RECENT ADVANCES IN BIOLOGICAL SCIENCES

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PREFACE

Biological sciences aim at the study of life and living organisms, their life cycles, adaptations, and the environment. ‘Recent Advances In Biological Sciences’ describes recent progress in various biological sciences, such as microbiology, molecular biology, zoology, and botanic. This book consists of a total of eight chapters, each of which has been contributed by highly qualified professionals in the respective fields of research.

I am proud to present this book, which contains recently updated information on various research areas and techniques in the biological sciences, which will benefit many researchers from different life science institutions around the world.

We would like to thank Iksad Publishing house for their devoted work to bringing together valuable scientists, editing, and publishing the book named **Recent Advances In Biological Sciences**.

With my regards

Res. Assist. Dr. M. Burcu KÜLAHCI

CHAPTER 1

ORIJIN OF INFECTIOUS DISEASES AND COVID-19

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INTRODUCTION

Mankind has faced infectious diseases repeatedly throughout its evolutionary history. Some of these diseases that recently affected human populations are black plague, smallpox, tuberculosis, Spanish flu, Russian flu, AIDS, Ebola, Asian flu, Avian flu, Hong Kong flu, SARS, swine flu, MERS, Zika, and finally human coronavirus disease (COVID-19). Bacteria, viruses, fungi, and worms/helminths are among the pathogens that cause infectious disorders (Kotra, 2007). For humans, pathogens transmission may occur by other people, animals, or environmental sources. As infective organisms, like humans, benefit from modern transportation facilities, infectious diseases are much faster and imminent dangers than before. In the classification of infectious diseases, the concepts of endemic, outbreak, epidemic, and pandemic appear. The amount of disease found only in a particular population is called the endemic level of the disease. An outbreak is defined as an unpredictable increase in the number of people showing a health condition or the occurrence of cases in a new region. When the disease spreads over a wider geographical area, the concept of epidemic emerges. When the epidemic spreads all over the world, the disease is now defined as a pandemic disease (Piret and Boivin, 2020). Throughout human history, epidemics and pandemics have constantly changed people's lifestyles due to their striking effects and still continue to do so. The COVID-19 pandemic we are currently experiencing is also changing everyone's lives in a remarkable way, and its effects on the future cannot be fully predicted. Learning about the factors, progress, and

effects of COVID-19 and past epidemics will at least help us to be prepared for infectious diseases.

1. BRIEF HISTORY

The first records of infectious diseases that caused great epidemics in human history go back to 430 BC. In this section, information about some of the major pandemics that have occurred to date is given. These pandemics are given in Figure 1.

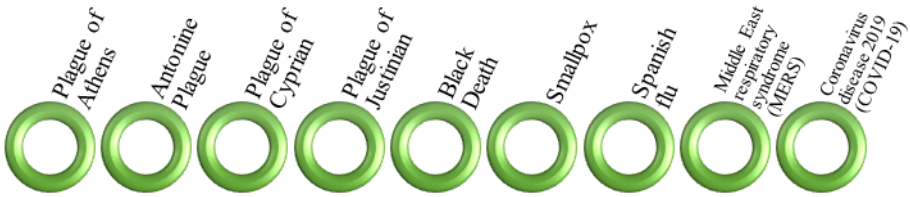


Figure 1: Some Of The Major Pandemics That Have Occurred In The World

The first pandemic in human history was the plague of Athens, which appeared in 430 BC during the Peloponnesian War between Athens and Sparta and lasted for five years. According to the records, 100,000 people, who make up 25% of the city's population, died during this epidemic. As a result of the researches, this epidemic may have been caused by smallpox or typhus (Littman, 2009).

The Antonine Plague, also known as the Plague of Galen between 165 and 180 BC, is an ancient epidemic that was carried into the Roman Empire by soldiers returning from expeditions. Symptoms of the disease included fever, diarrhea, vomiting, thirst, swollen throat, and cough. It was stated that during this epidemic, which is considered one of the biggest plague epidemics, 2 thousand people per day and 30

percent of the total population of the empire (5 million) died (Littman and Littman, 1973).

The plague of Cyprian, seen between 249-270, was recorded as a type of hemorrhagic fever that probably affected the entire Roman Empire. During this epidemic, 1 million people lost their lives all over the world (Alfani and Murphy, 2017).

