be used in varous industries for bioremediation purposes. Hydrocarbon-degrading euryhaline microbial consortia comprising the genera *Marinobacter*, *Bacillus*, *Erwinia*, which can be capable of treating oily waste under saline conditions, have been isolated for the first time from crude oil and mangrove sediments (Piedad Díaz et al., 2000). Halophilic archaea can biologically degrade organic pollutants (Sekar and Kim, 2020). Similarly, haloversatile microorganisms can be useful in the bioremediation of environments exposed to saline and hypersaline pollution. Their ability to remain active under high salt concentrations enables them to degrade pollutants in conditions unsuitable for many conventional microorganisms. Hydrocarbons, organic pollutants, agricultural chemicals, and industrial waste compounds are among the potential targets.

8.2. Wastewater Treatment

It is known that discharging such wastewaters characterized by high salinity and high organic matter content without undergoing pre-treatment adversely affects aquatic life, drinking water potability, and agricultural productivity (Lefebvre and Moletta, 2006). Since the treatment of saline wastewater is hindered by issues related to salinity and turbidity, the use of halophilic aerobic or anaerobic organisms in biological treatment processes is a focal point for researchers (Lefebvre and Moletta, 2006). The existence of halophilic bacteria capable of removing heavy metals such as arsenic, cadmium, and mercury from saline wastewater via bioremediation is known (Sekar and Kim, 2020). While conventional microorganisms can be brought under control under saline conditions, haloversatile microbial communities can persist by remaining metabolically active under these conditions.

8.3. Food Biotechnology

Haloversatile microorganisms and their enzymes may contribute to food fermentation, preservation, flavor development, and food-processing under saline conditions.

8.4. Agriculture

Haloversatile microorganisms may have the potential to be beneficial plant-associated microorganisms. Some strains can produce compounds that promote plant growth or enhance the tolerance of plants to saline environments. Possible mechanisms include: Production of indole-3-acetic acid, ACC deaminase activity, phosphate solubilization, siderophore production, improvement of nutrient availability, production of osmoprotective compounds are among the possible mechanisms. The development of microbial inoculants for saline soils represents an emerging field of research.

8.5. Pharmaceutical and Biomedical Biotechnology

Halophilic and halotolerant microorganisms produce a wide variety of secondary metabolites, pigments, antimicrobial compounds, and other bioactive molecules. It has been reported that extremophile enzymes can prevent alterations in protein structure, thereby potentially enabling the treatment of neurodegenerative diseases such as Alzheimer's, Huntington's, and Parkinson's (Uppar et al., 2024). Although many discoveries are still in the early stages, saline environments represent promising sources of chemical diversity.

9. Bioactive Compounds

In addition to enzymes, haloversatile microorganisms can produce structurally and functionally diverse secondary metabolites. These compounds may include: antimicrobial molecules, pigments, antioxidants, extracellular polysaccharides, osmoprotective compounds, surfactants, polyhydroxyalkanoates are among the secondary metabolites of haloversatile microorganisms. Euryhaline cyanobacteria *Synechococcus* sp. (isolate PCC7002) was able to produce glycogen, which can be used as biofuel in biotechnological processes (Aikawa et al., 2014).

10. Current Challenges

Using omic methods, researchers have been able to uncover the genetic characteristics of *Synechococcus*—a unicellular, fully marine cyanobacterium capable of persisting across a range of salinities—and how it survives under salinity fluctuations (Xia et al., 2023). Despite the growing body of knowledge regarding salt-adapted microorganisms, several important questions remain unanswered. First of all, the diversity of microorganisms inhabiting saline environments has not been fully characterized because it is difficult to cultivate many organisms using traditional methods. Secondly, the molecular mechanisms responsible for rapid adaptation to changing salinity are not fully understood. Although most studies focus on either low or high salinity conditions, natural environments often exhibit continuous fluctuations.

Moreover, the relationship between salt tolerance and other stress responses such as desiccation, nutrient limitation, pH changes, oxidative stress, temperature stress requires further investigation. Various potentially valuable metabolites and enzymes have not yet been discovered. Enzymes and microorganisms with promising characteristics should be used in industrial applications.

11. Future Perspectives

Future research on haloversatile microorganisms is likely to focus increasingly on their functional diversity rather than merely their taxonomic classification. A promising aspect is the integration of multi-omics approaches combines data from transcriptomics, genomics, proteomics, metabolomics. Integrating transcriptomics, genomics, proteomics, metabolomics can provide a more comprehensive understanding of microbial responses to salinity. Under conventional cultivation conditions, many biosynthetic pathways remain inactive. Advanced cultivation methods that leverage complex physicochemical properties can help researchers overcome the barrier of inaccessibility and obtain microorganisms. A promising synthetic biology method that aims to produce microbial metabolites on a large scale by utilizing metabolic pathways and genetic information for biotechnological applications. The discovery of new extremophiles is also expected to remain an important area of research. Enzymes capable of functioning under combinations of high salt concentrations, extreme pH, high temperatures, or environmental stress factors can possess significant industrial value. Finally, saline environments should be regarded not only as extreme ecosystems but also as reservoirs of genetic and biochemical diversity.

12. Conclusion

Haloversatile microorganisms represent a fascinating group of extremophilic and stress-adapted bacteria, archaea, eukarya characterized by their ability to survive and maintain metabolic activity across wide ranges of salinity. Their success in fluctuating saline environments results from a combination of ion homeostasis, osmotic regulation, membrane shape changes, compatible-solute accumulation, protein adaptation, antioxidant defense, and flexible metabolic regulation. Halo-versatile microorganisms are considered valuable sources of salt-tolerant enzymes, metabolites, osmoprotective compounds, and bioactive metabolites capable of functioning under saline conditions; they hold significant potential in the fields of biotechnology, agriculture, food processing, bioremediation, and industrial biocatalysis. Studies on haloversatile microorganisms also contribute to various questions regarding microbial evolution, environmental resilience, and the limits of life. Recent advances in evolutionary biology including genomics, high-throughput

metagenomic sequencing, microbial single-cell sequencing, metabolomics, synthetic biology, and high-throughput microbial cultivation and isolation are likely to reveal large diversity of unknown haloversatile microorganisms. In conclusion, the study of haloversatility demonstrates that microbial survival is not merely a matter of resisting extreme conditions. Rather, it reflects a dynamic capacity to sense environmental change, reorganize cellular physiology, and maintain biological activity under fluctuating conditions. This extraordinary adaptability makes haloversatile microorganisms both scientifically intriguing and promising sources for future biotechnological innovations.

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Chapter 2

CELLULAR AND MOLECULAR MECHANISMS IN PELVIC ORGAN PROLAPSE: EXTRACELLULAR MATRIX, OXIDATIVE STRESS, INFLAMMATION, AND CELLULAR SENESCENCE

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1. Introduction

Pelvic organ prolapse (POP) is a multifactorial pelvic floor disorder characterized by the downward displacement of pelvic organs, including the bladder, uterus, vagina, or rectum, from their normal anatomical positions. It may adversely affect women's quality of life, urinary and bowel function, and sexual health (Haylen et al., 2010; Jelovsek et al., 2007). POP frequently coexists with urinary incontinence and other pelvic floor disorders and is considered an important component of women's pelvic floor health (Milsom et al., 2012). The association between pelvic organ prolapse and overactive bladder is also clinically relevant, and the two conditions may coexist in the same patient (de Boer et al., 2010).

Numerous risk factors contribute to the development of POP, including advanced age, pregnancy and childbirth, particularly vaginal delivery, obesity, chronic constipation, chronic cough, and increased intra-abdominal pressure (Jelovsek et al., 2007; Memon & Handa, 2013). However, the fact that women exposed to similar mechanical and obstetric risk factors may differ considerably in the development and severity of prolapse suggests that the disease cannot be explained solely by mechanical failure of pelvic support. These differences highlight the potential roles of the biological properties of pelvic support tissues, extracellular matrix (ECM) metabolism, fibroblast function, collagen and elastin homeostasis, hormonal environment, and cellular aging processes in the pathogenesis of POP.

The integrity of the pelvic support system depends on the coordinated function of the pelvic floor muscles, fascia, ligaments, and connective tissue components within these structures. The mechanical strength and elasticity of connective tissue are largely determined by the composition and organization of the ECM. The ECM is a dynamic biological structure composed of collagen, elastin, proteoglycans, glycoproteins, and various matrix-associated molecules. Rather than serving solely as a passive strüktürele scaffold, the ECM actively regulates cell proliferation, differentiation, migration, metabolism, and cellular responses to mechanical stimuli (Theocharis et al., 2016; Bonnans et al., 2014).

Fibroblasts within pelvic support tissues are key cellular components involved in ECM synthesis, remodeling, and repair. Maintenance of the balance between collagen and elastin synthesis and their degradation is essential for preserving the mechanical integrity of the tissue. Matrix metalloproteinases (MMPs) participate in the degradation of ECM components, whereas tissue inhibitors of metalloproteinases (TIMPs) regulate and restrict MMP activity (Nagase et al., 2006). Accordingly, alterations in the MMP/TIMP balance may

influence ECM turnover and, consequently, the biomechanical properties of pelvic support tissues.

Collagen is one of the principal structural proteins determining the tensile strength of pelvic support tissues. In particular, type I and type III collagen play important roles in maintaining the mechanical properties of pelvic connective tissue (Chen et al., 2007; Ricard-Blum, 2011). Alterations in collagen synthesis, fibril formation, cross-linking, and proteolytic degradation may modify the mechanical capacity of the tissue. Similarly, elastin enables tissues to return to their original configuration following stretching and therefore plays an important role in the adaptation of pelvic support tissues to repeated mechanical loading (Sherratt, 2009).

Aging and menopause are also of particular importance in understanding the biology of POP. Aging may be accompanied by alterations in fibroblast proliferation, ECM synthesis, and tissue repair capacity; disruption of collagen and elastin fiber organization; and increased oxidative stress and cellular senescence (López-Otín et al., 2013; Gorgoulis et al., 2019). In addition, the decline in estrogen levels associated with menopause may influence the metabolism and tissue homeostasis of pelvic connective tissues (Robinson & Cardozo, 2003).

Recent research into the biological basis of POP suggests that, in addition to ECM alterations, processes such as oxidative stress, mitochondrial dysfunction, inflammation, and cellular senescence should also be considered. Excessive production of reactive oxygen species (ROS) may affect fibroblast function, ECM turnover, and inflammatory signaling pathways (Sies & Jones, 2020). The bidirectional relationship between oxidative stress and inflammation may contribute to the persistence of chronic tissue injury (Reuter et al., 2010). Furthermore, cellular senescence occurring in aged or stressed cells and the senescence-associated secretory phenotype (SASP) have been associated with chronic inflammatory microenvironments and ECM remodeling (Coppé et al., 2010; Gorgoulis et al., 2019).

Therefore, the biological basis of POP should be considered within the framework of complex interactions among mechanical loading, ECM homeostasis, fibroblast function, collagen and elastin metabolism, hormonal alterations, oxidative stress, mitochondrial function, inflammation, and cellular senescence. This chapter examines the cellular and molecular basis of POP, with particular emphasis on ECM and connective tissue biology, the MMP/TIMP system, aging and menopause, oxidative stress, mitochondrial dysfunction, inflammation, cellular senescence, genetic and epigenetic mechanisms, and mechanotransduction.

2. Cellular and Molecular Structure of Pelvic Support Tissues

2.1. Fibroblasts

Fibroblasts are among the principal cells of connective tissue and are responsible for the synthesis of collagen, elastin, proteoglycans, and various glycoproteins. These cells also contribute to the continuous remodeling of the ECM, thereby helping maintain the mechanical and biological properties of tissues (Theocharis et al., 2016).

Fibroblasts are not merely ECM-producing cells; they can also sense changes in their mechanical environment and modify their cellular responses accordingly. Tissue tension, matrix stiffness, growth factors, inflammatory mediators, and hormonal signals can all influence fibroblast behavior (Humphrey et al., 2014). Therefore, alterations in fibroblast function may represent an important mechanism capable of affecting the structural integrity of pelvic support tissues.

Changes in collagen metabolism and proliferative characteristics of fibroblasts have been reported in POP tissues (Moalli et al., 2005; Chen et al., 2007). However, it remains unclear whether these alterations in fibroblast function contribute to the initiation of prolapse or arise as a consequence of chronic mechanical stress imposed on prolapsed tissues. Thus, although fibroblast biology represents an important area of POP research, it remains an evolving field of investigation.

2.2. Collagen

Collagen is one of the major structural proteins contributing to the mechanical strength of pelvic support tissues. In particular, type I and type III collagen are important determinants of the biomechanical properties of pelvic connective tissue (Chen et al., 2007; Ricard-Blum, 2011).

Type I collagen provides greater tensile strength, whereas type III collagen is associated with more compliant connective tissue properties. Changes in the relative amount, distribution, and fibrillar organization of these two collagen types may alter the mechanical behavior of the tissue.

Collagen metabolism is not determined solely by the rate of collagen synthesis. The formation and cross-linking of collagen fibrils, their structural organization, and their proteolytic degradation also contribute to tissue integrity. Therefore, POP research should consider not only total collagen content but also the distribution of collagen types and the organization of collagen fibrils.

Studies investigating ECM remodeling in pelvic organ prolapse indicate that collagen metabolism may be altered in prolapsed tissues. Moalli et al.

(2005) reported that ECM remodeling in prolapsed tissue may be associated with alterations in collagen metabolism. These findings suggest that POP may represent not merely a loss of anatomical support but also an active process of connective tissue remodeling.

2.3. Elastin

Elastin is a major ECM protein that enables tissues to return to their original configuration following stretching. The integrity of elastic fibers is important for enabling pelvic support tissues to adapt to repeated mechanical loading throughout life.

The synthesis, organization, and degradation of elastic fibers may be influenced by aging, hormonal alterations, and proteolytic activity (Sherratt, 2009). Disturbances in elastin metabolism may reduce the elastic properties of pelvic support tissues and alter their resistance to mechanical loading.

The substantial mechanical stress imposed on pelvic support tissues during pregnancy and childbirth is thought to contribute to injury of elastic fibers. The decline in tissue repair capacity associated with aging may further contribute to the accumulation of such damage. However, whether alterations in elastin represent a primary initiating factor in POP or a consequence of chronic mechanical stress remains uncertain and requires further longitudinal investigation.

3. Extracellular Matrix Homeostasis and Pelvic Organ Prolapse

The ECM is a dynamic structure that is continuously synthesized, degraded, and reorganized. Under normal conditions, a tightly regulated balance exists between ECM synthesis and degradation. Disruption of this balance may result in alterations in tissue architecture, stiffness, and mechanical properties (Bonnans et al., 2014; Theocharis et al., 2016).

MMPs are proteolytic enzymes that degrade various components of the ECM. TIMPs, in contrast, restrict MMP activity and contribute to the regulation of ECM degradation (Nagase et al., 2006). The MMP/TIMP balance is therefore an important regulator of connective tissue homeostasis.

Alterations in collagen metabolism and MMP activity have been reported in POP tissues (Moalli et al., 2005; Chen et al., 2007). These alterations may affect collagen turnover and, consequently, the mechanical properties of pelvic support tissues. However, the fact that findings regarding MMP activity and collagen metabolism are not entirely consistent across studies suggests that POP cannot be explained by a single proteolytic mechanism.

The ECM also provides biological signals to cells through its role as an active cellular microenvironment. Changes in ECM structure may modify

intracellular signaling pathways through integrins and other cell–matrix interactions (Humphrey et al., 2014). Thus, ECM degradation does not simply result in passive tissue loss; it may also alter fibroblast behavior and the subsequent production of new ECM.

From this perspective, disruption of ECM homeostasis in POP may be conceptualized as a self-reinforcing process. Mechanical stress may induce alterations in the ECM; the altered ECM may modify cellular responses; and these changes in cellular behavior may, in turn, reshape ECM synthesis and degradation.

4. Effects of Aging and Menopause on Pelvic Support Tissues

Aging is an important epidemiological risk factor for POP. However, in addition to chronological age itself, the cellular and molecular changes occurring during the aging process are also likely to be relevant (Jelovsek et al., 2007; López-Otín et al., 2013).

Aging may lead to alterations in fibroblast proliferation, ECM synthesis, and tissue repair capacity. The organization and cross-linking of collagen fibers may change, while elastic fibers may undergo fragmentation and functional deterioration (Sherratt, 2009). In addition, oxidative stress and cellular senescence may increase in aging tissues.

In women, menopause is accompanied by a substantial decline in estrogen levels. Estrogen is one of the hormones involved in connective tissue metabolism and tissue homeostasis. The presence of estrogen receptors in pelvic tissues suggests that hormonal alterations may influence the biology of pelvic support tissues (Robinson & Cardozo, 2003).

Reduced estrogen levels have been proposed to alter fibroblast activity, collagen metabolism, tissue hydration, and ECM organization. However, the relationship between menopause and POP should not be attributed solely to estrogen deficiency. Chronological aging, obstetric history, genetic susceptibility, obesity, and chronic mechanical loading should be considered together.

Accordingly, menopause should not be regarded as an independent cause of POP but rather as one of several factors that may influence age-related biological changes in pelvic support tissues.

5. Oxidative Stress and Pelvic Organ Prolapse

Oxidative stress is a cellular state resulting from an imbalance between the production of reactive oxygen species and the capacity of antioxidant defense systems. Although ROS are required for physiological cellular signaling at controlled levels, excessive ROS production may cause oxidative damage to

lipids, proteins, DNA, and cellular organelles (Sies & Jones, 2020).

During aging, increased ROS production and reduced antioxidant defense capacity may contribute to the accumulation of oxidative stress in various tissues (López-Otín et al., 2013). Pelvic support tissues may likewise represent tissues in which oxidative stress warrants investigation because of their exposure to aging-related changes and repeated mechanical loading.

Oxidative stress may affect fibroblast function and ECM metabolism. ROS can modify cellular signaling pathways and thereby influence MMP activity, inflammatory responses, and cellular senescence (Sies & Jones, 2020; Reuter et al., 2010).

In this context, oxidative stress should not be considered solely as a process responsible for ECM damage. Bidirectional interactions exist among oxidative stress, inflammation, and mitochondrial dysfunction. Increased ROS may activate inflammatory signaling pathways, whereas inflammatory responses may further increase mitochondrial stress and ROS production.

Nevertheless, evidence concerning the role of oxidative stress in POP remains more limited than the evidence concerning ECM and collagen biology. Therefore, oxidative stress should not be regarded as an established single cause of POP but rather as a potential mechanism contributing to the biological heterogeneity of the disease.

6. Mitochondrial Dysfunction and Cellular Energy Metabolism

Mitochondria are central organelles involved not only in ATP production but also in ROS metabolism, calcium homeostasis, apoptotic signaling, and cellular stress responses (Murphy, 2009).

