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İÇİNDEKİLER

CHAPTER 1

POLLEN IN THE CAVES

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Introduction

According to the definition of the International Union of Speleology, caves are natural underground spaces accessible to humans. The reason for the widespread use of this definition is that only the parts of caves that people can enter are known and can be studied scientifically. However, there are various definitions of caves according to different branches of science. In the field of biology, a cave is defined as an environment where at least part of it is in constant darkness, has a turbulent water presence, and is inhabited by species with weak pigmentation. However, the most appropriate definition is the ecological one. According to the ecological definition, caves are naturally or artificially formed as a result of various processes, providing suitable conditions for some life forms, having water or air currents periodically or continuously, and often accumulating substrate (Lazaridis, 2022).

Caves are environments with unique physical conditions and a mostly stable structure. The ecosystems in caves differ from those on the ground, making them distinct ecosystems. They harbor a biodiversity of over 50,000 species worldwide. Some of the species that can be found in caves permanently or during certain periods include fungi, crustaceans, worms, insects, special fish species, bats, bears, hyenas, foxes, and badgers (Medellin et al., 2017; Zhang et al., 2018; Catalano et al., 2024; Duñó-Iglesias et al., 2024; Fei et al., 2024; Marciszak et al. 2024; Ogórek et al., 2024). Some of these species are cave-dependent, some spend part of their lives outside using caves as roosts or nests, and some visit caves accidentally or for various reasons (Coppari et al., 2024; Manenti et al., 2024).

For centuries, caves have served as shelters for numerous organisms. The prolonged use of these caves by various species, combined with the effects of physical events, has led to the accumulation of sediment within them, which includes pollen (Gerasimenko et al., 2019). Pollen consists of reproductive cells produced in the male reproductive organs of plants and carries male gametes. For fertilization to occur in flowering plants, pollen must reach the female flower. The transportation of pollen occurs through various vectors shaped by the evolutionary processes of plants. These vectors can be classified into two categories: biotic and abiotic. Biotic vectors include insects, birds, and mammals, while abiotic vectors encompass water and wind (Pacini, 1992; Erdtman, 2023). Pollen delivered to caves by either biotic or abiotic means complements each other in terms of palynological data and represents the surrounding vegetation (Hunt & Fiacconi, 2018).

Pollen is one of the best-preserved and most fossilizable components of plants. It can remain intact for many years, even in the harshest envi-

ronments, due to the durability of the exine layer (Durska, 2016). Cave environments also provide favorable conditions for the preservation of plant remains, including pollen, as both temperature and humidity tend to be more stable. Consequently, palynological studies conducted in caves yield highly accurate results when the data obtained are taphonomically reliable (McGarry & Caseldine, 2004; Bogdanowicz et al., 2020).

Pollen entry mechanisms into the caves

Studies have shown that pollen belonging to anemophilous plant taxa are found in high concentrations near the entrances of the caves, while pollen from zoophilous taxa becomes more dominant as you move inland. This shift may be attributed to the limited reach of air currents into the cave interior due to its structure. In such cases, air currents are likely the primary influencing factor. However, when the cave structure restricts air currents, water or animals may play a more significant role. Despite this, several uncertainties persist due to the lack of comprehensive studies. In some caves, pollen concentrations decrease as one ventures deeper, while in others, these concentrations display irregularities at various locations within the cave. (Yang et al., 2021). Therefore, it is very important to thoroughly investigate the structure of the cave under study, to identify any air currents, to understand and analyse the vegetation surrounding the cave and the mechanisms of pollen transport into the cave.

We can categorize the mechanisms into two situations: the first involves pollen carried by the cave's structure and opportunities created by meteorological conditions. The second situation pertains to transport by animals.

Abiotic Mechanisms

Since the structure and mechanisms of each cave may vary, and unique itself, the cave environment and conditions should be thoroughly evaluated, taking all factors into consideration.

Air flow- Factors to consider in airborne pollen transport include the number of cave entrances and the distance air currents can travel within the cave (McGarry & Caseldine, 2004). Airflow in caves can be influenced by various factors. In caves with multiple entrances, air currents are typically caused by differences in air density between the surface and the cave, which are influenced by temperature and humidity. For example, Figure 1 depicts a cave with a third entrance, in addition to two adjacent main entrances. Atmospheric pressure differentials can impact airflow in caves with a single entrance. Additionally, streams within the cave can also affect

air currents. Depending on the cave's structure and formation, air currents may vary due to various factors (Lauritzen, 2018). Consequently, airborne pollen can enter caves in diverse ways, leading to accumulation in various locations.

Figure 1. *A cave with two distinct main entrances (on the left) and a third entrance (on the right) situated in the upper section of the cave.*

Active or filtered water- The study of sediments found in flooded caves has shown their suitability to fill many prehistoric gaps (Steele et al., 2023). Pollen reaching the caves by water is either filtered into the cave by surface waters (Figure 2) or in caves with active water flow, an input with flowing waters is formed (Figure 3). While percolating water often forms stalactites and/or stalagmites in fossil caves (Figure 4), active water flow forms sediment periodically or continuously. The content of this sediment is also very rich in terms of pollen data (McGarry & Caseldine, 2004; Reis et al., 2023). Since the water deposits in the cave may also be associated with or in contact with various other factors, it would be appropriate to evaluate them from a multidimensional perspective.

Figure 2. *Inflow of filtered surface water into the cave.*

Figure 3. A cave with periodically active water flow. *The pools formed when the water flow ceased (on the left) and, the active water flow from the main entrance of the cave (on the right).*

Figure 4. *Stalactites (on the left) and stalagmites (on the right) formed by filtered water in the caves.*

Biotic Mechanisms

Animals- Animal transport of pollen grains into caves involves slightly more complex mechanisms. The animals that can be found in the caves (like birds, foxes, bats, lynxes and skunks) provide a pollen input to the caves. In addition, the photographs in Figure 5 display images and remains of various animals encountered during the cave visits. Materials brought by animals for nest construction or by predator animals bringing their prey to their nests inside the caves also contribute to the pollen input (Figure 6). Additionally, pollen grains can be brought by the animals through direct contact, or it may be carried by their digestive system (Figure 7) as a result of their dietary habits (McGarry & Caseldine, 2004; Omar & Castillo-Gamez, 2017; Reis et al., 2023).