The plague of Justinian, which occurred between 541 and 543 and is traditionally regarded as the first of three human plague pandemics, traveled over the Mediterranean basin from Central Asia or Africa to Europe, killing an estimated 100 million people. This pandemic is thought to be caused by the bacterium *Pasteurella pestie* (later referred to as *Yersinia pestis*) (Wagner et al., 2014).

In more recent history, a significant epidemic disease known to spread from China to the world is the Black Death (14th century) (Benedictow, 1992). This epidemic, which caused the death of approximately 75-125 million people between 1347 and 1351 in North Africa and Europe (Shaw and Taylor, 2020). The plague is caused by a bacterium called *Y. pestis*, the main source of which is wild rodents (Wheelis, 2002). *Bacteria carried by fleas can affect both humans and animals.* A variant of bubonic plague is named after the swollen lymph nodes (bubs) caused by the disease (Hinnebusch, 1997). From China, the plague also traveled west, reaching Crimea, Istanbul, then spreading to the Balkans, and from there to the Adriatic, Italy and the rest of Europe, and Africa, and Damascus. Plague spread from here to Anatolia (Ayalon, 2015).

In the Ottoman landscape, the Black Death affected the Ottomans until the middle of the 19th century. Ten thousand people died in Istanbul in the year 1409/1410 (Marien, 2009). The water vole, shore vole, ground squirrel, and maybe the Persian jird, known in Iran, were all mentioned as plague carriers by the seventeenth-century Ottoman traveler Evliya Çelebi.

Smallpox, a viral epidemic that caused the death of 300 million people in the last 100 years before being eradicated in 1979 in the 20th century, spread from China to the world in the 1500s (Needham and Lu, 2000). This disease is caused by the variola virus and causes pus-filled blisters (pustules) to form on the skin (Lofquist et al., 2003). This cureless disease as a biological weapon against Native Americans in the 18th century (Patterson and Runge, 2002). The inoculation against smallpox has been applied for centuries in Ottoman lands (first vaccine usage), the wife of the British ambassador was observed in Istanbul in 1721, then send the method to England. The modern smallpox vaccine was developed in the 20th century (Plotkin, 2011).

Despite the use of vaccines and various antibiotics worldwide, tuberculosis (TB), one of the oldest diseases of humanity, is still the leading cause of death among infectious diseases (Smith, 2003; Talbot and Raffa, 2015; Fitzgerald and Haas, 2005; Moule and Cirillo, 2020). Every year, two million people die due to tuberculosis, an epidemic that causes the death of millions of people. The microorganism that causes TB is *Mycobacterium tuberculosis.* While *Mycobacterium* species were originally found in the soil, some species evolved to live

in mammals such as cows and were transmitted to a new host, humans, due to the domestication of cattle (Smith, 2003).

The age of antibiotics began in the 1940s with Schatz and Waksman's use of streptomycin in the treatment of tuberculosis, which was followed by the use of numerous other antibiotics (Smith, 2003). A vaccine strain attenuated by the successful passage of a virulent *M. bovis* strain was produced by Calmette and Guerin in Paris in 1920, which later introduced the BCG vaccine, but these vaccines failed to eradicate TB (Smith, 2003).

Spanish flu pandemic, an outbreak that claimed the lives of as many as 50 million people between 1918 and 1919. The Spanish flu was a type A form of influenza virus that the first host is a bird (bird flu), then, it was transmitted to mammals. The virus that causes the disease is H1N1, which also caused Swine Flu in 2009. The origin of the H1N1 virus, which caused the loss of 150,000 to 575,000 people in a year between 2009 and 2010, in China, as stated in Mark Humphries' book "The Last Plague" (Humphries, 2013) and other publications. Unusually, young adults died from infection and the majority lost their lives of pneumonia caused by secondary bacterial infections (Hobday and Cason, 2009). Vaccines, antiviral drugs, and antibiotics are used to control avian influenza and also deal with secondary infection.

In 1960, approximately 500 patients with flu-like symptoms were reported as cases of coronavirus disease. 17-18 cases were confirmed to be infected with the coronavirus strain (Kumar et al., 2020). Later, coronavirus strains have been identified in several avian hosts and

some mammals, including bats, dogs, camels, mice, and cats (Lu et al., 2020). The virus is believed to have passed from bats to other creatures and the second infection was encountered in China in 2002, causing the death of more than 8000 humans. The virus disappeared by 2004. Then, in September 2012, a new coronavirus strain caused Middle East respiratory disease (MERS) in Saudi Arabia. For MERS transmitted from camels to humans, 2494 cases and 858 deaths have been recorded.