Aging may be associated with mitochondrial DNA damage, alterations in electron transport chain function, reduced mitochondrial biogenesis, and impairment of mitochondrial quality-control mechanisms (López-Otín et al., 2013; López-Otín et al., 2016). These changes may reduce the energy-producing capacity of cells while increasing ROS production.

Fibroblasts within pelvic connective tissues require sufficient energy for ECM synthesis, proliferation, and tissue repair. Therefore, mitochondrial dysfunction may potentially affect the regenerative capacity of fibroblasts. However, direct evidence regarding mitochondrial biology in POP tissues remains limited. Accordingly, mitochondrial dysfunction should be considered not as a proven primary mechanism of POP but as a potential biological mechanism associated with aging and oxidative stress.

Future studies investigating mitochondrial DNA damage, ATP production, oxidative phosphorylation, mitochondrial biogenesis, and

mitophagy simultaneously in POP tissues may help address current knowledge gaps in this field.

7. Inflammation and Cellular Signaling Pathways

Inflammation is a physiological response to tissue injury; however, prolonged or dysregulated inflammatory activity may disrupt connective tissue homeostasis. Inflammatory mediators such as TNF-α, IL-1β, and IL-6 can influence fibroblast behavior and ECM metabolism (Lawrence, 2009; Reuter et al., 2010).

NF-κB is a central signaling pathway involved in the regulation of inflammatory responses. Oxidative stress and inflammatory stimuli can increase NF-κB activation, thereby altering the expression of various inflammatory genes (Lawrence, 2009).

TGF-β is an important regulator of fibroblast activation, collagen synthesis, and ECM remodeling. Alterations in the level or duration of TGF-β signaling may influence the nature of tissue repair and ECM accumulation (Frangogiannis, 2020).

A bidirectional relationship exists between oxidative stress and inflammation. ROS can activate inflammatory signaling pathways, whereas the metabolic activity of inflammatory cells can further increase ROS production (Reuter et al., 2010). This interaction may contribute to the development of a chronic, low-grade inflammatory microenvironment in aging tissues.

Although the role of inflammation in POP has been investigated, current evidence does not demonstrate that inflammation alone is a determinant of the disease. A more plausible biological model is a multifactorial process in which mechanical stress, oxidative stress, hormonal alterations, and ECM remodeling interact with inflammatory responses.

8. Cellular Senescence and Pelvic Organ Prolapse

Cellular senescence is a state in which cells permanently lose their proliferative capacity while remaining metabolically active. Although senescence is a natural component of aging, it can also be triggered by DNA damage, oxidative stress, telomere shortening, and various forms of cellular stress (Gorgoulis et al., 2019). Senescent cells may develop a secretory phenotype known as the senescence-associated secretory phenotype (SASP). SASP consists of various molecules, including inflammatory cytokines, chemokines, growth factors, and proteolytic enzymes (Coppé et al., 2010).

SASP has the potential to alter ECM homeostasis. In particular, increases in MMPs and inflammatory mediators may modify the function of neighboring cells and affect tissue remodeling (Coppé et al., 2010).

This mechanism is particularly noteworthy in the context of biological changes associated with aging in POP. Theoretically, the accumulation of senescent fibroblasts in pelvic support tissues may contribute to reduced fibroblast proliferation, altered ECM synthesis, and the development of a chronic inflammatory microenvironment.

However, direct confirmation of this hypothesis in POP requires systematic investigation of senescence markers, such as p16^INK4a, p21, SA-β-gal, and SASP components, in prolapsed tissues. Therefore, cellular senescence should not be regarded as an established mechanism of POP, but rather as an important area of investigation that may help explain the biological basis of age-related prolapse.

9. Genetic and Epigenetic Mechanisms

Evidence suggests that genetic susceptibility may play a role in the development of POP. In particular, differences in genes involved in collagen metabolism, ECM organization, and connective tissue biology may influence the mechanical properties of pelvic support tissues.

However, genetic susceptibility should be considered in interaction with environmental and mechanical factors. Among individuals with similar genetic characteristics, differences in obstetric history, age, hormonal status, and mechanical loading may contribute to the development of different clinical phenotypes.

Epigenetic mechanisms regulate gene expression without altering the DNA sequence. DNA methylation, histone modifications, and microRNAs are among the major components of this regulatory system (Egger et al., 2004).

MicroRNAs can regulate the expression of genes involved in ECM synthesis, MMP activity, inflammation, and cellular aging. Therefore, the use of microRNA profiles as biomarkers in POP and their potential application in identifying molecular subtypes of the disease represent important areas for future investigation. Nevertheless, molecular studies with adequate sample sizes that compare different clinical stages and phenotypes are required to clarify the specific role of epigenetic mechanisms in the pathogenesis of POP.

10. Mechanical Stress and Mechanotransduction

Pelvic support tissues are exposed to repetitive mechanical forces throughout life. Pregnancy and childbirth, chronic constipation, chronic coughing, obesity, and heavy physical loading may increase the mechanical load imposed on the pelvic floor (Jelovsek et al., 2007; Memon & Handa, 2013).

Mechanical stress does not merely affect the physical integrity of tissues; it can also alter cellular behavior. The process by which cells sense

mechanical stimuli and convert them into biochemical signals is known as mechanotransduction (Humphrey et al., 2014).

Integrins, focal adhesion complexes, the actin cytoskeleton, and mechanosensitive pathways such as YAP/TAZ play important roles in linking mechanical stimuli to intracellular responses. These systems may influence fibroblast proliferation, differentiation, and ECM production (Humphrey et al., 2014).

Chronic mechanical stress may alter fibroblast behavior and ECM synthesis, resulting in long-term remodeling of pelvic support tissues. Thus, a bidirectional relationship may exist between mechanical loading and ECM metabolism.

Within this model, mechanical stress should not be viewed solely as a factor that physically strains tissues; it may also function as a biological signal that alters cellular behavior and consequently affects ECM homeostasis.

11. Interactions Among Biological Mechanisms in Pelvic Organ Prolapse

The biological basis of POP is too complex to be explained by a single molecule, cell type, or signaling pathway. The development of the disease is thought to involve interactions among mechanical, hormonal, metabolic, inflammatory, and cellular processes.

When these processes are considered collectively, aging and hormonal changes may influence fibroblast function and ECM metabolism. Consequently, disruption of collagen and elastin homeostasis may alter the mechanical properties of pelvic support tissues. Tissues with altered mechanical properties may, in turn, become more susceptible to repeated mechanical loading.

In parallel, mechanical stress may increase cellular stress responses and affect ROS production and inflammatory signaling pathways. Increased oxidative stress may influence MMP activity, inflammation, and cellular senescence. Collectively, these processes may further alter ECM remodeling.

During aging, increased DNA damage, oxidative stress, and telomeric stress may trigger cellular senescence. Through SASP, senescent cells may secrete inflammatory mediators and proteolytic enzymes, thereby altering the function of neighboring cells and ECM homeostasis (Coppé et al., 2010; Gorgoulis et al., 2019).

Accordingly, the following interconnected biological network may be proposed for POP:

Aging + Hormonal alterations
↓
Alterations in fibroblast function
↓
Alterations in collagen/elastin metabolism
↓
Disruption of ECM homeostasis
↓
Changes in the mechanical properties of pelvic support tissues

In parallel:

Repetitive mechanical stress
↓
Cellular stress and mechanotransduction
↓
Increased ROS production
↓
Oxidative stress
↓
Activation of inflammatory signaling pathways
↓
Alterations in the MMP/TIMP balance
↓
ECM remodeling

During aging:

DNA damage + oxidative stress + telomeric stress
↓
Cellular senescence
↓
SASP
↓
Inflammatory microenvironment + ECM alterations
↓
Reduced tissue regenerative capacity

This biological network demonstrates that POP is not a disease with a single underlying cause and that mechanical injury and tissue biology cannot be considered independently. For example, mechanical injury occurring

during childbirth may result in persistent structural alterations when adequate tissue repair does not occur. Aging and hormonal changes may further influence the capacity of tissues to repair themselves. Processes such as oxidative stress, inflammation, and cellular senescence may also contribute to the persistence of inadequate tissue repair.

Accordingly, POP may be regarded as a complex tissue disorder arising at the intersection of mechanical loading and biological disruption of connective tissue homeostasis.

12. Future Research Directions

Future POP research should address not only anatomical changes but also the cellular and molecular processes underlying the disease.

One of the major priorities is ECM biology. Comprehensive assessment of collagen types, elastin, the MMP/TIMP system, and fibroblast function may contribute to a more detailed understanding of tissue remodeling in prolapsed tissue.

Oxidative stress and mitochondrial biology also represent important areas of investigation. Characterizing mitochondrial DNA damage, mitochondrial biogenesis, mitophagy, antioxidant defense, and energy metabolism in POP tissues may improve understanding of the biological relationship between aging and prolapse.

Cellular senescence is particularly relevant to elucidating the biological mechanisms underlying prolapse in older women. Comparing p16^INK4a, p21, SA-β-gal, and SASP-associated molecules between prolapsed and healthy pelvic tissues may help test this hypothesis.

Epigenetic mechanisms and microRNAs are also potential areas for understanding the molecular heterogeneity of POP. Emerging approaches, including single-cell RNA sequencing, proteomics, metabolomics, and spatial transcriptomics, may help characterize the distribution of different cell types and molecular pathways within prolapsed tissues.

Tissue engineering and regenerative medicine may also become important areas of future research. Approaches involving extracellular matrix-based biomaterials, stem cells, and cellular signaling modulators are being investigated for the biological reconstruction of pelvic support tissues. However, the translation of these approaches into clinical practice requires detailed evaluation of biocompatibility, safety, mechanical durability, and long-term outcomes.

Future studies should consider not only the presence of prolapse but also its anatomical location and stage, menopausal status, obstetric history,

obesity, and accompanying metabolic characteristics. Such an approach may contribute to determining whether POP can be classified into distinct molecular subgroups.

13. Conclusion

Pelvic organ prolapse is a multifactorial process that affects anatomical support structures but has a considerably broader biological basis. The integrity of pelvic support tissues depends on the balanced interaction among fibroblast function, collagen and elastin metabolism, extracellular matrix homeostasis, and the mechanical environment.

Biological changes associated with aging and menopause may disrupt this homeostasis. When oxidative stress, alterations in mitochondrial function, inflammation, and cellular senescence are superimposed on these changes, the capacity of tissues to regenerate and adapt to mechanical loading may be reduced.

Current evidence supports the importance of ECM and collagen metabolism in the biology of POP. In contrast, evidence regarding the specific roles of oxidative stress, mitochondrial dysfunction, and cellular senescence in POP remains more limited. Therefore, some of these mechanisms should be regarded as biological processes supported by existing evidence, whereas others should be considered research hypotheses that require further investigation in the context of POP.

To understand the biology of POP, these processes should be evaluated not independently but in terms of their interactions. In particular, investigation of the relationships among ECM remodeling, the MMP/TIMP balance, oxidative stress, mitochondrial function, inflammation, and cellular senescence may contribute to a better understanding of the molecular basis of prolapse.

In conclusion, examining pelvic organ prolapse from a biological perspective provides an opportunity to understand the cellular and molecular processes underlying the anatomical changes observed clinically. The integration of molecular biology, cell biology, biomechanics, and regenerative medicine approaches may contribute to a more comprehensive understanding of the biological heterogeneity of POP in the future.

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Chapter 3

ACOUSTIC STIMULI IN PLANTS: MECHANOPERCEPTION, PHYSIOLOGICAL AND DEVELOPMENTAL RESPONSES, AND HORMONAL REGULATION

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Introduction

Sound is a mechanical wave generated by a vibrating source and propagating through air, water, or solid media as pressure fluctuations and particle motion. From the perspective of plants, sound should therefore be considered not as a stimulus perceived directly through an auditory organ, but as an environmental signal capable of generating mechanical stress on plant surfaces, the cell wall–plasma membrane interface, and mechanosensitive perception systems. This approach requires the effects of sound in plants to be addressed not merely as an "auditory" phenomenon, but within the framework of mechanoperception and mechanotransduction (Demey, Mishra, & Van Der Straeten, 2023; Hassanien, Hou, Li, & Li, 2014; Kinsler, Frey, Coppens, & Sanders, 2000; Pagano & Del Prete, 2024).

From an acoustic perspective, sound is a mechanical wave phenomenon that can be described by parameters such as frequency, period, amplitude, sound pressure, energy density, and acoustic intensity. Frequency refers to the number of repetitions of a vibration or wave per unit time; period denotes the time required to complete one full oscillation; amplitude indicates the magnitude of the vibration; sound pressure is the measurable deviation produced by an acoustic wave in the ambient pressure of the medium; energy density refers to the amount of sound energy per unit volume; and acoustic intensity denotes the flow of this energy through a given area. As these definitions indicate, amplitude, sound pressure, energy density, and acoustic intensity are related to one another, but they are not identical physical quantities (Kinsler et al., 2000). Erbe, Duncan, Hawkins, Terhune, and Thomas (2022) also emphasize that terms such as sound pressure, sound exposure, particle velocity, sound speed, frequency modulation, amplitude modulation, frequency weighting, power spectral density, and one-third-octave band level should be systematically defined in bioacoustic and environmental acoustic studies.

The nature of the vibrational effect produced by sound in plants depends on these physical properties of the applied sound. While frequency determines how often the mechanical oscillation generated in plant tissues is repeated, amplitude- and sound pressure-related level properties may determine the magnitude of this oscillation and the strength of the resulting mechanical effect. By contrast, the decibel (dB) is not frequency, amplitude, or sound pressure itself, but a logarithmic scale used to express the level of such acoustic quantities. Robin and Plante (2022) clearly demonstrate that the dB is used in acoustics primarily to express ratios and level differences. Therefore, although a high dB value often indicates a stronger acoustic stimulus, it does not by itself fully explain the physical character of the resulting vibration. Moreover, the vibration pattern that emerges in a plant is shaped not only by frequency

and level, but also by the waveform, spectral composition, continuity of the sound, and its mode of transmission through air, water, or substrate. Thus, plant responses should be considered in relation not only to the applied sound parameters, but also to the specific vibration regime generated by these parameters within the tissue.

These distinctions are particularly important for the characterization of naturally derived complex sounds. Structurally, sounds can be considered as simple/pure or complex. Simple or pure sounds ideally consist of a single frequency component and exhibit a periodic waveform. Complex sounds, by contrast, consist of a temporally varying combination of different frequency and amplitude components. Most natural environmental sounds and animal sounds have a complex structure. In the acoustic characterization of such sounds, dominant frequencies, frequency bands, amplitude pattern, continuity/intermittency, modulation properties, and spectral energy distribution should be evaluated together. Therefore, in environmental and biological sound studies, acoustic parameters such as sound pressure, sound exposure, particle velocity, sound speed, frequency/amplitude modulation, frequency weighting, power spectral density, and one-third-octave band level should be clearly defined and reported (Erbe et al., 2022).

The fact that sound is a mechanical wave requires it to be considered in living systems not only as an auditory phenomenon, but also as a potential mechanical stimulus. Since plants can respond to mechanical environmental signals such as touch, wind, rain, and similar stimuli, sound vibrations may also function as signals that can be perceived by plant structures and affect physiological processes (Ali, Tyagi, Park, & Bae, 2024; Chowdhury, Lim, & Bae, 2014). Studies in this field indicate that sound applications may be associated with various biological responses ranging from seed germination and seedling growth to hormone activities (Chowdhury et al., 2014; Hassanien et al., 2014).

Within this framework, it is also evident that plant responses to acoustic stimuli are not limited to changes in growth and development. Veits et al. (2019) showed that flowers of *Oenothera drummondii* responded within only 3 min to honeybee flight sounds and low-frequency synthetic sounds covering pollinator wingbeat frequencies by increasing nectar sugar concentration. Laser vibrometry measurements revealed that this response was associated with mechanical vibration of the petals in response to low-frequency pollinator sounds, whereas similar vibration and nectar responses did not occur at high frequencies. These findings show that acoustic stimuli can induce rapid functional physiological/biochemical responses in plants.

Gagliano, Grimonprez, Depczynski, and Renton (2017) further demonstrated that the effect of sound is not limited to aerial organs and that the root system may also use acoustic vibrations as directional cues. In their study, roots of *Pisum sativum* seedlings showed an orientation similar to that observed toward an actual water source even when they were exposed only to the live sound of water flowing through a pipe, without direct access to water. The authors suggested that these findings indicate that roots may use acoustic vibrations generated by water as long-distance directional cues, whereas moisture signals may provide more precise targeting when the root approaches the source.

The effects of sound may vary depending on plant species, developmental stage, sound level and frequency, sound type (simple/pure sound, complex sound, music, noise, or biologically derived natural sound), the mode of stimulus transmission (airborne sound waves or substrate/organ vibrations), and exposure duration (Chowdhury et al., 2014; Collins & Foreman, 2001; Hassanien et al., 2014; Son, Jang, Mathevon, & Ryu, 2024; Veits et al., 2019). This variability makes it necessary to evaluate the possible mechanisms of sound perception in plants, the different physiological/biochemical responses, and the existing experimental limitations together.

Within this framework, this chapter focuses on possible mechanisms of sound perception and mechanotransduction in plants and evaluates current evidence on seed germination, seedling and vegetative growth, photosynthesis, respiration, and phytohormonal responses. It also considers methodological factors that may contribute to variation among reported physiological and developmental responses and discusses research directions needed to clarify how acoustic parameters and exposure conditions are related to these biological outcomes.

Sound Perception and Possible Mechanisms of Action in Plants

One of the main points commonly indicated by the current literature is that sound perception in plants cannot be explained through a single receptor or a single organ (Chowdhury et al., 2014; de Melo, 2023; Demey et al., 2023; Mishra, Ghosh, & Bae, 2016). Current evidence suggests that sound primarily acts as a mechanical stimulus in plants and that physical strain generated on plant surfaces, particularly at the cell wall–plasma membrane interface, may initiate early signaling events through mechanosensitive ion channels and wall-associated sensing systems (de Melo, 2023; Demey et al., 2023; Mishra et al., 2016). This view is also consistent with general studies on plant mechanosignaling, which indicate that the cell wall, plasma membrane, and cytoskeletal connections may contribute to the transfer of mechanical effects into the cell, and that mechanical stimuli can initiate multi-layered cellular responses through ion channels, Ca^{2+} and ROS signaling, receptor-like kinases, hormone networks, and gene expression (Braam, 2005; Kouhen,

Dimitrova, Scippa, & Trupiano, 2023; Sparke & Wünsche, 2020). Indeed, it has been reported that cell wall–plasma membrane connections may contribute to the transmission of mechanical integrity signals, and that mechanically induced actin reorganization and phosphorylation changes may function in plant movement responses (Hagihara & Toyota, 2020; Yoneda, Ohtani, Katagiri, Hosokawa, & Demura, 2020).