Figure 5. *Wide variety of animals can visit or live in the caves.*

Recent studies have focused more on palynological data obtained from coprolites of animals, found in caves (Figure 6). Coprolites complement paleobotanical records as they also contain entomophile taxa (Djamali et al., 2011; Beck et al., 2019; Ochando et al., 2020). The study of hyena coprolites from caves has yielded very successful results (Gil-Romera et al., 2014; Gatta et al., 2016; Gross et al., 2023). However, guano from bats has attracted more attention of palynologists. Guano has been shown to be richer in pollen content. Especially insectivorous bats are expected to have a high pollen content in the guano due to the digestion of the insects they feed on, grooming and the inclusion of pollen in their fur in the digestion, which is supported by copropalynological studies. At the same time, the periodic increase in insect populations can enable periodic monitoring as they represent plants that show periodic pollination (Carrión et al., 2006; Leroy & Simms, 2006; Basumatary & Tripathi, 2021; Tsalickis et al., 2021).

Figure 6. *Hunting remains (bird feathers on the left and bones on the right) inside the caves brought by predators.*

Figure 7. *Some mammal faeces found in caves (bat on the left, marten on the right).*

The human visits- Since pollen is present in the atmosphere, on surfaces, and in various environments, it is both possible and natural for it to contaminate humans. After spending time outdoors, we inadvertently transfer pollen into indoor spaces via our clothing and footwear (Jantunen & Saarinen, 2011). Cave tourism is becoming more widespread day by day and caves are being opened to tourism. Caves, as closed ecosystems, can also become contaminated with pollen brought in by humans. With each visit, pollen from the surrounding vegetation is introduced into the cave environment. Furthermore, in caves located near human settlements, pollen from agricultural plants can enter both through human activity and via airborne transport. If not carefully monitored, this contamination may lead

to misleading results in palynological studies (Buosi et al., 2015; Revelles et al., 2022). It is important to recognize that caves have been utilized for various purposes throughout history, including tourism, mushroom cultivation, and guano collection. Additionally, it should be noted that natural caves have suffered significant damage due to poor management practices, which can be challenging to remediate (Gillieson, 2011). As illustrated in Figure 8, ferns that do not naturally belong to the cave environment have established themselves due to the influence of artificial light. The impact of human activity on cave ecosystems is becoming increasingly significant.

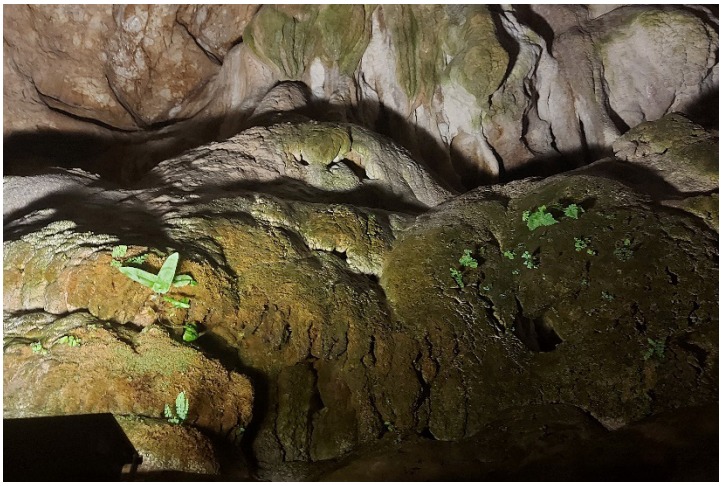

Figure 8. *Ferns and mosses that thrive in cave environments under the influence of artificial light in show caves.*

Conclusion

If we summarize these mechanisms based on the studies and data collected, we can categorize them also into three distinct categories:

- Airborne pollen input varies based on the cave's structure, the influence of air currents, and the number of entrances to the cave.
- Waterborne transport refers to the movement of water through flowing sources within a cave, as well as the infiltration of water into the cave system.
- Animal transport occurs through fur, digestive tracts, and any materials carried by animals that utilize the caves as nesting sites.

Depending on the cave's structure and ecosystem, these mechanisms may interact with one another.

Palynological data obtained from caves are particularly valuable in regions where historical palynological information is limited. The accuracy and reliability of data derived from cave sediments have been well established. This data reflects the flora present in the area (Gerasimenko et al., 2022). The composition of sediment or coprolites may vary depending on the cave's structure and ecosystem. In some instances, there may be no active water input or living organisms within the studied cave. However, the presence of any sedimentation mechanism provides researchers with interpretable evidence regarding the cave's ecological history. Therefore, regardless of the specific mechanisms involved, caves that have formed or continue to form over many years offer highly valuable palynological data.

Given the numerous factors involved in palynological studies conducted in caves, researchers often employ specific techniques to guide their investigations. In certain caves, particular pollen species may be introduced into the cave ecosystem by specific animal species, resulting in a significant representation of those taxa. Conversely, it has been observed that anemophilous taxa are predominantly represented in caves with simple morphological structures, while the complexity of pollen taphonomy tends to increase in more intricate caves, especially those with human or animal access. Furthermore, pollen data collected from guano may be obscured by anemophilous taxa that cover the guano due to specific physical conditions in certain instances. In light of these considerations, it is essential for the palynologist conducting the study to have a thorough understanding of the regional pollen rain and vegetation, as well as to evaluate the aforementioned factors and mechanisms (Carrión et al., 2022). All or some of the potential mechanisms for pollen introduction may interact with one another, making it beneficial to consider these possibilities when interpreting the collected data.

The coprolites discovered in the cave help bridge gaps in the pollen data from specific periods, as they are believed to contain pollen from entomophilous plant taxa (Ochando et al., 2024). However, resources such as guano are being depleted due to mining activities, which undermines both their biological value and their significance as data sources (Medellin et al., 2017). In addition to the challenges posed by environmental pollution, a significant accumulation of heavy metals has been observed in guano and, consequently, in caves (Forray et al., 2024). Research on the potential negative impacts of human activities on cave ecosystems is limited, and the effects on the relevant mechanisms remain unclear.

In conclusion, palynological studies conducted in caves are both labour-intensive and challenging. Researchers must evaluate each cave from a unique perspective and consider the specific possibilities for each site individually. Furthermore, fieldwork for these studies often occurs under dif-

ficult conditions, which can vary significantly depending on the geological structure of the region. These factors may contribute to the scarcity of comprehensive and robust palynological research in caves. Nevertheless, every study in this field is valuable and enhances our limited understanding.

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CHAPTER 2

MYRTUS (*MYRTUS COMMUNIS L.*) PLANT: ACTIVE INGREDIENTS, ANTIOXIDANT AND ANTIMICROBIAL EFFECTS

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Abstract

Myrtle (*Myrtus communis* L.) is a characteristic plant of the Mediterranean climate and has an important place in traditional medicine and modern phytotherapy. Myrtle is a shrub-shaped plant from the Myrtaceae family, maquis group. It can grow up to 2-3 meters tall and is a plant with very dense leaves, sometimes in the form of a small tree, seen especially in coastal areas where the Mediterranean climate prevails. Its leaves are light, bright green and its old leaves are dark green, leathery, egg- and spear-shaped with pointed tips and entire edges. Its flowers are white, oval or egg-shaped, consisting of five parts and white with small knobs at the ends. Its fruits are round or egg-shaped, pea-sized, bluish black or rarely white. While unripe fruits are bitter (astringent), ripe fruits are sweet. It is a drought-resistant plant. It blooms throughout the summer (June-September). In this study, the general characteristics, phenolic contents and biological activities of the Myrtle plant were compiled. It is thought that the plant may be an important natural resource.