Last but not the least, a novel coronavirus to emerge in this century from China in December 2019. This new virus, called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is the causative agent of COVID-19 (Huang et al., 2020; Zhou et al., 2020; Zhu et al., 2020). It was reported to appear in Wuhan, China in December 2019, and COVID-19 was declared a global pandemic by the World Health Organization on March 11, 2020 (Song et al., 2020). 4.979.421 *people* have died from the COVID-19 outbreak (for now, October 28), there are 245 million 373,039 confirmed cases in the world. However, the actual number of cases is estimated to be five to twenty times higher than this number (Noh and Danuser, 2021). The disease initially presents with normal flu-like symptoms. However, this virus then causes very severe lung damage. However, the disease may progress worse in people with different clinical histories: advanced age, high blood pressure, diabetes, etc.

2. DRUGS FOR INFECTIOUS DISEASES

2.1. Antibacterial Drugs

The discovery of antibiotics was one of the greatest achievements of the 20th century. Antibiotics are chemicals that inhibit bacterial growth by stopping bacterial cell division or killing them. Antibiotics are used for the treatment or prevention of infectious diseases in humans and animals (Dugassa and Shukuri, 2017). Antibiotics show their effects by targeting protein synthesis, nucleic acid synthesis, metabolic compound synthesis, bacterial cell wall, and cell membrane (Zaman et al., 2017). The mechanisms of action of antibiotics are shown in Figure 2.

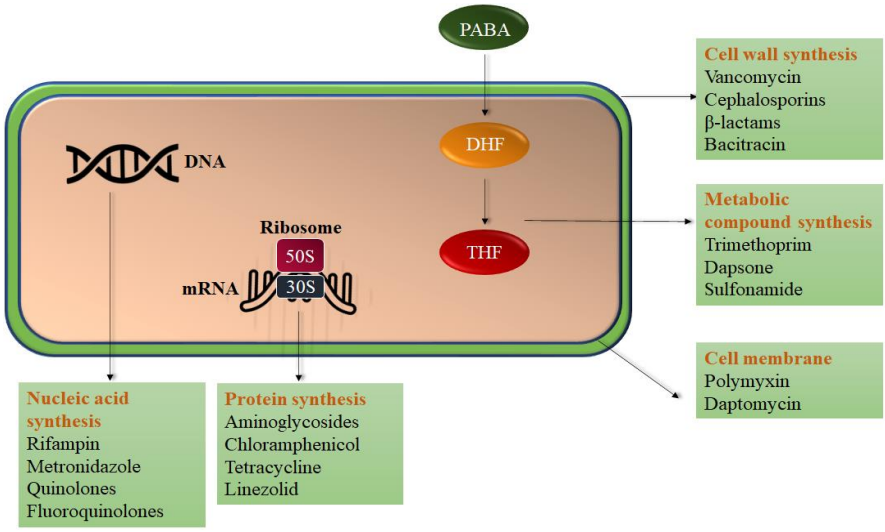
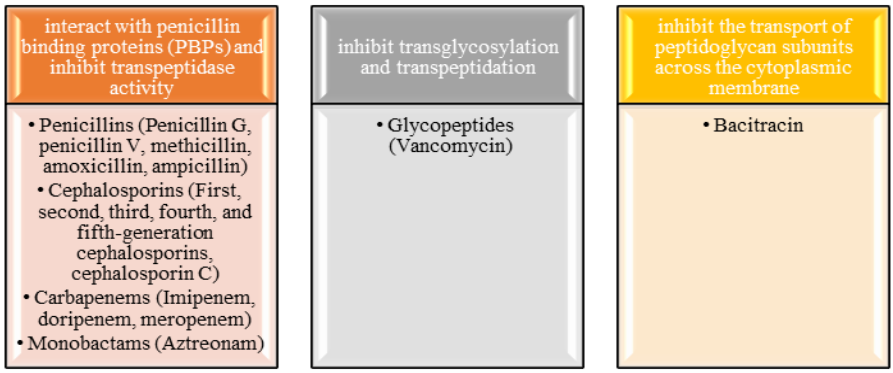