The organ-level distribution of sound perception also does not appear to be unidimensional. Airborne acoustic stimuli may generate more pronounced mechanical vibrations, particularly in aerial organs with broad surfaces, such as leaves and flowers. Trichomes also emerge as candidate structures in the perception of mechanical force and its conversion into cellular responses. Zhou et al. (2017) showed that mechanical stimulation in *Arabidopsis thaliana* trichomes caused force focusing in the pliant zone of the trichome stalk and the adjacent podium region, while inducing cytosolic Ca^{2+} oscillations and apoplastic pH changes in the skirt cells surrounding the podium. Matsumura et al. (2022) further reported that trichomes in *A. thaliana* can perceive raindrop-like mechanical stimuli and generate Ca^{2+} waves spreading to surrounding tissues, together with defense-related gene expression responses involving CAMTA3 and MAPK. In addition, Liu et al. (2017) biophysically proposed that *A. thaliana* leaf trichomes may act as acoustic antennae, while Peng et al. (2022) calculated that certain acoustic stimuli could generate mechanical stress in tomato trichomes at levels capable of activating or modulating mechanosensitive ion channels. These findings indicate that trichomes are strong candidate structures for the mechanical transduction of airborne sound waves. The frequency-selective vibration of petals in response to low-frequency pollinator sounds in *Oenothera drummondii* flowers reported by Veits et al. (2019), together with the rapid increase in nectar sugar concentration, also supports the idea that aerial organs can convert sound-induced vibrations into physiological outputs.

By contrast, vibrational stimuli transmitted through substrate or water appear to be perceived by the root system. Rodrigo-Moreno et al. (2017) showed that 200 Hz sound application induced positive phonotropism in *A. thaliana* roots and that this response was associated within minutes with increased cytosolic Ca^{2+}, ROS production, and changes in K^+ fluxes. The authors suggested that these early responses may be related to Ca^{2+} influx, Ca^{2+} release from internal stores, NADPH oxidase-mediated ROS production, and activation of outward-rectifying K^+ channels. These findings indicate that roots do not merely display directional growth responses, but can also respond to sound stimuli at the level of early cellular signaling. Nevertheless, the specific receptor system through which sound-induced mechanical effects are initiated at the cellular level has not yet been definitively identified. Although current evidence highlights mechanosensitive ion channels and sensing components at the cell wall–plasma membrane interface as

candidate structures, their specific roles in sound perception have not been directly demonstrated. Taken together, these findings support a conceptual framework in which candidate perception structures and early mechanotransduction events constitute the initial stages of a broader sequence linking acoustic stimuli to downstream biological responses (Fig. 1).

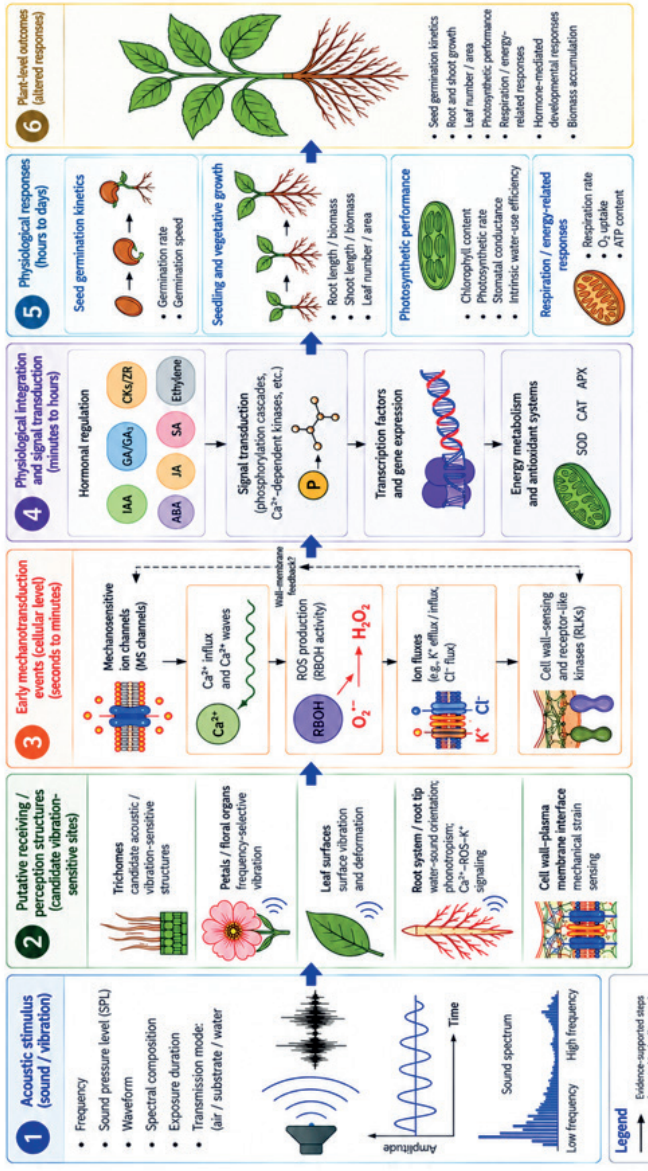


Fig. 1. Conceptual model of mechanoperception and physiological–developmental responses to acoustic stimuli in plants.

Sound and vibration stimuli differing in frequency, sound pressure level, waveform, spectral composition, exposure duration, and transmission mode may generate mechanical deformation and tissue vibration. Trichomes, petals and floral organs, leaf surfaces, the root system/root tip, and the cell wall–plasma membrane interface are presented as putative vibration-sensitive structures rather than confirmed sound-specific receptors. Early mechanotransduction may involve mechanosensitive ion channels, Ca^{2+} influx and waves, RBOH-associated ROS production, ion fluxes, and cell wall–sensing/RLK-related signaling. These events may subsequently interact with hormonal regulation, phosphorylation-dependent signaling, transcriptional regulation, energy metabolism, and antioxidant systems. At the physiological and developmental levels, acoustic stimulation may be associated with changes in seed germination kinetics, seedling and vegetative growth, photosynthetic performance, respiration and energy-related processes, and phytohormonal responses. Solid arrows represent evidence-supported steps, whereas dashed arrows indicate hypothesized or incompletely resolved relationships. Not all mechanistic links shown have been demonstrated directly for sound and some are inferred from broader plant mechanosignaling models (de Melo, 2023; Demey et al., 2023; Sparke & Wünsche, 2020).

Abbreviations: ABA, abscisic acid; APX, ascorbate peroxidase; ATP, adenosine triphosphate; Ca^{2+}, calcium ion; CAT, catalase; CKs, cytokinins; Cl^{-}, chloride ion; GA, gibberellin; GA_3, gibberellic acid; H_2O_2, hydrogen peroxide; IAA, indole-3-acetic acid; JA, jasmonic acid; K^{+}, potassium ion; MS channels, mechanosensitive ion channels; $O_2\bullet^{-}$, superoxide radical; P, phosphorylation; RBOH, respiratory burst oxidase homolog; RLKs, receptor-like kinases; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase; SPL, sound pressure level; ZR, zeatin riboside.

When these models are evaluated together, sound perception in plants can be considered within the broader framework of mechanical signaling; however, current explanations remain limited with respect to the organ-level sites of initiation and the specific vibration patterns generated by acoustic parameters. Sparke and Wünsche (2020) emphasize the general framework of mechanosignaling, de Melo (2023) highlights the cell wall–plasma membrane interface and early signaling events, and Demey et al. (2023) present both experimentally supported and hypothesis-based components of sound responses (Fig. 1). Nevertheless, current models do not yet sufficiently explain how the physical properties of sound, such as frequency, amplitude, waveform, and spectral composition, generate specific vibration patterns in plant tissues or how these patterns are causally linked to biological responses. Therefore, leaves, flowers, and trichomes should be more explicitly considered in airborne sound perception, whereas the root system should be more clearly

evaluated in relation to vibrations transmitted through substrate or water.

Current evidence particularly highlights the Ca^{2+} and ROS axis in the conversion of this initial physical effect into cellular signaling. Sound-induced mechanical strain may activate mechanosensitive channels in the plasma membrane and initiate an increase in cytosolic Ca^{2+}; subsequently, downstream signaling networks are thought to be activated through calcium-dependent protein kinases, calmodulin-like proteins, NADPH oxidases, and ROS production (de Melo, 2023; Demey et al., 2023; Ghosh et al., 2016; Matsumura et al., 2022; Mishra et al., 2016; Zhou et al., 2017). The early ROS response and changes in RBOH gene expression reported by Ali, Park, Tyagi, and Bae (2026) also support this framework. These early signaling events appear to be subsequently integrated with hormone responses, transcription factors, and reprogramming of gene expression (de Melo, 2023; Ghosh et al., 2016). However, direct molecular evidence for the acoustic antenna-like function of trichomes and for the cellular components initiating sound perception in roots remains limited; therefore, the current framework needs to be tested through stronger experimental studies in terms of organ-specific perception sites and molecular transduction steps (Demey et al., 2023; Son et al., 2024).

The study conducted by Sardari, Ghanati, Mobasheri, and Hajnorouzi (2024) on suspension-cultured *Nicotiana tabacum* cells is particularly important because it provides cellular evidence for a sound-level-dependent Ca^{2+} response to sound. The authors exposed cells to sinusoidal sound waves at a constant frequency of 1000 Hz and sound levels of 60, 75, and 90 dB for 15–60 min, while controlling sound level and its spatial homogeneity at the level of the plates containing the cells. Sound application increased total and cytosolic free Ca^{2+} levels, particularly at 75 and 90 dB. These findings provide controlled cellular-level support for the involvement of Ca^{2+} in early responses to acoustic stimulation.

Effects of Sound on Seed Germination

Studies on the effects of sound and vibration on seed germination indicate that this response is not uniform, but varies depending on sound type, frequency, sound level, exposure duration, and transmission medium. Studies using controlled artificial vibration and single-frequency stimuli show that sound does not always increase the final germination percentage; in some cases, it primarily affects germination kinetics. Uchida and Yamamoto (2002) reported that sinusoidal vibration applied to *Arabidopsis thaliana* seeds at 40–120 Hz with a maximum amplitude of 0.42 mm accelerated germination, but did not markedly alter the total germination percentage by day 5. The fact that the effect became evident at approximately 70 m s^{-2} in terms of maximum acceleration and reached saturation at higher accelerations suggests that the

acceleration level generated in the seed may be more decisive than frequency or amplitude alone. Mechanistically, the substantial loss of the vibration effect in the *pgm* mutant suggests that amyloplasts may function as possible sensing structures, whereas the disappearance of the promotive effect in the ethylene-insensitive *etr1-3* mutant and in the presence of AVG indicates that an ethylene-mediated component may be involved in the vibration response during germination. Similarly, Bochu et al. (2003) identified the combination of 400 Hz and 106 dB as the most favorable condition in rice seeds, whereas they reported that the effect could become inhibitory or harmful at 4 kHz and 111 dB. The researchers related this pattern to the function of sound as a biomechanical stimulus at specific frequency and sound-level thresholds and emphasized the possible roles of membrane fluidity, Ca^{2+}-related processes, and changes in metabolic activity. Cai, He, Zhu, and Wang (2014) also reported that mean germination time decreased in all sound-treated groups in mung bean seeds exposed to 1000–2500 Hz and 80–100 dB(A), with this effect becoming more pronounced in the higher frequency bands. Chuanren et al. (2004) showed that a 1000 Hz, 100 dB sine wave increased germination percentage and reduced mean germination time in *Echinacea angustifolia* seeds; however, they emphasized that this effect should be evaluated within a multiple pretreatment system that included dormancy-breaking treatments such as chilling, seed coat removal, GA_3, and continuous light.

Studies using white noise, music, and complex sounds also indicate that the germination response may depend not only on frequency and sound level, but also on the spectral and temporal organization of the signal. Vicient (2017) compared different frequency and white noise treatments at 80 dB in *Zea mays* seeds and reported that especially 300 Hz and white noise increased germination percentage, relating this effect to physical alteration of the pericarp and facilitated water/oxygen uptake. Das and Ghosh (2022) showed that both Indian classical music and natural music accelerated germination in *Pisum sativum* seeds, but that Indian classical music produced a stronger effect; they explained this difference through acoustic complexity features such as waveform, power distribution, and temporal correlation. Azgomi, Iranbakhsh, Majd, Ebadi, and Oraghi Ardebili (2023) reported that Iranian music, rock music, and urban traffic noise applied at 80 dB all increased germination in *Satureja hortensis* seeds, with the strongest effect observed under rhythmic-harmonic Iranian music. These findings suggest that germination responses may vary according to the rhythmic, harmonic, and temporal properties of sound.

The literature on natural environmental sounds remains extremely limited. Makris and Navarro (2026) subjected rice (*Oryza sativa*) seeds to controlled drop-impact experiments in shallow water and characterized

the impulsive acoustic fields generated by raindrops striking water or soil surfaces. The soil-surface component of the study was evaluated mainly at the level of acoustic measurement and modeling. The study proposed that low-frequency components, particularly in the 10–100 Hz range, and the statolith displacements generated by these components may be decisive for the germination response. Under conditions in which the estimated statolith displacement was 200–600 nm, germination rate increased by 24–37% relative to the control; the effective threshold was suggested to be approximately 30 nm, and the response was argued to involve gravitropic mechanisms through statolith–membrane contact dynamics. However, these interpretations should be considered in light of the fact that they are largely based on physical modeling and calculations of statolith displacement and are not supported by direct molecular or cellular biomarker measurements.

Overall, this body of literature indicates that sound and vibration often affect germination kinetics rather than the final germination percentage. Positive effects emerge under specific frequency, sound level, duration, and structural acoustic conditions, whereas the effect may weaken or become inhibitory under unsuitable or excessive conditions. This variability has been associated with different explanatory levels, including membrane fluidity, Ca^{2+}-related early signaling processes, ethylene responses, amyloplast/statolith-based mechanical perception, physical changes at the pericarp level, and spectral-temporal complexity. Therefore, the germination literature shows that the effect of sound should be evaluated not only in relation to frequency and sound level, but also together with sound type, exposure duration, transmission medium, and the level of mechanical displacement or acceleration generated in the seed.

Effects of Sound on Seedling Growth and Vegetative Development

The effects of sound applications on seedling growth and vegetative development have been evaluated through leaf area and number, biomass, root activity, meristematic activity, and, in some studies, harvest yield. As in germination studies, the seedling growth response appears to vary depending on the physical and structural properties of sound, as well as plant species and developmental stage.

Single-frequency or narrow-frequency-range sound applications can produce distinct but threshold-dependent responses in seedling growth. Bochu et al. (2003) reported that 400 Hz and 106 dB increased plant height and root parameters in rice, whereas root length was suppressed at 4 kHz. Cai et al. (2014) showed that root length increased only under specific frequency–sound level combinations in mung bean seedlings and that root and shoot responses may differ even under the same acoustic conditions. Weinberger

and Measures (1979) reported that only the 5 kHz–92 dB treatment increased plant height, root number, and root/shoot dry weight in wheat, whereas the positive effect disappeared at 105–120 dB and morphological abnormalities appeared. These findings indicate that the sound response in seedling growth depends on the frequency–sound level combination, application period, and organ evaluated.

Studies focusing on root development show that, under suitable acoustic conditions, sound can affect not only morphological growth but also root activity and meristematic activity. Yi, Bochu, Xiujuan, Chuanren, and Xiaocheng (2003) reported that 1000 Hz and 100 dB sound application in *Chrysanthemum* seedlings increased root fresh weight, root length, and root activity, particularly after 9 days of exposure, and related this increase to plasma membrane H^+-ATPase activity and Ca^{2+}-associated regulatory processes. Kim, Lee, Kim, and Jeong (2021) showed that 100 Hz and 100 Hz + 9 kHz dual-frequency treatments increased primary root length in *A. thaliana*. Increased expression of genes associated with auxin transport and biosynthesis, together with suppression of cytokinin and ethylene signaling, indicates that the root growth response may be shaped together with hormonal balance.

Studies using specific acoustic frequency technologies, such as Plant Acoustic Frequency Technology (PAFT), have mostly reported positive effects on leaf development and biomass accumulation. Zakariya, Rivai, and Aini (2017) reported that a PAFT system operating with a 2000 Hz square wave at 80–100 dB increased plant height, leaf area, and fresh and dry weight in *Brassica rapa* var. *parachinensis*, with the most pronounced increases observed in leaf area and dry weight. Qi, Teng, Hou, Zhu, and Liu (2010) similarly reported that acoustic frequency application in the 100–2000 Hz range increased leaf area at different developmental stages in strawberry, resulted in approximately one-week earlier flowering/fruit formation, and reduced disease incidence, although no significant increase in yield was observed. Hou and Mooneyham (1999) reported increases in fresh weight and yield in spinach and lettuce under Agri-wave technology applications in which sound waves and micronutrient fertilizer were used either together or separately. In lettuce, sound alone increased mean fresh weight under greenhouse conditions by 29.92% relative to the control, whereas the sound + fertilizer combination produced a 44.10% increase. However, particularly when sound is applied together with fertilizer or other device-based inputs, attributing the growth response solely to the acoustic stimulus should be done with caution.

Studies using music and complex sounds have also shown mostly positive effects on seedling growth, although the responses vary depending on sound structure. Ekici, Dane, Mamedova, Metin, and Huseyinov (2007) reported that

two different musical stimulus groups increased root elongation and mitotic activity in the root apical meristem of adventitious roots of *Allium cepa*; the second group, which contained broader musical intervals and rhythmically changing lyrics, produced a stronger response. Hendrawan, Putra, Sumarlan, and Djoyowasito (2020) reported that Javanese gamelan music produced the best growth responses in red lettuce at 3–5 kHz with a 2 h exposure, whereas some exposure durations in the 11–13 kHz range resulted in the lowest values for fresh/dry weight, leaf area, and plant height. Azgomi et al. (2023) compared Iranian music, rock music, and urban traffic noise in *Satureja hortensis* seedlings and reported that Iranian music and rock music applied at 80 dB increased root and hypocotyl development more prominently, whereas urban traffic noise produced more limited responses. In the same study, leaf area in 30-day-old seedlings increased under all sound treatments, while the strongest response in terms of root and shoot fresh weight was observed under Iranian music. These findings indicate that it is not sufficient to label complex sounds simply as "music" or "noise"; rhythm, harmonic structure, frequency range, exposure duration, and temporal pattern may all shape the growth response. As an example of combined applications involving a natural sound component, Zhu, Jiang, and Shen (2011) applied a mixture of classical music and insect sounds in the 3–6 kHz range and reported that plant height increased in tomato, melon, eggplant, and mini-cucumber, in which height was evaluated. However, because insect sounds were used together with classical music, it remains unclear whether the observed effect originated from the biological sound component, the musical structure, or the total acoustic stimulus in the 3–6 kHz range.