Keywords: Antimicrobial, Antioxidant, Medicinal plants, Myrtle, traditional medicine

Introduction

Natural products have been used by people to combat many diseases since ancient times (Sevindik et al., 2017). Especially mushrooms, plants and animals are important natural products. Plants are used by people for different purposes (Sevindik et al., 2018; Gürgen et al., 2020). These areas of use include food, shelter, heating, equipment production, and disease control (Mohammed et al., 2022). The medical use of plants is quite common (Mohammed et al., 2019). Many studies have shown that plants have different biological activities such as antioxidant, anticancer, antiproliferative, antiaging, antimicrobial, and DNA protective (Akgül et al., 2020; Mohammed et al., 2021; Çömlekçioğlu et al., 2022; Doğan et al., 2023; Kalkan et al., 2023; Mohammed et al., 2023; Sevindik et al., 2023; Akkaya et al., 2024). In our study, the general characteristics, usage areas, and biological activities of Myrtus communis L. were compiled. Myrtle plant (*M. communis*) is a perennial, green in summer and winter, shrub-shaped plant belonging to the Myrtaceae family and usually short, but sometimes can grow up to 1-3 m in height. It is naturally distributed in the Mediterranean region, the Middle East, and the temperate regions of North America and Australia (Baytop, 1999). *M. communis*, which grows wild in the coastal areas of Tunisia, Morocco, Turkey and France, is cultivated in countries such as Iran, Spain and Italy (Yıldırım et al., 2013). *M. communis*, one of the typical natural plants of the Mediterranean Basin, is naturally found in

the provinces of Adana, Antalya, İçel, Çanakkale, İstanbul, Zonguldak, Sinop, Ordu, Trabzon, İzmir, Samsun, Muğla and Hatay in our country (Söke & Elmacı, 2015). Although *M. communis* is generally known as "Mersin" in our country, it is also known as "murt", "hambeles" and "adi mersin", especially on the southern coasts, and its leaves are called "bahar" in some places (Yeğin & Uzun, 2015). The volatile oil obtained from the myrtle plant is widely used in medicine and the pharmaceutical industry due to the components it contains in its composition. The leaves and fruits of the myrtle plant are used in the production of two famous liqueurs called "Mirto Rosso" and "Mirto Bianco" on the island of Sardinia, Italy, and also in folk medicine due to their astringent and balsamic properties. In the past, the ripe fruits were used as a nutritional supplement due to their rich vitamin content, and the decoction of the fruits and leaves was used for delicate washing of newborn babies. Today, the decoction of the leaves is used against respiratory diseases (Flamini et al., 2004).

The leaves of the myrtle plant contain tannin (14-19%), volatile oil (0.3-0.5%) and bitter substances. Its fruit also carries tannin, volatile oil, sugars and organic acids (malic and citric acid). Myrtle leaves and fruits are used internally as an antiseptic for constipation, urinary tract diseases and chest diseases, and externally as a wound healer. Myrtle essential oil is used in the food and perfume industry due to the terpenes it contains, and internally against bronchitis, tuberculosis and diabetes due to its antiseptic, blood-thinning and sedative effects. It is stated that when consumed in high amounts, it irritates the respiratory system and causes miscarriages in pregnant women due to uterine contractions (Baytop, 1999). When looking at the studies on the chemical composition of myrtle essential oil, studies conducted in Morocco (Farah et al., 2006) and Tunisia (Bouzouita et al., 2003) reported the main component of its essential oil as 1.8-cineole. Studies conducted in Iran (Yadegarinia et al., 2006), Tunisia and France (Curini et al., 2003) determined the main component of its essential oil as α-pinene.

Active Compounds

Myrtle essential oil can be obtained from different parts of myrtle such as myrtle leaves, flowers and fruits, and in addition to traditional methods such as hydrodistillation, various methods such as supercritical fluid extraction, microwave extraction and enflorage are used in its production. It has been determined that myrtle essential oils are rich in tannins, flavonoids, phenolic compounds and fatty acids (Aleksic & Knezevic, 2014; Bekhechi et al., 2019). Myrtle extracts can be obtained by extraction methods such as Soxhlet, maceration, boiling, brewing and membrane technology using various solvents such as water, ethanol, methanol and ethyl acetate (Aleksic

& Knezevic, 2014). The physical properties, nutritional content and phenolic and antioxidant properties of myrtle, which can be found in different morphologies, may vary depending on the location and conditions of the myrtle, season, analyzed parts, color and whether it is grafted or wild. Myrtle fruit samples were collected from different locations, including coastal and inland Spain, and as a result of the study, it was stated that the location had an effect on the chemical content and morphological characteristics of myrtle (González-de-Peredo et al., 2019). It was determined that myrtle fruits grown in coastal areas were richer in bioactive components such as anthocyanins than inland areas. The chemical composition of essential oils obtained by hydrodistillation in cultivated and naturally growing myrtles in Spain was examined by GC/MS. More than a hundred compounds were detected in the oils, but only about fifty of them could be identified and their amounts were determined. The main components were determined to be α-pinene (8.05-8.18%), 1,8-cineol (15.14–29.89%), linalool (0.5-8.3%) and myrtenyl acetate (32.90-35.90%) (Boelens & Jimenez, 1991). The chemical composition of essential oils obtained from the leaves and leaves+branches of Myrtus communis L. obtained from Turkey was examined by GC/MS technique and eighty-six components representing 97.9% of the total leaf oil and 115 components representing 95.5% of the total leaf+branch oil were identified. The main components were determined as 1,8-cineole (18.3% and 10.5%), linalool (16.3% and 18.6%) and myrtenyl acetate (14.5% and 10.8%). It was reported that Turkish myrtle oil is rich in 1,8-cineole, linalool and myrtenyl acetate, and also that Turkish myrtle oil contains significant amounts of a-pinene, limonene, linalyl acetate, an-terpineol and geranyl acetate *(Özek et al., 2000). The* chemical composition and antibacterial activity of Algerian myrtle essential oil were investigated and it was reported that Myrtus communis L. leaf essential oils were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) and contained a total of thirty-four components constituting 95% of the oil, the main compounds being limonene (23.4%), linalool (15.4%), geranyl acetate (10.9%), α-pinene (10.7%), linalyl acetate (8.2%) and 1,8-cineole (6.6%) (Hennia et al., 2015).