Figure 2: The Mechanisms of Action of Antibiotics

2.1.1. Antibiotics Targeting Cell Wall

Bacterial cell wall components are attractive antibacterial targets due to the lack of analogs in human cells, thereby ensuring target selectivity. Various antibacterial drugs make cells more susceptible to osmotic lysis by blocking different steps in the biosynthesis of peptidoglycan. Peptidoglycan, which is the basic element of the bacterial cell wall, is synthesized of four stages: synthesis of precursors in the cytoplasm, transport of these lipid-dependent precursors across the cytoplasmic membrane, glycan units attached to the cell wall, and transpeptidation binding and maturation (Mc Dermott et al., 2003). β-lactams, glycopeptides, and bacitracin are among the antibiotics that inhibit different stages of the cell wall biosynthesis of bacteria (Dowling et al., 2017). Drugs inhibiting bacterial cell wall synthesis are divided into 3 main groups according to their mechanism of action. Antibiotics in the first group interact with penicillin-binding proteins (PBPs) and inhibit transpeptidase activity. Antibiotics in the second group inhibit transglycosylation and transpeptidation. The antibiotics in the third group inhibit the transport of peptidoglycan subunits across the cytoplasmic membrane. The names and mechanisms of action of drugs inhibiting bacterial cell wall synthesis are shown in Table 1.

Table 1: Antibiotics That Inhibit Bacterial Cell Wall Synthesis



2.1.1. Antibiotics Targeting Cell Membrane

Cyclic polypeptides (colistins and polymyxins) in this group are branched polypeptides containing both hydrophobic and cationic amino acids. Due to this amphipathic feature, polymyxin and colistins change the permeability of the cell membrane and act as detergents. They are more effective on Gram-negative bacteria as they can hardly pass through the thick peptidoglycan layer on the Gram-positive cell wall (Epand et al., 2016).

2.1.2. Antibiotics Targeting Protein Synthesis

Bacterial ribosomes consist of two subunits of rRNA (the 30S and 50S) and proteins. Antibiotics that inhibit protein synthesis generally show their effect by binding to these subunits. Antibiotics in this group are aminoglycosides (binds to the 30S subunit of the ribosome), tetracyclines and alkylaminocyclines (inhibits the binding of aminoacyl tRNA to the A site of the ribosome), fusidic acid (inhibits

the transport of peptidyl tRNA from the A site of the ribosome to the P site), mupirocin (inhibits protein synthesis by binding to bacterial isoleucyl tRNA synthetase), macrolides (targets 50S subunit of ribosome), lincosamides (targets 50S subunit of ribosome), streptogramins (targets 50S subunit of ribosome), chloramphenicol and thiamphenicol (targets 50S subunit of ribosome) (McCoy et al., 2011).

2.1.3. Antibiotics Targeting Nucleic Acid Synthesis

Fluoroquinolones in this group show their effect by inhibiting topoisomerase activity. Ansamycins, on the other hand, inhibit the initiation step of transcription by acting on RNA polymerase (Bhattacharjee, 2016).

2.1.4. Antibiotics Targeting Biological Metabolic Compound Synthesis

Sulfonamides, the most important antibiotics in this group, are derived from p-aminobenzenesulfonamide, which is a necessary factor for folic acid synthesis in bacteria. In this way, it shows its effect by inhibiting the synthesis of tetrahydrofolate (Kapoor et al., 2017).

2.2. Antifungal Drugs

Fungi are eukaryotic cells like human cells. Therefore, drugs used in antifungal therapy generally do not show selective toxicity and also damage human cells. However, a few drugs show selective toxicity to these organisms more effectively by inhibiting metabolic pathways

found only in fungi. Antifungal drugs generally show their effects by inhibiting cell wall synthesis, ergosterol synthesis, nucleic acid synthesis, membrane functions, and microtubule formation (Prasad et al., 2016).

2.3. Antiviral Drugs

Antiviral drugs act by targeting viruses or host cell factors. Many of these drugs work by interfering with the viral genome replication. Other antiviral drugs inhibit the attachment of the virus to the receptors on the cell surface and its entry into the cell, enzymes such as polymerase, protease, integrase, and reverse transcriptase (Kausar et al., 2021).