Overall, studies on seedling growth and vegetative development show that sound applications can affect root, shoot, leaf, and biomass parameters, but this effect should not be interpreted as a uniform and generalizable growth promotion. In single-frequency, narrow-band, or device-controlled acoustic applications, the response varies depending on the combination of frequency and sound level, exposure duration, and the organ evaluated, whereas unsuitable frequencies, high sound levels, or unfavorable frequency–duration combinations may weaken the growth response, suppress root development, or cause morphological abnormalities. In music and complex sound applications, it is often unclear whether the response originates from dominant frequencies, harmonic/rhythmic structure, or the overall sound pattern. Therefore, the available evidence indicates that the effect of sound on seedling growth should be evaluated as an acoustic/mechanical response that is sensitive to organ level, developmental stage, and application design.

Effects of Sound on Photosynthesis and Respiration

The effects of sound applications on photosynthesis have been evaluated through chlorophyll and carotenoid contents, stomatal aperture, net photosynthetic rate, transpiration, stomatal conductance, and chlorophyll fluorescence parameters. Respiration-related effects have been examined through indicators of respiration and energy metabolism, including O_2 uptake, ATP content, and postharvest respiration rate. Controlled frequency applications show that the effects of sound on photosynthetic pigments and primary metabolism are not always unidirectional. Calderón, Almagro, Martínez-Calderón, and Ferrer (2024) reported that sound vibrations applied to tomato plants at 100, 500, and 1000 Hz, 90 dB, for 3 days reduced chlorophyll a, total chlorophyll, and carotenoid contents, particularly on day 3; by contrast, soluble protein and sugar contents tended to increase, although these changes were not statistically significant. This result indicates that sound application may affect pigment balance and processes related to carbon metabolism in different directions. In contrast, Ghosh et al. (2016) reported that especially the 500 Hz and 80 dB treatment in *A. thaliana* altered numerous transcripts and proteins associated with light reactions, carbon metabolism, glycolysis, the TCA cycle, and ATP synthesis. These findings suggest that sound vibrations may affect photosynthesis and energy metabolism not through a single parameter, but within the broader reorganization of primary metabolism.

Studies using PAFT and acoustic frequency technologies have mostly reported positive effects on chlorophyll, gas exchange, and PSII performance. Meng, Zhou, Zheng, and Gao (2012) reported that PAFT application at 40–2000 Hz and 100 dB in strawberry increased chlorophyll content, net photosynthetic rate, transpiration, stomatal conductance, and chlorophyll fluorescence indicators such as Fv/Fm, NPQ, and ΦPSII. Qi et al. (2010), also in strawberry, showed that acoustic frequency application in the 100–2000 Hz range increased transpiration and stomatal/H_2O conductance in most developmental periods, but that the significant increase in photosynthetic rate appeared particularly during the fruiting stage; no significant difference was observed in intercellular CO_2 concentration. Taken together, these two studies indicate that acoustic frequency applications may affect photosynthetic performance in strawberry, although the response varies according to developmental stage and the indicator evaluated.

Jusoh et al. (2023) evaluated the relationship between sound applications and leaf gas exchange in rice (*Oryza sativa* L.). In rice seedlings of the MR219 cultivar, sound treatments reported in the 350–380 Hz frequency range and reported sound levels of 60–78 dB, varying with speaker distance, were

compared; net photosynthetic rate, stomatal conductance, and intrinsic water-use efficiency were among the parameters measured. The 357 and 350 Hz treatments increased net photosynthetic rate relative to the control, whereas the highest intrinsic water-use efficiency was recorded under the 359 Hz treatment. These findings indicate that acoustic stimuli may affect leaf-level photosynthetic performance and intrinsic water-use efficiency. However, because the control plants were maintained in a separate greenhouse and frequency and reported sound level changed together as a function of distance, the findings should be considered not as definitive mechanistic evidence, but as a physiological example of the effects of sound quality on photosynthesis and intrinsic water-use efficiency.

Studies conducted on leafy vegetables also show that acoustic applications can induce responses related to chlorophyll content and stomatal behavior. Zakariya et al. (2017) reported that a 2000 Hz square-wave PAFT application at 80–100 dB increased stomatal aperture and chlorophyll contents in *Brassica rapa* var. *parachinensis*. Wang and Xiao (2023) showed that acoustic frequency application at approximately 60 Hz and 80 dB increased chlorophyll content and shoot biomass in lettuce. In that study, chlorophyll content was higher than the control under all electronic, rock, and classical music treatments, with the strongest increase observed under electronic music. This finding suggests that the photosynthetic pigment response may depend not only on the presence of sound, but also on its structural properties and spectral/temporal pattern.

Music and complex sound applications have been evaluated in relation to photosynthesis-related indicators mainly at the levels of stomatal aperture, chlorophyll index, and O_2 uptake. Prasetyo and Raju (2022) reported that violin music in the 550–1200 Hz range increased stomatal aperture, SPAD chlorophyll index, and fresh weight above the control in green mustard; the highest stomatal aperture was obtained at 80–85 dB, whereas the highest SPAD value and fresh weight were obtained at 70–75 dB. This finding shows that photosynthetic or growth responses do not increase linearly with increasing sound level. Qin, Lee, Choi, and Kim (2003) reported that the effects of green music and 20 kHz ultrasound on O_2 uptake in Chinese cabbage and cucumber varied according to species and developmental stage. In Chinese cabbage, green music produced the highest O_2 uptake in both 15-day-old seedlings and 70-day-old mature plants, whereas in cucumber, green music resulted in higher but statistically non-significant O_2 uptake in 15-day-old seedlings. In mature cucumber, however, 20 kHz ultrasound produced a significant and more pronounced increase.

Although the available data on respiration and energy metabolism remain limited, the direction of the sound effect appears to vary according to tissue

context. Yang, Wang, and Duan (2003) reported that sinusoidal sound waves affected ATP content in *Actinidia chinensis* callus depending on frequency and sound level, and that the 1000 Hz and 100–105 dB range was the most effective condition for ATP accumulation. However, because O_2 consumption, CO_2 production, or mitochondrial respiration rate was not directly measured, these findings represent indirect evidence at the level of energy metabolism rather than direct evidence for respiration. By contrast, Kim et al. (2015) showed that 6 h exposure of harvested tomato fruit to a 1 kHz sound wave reduced respiration rate during storage and delayed color change and softening. This indicates that sound can increase ATP accumulation in callus tissue, whereas in postharvest fruit tissue it can suppress respiration and ripening processes.

Overall, sound applications can affect photosynthesis- and respiration-related processes at the levels of chlorophyll content, stomatal behavior, gas exchange, PSII activity, carbon flux, and energy metabolism. However, this effect is neither linear nor unidirectional; while chlorophyll content, net photosynthetic rate, and PSII performance increase in some applications, pigment levels may decrease under certain frequency/duration combinations or responses may weaken at high sound levels. In terms of respiration, O_2 uptake and ATP content may increase, whereas postharvest fruit tissue may exhibit a reduced respiration rate. Therefore, the available evidence indicates that the effects of sound on photosynthesis and respiration vary depending on the physiological indicator measured, the organ or tissue examined, and the properties of the applied acoustic stimulus.

Effects of Sound on Plant Hormones

The effects of sound applications on plant hormones have been evaluated through growth- and development-related hormones (IAA, GA, and cytokinins), stress and defense hormones (ABA, JA, and SA), and ethylene biosynthesis associated with the ripening process.

Controlled frequency applications show that growth hormones and stress hormones may not respond in the same direction. Bochu, Jiping, Biao, Jie, and Chuanren (2004) reported that 1.4 kHz and 95 dB sound application increased IAA levels while decreasing ABA levels in mature *Chrysanthemum* callus, and they associated this change with accelerated recovery and differentiation in callus tissue. Ghosh et al. (2016), in *A. thaliana*, reported that 500 Hz and 80 dB treatment increased IAA and GA_3 levels particularly at 24 h, that JA increased mainly at 48 h, and that SA remained elevated at all time points, with the strongest increase occurring at 48 h. By contrast, no significant change was detected in ABA. Together, these two studies indicate that IAA/GA_3 increases may occur under controlled sound applications, whereas the ABA response is neither stable nor unidirectional.

PAFT/pulsed sine-wave applications indicate that hormonal responses may vary according to exposure duration and developmental stage. Hassanien, Li, and Hou (2020) applied sound to strawberry plants from the four-true-leaf stage onward in the 0.1–1 kHz frequency range and at 70–100 dB, comparing daily exposure durations of 2 and 5 h. In this study, GA levels increased at some stages, ZR showed a significant increase only at day 75 under the 2 h treatment, and JA increased at day 60 under the 5 h treatment. No consistent change was observed for IAA. The most striking response was observed in ABA; under the longer 5 h exposure, ABA levels exceeded the control at days 60 and 90. This pattern suggests that long-term sound exposure may produce a stress-like hormonal profile through ABA accumulation.

Music and complex sound applications have mainly been evaluated through growth hormones. Zhu et al. (2011) reported that an acoustic application consisting of a mixture of classical music and insect sounds in the 3–6 kHz range generally increased IAA content in cucumber, tomato, eggplant, cherry tomato, melon, and cowpea. However, because the stimulus consisted of both classical music and insect sounds, it remains unclear whether the effect was caused by a specific frequency component, the biological sound component, or the overall acoustic pattern. Rout, Swain, and Padhi (2022) reported that synchronized sound waves in the form of Indian classical ragas increased IAA and GA_3 levels in *Chamaecostus cuspidatus* and *Stevia rebaudiana*. However, the limited information on frequency, sound level, exposure duration, and statistical significance requires this study to be interpreted with caution.

Environmental/anthropogenic noise differs clearly from controlled sound and music applications in terms of hormonal responses. Kafash, Khoramnejadian, Ghotbi-Ravandi, and Dehghan (2022) exposed *Tagetes patula* and *Salvia splendens* plants for 16 h per day over 15 days to a stimulus generated from real traffic noise, with an equivalent sound level of approximately 73 dB and a dominant frequency of approximately 250 Hz. This application caused decreases in growth-related hormones and very strong increases in stress hormones. In *S. splendens*, zeatin, IAA, GA_3, and SA decreased, whereas ABA and JA increased by 1408.09% and 734.92%, respectively. In *T. patula*, zeatin, IAA, GA_3, and SA also decreased, whereas ABA and JA increased by 911.83% and 256.03%, respectively. Changes of this magnitude indicate that long-term and complex traffic noise should not be placed in the same biological category as PAFT, controlled frequency, or music applications, and that it may generate a stress-dominated hormonal profile rather than a growth-promoting hormonal pattern.

In postharvest fruit tissue, hormonal responses emerge along the ethylene and ripening axis, unlike the growth- or stress-hormone responses observed in vegetative tissues. Kim et al. (2015) applied a 1 kHz sound wave to harvested

tomato fruit for 6 h and showed that ethylene production decreased relative to the control during storage. This study shows that sound can delay ripening in postharvest fruit tissue by suppressing ethylene biosynthesis.

Overall, the effects of sound applications on plant hormones are not unidirectional. IAA and GA_3 may increase under some controlled frequency, music, or complex sound applications, but this increase is not consistent across all studies. The ABA response is more variable; it may decrease or show no significant change under controlled sound applications, whereas it may increase under long exposure durations or stimuli with a more pronounced stress character, such as traffic noise. JA and SA responses also differ depending on whether the stimulus is a controlled acoustic application or an environmental noise with stress-like characteristics. Therefore, the hormone literature indicates that sound can affect hormone metabolism and hormonal signaling; however, whether this effect emerges as growth-promoting, stress-inducing, or ripening-delaying depends on the physical and structural characteristics of sound, exposure duration, plant species, developmental stage, and the organ or tissue evaluated.

Methodological Considerations and Future Directions for Physiological and Developmental Studies

Although the current literature provides important findings on the effects of sound on plants, there is marked heterogeneity among studies in terms of experimental setup, acoustic description, exposure duration, sound source, exposure environment, and the biological parameters measured. Stimuli such as single-frequency sound, sine waves, mechanical vibration, PAFT devices, music, white noise, traffic noise, rain sound, bee sound, and herbivore chewing vibrations represent physically different forms of exposure; therefore, evaluating all these studies as a single type of "sound effect" would be misleading. Generalizations regarding physiological and developmental responses should therefore be made by considering the physical nature of the stimulus and its mode of exposure (Appel & Cocroft, 2014; Azgomi et al., 2023; Chowdhury et al., 2014; Hassanien et al., 2014; Veits et al., 2019).

Although frequency and sound level are reported in a substantial proportion of studies, the complete acoustic characterization of the applied sound is often limited. In complex sounds in particular, dominant frequency, power spectral density, frequency bands, amplitude/time pattern, waveform, modulation properties, and spectral composition have not been reported in detail. In addition, it is not always clear which weighting was used for the dB value, at what distance from the sound source it was measured, what the actual exposure at the plant surface was, or whether the sound field was homogeneous within the experimental setup. Factors such as sound insulation, background

noise, reverberation, standing wave formation, internal reflections within the chamber, use of absorbent surfaces, and speaker position may also alter the actual acoustic exposure at the plant level. Therefore, it cannot be assumed that plants in two studies with the same nominal frequency and dB value were exposed to the same mechanical/acoustic stimulus (Erbe et al., 2022; Kinsler et al., 2000; Makris & Navarro, 2026; Robin & Plante, 2022).

Additional physical effects that may be generated by equipment such as speakers, amplifiers, PAFT devices, ultrasonic sources, or piezoelectric actuators should also be considered. These devices not only produce sound, but may also generate side effects such as heat, air movement, electromagnetic fields, mechanical vibration, or device noise. Therefore, the use of only a silent control group may not be sufficient in all cases. Stronger experimental designs should include, together, sham controls in which the device operates without acoustic exposure to the plant, controls in which vibration originating from the speaker or actuator is isolated, background noise controls, and measurements of temperature, humidity, and air movement. Studies such as Appel and Cocroft (2014), which address vibration transmission in a controlled manner, provide methodologically valuable examples in this respect, whereas in many speaker- or PAFT-based studies it is not clear to what extent device-related additional effects were separated from the acoustic stimulus (Appel & Cocroft, 2014; Meng et al., 2012; Qi et al., 2010; Zakariya et al., 2017).

The mode of sound transmission also represents an important methodological distinction. Airborne sound pressure waves, substrate or platform vibrations, acoustic waves in water, and mechanical vibrations transmitted directly to tissue may produce different physical effects on plants. Even in studies using speakers, mechanical vibration may be transmitted through the shelf, container, nutrient medium, or substrate in which the plant is placed. By contrast, in controlled vibration studies, quantities such as amplitude, acceleration, displacement, or particle motion may be more decisive for the biological response. Therefore, sound pressure level should not be directly equated with the actual mechanical displacement or acceleration generated in plant tissues (Erbe et al., 2022; Kinsler et al., 2000; Makris & Navarro, 2026; Uchida & Yamamoto, 2002).

In the current literature, sound applications have been evaluated in different experimental systems, including seed germination, seedling growth, callus development, and postharvest fruit ripening. Therefore, results should be interpreted by considering the plant material used in the relevant study, the characteristics of the applied sound, exposure duration, developmental stage, and measurement time. The time dimension is particularly important in studies aiming to distinguish early signaling responses from later physiological, developmental, or metabolic responses; studies such as Ghosh

et al. (2016), Ali et al. (2026), Calderón et al. (2024), and Hassanien et al. (2020) show that sound responses can vary over time.

The biological meaning and interpretive limits of the measured indicators should also be considered. An increase in chlorophyll content or SPAD value does not by itself demonstrate an increase in net photosynthetic rate; it should be interpreted together with direct physiological measurements such as gas exchange, chlorophyll fluorescence, or carbon assimilation. Similarly, changes in hormone-related gene expression should not be considered directly equivalent to changes in hormone content. More generally, physiological and developmental responses should be interpreted by considering whether the measured parameter directly represents the biological process under discussion and whether apparently related indicators respond in the same direction under the applied acoustic conditions. Therefore, when interpreting studies such as Ghosh et al. (2016) and Kim et al. (2021), the extent to which the measured indicator represents the relevant biological process should be explicitly considered.

Taken together, these limitations indicate that the available findings support the ability of sound to induce physiological and developmental effects in plants; however, these effects should be interpreted by considering the physical nature of the applied stimulus, the actual exposure conditions, and the representative strength of the measured biological indicator. Therefore, existing studies should be evaluated not only in terms of the direction of their results, but also together with the acoustic setup, exposure mode, and measurement strategy used.

The dissection of natural and complex sounds is one of the priority research areas. Stimuli such as bee sounds, insect sounds, rain sounds, flowing water sounds, herbivore chewing vibrations, traffic noise, and music have spectral and temporal properties that cannot be represented by a single frequency value. Therefore, biologically meaningful sounds, particularly bee wingbeat sounds or herbivore feeding vibrations, should be examined through comparisons among whole recordings, dominant frequencies, filtered frequency bands, and synthetic pure tones (Appel & Cocroft, 2014; Azgomi et al., 2023; Makris & Navarro, 2026; Veits et al., 2019).

The initial sites of sound perception at the organ, tissue, and cellular levels also represent an important research gap. Trichomes, floral organs, the root tip, the cell wall–plasma membrane interface, and mechanosensitive ion channels emerge as candidate structures in sound/vibration perception. Rodrigo-Moreno et al. (2017) showed that 200 Hz sound application induced positive phonotropism in *Arabidopsis thaliana* roots and that this response was associated within minutes with increased cytosolic Ca^{2} , ROS

production, and changes in K^+ fluxes. Zhou et al. (2017) and Matsumura et al. (2022) demonstrated that trichomes can convert mechanical stimuli into cellular Ca^{2+} responses and defense-related signaling processes. However, it remains unclear which organ or cell types perceive different sound/vibration types and which molecular transducers connect these mechanical effects to the signaling network (Demey et al., 2023; Matsumura et al., 2022; Rodrigo-Moreno et al., 2017; Zhou et al., 2017).

At the mechanistic level, sound applications have been reported to affect Ca^{2+}, ROS, hormones, gene expression, and energy metabolism, but the causal sequence linking these responses to physiological and developmental outcomes remains unclear in most studies. It remains unresolved whether ROS accumulation represents an early signal, an indicator of oxidative stress load, or an intermediate component of downstream signaling; whether hormone changes are early components of perception or secondary outcomes; and whether gene expression changes lead to stable, measurable phenotypic consequences. Therefore, multi-level experimental designs combining time-series experiments, mutant and inhibitor applications, omics approaches, and direct physiological measurements are required (Ali et al., 2026; de Melo, 2023; Demey et al., 2023; Ghosh et al., 2016).