Antioxidant Effect

Free radicals are oxidant compounds produced as a result of metabolic activities (Sevindik et al., 2016). The antioxidant defense system is responsible for suppressing these oxidant compounds (Akgül et al., 2016a). However, in some cases, the antioxidant defense system is insufficient to suppress oxidant compounds. In this case, oxidative stress occurs (Akgül et al., 2016b). As a result of oxidative stress, diseases such as cancer, diabetes, Parkinson's, Alzheimer's, and cardiological disorders can be seen

(Sevindik, 2019; Islek et al., 2021; Saridogan et al., 2021; Sevindik, 2021). In this case, supplemental antioxidants can reduce the effects of oxidative stress (Sevindik, 2018). Plants are important natural products with these properties (Seğmenoğlu et al., 2024). The antioxidant potential of the myrtle plant was compiled in our study. The myrtle plant reduces cell damage by cleaning free radicals. Antioxidant activities are due to flavonoid and phenolic components *(Çömlekçioğlu et al., 2024).* Studies available in the literature have shown that myrtle extracts and essential oils have effective antioxidant activity thanks to the flavonoids (quercetin), phenolic acids, tannins and α-tocopherols found in their structure (Gorjian et al., 2021). It has been determined that the antioxidant activity of myrtle essential oils determined by the DPPH method varies between 100-768 µg/mL (IC50) (Hennia et al., 2019). It is seen that myrtle extract or essential oil contents may vary depending on various factors such as the analyzed parts of the plant, the region where it grows, whether the parts used are fresh or dried, the solvent used, and the analysis method. Antioxidant activity may also vary depending on the type of solvent, the method used in obtaining the extract or essential oil, or the method used in determining antioxidant activity. In a study examining the antioxidant activity of different extracts of myrtle leaves and fruits, the most effective antioxidant activity was detected in methanol, water, ethanol and ethyl acetate extracts, respectively (Amensour et al., 2015). In a study examining the antioxidant activity of ethanol, ethyl acetate, chloroform and water extracts of myrtle leaves, different methods such as DPPH, ABTS+, hydroxyl radical trapping power and metal chelation were used. According to the DPPH and ABTS+ analysis results, it was determined that the most effective extract was ethyl acetate (IC50= 0.004 mg/mL), followed by methanol (0.006 mg/mL), chloroform (0.009 mg/mL) and water (0.021 mg/mL) extracts, respectively. According to the hydroxyl radical trapping power analysis results, the highest antioxidant activity was detected in ethyl acetate, chloroform, methanol and water extracts, respectively; As a result of metal chelation analysis, the most effective extracts were revealed to be methanol (IC50= 0.39 mg/mL), water (0.403 mg/mL), chloroform (3.05 mg/mL) and ethyl acetate extract (16.05 mg/mL), respectively. While the reason why antioxidant activity varies according to the solvent used is the polarity of the solvent, the reason why different methods give different results is that the mechanisms of action of the methods are different. While the electron retention ability of antioxidant compounds was determined in DPPH and ABTS+ analyses, the ability of antioxidant compounds to capture heavy metal ions was investigated in metal chelation analysis (Bouaziz et al., 2015). In a study examining the antioxidant activity of myrtle extracts obtained by supercritical extraction and traditional extraction methods, it was determined that the concentration of flavonol glycosides in the extracts obtained by superc-

ritical extraction was higher than that obtained by the traditional method and, accordingly, the antioxidant effect was higher in the extracts obtained by supercritical extraction (Pereira et al., 2016). In the studies conducted, it was determined that there was a difference between the antioxidant activities of wild-growing myrtles and cultivated myrtles. In a study conducted by *(Çakmak et al., 2021), it was reported* that wild myrtles had higher antioxidant activity than cultivated myrtles, the most effective antioxidant activity was seen in wild myrtles with an IC50 value of 39.21 µg/mL, and the IC50 value in cultivated myrtles was 57.50 µg/mL.

Antimicrobial Effect

In recent years, microbial diseases have been increasing (Sevindik et al., 2024). The main reasons for this are the increase in the number of resistant microorganisms due to unconscious antibiotic use (Eraslan et al., 2021; El-Chaghaby et al., 2024). Researchers have focused on the discovery of new antimicrobial drugs. Plants are among the important natural resources with these properties (Mohammed et al., 2024). In our study, the antimicrobial activities of the myrtle plant were compiled. In some studies on the antibacterial effects of myrtle extracts and essential oils on Gram-positive and Gram-negative bacteria, it was determined that Gram-positive bacteria were more sensitive to myrtle extracts and essential oils than Gram-negative bacteria (Toauibia, 2015). In the study conducted by (Ben Hsouna et al., 2014), the inhibitory effect of myrtle leaf essential oil was investigated and it was determined that the most effective antimicrobial activity was seen in *B. cereus* and *S. aureus* with a minimum inhibitory concentration (MIC) value of 0.625 mg/mL. As a result of the antimicrobial study of Algerian myrtle essential oil, they stated that myrtle oil showed good antibacterial activity against *Staphylococcus aureus, Proteus mirabilis* and *Kleibsiella pneumonia*, but it was not active against *Pseudomonas aeruginosa*, on the contrary, depending on the strains (Hennia et al., 2015). In a study examining the inhibitory effect of myrtle leaf ethanol extract on different microorganisms, it was determined that the highest inhibitory effect was seen in *Bacillus subtilis* and *S. aureus* (125 µg/mL), and the lowest effect was seen in *P. aeruginosa* (8000 µg/mL) (Raeiszadeh et al., 2018). In a study, it was determined that the ethanol extract of myrtle leaf provided effective antimicrobial activity on Gram-positive microorganisms (inhibition zone = 9-25 mm), but did not show an inhibitory effect on Gram-negative bacteria (Mir et al., 2020). In another study, it was determined that water and methanol extracts of myrtle leaves had an inhibitory effect on *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae*, methicillin-resistant *S. aureus* and *Escherichia coli*, and the inhibitory effect increased as the extract concentration increased (Abdulqawi &

Quadri, 2021). It was determined that myrtle flower essential oil created the widest inhibition zone for *Bacillus cereus* and *Listeria monocytogenes* with 22 mm, and the narrowest inhibition zone for E. coli (14 mm) (Dhifi et al., 2020). It was determined that the microorganism on which the essential oil obtained from myrtle branches, leaves and flowers was most effective was *S. aureus* with an average inhibition zone diameter of 17.85 mm, and the lowest inhibitory effect was observed in *Serratia liquefaciens* with an average inhibition zone diameter of 6.33 mm (Mohamadi et al., 2021).