3. CORONAVIRUS MICROBIOLOGY AND GENOME

Viruses are not living organisms. The virus consists of nucleic acid, which is DNA or RNA, and surrounding proteins. When the virus enters the cell, it makes a large number of molecules like itself, then the new viruses that are formed explode the cell and invade other cells. Coronaviruses are the largest group of viruses infecting humans and animals which causes respiratory, hepatic, and neurological diseases. Infections are transmitted via respiratory and fecal-oral routes. The group belongs to the family of *Coronaviridae* (Liu et al., 2016). Coronaviruses have been classified into 39 species in 27 subgenera, five genera, and two subfamilies (Siddell et al., 2019). Coronaviruses subtypes are alpha, beta, gamma, and delta. Furthermore, each of these subtypes has many serotypes. Some of

these affect humans, while others affect other animals (Kumar et al., 2020). The reasons why coronaviruses are so diverse are the infidelity of RNA-dependent RNA polymerase, unique random template switching during RNA replication, and extra plasticity in hosting and replacing genes due to their large genomes (Woo et al., 2009).

Coronaviruses are particles whose single-stranded RNA genome surrounded by a nucleocapsid protein (N) is packaged by an envelope containing three structural proteins called “spike protein (S), membrane protein (M), and envelope protein (E)”. However, hemagglutinin esterase protein can also be found in the envelope of some coronaviruses (Mousavizadeh and Ghasemi, 2021). The virus genome length is approximately 26.4–31.7 kb, the largest among all known RNA viruses (Masters, 2006). The large genome possessed by these family members provided extra flexibility for inserting and modifying genes. The viruses' G + C contents range from 32% to 43% (Won et al., 2010). The 5' and 3' ends of the virus genomes contain short untranslated regions and the gene order in the coding regions is 5'-replicase ORF1ab, spike (S), envelope (E), membrane (M), nucleocapsid (N)-3'. According to Won et al., (2010) each subgroup has a variable number of additional ORFs, but Mousavizadeh and Ghasemi (2021) stated that a typical CoV genome contains at least six ORFs (except for Gammacoronavirus). The 16 nsp (nsp1-16) are encoded by the first ORFs (ORF1a/b). ORF1a and ORF1b contain a frameshift between them, producing the two polypeptides pp1a and pp1ab. These polypeptides are cleaved into 16 nsp by virally encoded

chymotrypsin-like protease (3CLpro) or parent protease (Mpro) and one or two papain-like proteases (Mousavizadeh and Ghasemi, 2021). Viral genes code for spike (S), envelope (E), membrane (M), and nucleocapsid (N) which are major proteins (Wang et al., 2020). The structural membrane (M) glycoprotein crosses the membrane bilayer three times, leaving a short NH_2-terminal domain outside the virus and a long COOH terminus (cytoplasmic domain) inside the virion (Haan et al., 1998). Spike protein (S), a type I membrane glycoprotein composed of peplomers, is a major inducer of neutralizing antibodies. Membrane and envelope proteins are small transmembrane proteins associated with the envelope and play an important role in the formation of virus particles (Mousavizadeh and Ghasemi, 2021).

The structure of SARS-CoV-2 is shown in Figure 3.

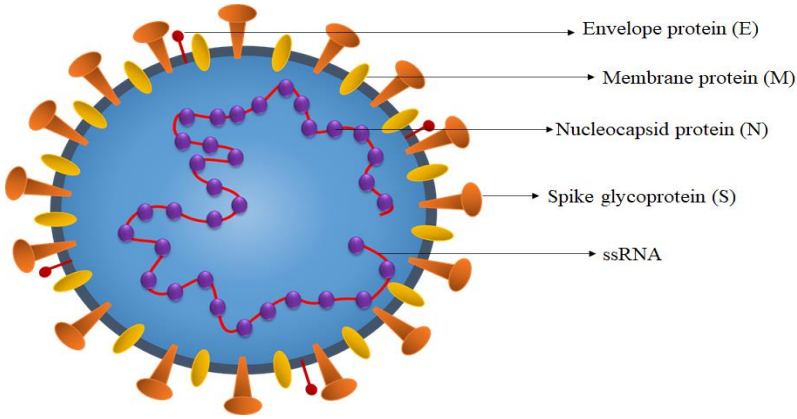


Figure 3: The Structure of SARS-CoV-2

4. SPREADING OF INFECTIONS

Viruses spread in different ways. Some viruses spread through the air droplets like influenza, while others by contact with an object that has viruses on it. Another way is spreading the infections through body fluids and blood such as HIV, hepatitis B, and hepatitis C. Some germs are spread from contaminated food or water, such as Salmonellosis, Listeriosis, and Toxoplasmosis. Some germs are transmitted by insects or other animals. The diseases spread by the insect are yellow fever, chikungunya virus, dengue fever), bacteria (plague, Lyme disease), parasites (malaria, filariasis, leishmaniasis, sleeping sickness).