The threshold values for stimulatory or inhibitory effects of sound have not been systematically determined according to plant species and, especially in cultivated plants, the targeted production output. Some frequency–sound level–duration combinations increase germination, growth, photosynthesis, or metabolic activity, whereas higher sound levels, long exposures, or unsuitable frequencies may be associated with inhibitory responses. Although this pattern suggests the possibility of a hormetic response, it remains unclear which combinations are stimulatory and which are stress-inducing. Therefore, future studies should include dose–response experiments in which frequency, sound level, and exposure duration are varied together, comparisons of short- and long-term exposure, and reversibility analyses (Bochu et al., 2003; Heidari, Kafi, Kalatejari, Shafaghatian, & Mollakarimi, 2020; Uchida & Yamamoto, 2002; Weinberger & Measures, 1979).

The transferability of laboratory findings to greenhouse or field conditions is also poorly understood. Experimental designs that bridge controlled laboratory experiments with semi-controlled greenhouse and field studies are required; in terms of agricultural application potential, the effects of sound should be evaluated beyond growth promotion, including yield and quality (Hou & Mooneyham, 1999; Minh & Bui, 2026; Qi et al., 2010), as well as postharvest durability (Kim et al., 2015).

Taken together, future studies should aim to determine which acoustic components lead to which physiological and developmental outcomes, through which perception structures and early signaling processes, and under which exposure conditions these responses become stimulatory, neutral, or inhibitory. This requires precise acoustic characterization, appropriate experimental controls, explicit consideration of transmission mode, dose–response and time-resolved experimental designs, and comparisons among developmental stages, organs, and physiological endpoints.

Conclusion

The current literature indicates that sound and vibration stimuli can affect germination, seedling growth, photosynthesis, respiration, and hormone balance in plants. These findings show that sound is not merely a passive environmental background factor for plants, but can be considered, under certain conditions, as an environmental stimulus capable of eliciting biological responses through mechanoperception and cellular signal transduction (Chowdhury et al., 2014; Demey et al., 2023; Ghosh et al., 2016; Hassanien et al., 2014).

Nevertheless, the effects of sound on plants are not linear, unidirectional, or universal. Appropriate combinations of frequency, sound level, and exposure duration may support germination rate, root and shoot development, chlorophyll content, or photosynthetic performance in some studies, whereas high sound levels, unsuitable frequencies, long-term exposure, or complex sounds with the characteristics of environmental noise may be associated with growth inhibition, reduced pigment levels, or unfavorable physiological and hormonal responses. Therefore, the effect of sound should not be reduced to a single statement such as "promotes growth" or "induces stress", but should be evaluated together with the physical nature of the stimulus, plant species, developmental stage, organ/tissue level, and the biological indicator measured (Azgomi et al., 2023; Bochu et al., 2003; Calderón et al., 2024; Weinberger & Measures, 1979).

From a mechanistic perspective, the available findings suggest that sound-induced mechanical effects may generate multi-layered responses through the cell wall–plasma membrane interface, mechanosensitive channels, Ca^{2} and ROS signaling, hormone regulation, energy metabolism, and gene expression. However, it remains unclear which components of this chain represent primary perception and which represent secondary responses. In particular, the causal links between the actual vibration regime generated by sound in plant tissues and subsequent physiological and developmental responses remain incompletely resolved (de Melo, 2023; Demey et al., 2023; Mishra et al., 2016; Son et al., 2024).

In conclusion, sound should be considered not merely as a simple external stimulus affecting plant growth, but as an environmental signal that can influence mechanoperception, signal transduction, physiological regulation, and plant development. This perspective will contribute not only to a more reliable understanding of the role of sound in plant biology, but also to a more scientifically grounded evaluation of the potential uses of controlled acoustic applications in plant production and development.

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Chapter 4

MICROBIAL DIVERSITY ON SALTED AND SOAKED HIDES AND SKINS USED IN LEATHER INDUSTRY

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I. Overview of hides and skins structure and microorganisms

I.a. The general structure of the hide and skin

Skin is a three-layered functional barrier between the body and the environment. It assists homeostasis, prevents fluid loss, regulates body temperature, and protects the animals from physical and mechanical injuries, toxic chemicals, and pathogenic microorganisms (Bailey, 1992; Ibrahim et al., 2022). The skin structure is composed of the epidermis, dermis (corium), and subcutaneous layer. The epidermis, found on the outermost layer of skin, is a waterproof protective barrier. This layer contains the stratum basale, stratum granulosum, stratum spinosum, stratum lucidum, and stratum corneum (keratinocytes), as well as Merkel cells, Langerhans cells, and melanocytes (non-keratinocytes) (Monteiro-Riviere et al., 2008). The inner layer, dermis (corium), consists of irregular and dense connective tissue. The dermis contains elastic fibers, reticular fibers, collagen, fibroblasts, mast cells, macrophages, blood vessels, nerves, sebaceous and sweat glands, arrector pili muscles, and hair follicles. While papillary layer of the dermis is thin and composed of loose connective tissue, the lower reticular layer of the dermis is thicker and consists of irregular dense connective tissue containing fewer cells and more fibers (Monteiro-Riviere et al., 2008). In this dense fibrous connective tissue layer, collagen fibers are held in bundles by other associated protein tissues made up of reticulin (Elliot et al., 1990). During the tanning process, the durability of the leather was enhanced by stabilizing the triple-helix structure of the collagen matrix (Onem et al., 2017). After tanning, the final leather consists of a three-dimensional weave of collagen fiber bundles, which are responsible for the stability, strength, and flexibility of the leather (Elliot et al., 1990; Haines, 1984; Brown et al., 2012; Onem et al., 2017). The hypodermis that lies beneath the dermis is a layer of adipose tissue that contains fat-storing cells and blood vessels (Urry et al., 2022). While larger and heavier skins obtained from buffalo, cattle, camels, or horses are called hides, smaller and lighter skins obtained from goats, sheep, or pigs are called skins (Elliot et al., 1990; Covington, 2009). Hair, wool, and epidermis are removed from the skin at begining of leather processing (Covington, 2009). A part of the skin called the grain, that extends from outer surface to base of hair root, is an extremely important part of leather quality (Haines, 1984).

I.b. Microorganisms found on fresh hides and skins

Hides and skins harbor both resident microorganisms associated with the animal and microorganisms acquired from air, water, soil, feed, dust, feces, and barn surfaces (Birbir and Ilgaz, 1996; Birbir and Ulusoy, 2015). While the animal is alive, normal microbiota of the skin is closely associated with the animal's immune, endocrine and nervous systems as well as antimicrobial

substances (free fatty acids, antimicrobial peptides), and all of these control microbial activities on the skin (Madigan et al., 2022). After the flaying process, hide/skin containing water, fat, structural proteins (collagen, elastin, keratin), non-structural proteins (globulins, albumins, mucoids, mucins), and amino acids (asparagine, aspartic acid, alanine, glutamic acid, glutamine, arginine, cysteine, glycine, histidine, hydroxylysine, hydroxyproline, isoleucine, leucine, lysine, phenylalanine, proline, serine, methionine, valine, threonine, tyrosine, and tryptophan) and mineral salts (Highberger, 1956; Hien, 2006; Covington, 2009) is an ideal growth medium for the normal flora and contaminant microorganisms. Following slaughter, the absence of the host's physiological defenses allows proteolytic and lipolytic microorganisms to proliferate and degrade proteins and lipids present in the hide and skin. Also, the breakdown of carbohydrates to sugar occurs on the hides and skins. Finally, the hydrolysis reactions of the macromolecules cause the putrefaction of the hides/skins (Covington, 2009; Khambhaty and Samidurai, 2026). Various bacterial species have previously been isolated from fresh animal hides by researchers. Moreover, the presence and hydrolytic activities of different microorganisms on both fresh and salted hides/skins were reported in the previous studies (Venkatesan et al., 1970; Birbir and Ilgaz, 1996; Shede et al., 2008) (Table 1). The presence of *Achromobacter liquefaciens*, *Bacillus megaterium*, *B. cereus*, *B. subtilis*, *Brevibacterium insectiphilum*, and *Staphylococcus aureus* on freshly slaughtered goat skins (Venkatesan et al., 1970), and gelatinase positive *Bacillus*, *Micrococcus*, and *Staphylococcus* species on freshly slaughtered cattle hides were previously mentioned (Birbir and Ilgaz, 1996). The bacterial population of unpreserved raw buffalo hide stored at ambient conditions for 195 hours was examined by Shede et al. (2008). It was reported that all species were proteolytic and lipolytic. The researchers noted an increase in the total viable bacteria of unpreserved raw buffalo hide from 6.1 log CFU/g to 8.8 log CFU/g at 24 h and 9.8 log CFU/g at 96 h. Then, the total viable count of bacteria declined to 9.5 log CFU/g at 120 h and 7.5 log CFU/g at 144 h. In that study, hydroxyproline from collagen and tyrosine from structural and non-structural proteins were released during 144 h of storage. Moreover, bad odor, hair loss, and complete liquefaction of the hide were stated as signs of hide putrefaction caused by enzymes from proteolytic and lipolytic bacteria and their metabolic activities.

Table 1. Microorganisms found on raw skins/hides

Source	Isolated species		Reference
Freshly slaughtered goat skins	*Achromobacter liquefaciens*, *Bacillus cereus*, *B. megaterium*, *B. sphaericus*, *B. subtilis*, *Brevibacterium insectiphilum*, *Flavobacterium lactis*, *Kurthia variabilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*	Bacteria	Venkatesan et al., 1970
Freshly slaughtered cattle hides	*Bacillus cereus*, *B. megaterium*, *B. sphaericus, B. subtilis*, *Kurthia variabilis*, *Micrococcus roseus*, *Staphylococcus aureus*	Bacteria	Birbir and Ilgaz, 1996
Unpreserved raw buffola hide stored for 195 hours	*Bacillus cereus*, *B. sphaericus*, *Aeromonas caviae*, *Escherichia coli*, *Proteus mirabilis*, *P. vulgaris*, *P.penneri*, *Myroides odoratimimus*, *Brevibacterium luteolum*, *B. otitidis*, *Staphylococcus sciuri*, *Weeksella virosa*	Bacteria	Shede et al., 2008

I.c. Microorganisms found on salted or brine-cured hides and skins

In the leather industry, due to curing processes, preservative salts can introduce halotolerant, haloversatile, moderately halophilic, and slightly halophilic bacteria, as well as extremely halophilic archaea and fungi, to raw hides. These microorganisms have been classified according to their optimal growth rates at different salt concentrations: a) *non-halophilic bacteria* that grow in environments containing less than 1% NaCl; b) *halotolerant non-halophilic bacteria* capable of tolerating high salt concentrations (10% or 15% NaCl) (Yilmaz and Birbir, 2019); c) *slightly halophilic bacteria* that grow in environments containing 1 to 3% NaCl; d) *moderately halophilic bacteria* that grow in environments containing 3 to 15% NaCl; e) *extremely halophilic archaea* that grow in environments containing 15 to 25% NaCl or 30% NaCl (Kushner and Kamekura, 1988; Yilmaz and Birbir, 2019). Haloversatile bacteria are able to tolerate a broad range of NaCl (from 0 to 17.5%). They can grow in media with 1.17%-2.92% NaCl concentration but also can grow in medium without NaCl (Kushner, 1978; Grant et al., 1998). While halotolerant and haloversatile bacteria can grow in both saline and non-saline environments, slightly halophilic bacteria, moderately halophilic bacteria, and extremely halophilic archaea cannot grow in non-saline environments. Traditionally, raw hides and skins have been preserved with 95% saturated salt solution or 40%-50% salt (w/w) on rawhide weight (Bailey, 1992; Sarker et al., 2020). Forty percent of sodium chloride is mostly used in the curing of hides and skins but an additional salting process may be required in hide dealers' and tannery premises and sodium chloride amount may increase to fifty percent (green weight) (Buljan and Kral, 2015). The main goal of the curing of the hide or skin is to temporarily preserve freshly slaughtered hide or skin until it can be processed into a final product (Bailey, 1992). These methods remove water

from raw skins and hides to prevent microbial growth, activity, and damage during storage and transport.

The preservation methods may be applied in slaughterhouses, hide dealers' premises, and tanneries. Occasionally, an antimicrobial agent may be used in this salt curing method. In the salt pack curing method, freshly flayed hides and skins are cooled and spread out (lying down hair side to the floor), and uniformly covered with salt. Then, the hides and skins are stacked, sandwiched with additional salt, and left to drain for almost one week until the salt reaches saturation. Finally, the hides and skins are resalted and packed for long-term storage or transport (Buljan and Kral, 2015). In brine curing, hides and skins are placed in raceways, and brine solution fully diffuses into the hides/skins for 12 hours. After the brine curing, additional salt may be sprinkled to reduce the moisture content of the hides and skins (Buljan and Kral, 2015). Kallenberger (1985) emphasized that moisture content is important for cured hides due to raw stock quality and cost. The researchers reported that cured hides containing higher than 48% moisture would deteriorate much more rapidly than the hides with less than 48% moisture. In addition, Bailey (1992) reported that the recommended moisture content, ash content, and salt saturation of salted hides should be between 40-48%, 14-48 %, and ≥ 85%, respectively. Despite salt curing application to hides, Birbir and Ilgaz (1996) also isolated different bacterial species from one week old 15 salted hides and two months old 25 salted hides collected from tanneries of Türkiye (Table 1). In that study, all *Bacillus* species, *Micrococcus luteus*, *Staphylococcus aureus*, and *S. epidermis* were found to be protease positive (Birbir and Ilgaz, 1996). Moisture contents, ash contents, ash/moisture ratios, brine saturation values of one week old salted cattle hides and two months of salted cattle hides were respectively ranged from 36.00 to 57.50% and from 31.00 to 40.51%; from 4.01 to 14.51% and from 6.34 to 10.09%; from 11.13 to 32.70 and from 16.55 to 28.53%; from 31.07 to 92.67% and from 46.12 to 79.48%. In addition, pH values, nitrogen contents, hide substances values of one week old salted cattle hides and two months of salted cattle hides were respectively ranged from 7.11 to 7.55 and from 7.23 to 8.00; from 4.67 to 7.68% and from 4.26 to 7.29%; from 26.27 to 43.18%; from 23.94 to 40.96% (Birbir and Ilgaz, 1996). In the histological examination of one week old salted hides and two-month-old salted hides, 30% of the one week old salted hides and 36% of two-month-old salted hides were observed in poor condition (Birbir and Ilgaz, 1996). Due to autolysis, bacterial attack, and degradation, separation of fiber bundles in corium were detected at the two-month-old salted hides (Birbir and Ilgaz, 1996).

Totally 396 isolates of Gram-positive bacteria were obtained from 10 salted cattle hides (Aslan and Birbir, 2011a) (Table 2). In that study, 68%, 70% and 57% of the Gram-positive isolates were found to be lipolytic, proteolytic

and both proteolytic and lipolytic, respectively (Aslan and Birbir, 2011a). The dominance of endospore-forming *Bacillus* species on the salted hides may be closely related to contamination of the hides with soil and preservation salt (Aslan and Birbir, 2011a). The contamination of preservation salts with *Bacillus* species has been shown in the previous study (Yılmaz and Birbir, 2019). The researchers stated the isolation of 83 proteolytic halotolerant *Bacillus* strains belonging to 12 *Bacillus* species from 30 curing salt samples collected from 14 leather factories in Türkiye (Yılmaz and Birbir, 2019) (Table 2). That study proved that curing salt containing halotolerant bacteria that may contaminate hides and skins during the curing process. Furthermore, 256 isolates of Gram-negative bacteria were recovered from the salted hides (Aslan and Birbir, 2012) (Table 1). Proteolytic, lipolytic, both proteolytic and lipolytic Gram-negative isolates on the salted hides were detected as 68%, 52%, and 43%, respectively (Aslan and Birbir, 2012). Although the salt saturation values of those hides were 100%, a wide variety of Gram-positive and Gram-negative bacteria were recovered from the salted hides (Aslan and Birbir 2011a; Aslan and Birbir 2011b; Aslan and Birbir, 2012) (Table 2). The salted hides contained fairly high numbers of bacteria (10^5-10^8 CFU/g), proteolytic (10^4-10^7 CFU/g), lipolytic bacteria (10^4-10^7 CFU/g), respectively (Aslan and Birbir, 2011b).

The members of the family *Enterobacteriaceae* were also investigated at five salted cattle hides and five salted sheepskins. In that study, 27 bacterial strains from salted cattle hides and 28 bacterial strains from salted sheepskins were isolated (Ulusoy and Birbir, 2015) (Table 2). Total viable cell counts of *Enterobacteriaceae*, proteolytic *Enterobacteriaceae*, lipolytic *Enterobacteriaceae*, each species belonging to *Enterobacteriaceae* on the salted hides and sheepskins were respectively found as between 10^4-10^5 and 10^5-10^6 CFU/g; 10^3-10^5 and 10^5-10^6 CFU/g; 10^2-10^5 and 10^4-10^5 CFU/g; and 10^2-10^5 and 10^3-10^5 CFU/g (Ulusoy and Birbir, 2015). The presence of the members of *Enterobacteriaceae* family on cattle hides and sheepskins may be associated with the animal itself, fecal contamination, soil, feed, and water. While 45% of the isolates produced protease enzyme, lipase enzyme was produced by 30% of the isolates. That study results showed that decomposition processes of salted hides and skins by proteolytic and lipolytic strains of *Enterobacteriaceae* were inevitable. Also experimental results demonstrated that the isolates had catabolic activity to break down carbohydrates which were related to hide and skin deterioration (Ulusoy and Birbir, 2015).

Halotolerant bacteria have been commonly found in salted hides and skins cured in different countries. In the previous studies, researchers emphasized that rapid-growing halotolerant bacterial strains causing hide damage were common in cured hides and brines (Kallenberger, 1984; Kallenberger and Lollar, 1986). The presence of halotolerant bacterial strains in ten salted hides

was also noted (Table 2). Total viable cell counts of halotolerant bacteria were found as between 10^5 CFU/g and 10^8 CFU/g in those salted hides (Aslan and Birbir, 2011a). Additionally, eight salted sheepskins contained halotolerant bacteria between 10^3 CFU/g and 10^7 CFU/g (Caglayan et al., 2015). Yilmaz and Birbir (2019) reported that contamination of hides and skins with halotolerant bacteria might be related to curing salt used in hide and skin preservation. In that study, seventy percent, twenty-three, and seven percent of the 30 curing salt respectively contained 10^5 CFU, 10^4 CFU, 10^6 CFU of halotolerant bacteria per gram of curing salt (Yılmaz and Birbir, 2019).