Conclusion

Today, the fact that medicinal plants are faced with an unlimited interest is increasing the search for alternative products and the search for creating value-added products that will take place in nutrition and treatment by using our idle plant resources in our country continues intensively. Especially the essential oil industry is developing very rapidly and the need for raw materials arises in parallel. The myrtle plant (*M. communis*), which is the subject of the research, is found densely in the Mediterranean flora of Turkey and forms the vegetation of the region. The myrtle (*M. communis*) plant has an important place in the field of health with its active ingredients and medical uses. With its biological activities and appropriate cultivation techniques, the myrtle plant offers more research and application opportunities in the future. More scientific studies will help us better understand the potential and effectiveness of the myrtle plant.

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CHAPTER 3

RUMEN FUNGI IN THE DEGRADATION OF PLANT CELL WALLS

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Abstract

The degradation of plant cell walls is a critical process for ruminants, primarily facilitated by the complex symbiotic relationship between the rumen microbiome and the host animal. This review explores the pivotal role of rumen fungi in the breakdown of lignocellulosic materials, highlighting their enzymatic capabilities and interactions with other rumen microorganisms. While rumen fungi are less abundant than bacteria, their contribution to plant cell wall degradation through the production of highly active fibrolytic enzymes is significant. These fungi not only possess the ability to form cellulosomes, enhancing their cellulolytic activity, but also demonstrate unique enzymatic profiles that allow them to degrade resistant plant cell wall components more effectively than their bacterial counterparts. Additionally, the review discusses the potential biotechnological applications of anaerobic fungi, including their use in improving ruminant feed efficiency, their role in biofuel production, and the challenges associated with their industrial use. The continuous culture of these fungi remains a challenge, yet their ability to produce a wide range of potent enzymes presents numerous opportunities for various industries. Understanding the specific contributions of rumen fungi to lignocellulose degradation and overcoming the challenges in their cultivation are essential for advancing both agricultural and industrial applications.

Keywords: Rumen fungi, ruminant, cellulase, xylanase, biotechnology

1. Structure of the Plant Cell Wall

The fibrous material of plant cell walls constitutes a fundamental component of the diet for many ruminants. In typical pastures, plant cell walls comprise 400-700 grams of one kilogram of dry matter (1). As a result, the rumen is exceptionally well-suited for fermenting plant cell walls. The cell walls of forage plants, which constitute the primary diet of ruminants, are more intricate than those of wood, largely due to their protein content and diverse structural carbohydrates. However, the composition of plant cell walls can differ significantly based on factors such as plant species, specific plant parts, type of tissue, plant maturity, and environmental conditions like temperature, light exposure, humidity, and soil nutrient levels (2).

All plant cell walls generally consist of a fundamental structure composed of cellulose microfibrils embedded within a matrix. This matrix typically contains polysaccharides of various structures, structural proteins, glycoproteins, and phenolic compounds (3). Plant cell walls constitute the primary stored carbon source in nature. The plant cell wall contains three major polymers: cellulose (insoluble fibrils made of β-1,4-glucan),

hemicellulose (non-cellulosic polysaccharides such as mannan and xylan), and lignin (a complex polyphenolic substance). These structures are collectively referred to as lignocellulose (4). Lignocellulose constitutes a significant portion, approximately 89-98%, of the dry weight of wood. The holocellulose fraction, which contains cellulose and hemicellulose, comprises 63-78% of the lignocellulose in various soft or hardwood trees, while lignin accounts for 15-38% (5).

1.1. Cellulose

Cellulose is a polymeric component that forms approximately 40% of plant material and is the most abundant polysaccharide on earth. Cellulose is a homopolymer composed of a chain of approximately 14,000 glucose units linked by β-1,4 glycosidic bonds. The amorphous form of cellulose, due to its fewer intramolecular hydrogen bonds, is relatively easier to degrade. However, crystalline cellulose, tightly packed within itself through hydrogen bonds, is more resistant to biological degradation due to its physical structure (6). The crystallinity of cellulose varies depending on its source and the pre-treatment it undergoes, usually falling within a 40% to 60% range. This supermolecular structure influences reactivity in a complex way, separating the hydroxyl groups in cellulose into highly reactive, easily accessible groups found in amorphous regions, and less reactive groups located in crystalline regions, which are almost unreachable (7).

The enzymatic hydrolysis of cellulose involves a complex sequence of reactions with several key steps: **1)** Enzymes migrating from the bulk aqueous phase to the cellulose particle surfaces, **2)** the adsorption of enzymes and the creation of enzyme-substrate complexes, **3)** the hydrolysis of cellulose, **4)** the movement of cellodextrins, glucose, and cellobiose from the cellulose particle surfaces back into the bulk aqueous phase, and **5)** the subsequent hydrolysis of cellodextrins and cellobiose into glucose within the aqueous phase (8). The breakdown of cellulose into glucose requires three main classes of enzymes: endoglucanases (EC 3.2.1.4; 1,4-β-D-glucan-4-glucanohydrolase) randomly cleave β-1,4-glycosidic bonds along the cellulose molecule, exoglucanases, including cellobiohydrolases (EC 3.2.1.91; 1,4-β-D-glucan cellobiohydrolase), attack the ends of the polyglucan chain and cleave off cellobiose or glucose units from these ends, and β-glucosidases (EC 3.2.1.21) hydrolyze cellobiose, releasing low molecular weight glucose (6).

1.2. Xylan

After cellulose, hemicelluloses are the second most abundant polysaccharides found in plants. β-1,4-xylan is a heteropolysaccharide with a

homopolymeric backbone consisting of 1,4-β-D-xylopyranose units. The backbone is composed of O-acetyl, α-L-arabinofuranosyl, α-1,2-glucuronic acid, or 4-0-methyl D-glucuronic acid components. Xylans can be characterized based on these components into linear homoxylan, arabinoxylan, glucuronoxylan, and glucuronoarabinoxylan (9).

β-1,4-xylan is typically found in the secondary cell walls of plants. The amount of xylan varies depending on the plant. Xylan acts as a protective barrier for cellulose against enzymatic breakdown. When xylan is extracted from the plant, cellulose becomes more vulnerable to cellulolytic hydrolysis (4). The degradation of xylan requires enzymes such as endo-1,4-β-D-xylanase (EC 3.2.1.8) and β-xylosidase (EC 3.2.1.37), which act on the main sugar chain depending on the type of xylan. Additionally, the removal of side groups requires the action of enzymes like α-glucuronidase (EC 3.2.1.131) and acetyl xylan esterase (EC 3.1.1.72) (10).