Many recent infectious diseases are caused by viruses, and their origin is nonhuman host reservoirs that are usually mammalian and sometimes avian (Taylor et al., 2001). The recent emerging infectious disease outbreaks are the Ebola virus disease (EVD) epidemic in West Africa, the Zika virus disease epidemic in America (Woolhouse et al., 2016), and SARS CoV-2 in the World. Domesticated and wild animals can carry harmful viruses and germs that can spread to people and cause illnesses called zoonotic diseases. Generally, Carnivora and Chiroptera spread RNA viruses to other hosts, while wild and domesticated species transmit both RNA and DNA viruses to other hosts (Wells et al., 2020). The novel coronavirus originated from the seafood market in Wuhan where racons, dogs, bats, snakes, and other animals sold and spread the world (Hafeez et al., 2020). The main

transmission route of COVID-19 is respiratory droplets (Sheeren et al., 2020).

RNA viruses have higher mutation rates than DNA viruses. (Lauring and Hodcroft, 2021). COVID-19 is rapidly changing due to virus genome type and replication enzyme characteristics. CT images of the lungs are used to aid in the diagnosis of COVID-19, but real-time polymerase chain reaction made from samples from the upper and lower respiratory tract is used for definitive detection of the virus.

5. HOW DOES THE CORONAVIRUS INFECT?

It enters the body through the mouth or nose, invading cells in the throat and lungs. Normally, when the virus enters the body, the defense system starts to work. However, this virus somehow makes copies of itself by blocking the defense system. Even if it is late, the small chemical weapons in our bodies begin to be secreted for defense. Therefore, our fever increases. Our germ-eating white blood cells rush to the occupied place.

The virus uses everything that does not belong to itself in the cell, for its benefit, the cell tries to commit suicide to get rid of it, but the virus prevents it. After making thousands of viruses, it kills the cell itself and invades neighboring cells with its new viruses. In the cell it enters, the message in the virus is read and the proteins necessary for the continuation and reproduction of the virus are made in line with the message. The virus has 24 proteins, versus 100,000 different proteins in the human cell, and these make many people helpless. Some of

these proteins work like photocopiers and make copies of the virus, while others act like scissors. Thus, it allows the proteins to be released and do their work. Normally, the human defense system stands up to the virus, but the viruses camouflage and prevent the attacks of the defense system. During an innate immune response to a viral infection, dendritic cells, adaptive T and B lymphocytes, macrophages, natural killer cells, and produce cytokines. Pattern recognition receptors (PRRs) identify the virus's numerous molecular structures. When PRRs attach to them, the inflammatory reaction against the virus that has entered the body is triggered, and some signaling pathways and, as a result, transcription factors are activated. Pro-inflammatory cytokines are produced for the expression of genes encoding adhesion molecules, inflammatory cytokines, and chemokines. Plasma proteins and leukocytes, which will fight the infection, come to the area where the virus is located (Ragab et al., 2020). During the cytokine storm, the level of proinflammatory cytokines IL-6, IL-1, TNF-α, and interferon increases. This increase results in acute respiratory distress syndrome, a systemic inflammatory response, multi-organ failure, and ultimately death. When the virus infects the body, a strong cytokine storm resulting from an unbalanced response can be very damaging to patients, although the inflammatory response plays an antiviral role (Shimizu, 2019).

6. WHAT ARE THE DISEASE AND ITS SYMPTOMS?

Some human symptoms begin to show 5 days after infection, after 12 days most infections appear. People can be removed from quarantine after 14 days if there are no symptoms. Some people can get the virus, show no symptoms, but spread the virus. Fever, dry cough, fatigue, shortness of breath, headache, nausea, and diarrhea may occur. Patients with the most severe symptoms are 60 years of age or older.

7. WHO IS IN DANGER?

Health workers, caregivers of the elderly and children, etc. Service sector employees, public officials who are intertwined with people such as soldiers and police, and people who cannot benefit from adequate nutrition and health services are more in danger.

8. HOW CAN WE BE PROTECTED?

We can protect ourselves from the virus by maintaining social distance, avoiding physical contact, protecting the elderly-chronic patients, washing our hands frequently with soap, not touching our faces, changing our clothes when we come from outside, using disinfectants, and wearing a mask if we have to go out. The most important thing is to get vaccinated.