In addition to halotolerant bacteria in curing salt samples, extremely halophilic archaea are found in curing salts obtained from marine and salt lakes and contaminate the hides and skins during the curing processes (Jordan-Lloyd et al., 1929; Stuart et al., 1933; Kallenberger, 1985; Kallenberger and Lollar, 1986; Birbir et al., 2004; Birbir et al., 2007) (Figure 1). In an earlier study carried out by Stuart et al. (1933), 34 of 35 samples of crude solar-evaporated salts and 25 of 39 open-plan evaporated salts contained red-colored microorganisms. The extremely halophilic archaea in red, red, blood-red, brick red, pink, pale pink, orange-red, pale-orange colors were isolated from the salt samples collected from Tuzkoy Salt Mine and Tuz Lake (Türkiye) (Birbir et al., 2004; Birbir et al., 2007). In a study conducted with 40 curing salt samples collected from various leather factories in Türkiye, between 10^2 and 10^5 CFU/g of extremely halophilic archaea were found in the salt samples. The viable cell counts of proteolytic and lipolytic extremely halophilic archaea were detected between 10^2 and 10^4 CFU/g (Berber and Birbir, 2010). The salted hide samples contained 10^2-10^6 CFU/g of proteolytic and lipolytic extremely halophilic archaea. These studies showed that salt-treated hides were contaminated with extremely halophilic archaea present in the salt used in the process (Berber and Birbir, 2010).

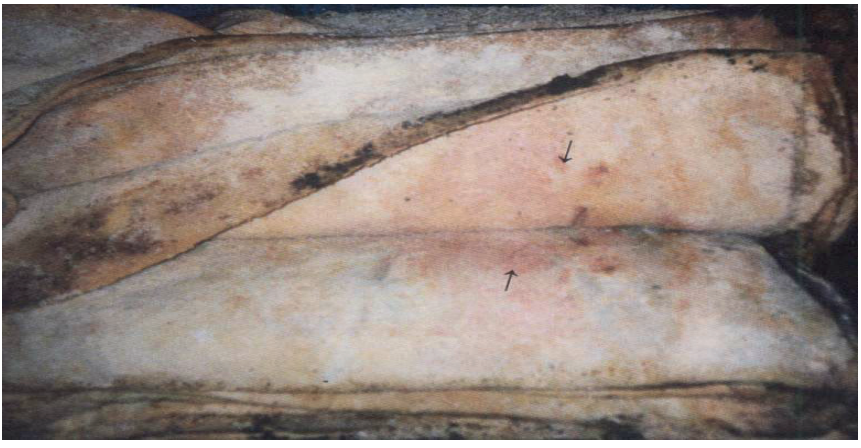


Figure 1. *Extremely halophilic archaea found on the salted hides.*

The importance of extremely halophilic archaea in the leather industry has been comprehensively elucidated by Kallenberger (1985) in a previous study. Kallenberger (1985) stated that extremely halophilic archaea were able to grow on proteinaceous substrates at both room temperature and high temperature. Kallenberger (1985) observed that high pH value detected in the liquid medium containing hide, 30% NaCl, and extremely halophilic archaea was related to protein degradation by these microorganisms. The researcher stated that contamination of hides with extremely halophilic archaea was due to the raceway brines. The researcher also explained that the brine cured hides were mostly stored in hot warehouses, trucks, and ships. Therefore, the hot conditions with moisture trapped by the folded, tightly packed hides offer an ideal growth medium for halophilic microorganisms. Kallenberger (1985) reported that extremely halophilic archaea, halotolerant bacteria, and moderately halophilic bacteria were found in the commercial raceways and cured hides. White, pink, and red colonies were isolated from the commercial raceways and cured hides. The researcher detected that extremely halophilic archaeal strains digested gelatin which is a denatured collagen material. Hence, it was concluded that these microorganisms have a high potential for hide damage (Kallenberger, 1985). The salted hides stored for one year were also investigated for collagenase producing-extremely halophilic archaea. In this study, the flesh side of the hide containing extremely halophilic archaea in high number was pink in color. When those hides were incubated at 35-40°C, malodor, hair slip, and severe grain damage were detected. Strong positive gelatin test results supported the digestion of collagen by the extremely halophilic archaea. Thus, the collagenolytic activity of extremely halophilic archaea on the cured hides was firstly proved by Kallenberger (1985).

The prevalence of extremely halophilic archaea and moderately halophilic bacteria on commercially cured hides and commercial raceways in the USA was also reported by Kallenberger and Lollar in 1986. In another study, the presence of halotolerant, slightly, moderately, and extremely halophilic microorganisms was investigated on 131 brine-cured hides collected from 34 hide curing facilities in the midwestern United States during ten months by Bailey and Birbir in 1993. Halotolerant, slightly, moderately halophilic bacteria, and extremely halophilic archaea were detected at 57%, 80%, 96%, and 94% of the hides, respectively. The viable cell counts of halotolerant, slightly, moderately halophilic bacteria, and extremely halophilic archaea were respectively found to be between 10^3 to 10^4 CFU/g, 10^4 to 10^7 CFU/g, 10^5 to 10^8 CFU/g, and 10^5-10^8 CFU/g. Ninety-four percent of hides contained extremely halophilic archaea. Fifty-three percent of 332 extremely halophilic archaeal isolates were protease positive. The growth of four extremely halophilic archaeal isolates was examined at temperatures ranging from 25°C to 55°C for 15 days. While the

growth of the isolates was slight between 25°C and 30°C, maximum growth was observed at 40°C. These results demonstrated that the usual brine raceway treatments do not prevent the growth of extremely halophilic archaea on brine-cured hides. The researchers reported that extremely halophilic archaea could grow in high numbers between 35°C and 42°C and cause deterioration of brine-cured hides at prolonged storage (Bailey and Birbir, 1993).

In another research, 35 salted hides contained respectively 91%, 86%, 37% and 29% of extremely halophilic archaea, moderately halophilic bacteria, slightly halophilic bacteria, and halotolerant bacteria. Red heat, hair slip, malodor, and sticky appearance, which are closely related to the microbial decomposition of the hides, were detected on those hides (Birbir, 1997).

Moderately halophilic bacteria were also examined on the salt-cured hides (Caglayan et al., 2015) (Table 2). The total count of moderately halophilic bacteria, the total count of proteolytic moderately halophilic bacteria, and the count of lipolytic moderately halophilic bacteria were determined in salted hide samples as 10^2-10^7 CFU/g, 10^1-10^5 CFU/g, and 10^5-10^6 CFU/g, respectively. While thirty-one, fifteen, thirty-one, thirty-eight percent of the isolates produced protease, lipase, urease, and DNase enzymes, fifteen, fifteen, and eight percent of the isolates produced amylase, caseinase, and pullulanase enzymes, respectively. The experimental results of that study showed that moderately halophilic isolates were able to break down macromolecules such as proteins and fats and contributed to hide putrefication (Caglayan et al., 2015).

In another study, 186 extremely halophilic archaeal isolates were obtained from eight salted hide samples (Bilgi et al., 2015) (Table 2). In that study, 63%, 26%, 25%, 15%, 8%, 7%, and 5% of the isolates used lactose, sucrose, galactose, fructose, maltose, mannitol, and trehalose, respectively. DNase enzyme was produced by all isolates but cellulase was not produced. Furthermore, 12%, 57%, 11%, 35%, 18% of the isolates respectively produced caseinase, gelatinase, amylase, esterase, and lipase (Bilgi et al., 2015).

Seventy-eight moderately halophilic bacterial strains were isolated from four salted sheepskins collected from a warehouse in Valencia, Spain (Akpolat et al., 2015) (Table 2). Red and yellow discolorations, bad odor, slimy layers, hair slips that were related to microbial activities were observed on the sheepskin samples. The pH values of these sheepskins were detected as between 7.95 and 8.90. While the total viable cell counts of moderately halophilic bacteria were detected as between 9×10^5 CFU/g and 1.1×10^8 CFU/g, total viable cell counts of extremely halophilic archaea were found between 9×10^5 CFU/g and 1.1×10^7 CFU/g (Akpolat et al., 2015). *Halococcus morrhuae* and *Halorubrum lipolyticum* showed both proteolytic and lipolytic activities. While *Acinetobacter johnsonii*, *Haloterrigena saccharevitans*, *Natrinema versiforme* showed lipolytic activity,

Halococcus dombrowskii, *Natrinema pellirubrum* had proteolytic activity (Akpolat et al., 2015).

Moderately halophilic isolates were isolated from 25 salted sheepskin samples (Caglayan et al., 2017) (Table 2). All isolates of moderately halophilic bacteria grew between pH 6 and 8 (Caglayan et al., 2017). The total viable counts of moderately halophilic bacteria, proteolytic moderately halophilic bacteria, lipolytic moderately halophilic bacteria ranged from 6.0×10^3 to 8.0×10^7 CFU/g, 4.5×10^2 to 4.7×10^6 CFU/g, 7.5×10^2 to 6.8×10^6 CFU/g, respectively. The values of salt saturation, moisture content, and ash content of the salted goat skins respectively ranged from 64% to 100%, 34% to 70%, and 11%-32%. Seventy-nine percent of the isolates were able to produce different enzymes. Sixty, 43%, 39%, 26%, 23%, 17%, 12%, 12%, 10% of the isolates were able to produce protease, lipase, β-galactosidase, amylase, caseinase, DNase, urease, cellulase, and lecithinase, respectively. Eighty-seven percent of the isolates produced NH_3 from peptone. Ammonia odor, related to protein catabolism, was detected on the salted sheepskins. The utilization of different sugars by the isolates as carbon and energy sources was common. The test results of the study proved that all strains isolated from different sheepskin samples had high catabolic activities to break down different carbon sources and use them as energy sources to grow (Caglayan et al., 2017).

Also, moderately halophilic bacterial isolates were recovered from 25 salted goatskin samples (Caglayan et al., 2018a) (Table 2). The total viable counts of moderately halophilic bacteria, lipolytic moderately halophilic bacteria, and proteolytic moderately halophilic bacteria, ranged from $7.0x10^3$ to 5.0×10^7 CFU/g, 3.0×10^2 to 7.5×10^6 CFU/g, and 1.5×10^2 to 8×10^6 CFU/g, respectively. The values of moisture content, salt saturation, and ash content of the sheepskins respectively ranged from 32% to 68%, 58% to 100%, and 12%-30%. The high numbers of moderately halophilic bacteria (proteolytic, lipolytic), high moisture content, low ash content, and low salt saturation values detected in some sheepskins were associated with inadequate preservation of these skins (Caglayan et al., 2018b). While catalase was produced by all isolates, protease, lipase, β-galactosidase, oxidase, urease, caseinase, amylase, lecithinase, and cellulase enzymes were produced by 87%, 64%, 59%, 56%, 46%, 28%, 26%, 8%, and 5%, respectively. Eighty-five percent of the isolates produced NH_3 from peptone. Ammonia odor related to protein catabolism detected on the salted goat skins. Utilization of different sugars by the isolates as carbon and energy source was common (Caglayan et al., 2018a).

Two novel species of Gram-positive moderately halophilic bacteria called *Thalassobacillus pellis* sp. nov., and *Salimicrobium salexigens* sp. nov. were isolated from salted hide samples (Sánchez-Porro et al., 2011; De la Haba et al., 2011) (Table 2). A novel species of moderately halophilic *Thalassobacillus pellis*

sp. nov. was Gram-positive, motile, endospore-forming rods. Cream-colored new species produced catalase, oxidase, protease, caseinase, DNase, pullulanase, phosphatase, and aesculinase enzymes. *Thalassobacillus pellis* sp. nov. produced acid from different carbon sources (D-fructose, D-glucose, glycerol, trehalose, sucrose, and D-xylose). Novel species also used different carbon and energy sources. Furthermore, amino acids such as L-serine, tryptophan, valine, L-lysine, L-threonine, isoleucine, L-alanine, phenylalanine, L-arginine, and glutamine were used by new species. While *Thalassobacillus pellis* sp. nov. was found to be susceptible to erythromycin (15 mg), sulfamethoxazole/trimethoprim (23.75/1.25 mg), novobiocin (30 mg), chloramphenicol (30 mg), cephalothin (30 mg), it was found to be resistant to nalidixic acid (30 mg), and neomycin (10 mg), kanamycin (30 mg), and penicillin G (10 U) (Sánchez-Porro et al., 2011). The other two moderately halophilic bacteria were proposed as a novel species of the genus *Salimicrobium*, with the name *Salimicrobium salexigens* sp. nov. The novel strains reduced nitrate to nitrite. DNase, phosphatase, catalase, oxidase were produced by those strains. Acid was produced by D-glucose, D-fructose, D-trehalose, maltose, and sucrose. D-mannitol, D-sorbitol, D-galactose, D-glucose, ribose, and acetate were used by *Salimicrobium salexigens* sp. nov. (De la Haba et al., 2011).

In another study conducted with five red heat-damaged salt-cured sheepskins, the isolation of bacterial and archaeal isolates was reported (Enquahone et al., 2020) (Table 2). Collagen degradation abilities of 17 isolates were also separately examined in the liquid medium containing only small pieces of sheepskin (Enquahone et al., 2020). While all isolates grew in the liquid medium, *Halococcus* sp. s2535 grew faster using sheepskin as a carbon and energy source than the other isolates. Therefore, *Halococcus* sp. s2535 was graded as excellent collagen degradation efficiency, *Nesterenkonia* sp. s2011 (*Nesterenkonia aethiopica*), *Marinococcus* sp. s2526 (*Marinococcus luteus*), and *Halococcus* sp. s2528 (*Halococcus dombrowskii*) were graded as very good degradation efficiency, six isolates (*Halomonas* sp. s106, *Salimicrobium* sp. s1026, *Nesterenkonia* sp. s203, *Nesterenkonia* sp. s2020, *Bacillus* sp. s10111, and *Bacillus* sp. s10112) were graded as good degradation efficiency and seven isolates were graded as low degradation efficiency (Enquahone et al., 2020).

Xiaoguang et al. (2022) explored how microbial communities change over time on wet-salted goatskins using sequencing and culturing methods in their study. They found that microbial diversity fluctuates, with salt-sensitive bacteria declining and halophilic archaea becoming dominant. Microorganisms gradually move from the surface into the inner layers to adapt to high-salt conditions. The genera of *Bacillus, Chromohalobacter, Psychrobacter*, *Salimicrobium*, *Salinicola*, *Ornithinibacillus*, and *Halomonas* were isolated from fresh goatskin samples (Xiaoguang et al., 2022).

Presence of antibiotic-resistant bacteria on salted hides and skins is significant important problem for both the leather industry and environment. Hence, the researchers examined antibiotic-resistant bacteria on salted hides and skins. The presence of multidrug-resistant bacteria on salted raw hides and sheepskins has been reported in a previous study (Birbir et al., 2016) (Table 2). In that study, the antibiotic resistance profiles of 27 salted cattle hide strains and 28 salted sheepskin strains belonging to the family *Enterobacteriaceae* were examined using twenty-four antimicrobial agents. While 45% of the isolates showed resistance against ceftriaxone, cefuroxime sodium, and ampicillin, 71%, 42%, 38%, 35%, 33%, 29, 25%, 25%, 20%, 16%, 16%, 13% of the isolates exhibited resistance against aztreonam, nalidixic acid, piperacillin-tazobactam, chloramphenicol, ceftazidime, ampicillin-sulbactam, amoxycillin-clavulanate, trimethoprim-sulfamethoxazole, cefoxitin, cephalothin, tetracycline, and tobramycin, respectively. Among the isolates, both *Klebsiella pneumoniae* ssp. *ozaenae* and *Serratia odorifera* that showed resistance to 12 different antimicrobial agents were found to be the most multidrug-resistant bacteria. While *Serratia rubidaea*, *Serratia marcescens*, and *Citrobacter lapagei* showed resistance to 7 different antimicrobial agents, *Enterobacter sakazakii*, *Serratia ficaria*, *Proteus penneri*, *Yersinia enterocolitica*, *Enterobacter cloacae*, *Raoultella ornithinolytica*, *Serratia plymuthica*, *Serratia liquefaciens*, *Citrobacter koseri*, and *Ewingella americana* showed resistance to 11, 10, 10, 9, 8, 6, 5, 4, 4, and 3 different antimicrobial agents, respectively. However, *Escherichia coli* and *Klebsiella oxytoca* were found to be susceptible to 23 and 22 antimicrobial agents, respectively. That study proved that multidrug-resistant enteric bacteria were common on each salted cattle hide and sheepskin sample due to fecal contamination. The most notable result of the study was the detection of isolates resistant to both antimicrobials of high importance and critically important antimicrobials of the highest priority (Birbir et al., 2016). Detection of antibiotic-resistant strains belonging to the family *Enterobacteriaceae* on the salted cattle hides and sheepskins may be related to using antibiotics for growth promotion, prevention, and treatment of diseases in animals (Mirzaagha et al., 2011; Van Den Bogaard and Stobberingh, 2000). The random use, overuse, and misuse of different antibiotics may also cause the occurrence of multidrug-resistant bacteria in animals (Birbir et al., 2016).

Moreover, the prevalence of multidrug-resistant moderately halophilic Gram-negative bacteria both salted sheepskin and goatskin samples cured in different countries was reported by researchers (Caglayan et al., 2020) (Table 2). Susceptibilities of 47 moderately halophilic Gram-negative skin isolates belonging to *Halomonas halodenitrificans*, *H. halmophila*, *H.venusta, H. zhanjiangensis*, *H. alkaliphila*, *H. eurihalina*, *Chromohalobacter beijerinckii, C. israelensis*, *C. canadensis*, *C. japonicus*, and *Idiomarina loihiensis* against 16 different

antimicrobial agents were examined. All isolates were resistant to amikacin (30μg), gentamicin (10μg), tobramycin (10μg), and cefadroxil (30μg) but all skin isolates were found to be susceptible to chloramphenicol (30μg), imipenem (10μg), meropenem (10μg), cefoxitin, (30μg), cefuroxime (30μg), ceftriaxone (30μg), amoxycillin/clavulanic acid (30μg), and piperacillin/tazobactam (110μg). Among the strains, only *Halomonas eurihalina* was found resistant to sulfamethoxazole-trimethoprim (25μg) but the other strains formed inhibition zones between 10 mm and 21 mm against the antimicrobial agent. While only *Chromohalobacter israelensis* was resistant against aztreonam (30μg), only *Chromohalobacter beijerinckii* was found to be resistant against ampicillin/sulbactam (20μg). The inhibition zones of *Halomonas halodenitrificans*, *Halomonas halmophila*, *Chromohalobacter japonicus*, *Idiomarina loihiensis* to ampicillin were respectively detected as 15-20 mm, 10-15 mm, 22-23 mm, 15 mm. However, the other strains were resistant to ampicillin (Caglayan et al., 2020).

Table 2. Microorganisms found on salted skins/hides and curing salts.