1.3. Lignin

Lignin is a complex, highly branched hydrophobic polymer formed by the random coupling of aromatic alcohol radicals. Thick cell walls that provide mechanical support are usually saturated with lignin. The deposition of lignin in plant cells begins in the middle lamella and extends into the primary and secondary cell walls. Tissues containing lignin are more resistant to mechanical and biological degradation than those that do not. Therefore, lignin primarily provides mechanical support to the plant structure and act as a barrier against enzymatic degradation and microbial breakdown (11, 12).

The degradation of lignin requires oxygen and does not occur rapidly under anaerobic conditions. Increasing the oxygen level in the medium enhances the activity of lignin-degrading enzyme systems and increases the production of hydrogen peroxide. Ligninase or lignin peroxidase enzymes are necessary for the breakdown of the lignin polymer. Manganese peroxidase is another enzyme involved in lignin degradation. These enzymes are thought to function as phenol-oxidizing enzymes and may also play a role in the production of hydrogen peroxide. Laccase or phenol oxidase (EC 1.10.3.2) enzymes are involved in the oxidation of phenols to phenoxy radicals (13).

In the cell walls of grasses, ferulic and *p*-coumaric acids, which are precursors of lignin, are phenolic compounds typically bound to lignin by ester and ether bonds along with arabinoxylan (14). *p*-Coumaric acid is more resistant to microbial degradation than ferulic acid. It has been reported that *p*-coumaric acid is more abundant in less digestible fibrils than ferulic acid and is located in a different structural environment within the

cell wall, and also exhibits higher toxicity under in vitro conditions (15). Phenolic compounds inhibit the growth of both cellulolytic and non-cellulolytic ruminal bacteria and the degradation of plant cell walls (16). Phenolic monomers reduce the adhesion of cellulolytic ruminal bacteria to fibril particles (18). Phenolic compounds can also affect enzyme activity (19).

1.4. Pectin and Proteins

Pectins generally consist of an α-1,4-D-polygalacturonate backbone connected to acidic polysaccharides. The galacturonan backbone contains L-rhamnose residues linked by α-1,2 bonds. Contrary to what has been dogmatically emphasized in recent decades, the presence of (1→2)-linked L-rhamnose is not responsible for causing significant bends in the backbone geometry. It is solely the side chains attached to these residues that are responsible for terminating or limiting the interchain interactions (20). Pectinolytic enzymes are categorized into two classes: pectin esterases and depolymerizing enzymes. Pectin esterase (EC 3.1.1.11) hydrolyzes the methyl ester bonds of pectin, converting it to pectic acid and methanol. Depolymerases are classified into five classes: endo-polygalacturonase (EC 3.2.1.15), exo-polygalacturonase (EC 3.2.1.67), endo- and exo-methoxy pectin lyase (EC 4.2.2.10), and endo pectin lyase (21).

Proteins associated with polysaccharides in plant cell walls play a role as enzymes in remodelling the developing cell wall or structurally. In primary walls, proteins account for 10% of the dry matter. Certain proteins, like “extensin” (a hydroxyproline-rich glycoprotein), polymerize within the cell wall to create a robust network. These proteins are notably upregulated when the plant faces pathogen attacks or in areas subjected to mechanical stress. These proteins are also found as monomers in growing cell walls, but after growth ceases, they are cross-linked to the wall by peroxidase action, and thus this action may provide a means for wall hardening (22).

2. Degradation of Plant Cell Walls in the Rumen

The digestive system of ruminants is highly adapted for the fermentation of plant material. While the host animal provides a suitable habitat for the growth of microorganisms, these microorganisms, in turn, supply the animal with protein, vitamins, and short-chain organic acids (23). Within the rumen ecosystem, the enzyme activity responsible for breaking down polysaccharides from cell walls is ten times greater than that observed in other known fermentation systems. In the rumen, polysaccharide-degrading enzymes such as endoglucanases and exoglucanases can be found on the cell surface or within the cytoplasm, while enzymes that break down

glycosides, such as β-glucosidase and β-fucosidase, are predominantly present in the rumen fluid. It has been suggested that these enzymes in the rumen fluid are released from the cytoplasm of rumen microorganisms as they degrade (24).

Several factors are crucial for the rapid breakdown of cellulose. One of these is the abundant synthesis of cellulase enzymes, which can be observed in the aerobic fungus *Trichoderma reesei*, though it is not a ruminal microorganism. Another important pathway for cellulose degradation is facilitated by rumen fungi. Rumen fungi synthesize enzymes in relatively small quantities but with very high specific activity. A third strategy is employed by cellulolytic rumen bacteria, which possess multi-enzyme systems for cellulose degradation known as cellulosomes, located on their cell surface. A cellulosome is a cell surface complex formed by the assembly of 18-20 proteins that bind to cellulose microfibrils, facilitating cellulose degradation. Several proteins within the cellulosome contain binding domains that allow for the physical attachment of the cell to cellulose (25). This strong attraction of the cellulosome to cellulose provides several key advantages: **1)** The cellulolytic enzymes are concentrated on the substrate, outcompeting other bacteria and their enzymes; **2)** The cellulolytic enzymes are protected from the proteases that are abundant in the rumen; **3)** The presence of multiple cohesin regions on the scaffolding protein and the binding of various cellulosomal enzymes to these regions increases the combinations of the cellulosome complex, enabling it to degrade a wide variety of plant cell walls; **4)** The combined action of cellulosome enzymes leads to more efficient degradation of plant cell walls. The assembly of the cellulosome complex is driven by carbon source-related gene expression, which dictates the specific enzymes required and their quantities (26).

Ruminants obtain their energy and vital nutrients from lignocellulose in plant matter through symbiotic relationship with the microbiome present in their rumen. The composition of this rumen microbiome is constantly shaped by the feed that the ruminant consumes and shifts in the bacterial community can significantly impact the host's productivity and overall health. Thus, ensuring a balanced diet for ruminants is crucial (27). Three predominant bacterial species are primarily responsible for cellulose degradation in the rumen: *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus*. These species share common characteristics that differentiate them from other ruminal cellulolytic bacteria and non-ruminal cellulolytic bacteria. Their most significant feature is their nutritional specialization. *F. succinogenes* and *Ruminococcus* species primarily utilize cellulose and its degradation products (cellodextrin) as growth factors (25). Although rumen bacteria are considered responsible for the majority of nutrient digestion in the rumen due to their numerical dominance and

metabolic diversity (28), rumen fungal enzymes may be more effective than rumen bacterial enzymes in the degradation of plant cell walls (24).

Several factors influence the rate and extent of plant cell wall hydrolysis in the rumen. The structure and composition of the substrate, such as lignin concentration (29), phenolic esters (30), and condensed tannins (31), affect the fermentation of the plant cell wall. Additionally, the characteristics of the rumen microflora, the population sizes of rumen microorganisms, and the interactions between these microorganisms or between them and their substrates also play a crucial role in rumen fermentation (32). The cellulolytic enzyme activity of rumen fungi has been found to be five times greater than that of dominant ruminal cellulolytic bacteria. In the absence of rumen bacteria, 62% of the material derived from grasses is broken down by rumen fungi (33).