9. LIFE CYCLE OF RNA VIRUSES; SARS-COV-2

The ssDNA, dsDNA, ssRNA, and dsRNA are the four main viral genomes. ssRNA viruses are further divided into 2 types: (+) ssRNA

(the infecting genome acts as mRNA) and (-) ssRNA. In some RNA and DNA viruses, viral mRNA synthesis occurs after entry into the host cell. The (–) ssRNA viruses enter the cell, then replicate by its RNA-dependent RNA polymerases to convert the antisense RNA to positive sense mRNAs, and viral protein synthesis proceed. The dsRNA viruses are transcribed by viral RNA-dependent RNA polymerases within the capsid, with the transcripts subsequently released into the cytoplasm for translation (Rampersad and Tennant, 2018). In retroviruses, which are (+) RNA viruses, the virion enzyme converts ssRNA to (+) dsDNA during infection. After the virus genome integrates into the host genome, it migrates to the cytoplasm, where it is transcribed and translated by cellular enzymes. Some ssRNA(+) transcripts found in the cytoplasm are packaged as new retrovirus virions (Rampersad and Tennant, 2018).

The initial steps of SARS-CoV-2 infection, coronavirus spike (S) protein binds to the cellular entry receptors, angiotensin-converting enzyme 2 (ACE2) together with host factors (such as the cell surface serine protease TMPRSS2), promote viral uptake (V'kovski et al., 2021) and fusion at the cellular or endosomal membrane (Figure 4). Following entry, coronaviruses inside the cell express genes and then, replicate their genomic RNA. The structural proteins are destined into endoplasmic reticulum (ER) membranes and to the Golgi apparatus. Finally, the virus is secreted from the cell by exocytosis (V'kovski et al., 2021).

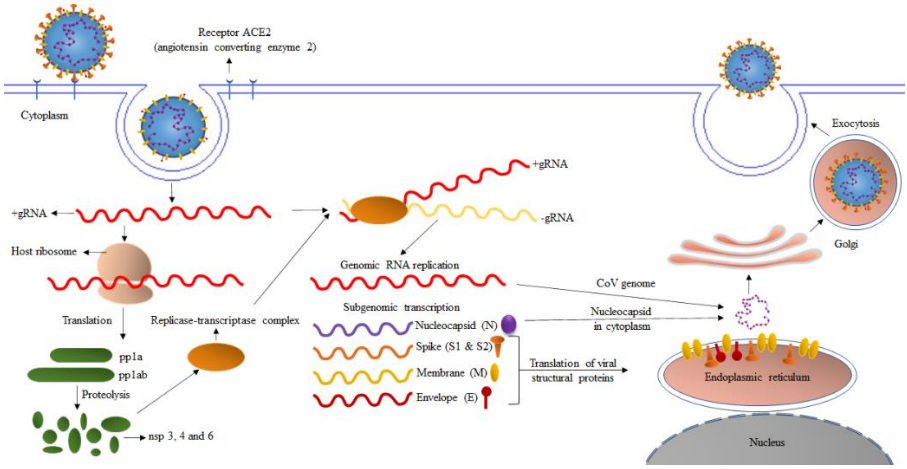


Figure 4: Life Cycle of SARS-CoV-2

10. VACCINE DEVELOPMENT FOR COVID-19

Vaccination is the first form of protection for the prevention of infectious diseases. Vaccines help reduce disease severity and transmission rates (Klompas**,** 2021). Under normal circumstances, vaccine development for human infectious agents and obtaining the final product traditionally take 15 to 20 years (Excler et al., 2021; Bregu et al., 2011). However, with the new vaccine platform technologies, this time can be shortened, making it possible to rapidly develop, test, and manufacture vaccines (van Riel and de Wit, 2020). This period has been shortened for vaccines developed for COVID-19, and some vaccines have been approved for emergency use by the FDA. Available human vaccines can be grouped into mRNA-based, virus-based, and protein-based vaccines. Virus-based vaccines are either live attenuated viruses or inactivated viruses that are no longer

harmful. Since fully inactivated viruses are not replicated, it is necessary to add adjuvants to the content of vaccines to stimulate the immune system. (van Riel and de Wit, 2020). Live attenuated virus vaccines are produced by cell culture. After the virus has lost its pathogenic properties, it is ready for further tests. Recommended vaccines are administered to small animals, usually mice, in preclinical studies, and their immune responses are measured. With these tests, immunity against the virus must be proven, otherwise, the vaccine will not be tested further. Toxicity studies are also carried out on animals to detect safety signals (Edwards and Orenstein, 2021). Once these steps are successful, clinical trials, consisting of three phases lasting another 5-10 years, begin to be tested in humans (Figure 5) (Deb et al., 2020).