Source	Isolated species		Reference
One week old salted hides	*Bacillus brevis*, *B. cereus*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. sphaericus*, *B. subtilis*, *Kurthia variabilis*, *Micrococcus candicans*, *M. luteus*, *M. roseus*, *M. rubens*, *Staphylococcus aureus*, *S. epidermis*	Bacteria	Birbir and Ilgaz, 1996
Two months old salted hides	*B. cereus*, *B. firmus*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. sphaericus*, *B. subtilis*, *Kurthia variabilis*, *Micrococcus candidus*, *M.luteus*, *M. roseus*, *Staphylococcus aureus*, *S. epidermis*	Bacteria	Birbir and Ilgaz, 1996
Five months old salted hides	*Halorubrum saccharovorum*, *H. tebenquichense*, *H. lacusprofundi*, *Natrinema pallidum*, *N. gari*	Extremely halophilic archaea	Yilmaz, 2010
Salt samples of Tuzkoy Salt Mine (Türkiye)	Genera of *Halobacterium*, *Haloarcula*, *Natrinema*, *Halorubrum*	Extremely halophilic archaea	Birbir et al., 2004
Salt samples of Tuz Lake (Türkiye)	Genera of *Halobacterium*, *Haloarcula*, *Halorubrum*	Extremely halophilic archaea	Birbir et al., 2007
Hide/skin curing salt samples	*B.amyloliquefaciens*, *B.atrophaeus*, *B.halotolerans*, *B.licheniformis*, *B.mojavensis*, *B.paralicheniformis*, *B.pumilus*, *B.safensis*, *B.siamensis*, *B.subtilis*, *B.tequilensis*, *B.velezensis*	Halotolerant bacteria	Yilmaz and Birbir, 2019

Salt samples collected from Camalti Saltern	*Exiguobacterium sibiricum*, *Bacillus subtilis* subsp. *stercoris*, *Bacillus haynesii*, *Paracoccus marcusii*, *Kocuria polaris*, *Acinetobacter radioresistens*, *Micrococcus aloeverae*, *Bacillus pumilus*, *Microbacterium maritypicum*, *Microbacterium aurantiacum*, *Brevibacterium frigoritolerans*, *Staphylococcus lentus*, *Paracoccus hibiscisoli*, *Staphylococcus hominis* subsp. *novobiosepticus*, *Bacillus velezensis*, *Staphylococcus equorum* subsp. *equorum*, *Bacillus safensis*, *Kocuria sediminis*, *Staphylococcus petrasii* subsp.*jettensis*, *Staphylococcus pasteuri*, *Bacillus thioparans*, *Staphylococcus warneri*, *Virgibacillus salarius*, *Bacillus paraflexus*, *Pseudomonas songnenensis*, *Microbacterium saccharophilum*, *Bacillus altitudinis*, *Staphylococcus saprophyticus* subsp.*saprophyticus*, *Gordonia alkanivorans*, *Micrococcus yunnanensis*, *Agrococcus lahaulensis*, *Kocuria rosea*, *Sanguibacter inulinus*, *Bacillus nealsonii*, *Staphylococcus cohnii* subsp. *urealyticus*, *Bacillus oryzaecorticis*, *Exiguobacterium artemiae*, *Exiguobacterium indicum*, *Bacillus siamensis*, and *Staphylococcus epidermidis*	Moderately halophilic bacteria	Caglayan, 2019

Table 2. Continue.

Hide/skin curing salt samples	*Bacillus subtilis subsp. stercoris*, *Kocuria sediminis*, *Paracoccus marcusii*, *Kocuria polaris*, *Micrococcus aloeverae*, *Microbacterium maritypicum*, *Bacillus haynesii*, *Brevibacterium frigoritolerans*, *Paracoccus hibiscisoli*, *Bacillus velezensis*, *Bacillus pumilus*, *Bacillus safensis*, *Staphylococcus petrasi subsp. jettensis*, *Staphylococcus hominis* subsp. *novobiosepticus*, *Staphylococcus lentus*, *Bacillus thioparans*, *Staphylococcus epidermidis*, *Acinetobacter radioresistens*, *Exiguobacterium sibiricum*, *Gordonia alkanivorans*, *Microbacterium aurantiacum*, *Staphylococcus pasteuri*, *Bacillus paraflexus*, *Microbacterium saccharophilum*, *Kocuria rosea*, *Staphylococcus saprophyticus* subsp. *saprophyticus*, *Micrococcus yunnanensis*, *Pseudomonas songnenensis*, *Bacillus nealsonii*, *Staphylococcus equorum* subsp. *equorum*, *Agrococcus lahaulensis*, *Sanguibacter inulinus*, *Virgibacillus salarius*, *Staphylococcus cohnii* subsp. *urealyticus*, *Bacillus altitudinis*, *Exiguobacterium artemiae*, *Bacillus siamensis*, *Exiguobacterium indicum*, *Bacillus oryzaecorticis*, *Staphylococcus warneri*	Haloversatile bacteria	Caglayan, 2023
Hide/skin curing salt samples	*B.amyloliquefaciens*, *B.atrophaeus*, *B.halotolerans*, *B.licheniformis*, *B.mojavensis*, *B.paralicheniformis*, *B.pumilus*, *B.safensis*, *B.siamensis*, *B.subtilis*, *B.tequilensis*, *B.velezensis*	Halotolerant bacteria	Yilmaz and Birbir, 2019

Hide/skin curing salt samples	*Staphylococcus epidermidis*, *S. warneri*, *Corynebacterium aurimucosum*, *S. caprae*, *S. pasteuri*, *Peribacillus frigoritolerans*, *S. haemolyticus*, *Lysinibacillus telephonicus*, *Kocuria rosea*, *Paenibacillus illinoisensis*, *K. polaris*, *Bhargavaea ginseng*, *Bacillus haynesii*, *B. sonorensis*, *B. licheniformis*, *Pseudomonas oryzihabitans*, *B. subtilis*, *B. piscis*, *S. hominis*, *Micrococcus yunnanensis*, *S. saccharolyticus*, *Pseudomonas iranica*, *Micrococcus luteus*, *S. capitis* subsp. *urealyticus*, *Corynebacterium minutissimum*, *B. paralicheniformis*, *S. taiwanensis*	Haloversatile bacteria	Abdulhusein et al., 2023
Salted hide	*Salimicrobium salexigens* sp. nov.	Moderately halophilic bacteria	De la Haba et al., 2011
Salted cattle hides	*Aerococcus urinae*, *A. viridans*, *Aneurinibacillus aneurinilyticus*, *Bacillus amyloliquefaciens*, *B. cereus*, *B. firmus*, *B. laterosporus*, *B. lentus*, *B. licheniformis*, *B. megaterium*, *B. mycoides*, *B. pumilus*, *B. subtilis*, *B. thuringiensis*, *Brevibacillus laterosporus*, *Enterococcus avium*, *E. casseliflavus*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *Geobacillus stearothermophilus*, *G. thermoglucosidiasius*, *Kocuira kristanea*, *K. varians*, *Lactococcus lactis*, *Paenibacillus pabuli*, *Staphylococcus aureus*, *S. capitis*, *S. caprae*, *S. chromogenes*, *S. cohnii*, *S. epidermidis*, *S. hominis*, *S. hyicus*, *S. intermedius*, *S. lentus*, *S. lugdunensis*, *S. sciuri*, *S. xylosus*, *S. warneri*, *Streptococcus acidominimus*, *S. bovis*, *S. pluranimalium*, *S. thermophilus*, *S. uberis*, *Virgibacillus panthothenticus*	Halotolerant bacteria	Aslan and Birbir, 2011a
Salted cattle hides	*Acinetobacter baumannii*, *A. calcoaceticus*, *A. haemolyticus*, *A. junii*, *A. lwoffii*, *Aeromonas caviae*, *A. hydrophila*, *Alcaligenes faecalis*, *Burkholderia gladioli*, *Citrobacter amalonaticus*, *C.ferundii*, *Comamonas testesteroni*, *Edwardsiella tarda*, *Enterobacter aerogenes*, *E. agglomerans*, *E. amnigenus*, *E. cloacae*, *E. gergoviae*, *E.intermedius*, *E. liquefaciens*, *E. sakazakii*, *Escherichia coli*, *Hafnia alvei*, *Klebsiella oxytoca*, *K.pneumoniae ssp. ozanae*, *Mannheimia haemolytica*, *Pasteurella multocida*, *P. pneumotropica*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *P.fluorescens*, *P. luteola*, *P. maltophila*, *P. paucimobilis*, *P. pseudoalcaligenes*, *P. putida*, *Salmonella choleraesuis ssp. arizonae*, *S. paratyphi A*, *S. typhimurium*, *Serratia marcescens*, *Sphingomonas paucimobilis*, *Stenotrophomonas maltophila*, *Vibrio fluvialis*, *V. vulnificus*, *Yersinia pseudotuberculosis*, *Y. ruckeri*	Halotolerant bacteria	Aslan and Birbir, 2012

Table 2. Continue.

Source	Isolated species		Reference
Salted cattle hides	*Cedecea lapagei*, *Enterobacter cloacae*, *E. sakazakii*, *Escherichia coli*, *E. vulneris*, *Ewingella americana*, *Klebsiella pneumoniaea ssp. ozaenae*, *K.* oxytoca, *Proteus vulgaris*, *Raoultella planticola*, *R. ornithinolytica*, *Serratia odorifera*, *S.liquefaciens*, *S. plymuthica*, *S.* rubidaea, *Yersinia enterocolitica*	Bacteria	Ulusoy and Birbir, 2015
Salted sheepskins	*Citrobacter koseri*, *Enterobacter cloacae*, *Escherichia coli*, *E. vulneris*, *Klebsiella pneumoniaea ssp.ozaenae*, *K. oxytoca*, *Proteus vulgaris*, *P. penneri*, *Raoultella planticola*, *Serratia ficaria*, *S. odorifera*, *S. liquefaciens*, *S.marcescens*, *S. plymuthica*, *S. rubidaea*, *Yersinia enterocolitica*	Bacteria	Ulusoy and Birbir, 2015
Salted hides	*Salimicrobium luteum*, *S. album*, *S. halophilum*, *Halomonas koreensis*, *H. elongata*, *H. halmophila*, *H. eurihalina*, *H.alimentaria*, *Marinococcus halophilus*, *Oceanobacillus picturae*, *Thalassobacillus devorans*, *Chromohalobacter salexigens*, *Alkalibacillus salilacus*	Moderately halophilic bacteria	Caglayan et al., 2015
Salted hides	*Natrialba aegyptia*, *Halococcus morrhuae*, *H. thailandensis*, *H. dombrowskii*, *Halovivax asiaticus*, *Natronococcus jeotgali*, *Haloterrigena thermotolerans*, *Natrinema pallidum*, *N. versiforme*, *Halobacterium noricense*	Extremely halophilic archaea	Bilgi et al., 2015
Salted sheepskins	*Alkalibacillus halophilus*, *Pseudomonas halophila*, *Acinetobacter johnsonii*, *Alkalibacillus salilacus*, *Salimicrobium salexigens*, *Marinococcus luteus*, *Staphylococcus equorum* subsp. *equorum*	Moderately halophilic bacteria	Akpolat et al., 2015
Salted sheepskins	*Halorubrum tebenquichense*, *H. saccharovorum*, *H. kocurii*, *H. terrestre*, *H. lipolyticum*, *Halococcus dombrowskii*, *H. qingdaonensis*, *H. morrhuae*, *Natrinema pellirubrum*, *N. versiforme*, *Halostagnicola larsenii*, *Haloterrigena saccharevitans*	Extremely halophilic archaea	Akpolat et al., 2015
Salted cattle hides	*Cedecea lapagei*, *Enterobacter cloacae*, *E. sakazakii*, *Escherichia coli*, *E. vulneris*, *Ewingella americana*, *Klebsiella pneumoniaea ssp. ozaenae*, *K. oxytoca*, *Proteus vulgaris*, *Raoultella planticola*, *R. ornithinolytica*, *Serratia odorifera*, *S.liquefaciens*, *S. plymuthica*, *S. rubidaea*, *Yersinia enterocolitica*	Bacteria belonging to *Enterobacteriaceae*	Birbir et al., 2016
Salted sheepskins	*Citrobacter koseri*, *Enterobacter cloacae*, *Escherichia coli*, *E. vulneris*, *Klebsiella pneumoniaea ssp. ozaenae*, *K. oxytoca*, *Proteus vulgaris*, *P. penneri*, *Raoultella planticola*, *Serratia ficaria*, *S. odorifera*, *S. liquefaciens*, *S. marcescens*, *S. plymuthica*, *S.rubidaea Yersinia enterocolitica*	Bacteria belonging to *Enterobacteriaceae*	Birbir et al., 2016

Salted sheepskins	*Alkalibacillus halophilus*, *Bacillus licheniformis*, *B. pumilus*, *B. tequilensis*, *B. safensis*, *B. siamensis*, *Chromohalobacter canadensis*, *C. japonicus*, *C. beijerinckii*, *C. israelensis*, *Halomonas halmophila*, *H. eurihalina*, *H. zhanjiangensis*, *H. venusta*, *H. alkaliphila*, *Salimicrobium salexigens*, *Gracilibacillus dipsosauri*, *Salinivibrio costicola* subsp. *alkaliphilus*, *Staphylococcus equorum* subsp. *equorum*, *S. cohnii* subsp. *cohnii*, *S. xylosus*, *S. lentus*, *S. saprophyticus* subsp. *saprophyticus*, *Salinicoccus roseus*, *Planococcus rifietoensis*, *Marinococcus luteus*, *M. tarijensis*, *Oceanobacillus picturae*, *Idiomarina loihiensis*	Moderately halophilic bacteria	Caglayan et al., 2017
Salted goatskins	*Staphylococcus saprophyticus* subsp. *saprophyticus*, *S. equorum* subsp. *equorum*, *S. arlettae*, *S. xylosus*, *Bacillus pumilus*, *B. licheniformis*, *Gracilibacillus dipsosauri*, *Salinicoccus roseus*, *Chromohalobacter beijerinckii*, *C. canadensis*, *Halomonas eurihalina*, *H. zhanjiangensis*, *H. venusta*	Moderately halophilic bacteria	Caglayan et al., 2018a

Table 2. Continue.

Salted goat skins	*Staphylococcus warneri*, *Alkalihalobacillus clausii*, *Solibacillus silvestris*, *Bacillus licheniformis*, *B. pumilus*, *Ornithinibacillus scapharcae*, *Kocuria rosea*, *Oceanobacillus polygoni*, *Virgibacillus proomii*, *Staphylococcus hominis* subsp. *hominis*, *Bhargavaea ginsengi*, *B. amyloliquefaciens*, *B. licheniformis*, *B. cereus*, *B. paralicheniformis*, *Staphylococcus nepalensis*, and *Micrococcus luteus. Staphylococcus warneri*, *Alkalihalobacillus clausii*, and *Solibacillus silvestris*	Haloversatile bacteria	Kose et al., 2024
Red heat-damaged salted sheepskins	*Halomonas halodenitrificans*, *Bacillus velezensis*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Salimicrobium jeotgalis*, *Salimicrobium salexigens*, *Nesterenkonia aethiopica*, *Nesterenkonia halophila*, *Nesterenkonia xinjiangensis*, *Nesterenkonia suensis*, *Marinococcus luteus*, *Marinococcus halobia*	Bacteria	Enquahone et al., 2020
Red heat-damaged salted sheepskins	*Halococcus dombrowskii*, *Halococcus morrhuae*, *Halococcus thailandensis*, *Halococcus qingdaonensis*	Archaea	Enquahone et al., 2020

I.d. Microorganisms found in soaking liquors and soaked hides treated with antimicrobial agents

Soaking, which is applied into pre-soaking and main-soaking, is first leather processing to remove curing salt, the major part of interfibrillar matter composed of non-collagenous proteins (globulins, elastin, and albumins), glycosaminoglycans (di-/polysaccharides), insecticides, and other antimicrobials (Mwinyihija, 2010; Buljan and Kral, 2015). This process also removes blood, dung, debris, and dirt from hides and skins. In this process, salted hides and skins are rehydrated and collagen fibers return to the original hydrated structure by reabsorbing water (Buljan and Kral, 2015).

In the study conducted using soaked hides, the numbers of viable bacterial cells were found as 10^7-10^8 CFU/g in 29 and 10^5-10^6 kob/g in 5 of the 34 soaked hides treated with antimicrobial agents. While the numbers of viable proteolytic bacterial cells were detected as 10^6-10^8 CFU/g in the 28 soaked hides and 10^4-10^5 kob/g in the 6 soaked hides, the numbers of viable lipolytic bacterial cells were 10^6-10^8 CFU/g in 31 and 10^4-10^5 CFU /g in the 3 soaked hides (Berber and Birbir, 2010). Proteolytic and lipolytic bacteria were not detected on two and one soaked hides, respectively. The numbers of viable proteolytic bacterial cells were detected as 10^5-10^6 CFU/g in the 8 soaked hides, 10^2-10^4 CFU/g in the 9 soaked hides, the numbers of viable lipolytic bacterial cells were 10^5-10^6 CFU/g in the 8 soaked hides and 10^1-10^4 CFU/g in the 10 soaked hides (Berber and Birbir, 2010).