In systems based on grazing, the mastication of plant material is essential. Plant particles must be reduced in size before reaching the rumen. It has been reported that the flow of food particles from the reticulorumen is a primary factor in controlling the intake and utilization of grasses by animals. Microbial degradation is required to weaken the structural barriers of plants and facilitate easier digestion. Rumen fungi play a significant role in the intake and passage of fibrils into the rumen by penetrating and wrapping around plant tissues with their rhizomycelium, altering the size and structural characteristics of plant particles (1). Traditionally, ruminant nutrition has been centered around evaluating various aspects such as animal performance, feed intake, fermentation characteristics, passage rate, diet digestibility, and nitrogen metabolism to assess how well a diet fulfills the animal's needs (34). On the other hand, significant relationships have been found between rumen microbial populations and the ruminant nutrition, and important advancements have been made in determining the rumen microbial ecosystem through culture-independent methods (35).

3. Degradation of Plant Cell Walls by Rumen Fungi

It has been suggested that the contribution of anaerobic fungi to the ruminal degradation of plant material, through their effective fibrolytic enzymes and their physical abilities to break down fibrous material via hyphae, might be more significant than that of cellulolytic rumen bacteria (36, 37). Although rumen fungi are fewer in number compared to bacteria and archaea, these fungi have been shown to represent approximately 20% of the rumen microbial biomass and 10-16% of the rRNA transcript abundance (38, 39). However, understanding how the carbohydrate-active enzymes of rumen fungi contribute to the degradation of plant cell walls in the rumen remains relatively limited compared to rumen bacteria. This

limitation may be due to the relative difficulty in culturing rumen fungi, which has led to insufficient research.

Another remarkable feature of rumen fungi is their ability to form cellulosomes, similar to rumen bacteria. Cellulosomes have been reported to increase cellulolytic activity by up to 12 times compared to free enzymes (40). Although cellulosomes belonging to rumen fungi were detected long ago (41), rumen fungal cellulosomes are still not fully understood (42). Unlike bacterial cellulosomes, which bind to the bacterial cell wall via dockerin-scaffold modules (43), rumen fungal cellulosomes can be found as free multi-enzyme complexes released into the extracellular matrix in addition to binding to the cell wall (44). Another feature that distinguishes rumen fungal cellulosomes from their bacterial counterparts is the presence of modules from glycoside hydrolase families 3, 6, and 45. Specifically, the β-glucosidase from glycoside hydrolase family 3 provides fungal cellulosomes with the ability to directly convert cellulose into fermentable monosaccharides. In contrast, Clostridial cellulosomes produce low molecular weight oligosaccharides (42).

Horizontal gene transfer between rumen fungi and rumen bacteria has been demonstrated, suggesting that horizontal gene transfer plays a significant role in the evolution of these fungi (45, 46). Additionally, it has been proposed that a common xylanase gene (*xynA*) found in rumen fungi may be responsible for the basal level of xylanase production in these fungi (47). Comparative analyses of DNA sequencing have shown significant homology between fungal xylanase and cellulase genes and bacterial xylanase and cellulase genes. Anaerobic fungal xylanase and cellulase genes do not contain introns (48). Furthermore, the existing homology between rumen fungi and bacteria has shown that horizontal gene transfer may occur between these microorganisms in different domains (49-51). The conditions in the rumen make it possible for gene transfer to occur within or between different groups of microorganisms. The large microbial population in the rumen increases the genetic diversity of rumen microorganisms. In addition, large amounts of aerobic and facultative anaerobic microorganisms pass through the rumen during the digestion of food. The abundant bacteriophages in the rumen facilitate gene transfer from these microorganisms (52).

Anaerobic gut fungi excel at breaking down crude lignocellulose by combining mechanical disruption with the action of a diverse set of biomass-degrading enzymes, which release large quantities of sugars into their surroundings (53). The combined action of CAZymes, whether functioning individually or as part of cellulosomes, along with the mechanical penetration of plant cell walls by fungal hyphae, leads to more effective breakdown of fibrous structures by anaerobic fungi (AF). This process not

only enhances the degradation of fibers but also improves access for other rumen cellulolytic and potentially proteolytic organisms (54). Hagen et al. (55) demonstrated in their study that the profile of carbohydrate-active enzymes (CAZymes) indicated a functional specialization unique to different domains. Bacterial communities were primarily focused on breaking down hemicelluloses, while fungi appeared to specialize in degrading more resistant cellulose structures. This was evidenced by the presence of various endo-acting and exo-acting enzymes from glycoside hydrolase (GH) families 5, 6, 8, and 48. Interestingly, enzymes from the GH48 were among the amplest CAZymes detected, with some also featuring dockerin domains, which are linked to fungal cellulosomes (55). On the other hand, the fate of lignin in anaerobic environments remains largely unknown. There is a significant knowledge gap regarding the interactions between anaerobic fungi and lignin. In this context, Lankiewicz et al. (56) demonstrated that anaerobic fungi can influence lignin either directly or indirectly, leading to the accumulation of lignin degradation products or a decrease in lignification in treated plant material. These findings change the perceptions of lignin degradation by anaerobes and provide opportunities for the advancement of decarbonization biotechnologies based on lignocellulose depolymerization (56). Additionally, the analysis of complete genome sequences will allow for the identification of genes that significantly accelerate functional discoveries in biological systems (57), such as lignocellulose degradation.

4. Anaerobic Fungi and Biotechnological Applications

The direct microbial supplementation of anaerobic fungi in animal feed has been investigated as a way to improve the utilization of low-quality feeds in both ruminant and non-ruminant livestock. The inclusion of anaerobic fungal cultures to the diets of different ruminants has been shown to enhance feed consumption, accelerate animal growth, boost feed efficiency, and increase milk yield. These studies indicate that introducing anaerobic fungi directly into animal feed can boost in vivo digestibility by improving rumen fermentation, increasing rumen microbial populations, and enhancing cellulolytic enzyme activities. On the other hand, adding only the enzymes produced by these fungi had no impact on rumen fermentation, emphasizing the necessity of using live fungal cultures as feed additives for ruminants (58). Hess et al. (59) reviewed that the studies have shown a positive relationship between fungal and bacterial concentrations, likely because AF hyphae physically break open plant tissues, increasing the surface area for colonization and nutrient access for other fiber-degrading microbes. This increased accessibility could also explain the rise in holotrich counts, a protozoa group involved in starch degradation, in response to AF dosing, while the overall ciliate protozoa population re-

mained unchanged (59). Studies have shown that using anaerobic fungal enzymes alone is more effective than live cultures when it comes to production in monogastric animals like pigs and poultry (60). Another approach in ruminant nutrition is the use of recombinant lactic acid bacteria. A gene encoding cellulase derived from anaerobic fungi was successfully expressed in lactic acid bacteria and used as silage inoculants for the pre-biological breakdown of plant biomass during ensiling. The results showed that recombinant lactic acid bacteria reduced NDF and ADF contents (61).