In order to control microbial growth and damage on hides and skins, antimicrobial agents have been used in the soaking process. In a study accomplished with the soak liquors, the numbers of viable bacterial cells were found as 10^6-10^7 CFU/g in 15 and 10^5 CFU/g in 4 of the 19 soak liquors treated with antimicrobial agents (Berber and Birbir, 2010). In addition, researchers examined the effectiveness of antimicrobial agents against bacteria in the soaking liquor in another study (Berber et al., 2010). Effectiveness of 0.4 g/l antimicrobial agent containing didecyldimethylammonium chloride (12.5%, w/v) and benzyl dimethyl ammonium chloride (12.5%, w/v) against bacteria in the soaking liquor containing 3.500 kg hide/13.000-liter water was examined at 18-20 °C during different exposure periods (10 min, 30 min, 60 min, 120 min, 180 min, 240 min, 300 min, 360 min, 420 min, and 480 min). The total bacterial counts, proteolytic bacterial counts, lipolytic bacterial counts increased respectively from 10^5 to 10^6 CFU/ml, 10^4 to 10^6 CFU/ml, 10^3 to 10^5 CFU/ml during 480 min exposure time. Due to the detection of high bacterial populations, the concentration of the antimicrobial agent was increased to 0.8 g/l in soak liquor. However, different Gram-negative bacteria belonging the genera of *Enterobacter, Pseudomonas* and *Vibrio* and Gram-positive bacteria belonging the genera of *Enterococcus, Lactococcus, Aerococcus*, *Kocuria*, *Staphylococcus* and *Micrococcus* were also isolated from the soaking liquor with 0.8 g/l of the antimicrobial agent (Table 3) (Berber et al., 2010). The presence of *Enterobacteriaceae* on soaked cattle hides and sheepskins treated with sodium dimethyl dithiocarbamate was also examined by researchers. In that study, different bacterial species belonging to the genera of *Enterobacter*, *Ewingella*, *Kluyvera*, *Morganella*, *Proteus*, *Providencia, Citrobacter*, *Cronobacter*, and *Serratia* were isolated from 10 oaked cattle hides and soaked sheepskins (Table 3) (Yazici and Birbir, 2018). Members of the family *Enterobacteriaceae* found on the soaked cattle hides and sheepskins showed resistance to the antimicrobial agent used in the soaking process. All isolates that were oxidase

negative and catalase-positive reduced nitrate to nitrite. Ninety-two percent of isolates catabolized glucose. Seventy-seven and seventy-three percent of the isolates utilized citrate and hydrolyzed lactose, respectively. Ninety-three and seventy-three percent of the isolates showed positive caseinase and ornithine decarboxylase activities, respectively. While 27% of the isolates produced lipase and protease enzymes, urease enzyme was produced by 42% of the isolates. Only 8% of the isolates produced H_2S. Thirty-one percent of the isolates showed positive arginine dihydrolase activity and produced NH_3 from peptone. Thirty-five percent of the isolates produced indol from tryptophan. Forty-two percent of the isolates utilized L-phenylalanine and L-alanine, respectively. Thirty-eight, twenty-three, twelve, fifteen percent of the isolates used L-proline, L-glycine, L-tyrosine, and L-lysine. Ninety-six, eighty-one, fifty, forty-six, forty-six, sixty-two, fifty, sixty-two, and fifty percent of the isolates utilized D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, and L-arabinose, respectively. That investigation highlighted catabolic activities of microorganisms belonging to the family *Enterobacteriaceae* found on the soaked cattle hides and sheepskins. All results confirmed that the species of *Enterobacteriaceae* found on soaked cattle hides and sheepskins treated with sodium dimethyl dithiocarbamate were able to break down hides and skins macromolecules and use those monomers to grow and damage to hides and skin structure (Yazici and Birbir, 2018).

The presence of multidrug-resistant bacterial strains of the family *Enterobacteriaceae* on the soaked skins and hides was reported in the previous research (Birbir et al., 2019) (Table 3). Susceptibilities of 13 soaked skin isolates belonging to species of *Proteus mirabilis*, *Enterobacter amnigenus*, *E. cloacae*, *Providencia rettgeri*, *Morganella morganii*, *Citrobacter freundii*, *C. koseri*, *Cronobacter sakazakii,* and 10 soaked hide isolates belonging to species of *Providencia rettgeri*, *Serratia marcescens*, *S. plymuthica*, *S. rubidaea, Citrobacter koseri*, *Cronobacter sakazakii*, and *Morganella morganii* were tested against 24 different antibiotics. Resistances of the skin isolates against aztreonam (92%), ampicillin (69%), ceftriaxone (69%), streptomycin (62%), cephalothin (62%), amoxicillin/clavulanate (62%), ceftazidime (61%), nalidixic acid (54%), ciprofloxacin (54%), chloramphenicol (46%), piperacillin/tazobactam (46%), cefuroxime sodium (46%), tetracycline (31%), sulfamethoxazole-trimethoprim (15%), cefoxitin (15%), ampicillin/sulbactam (15%), kanamycin (15%), tobramycin (8%), meropenem (8%), and imipenem (8%) were detected in that study. Resistances of the hide isolates to ampicillin (80%), amoxicillin/clavulanate (80%), tetracycline (70%), cefuroxime sodium (70%), streptomycin (60%), aztreonam (50%), ceftriaxone (40%), chloramphenicol (30%), nalidixic acid (30%), ampicillin/sulbactam (20%), piperacillin/tazobactam (20%), ciprofloxacin (20%), ceftazidime (20%), cephalothin (20%), cefoxitin (20%),

and kanamycin (10%) were determined. While all sheepskin isolates were found to be susceptible to norfloxacin, amikacin, gentamicin and ofloxacin, all cattle hide isolates were susceptible to tobramycin, ofloxacin, meropenem, sulfamethoxazole-trimethoprim, norfloxacin, amikacin, and gentamicin. All soaked sheepskin and cattle hide isolates showed resistance against critically important antimicrobials used in humans and animals (Birbir et al., 2019). Those studies showed that antimicrobial agents used on the soaked hides and in the soak liquors reduced bacterial numbers. However, the agents could not prevent the growth of all microorganisms on the soaked hides and soak liquors (Table 3).

Table 3. Microorganisms found on soaked skins/hides, soak liquors.

Source	Isolated species		Reference
Soaked cattle hides	*Citrobacter koseri*, *Cronobacter sakazakii*, *Ewingella american*, *Kluyvera intermedia*, *Morganella morganii*, *Providencia rettgeri*, *Serratia marcescens*, *S. plymuthica*, *S. rubidae*	Bacteria belonging to *Enterobacteriaceae*	Yazici and Birbir, 2018
Soaked sheepskins	*Citrobacter freundii*, *C. koseri*, *Cronobacter sakazakii*, *Enterobacter amnigenus*, *E. cloacae*, *Kluyvera intermedia*, *Morganella morganii*, *Proteus mirabilis*, *Providencia rettgeri*	Bacteria belonging to *Enterobacteriaceae*	Yazici and Birbir, 2018
Soaked sheep skins and cattle hides	Soaked skin isolates (*Citrobacter freundii*, *Citrobacter koseri*, *Cronobacter sakazakii*, *Enterobacter amnigenus, Enterobacter cloacae*, *Morganella morganii*, *Proteus mirabilis*, *Providencia rettgeri*) and soaked hide isolates (*Citrobacter koseri*, *Cronobacter sakazakii*, *Morganella morganii*, *Providencia rettgeri*, *Serratia marcescens*, *Serratia plymuthica*, *Serratia rubidae*)	Antibiotic-resistant bacteria beloning to *Enterobacteriaceae*	Birbir et al., 2019
Soak liquor with antibacterial agent	*Enterobacter gergoviae*, *E. sakazakii*, *E. amnigenus*, *E. cloacae*, *Enterococcus avium*, *E. faecium*, *Pseudomonas fluorescens*, *P. luteola*, *P. aeruginosa*, *P. putida*, *Vibrio parahaemolyticus*, *Lactococcus lactis ssp. lactis*, *Aerococcus viridans*, *Kocuria varians*, *Staphylococcus hominis*, *S. capitis*, *S. sciuri*, *S. xylosus*, *S. cohnii ssp. urealyticus*, *S. epidermidis*, *S. intermedius*, *S. haemolyticus*, *S. lugdunensis*, *S. cohnii ssp. cohnii*, *S. warneri*, *Micrococcus spp.*	Bacteria	Berber et al., 2010
Soak liquor sample with antibacterial agent	*Vibrio alginolyticus*, *Terribacillus halophilus*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*	Moderately halophilic bacteria	Caglayan, 2023

I.e. Damages caused by microorganisms on skins and hides

Microorganisms on the salted skins and hides may cause unpleasant odor, sticky layer, red heat, hair slip, fluid exudation, epidermis damage, grain damage, separation of collagen fibers, loss of tensile strength and elasticity of hides and skins, and finally disintegration of the skin structure (Tancous, 1964; Waldvogel and Swartz, 1969; Venkatesan and Nandy, 1970; Haines, 1984; Kallenberger, 1984; Tancous, 1964; Kallenberger and Lollar, 1986; Bailey and Birbir, 1993; Birbir and Ilgaz, 1996; Birbir et al., 2020). These conditions have been observed on the final leather as grain pin-prick, grain slip, grain sueding, grain peeling, loose grain break, blistering, emptiness, looseness, weakness, and holes (Tancous, 1964; Kallenberger, 1985). Lipolytic and proteolytic bacteria grown on the hides may also cause uneven dyeing onto leathers (Bitlisli et al., 2004).

Yellow, cream pink, and purple discolorations, sticky layer, red heat and hair slip on salted sheepskins are shown in Figures 2-5.



Figure 2. *Sticky appearance and yellow discolorations caused by microorganisms on flesh side of salted sheepskins.*

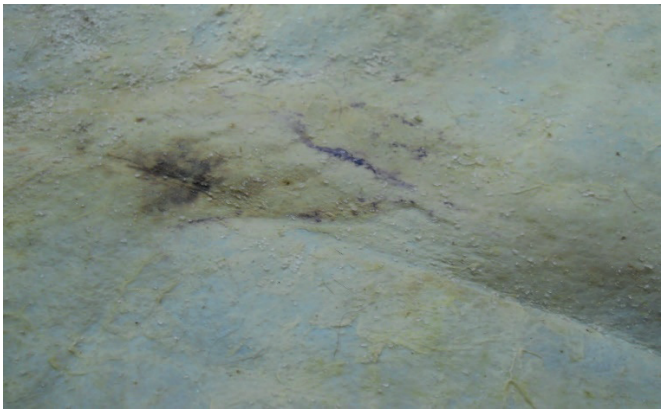


Figure 3. *Cream and purple discolorations caused by microorganisms on the flesh side of salted sheepskins.*



Figure 4. *Red heat caused by extremely halophilic archaea on the flesh side of salted skin.*



Figure 5. *Hair slip and slimy appearance caused by bacteria on the epidermis of salted skins.*

In the study by Berber and Birbir (2019), a questionnaire was also applied to leather technicians in the Istanbul Organized Leather Industrial Zone, Türkiye in both 2009 and 2018 to detect important problems on the salted and soaked skins/hides. The tanners stated that unwanted defects, including bacterial and fungal growth, red heat, unpleasant odour, preservation and flaying faults, spots, hair loss, decreased elasticity, holes, uneven dyeing, bloodstains, and burns due to manure were detected on skins/hides (Berber and Birbir, 2019).

Researchers examined the effects of three proteolytic extremely halophilic archaea, obtained from commercially brine-cured hides in the USA, on brine-cured hides. SEM micrographs proved that the mixed culture of proteolytic extremely halophilic archaea caused the suedeing effect on the brine-cured cattle hides. The top surface of the grain was partially removed and collagen fibers had been loosened up by those archaeal isolates causing red heat after 49 days of storage at 41°C (Bailey and Birbir, 1996).

In another study carried out by two extremely halophilic archaeal strains (*Haloferax gibbonsii* ATCC 33959 and *Haloarcula hispanica* ATCC 33960) and one moderately halophilic bacterial strain (*Halomonas elongata* ATCC 33173), most cells of the test strains attached to the hides after 30 minutes of exposure. The hides samples contaminated with *Haloferax gibbonsii* ATCC 33959 had unpleasant odor and hair slip. Those hides become thinner and very soft due to loss of hide substance after 45 days of storage at 40°C. SEM micrographs exhibited that the cells of both *Haloferax gibbonsii* ATCC 33959 and *Haloarcula hispanica* ATCC 33960 were found in the corium layer of the hides after 14 days of storage (Vreeland et al., 1998).

Three proteolytic and lipolytic extremely halophilic archaeal strains (*Haloarcula tradensis* 7T3, *Haloarcula salaria* AT1, and *Halobacterium salinarum* 22T6) were isolated from the sheepskins samples with red heat (Birbir et al., 2020). Then, freshly slaughtered sheepskins were separately brine-cured with *Haloarcula tradensis* 7T3, *Haloarcula salaria* AT1, and *Halobacterium salinarum* 22T6, and the mixed culture of those strains. Unpleasant odor and hair slip were detected on the sheepskin samples brine-cured with *Haloarcula salaria* AT1, *Halobacterium salinarum* 22T6, *Haloarcula tradensis* 7T3, and the mixed culture of those strains after five days of storage at 33ºC. After 16 days, sticky appearance and red heat were detected on the cured sheepskin samples. After 28 days, hole formations on these sheepskins samples were detected. The SEM micrographs showed that proteolytic *Halobacterium salinarum* 22T6, *Haloarcula salaria* AT1, and the mixed culture of the strains caused fibers in the corium to split and weaken, while *Haloarcula tradensis* 7T3 caused shredding of the fibers in corium after 47 days at 33°C. Experimental results of that study proved that *Haloarcula tradensis* (7T3), *Haloarcula salaria* (AT1), and *Halobacterium salinarum* (22T6), strains had protease activities that could be able to breakdown proteins in the corium of sheepskin causing loss of skin substance (Birbir et al., 2020). Besides, the utilization of amino acids found in collagen, elastin, and keratin also supported the growth and damage of microorganisms on the skins and hides (Figure 6).

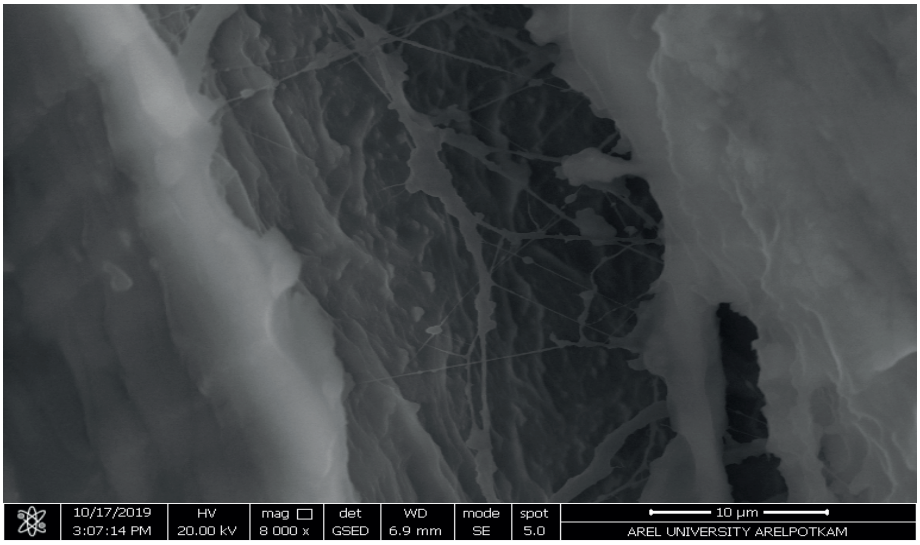


Figure 6. *Scanning electron micrograph of damaged sheepskin brine cured with mixed culture of extremely halophilic archaea and stored for 47 days at 33°C.*

As it is known, collagen types I and III contain hydroxylysine, alanine, hydroxyproline, arginine, glutamic acid, asparagine, aspartic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, tyrosine valine, threonine, phenylalanine, glycine, proline, and serine (Covington, 2009), Glutamic acid, isoleucine, glutamine, cysteine, glycine, hydroxyproline, leucine, lysine, phenylalanine, serine, proline, threonine, tyrosine, arginine, asparagine, alanine, aspartic acid, and valine are found in elastin. Keratin contains threonine, glutamine, glycine, tryptophan, histidine, aspartic acid, isoleucine, arginine, leucine, phenylalanine, lysine, methionine, proline, serine, tyrosine, alanine, asparagine, cysteine, valine, and glutamic acid (Covington, 2009). The researchers reported while *Haloarcula salaria* (AT1) used arginine, glycine, alanine, tyrosine, proline, hydroxyproline, methionine, serine, isoleucine, lysine, phenylalanine, leucine, valine, threonine, and histidine, *Halobacterium salinarum* (22T6) used arginine, glycine, alanine, tyrosine, proline, hydroxyproline, methionine, serine, isoleucine, lysine, phenylalanine, valine, threonine, and histidine. *Haloarcula tradensis* (7T3) utilized arginine, lysine, and histidine. Researches mentioned that test strains used the amino acids found in collagen, elastin, and keratin as nitrogen, energy and carbon sources for growth and damage (Birbir et al., 2020). Utilization of amino acids by moderately halophilic bacteria was also detected in the study carried out by one hundred thirty seven moderately halophilic bacteria belonging to the genera *Planococcus*, *Halomonas*, *Idiomarina*, *Salimicrobium*, *Marinococcus*, *Oceanobacillus*, *Bacillus*, *Salinicoccus*, *Staphylococcus*, *Salinivibrio*,

Gracilibacillus, Chromohalobacter, and *Alkalibacillus*. (Caglayan et al., 2018b). These isolates were recovered from the salted sheepskins (ninety isolates) and salted goat skins (forty-seven isolates). In that study, 90, 73, 66, 71, 56, 60 and 6 isolates of the sheepskins respectively used arginine, glycine, alanine, tyrosine, proline, hydroxyproline, and cysteine. Also, 47, 46, 45, 32, 31, and 25 isolates of the goat skins used proline, glycine, arginine, tyrosine, hydroxyproline, and alanine, respectively. Seven percent of the sheepskin isolates utilized all amino acids, and thirty-seven percent of the isolates utilized six amino acids. While five, four, three, and two amino acids were used by sixteen, eight, twenty-eight, and four percent of the sheepskin isolates, forty-five, twenty-one, four, and thirty percent of the isolates respectively used six, five, four, and three different amino acids (Caglayan et al., 2018b).

I.f. Washing solutions used for skins and hides

The former studies showed that the salt curing processes applied worldwide could not prevent the growth and damage of microorganisms on the salted hides and skins. Hence, leather scientists focused on finding effective antimicrobial washing solutions with different modes of action after flaying. To perform the curing process efficiently, the proliferation of bacteria on the hides/skins may prevent by washing after flaying. Haines (1984) reported that 50% of microorganisms on the fresh hides might be removed by continuous washing. Due to their contamination of hides with dirt, blood, and microorganisms, it has been suggested washing freshly slaughtered hides and skins with cold water by Bosilevac et al. (2004). The researchers also stated that washing with cold water reduces bacterial activity by decreasing the temperature of the hides/skins.

The investigators have developed effective disinfection treatments to reduce microbial load on the hides and skins and prevent microbial contamination of meat and hides/skins. Leather scientists suggested the treatment of hides and skins with cetylpyridinium chloride (Bosilevac et al., 2004), acetic acid, lactic acid, propionic acid, and chlorine (Dickson and Anderson, 1992), ozonated and electrolyzed oxidizing water (Bosilevac et al., 2005). Different spray wash solutions composed of a combination of antimicrobial agents such as dioctyl sulfosuccinate sodium salt-lactic acid, alkyltrimethylammonium bromide-lactic acid, alkyltrimethylammonium bromide-chlorhexidine di-gluconate, alkyltrimethylammonium bromide-chlorhexidine di-gluconate-H_2O_2-peracetic acid, alkyltrimethylammonium bromide-chlorhexidine di-gluconate-NaOCl-lactic acid (Long III et al., 2018a), spray wash treatment using cinnamaldehyde-lactic acid (Long III et al., 2018b), alkyltrimethylammonium bromide or chlorhexidine digluconate (Long III et al., 2019), sodium dichloroisocyanurate dehydrate (Sarker et al., 2020) have been suggested by researchers.

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