Due to the high efficiency of rumen fungi in enzyme production, significant research has focused on the use of enzymes of anaerobic fungi in several industries (62). The production of a wide range of potent polysaccharide-degrading enzymes by rumen fungi, makes them particularly attractive for various industries, such as brewing, food, textiles, paper, and biofuel production. Additionally, the ability of rumen fungi to produce enzymes from various carbon sources, including various lignocellulosic biomasses, is a significant advantage (63, 64). The ability of anaerobic fungi to break down cellulose and hemicellulose, thanks to their plant cell wall-degrading enzymes, currently surpasses that of certain commercial enzyme blends. Extracellular enzyme extracts from *N. patriciarum* and *Piromyces* sp. have demonstrated remarkable stability and a superior efficiency in degrading microcrystalline cellulose compared to commercial enzymes sourced from *Trichoderma reesei* and *Aspergillus niger* (65). In this context, rumen fungal xylanases in the paper industry may offer environmentally friendly methods for pulp processing (66). The use of *Orpinomyces* sp. PC-2 xylanase in pulp bleaching has been associated with reductions in chlorine use, decreases in lignin content, and improvements in the physical strength-related properties of pulp (67, 68).

Renewable, environmentally friendly energy from the anaerobic digestion of organic waste has garnered significant interest. It has been shown that the biological pretreatment of lignocellulosic waste with white and brown rot fungi can improve biogas production (69). Since biogas production occurs under anaerobic conditions, incorporating an aerobic pretreatment step raises the overall costs. However, directly adding anaerobic fungi to these bioreactors could remove the necessity for such aerobic pretreatment, potentially reducing expenses. The inclusion of anaerobic fungi in bioreactors has increased yield depending on the substrate and fungal species used (70). However, the current limitations of anaerobic fungi, particularly their inability to endure long-term survival in fermenters, prevent their use in commercial full-scale biogas production systems with existing technologies (71). Overall, the direct addition of cellulase-secreting microorganisms to the medium has been found to result in low yields, and it has

been suggested that treating cellulose biomass directly with cellulase may be a better solution (72).

A major hurdle in using anaerobic fungi for industrial or biotechnological purposes lies in the challenge of maintaining continuous cultures that efficiently produce fungal biomass or enzymes. The current methods for cultivating anaerobic fungi are both labor-intensive and costly, involving frequent transfers and batch cultures that are difficult to sustain. Approaches utilizing immobilized cells show more potential than conventional free-cell fermentations, although existing studies on the immobilization of monocentric and polycentric fungi have yet to yield commercially viable technologies (73-75). To make anaerobic fungi viable for industrial applications, it is crucial to develop effective storage carriers that can preserve their long-term viability. The discovery of a *Neocallimastix* strain with greater oxygen tolerance and stability under varying culture conditions, requiring fewer transfers, could enhance industrial processes. Additionally, recombinant DNA technology offers a promising pathway by enabling the production of rumen fungal enzymes in microorganisms that are easier to culture. (76, 77).

Recent years have seen growing interest in the ability of rumen fungi to produce bioethanol in a consolidated process. This interest is largely driven by their unique capacity to break down complex carbohydrates, such as cellulose and hemicellulose, into simpler sugars using specialized enzymes and cellulosomes (78). The enzyme composition produced by rumen fungi allows these fungi to process various lignocellulosic materials for the generation of bioethanol. It has been demonstrated that lignocellulosic biomass, such as wheat straw, bagasse (79), barley straw (36), sorghum fodder, and corn stover (80), can be converted into bioethanol by anaerobic fungi. Furthermore, the cellulase and xylanase enzymes synthesized by rumen fungi show higher activity than those commonly used in the industry, such as *Trichoderma reesei* (81), *Aspergillus oryzae*, and *Saccharomyces cerevisiae* (24). However, anaerobic fungi currently have a lower ethanol production capacity than filamentous fungi such as *Trichoderma reesei* and *Fusarium oxyporum* (78). The challenges in using anaerobic fungi for biofuel production can be overcome through genetic engineering, but several obstacles need to be tackled. These include the fungi's low GC content in their genome, the absence of suitable genomic tools for processing this low GC content, high AT content that hinders the identification of regulatory elements and complicates genetic engineering techniques like primer design and homologous recombination, and the limited understanding of the codon usage in these fungi, which hampers the successful expression of foreign metabolic pathways and proteins. (82).

5. Conclusion

Fungi represent a diverse collection of organisms with different lineages and lifestyles, playing crucial roles in ecosystems, biodegradation, pharmacology, and the food industry (83). Rumen fungi, in particular, are essential for breaking down plant cell walls and serve as key players within the complex rumen microbiome. Their unique enzymatic capabilities, particularly in producing fibrolytic enzymes, allow them to effectively break down resistant components of lignocellulosic materials. Rumen fungi can form cellulosomes, which enhance their cellulolytic activity, making them especially important in the digestion of tough plant fibers. Despite their relatively low abundance compared to bacteria, their contribution to the overall degradation process is substantial, as they possess high specific activity enzymes that target otherwise resistant plant material. This makes them indispensable for ruminants that rely on plant-based diets, ensuring efficient nutrient extraction and energy production.

The evolutionary adaptability of rumen fungi is highlighted by their potential for horizontal gene transfer with rumen bacteria, allowing them to acquire new functional traits that enhance their survival and efficiency in the rumen environment. This adaptability, combined with their ability to degrade complex polysaccharides like cellulose and hemicellulose, underscores their critical role in the rumen ecosystem. Furthermore, the synergistic interactions between rumen fungi and other rumen microorganisms contribute to a more efficient breakdown of plant cell walls, optimizing the fermentation process and ultimately improving the host's nutritional intake and health.

However, the industrial and biotechnological application of rumen fungi remains challenging due to difficulties in their cultivation and the maintenance of stable cultures. Despite these challenges, the enzymes produced by these fungi have shown great potential in various industries, including biofuel production, animal feed enhancement, and environmental management. To fully exploit these potentials, future research must focus on developing sustainable cultivation methods, improving the stability of fungal cultures, and further exploring the genetic and enzymatic diversity of rumen fungi. By overcoming these challenges, rumen fungi could play a pivotal role in advancing both agricultural practices and industrial processes, leading to more efficient and sustainable use of natural resources.

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