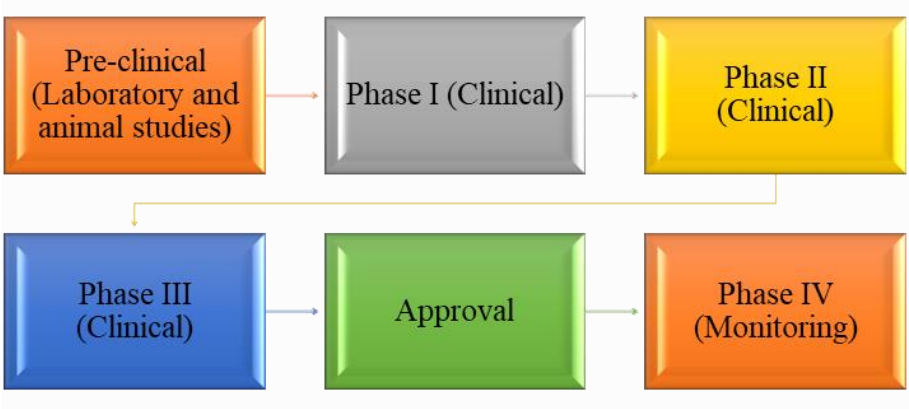


Figure 5: Vaccine Development Stages

No DNA vaccines have been licensed for use in humans, as DNA vaccines are not very good at inducing antibodies. Protein-based vaccines can be purified protein from a virus or virus-like particles. An adjuvant is added to them as well. mRNA vaccines have some advantages such as rapid construction of vaccine, no need for cell or animal substrates to manufacture, no need to enter the nucleus, production of a suitable number of protein antigen molecules per molecule of mRNA delivered, and no integration to DNA (Liu, 2019). Two COVID-19 mRNA vaccines were developed (Pfizer–BioNTech35 and Moderna36), both showing safety and high efficacy and now with US Food and Drug Administration (FDA) emergency use authorization (EUA) (Edwards and Orenstein,2021).

11. DRUG AND VACCINE EFFORTS

So far, remdesivir, an antiviral drug developed by the Gilead Inc. USA, and chloroquine (CQ)/hydroxychloroquine (HCQ), an antimalarial drug, have been approved by the US Food and Drug Administration (FDA) for the treatment of COVID-19. The antiviral drug remdesivir is a combination of two HIV drugs, lopinavir and ritonavir, lopinavir and ritonavir plus interferon beta and the antimalarial drugs chloroquine or hydroxychloroquine and acts by inhibiting RNA-dependent RNA polymerase. (Deb et al., 2020). Food and Drug Administration approved the first COVID-19 vaccine, called Pfizer-BioNTech in December 2020. According to the data of the World Health Organization, as of November 16, 2021, there are 194 COVID-19 vaccine candidates in preclinical development and 132 in

clinical development. Ten of the vaccines currently in clinical development are in phase IV. Three of these vaccines are viral vector-based (non-replicating), three are inactivated virus-based, three are RNA-based, and one is protein subunit-based vaccines (Table 2).

Table 2: COVID-19 Vaccines in Phase IV

Vaccine platform description	Type of candidate vaccine	Developers
Inactivated virus	CoronaVac; inactivated SARS-CoV-2 vaccine (vero cell)	Sinovac Research and Development Co., Ltd
Inactivated virus	Inactivated SARS-CoV-2 vaccine (Vero cell)	Sinopharm
Inactivated virus	Inactivated SARS-CoV-2 vaccine (Vero cell), vaccine name BBIBP-CorV	Sinopharm
Viral vector (non-replicating)	ChAdOx1-S - (AZD1222)	AstraZeneca + University of Oxford
Viral vector (non-replicating)	Recombinant novel coronavirus vaccine (Adenovirus type 5 vector)	CanSino Biological Inc./Beijing Institute of Biotechnology
Viral vector (non-replicating)	Ad26.COV2.S	Janssen Pharmaceutical
RNA based vaccine	mRNA-1273	Moderna + National Institute of Allergy and Infectious Diseases (NIAID)
RNA based vaccine	BNT162b2 (3 LNP-mRNAs), also known as "Comirnaty"	Pfizer/BioNTech + Fosun Pharma
Protein subunit	MVC-COV1901 (Spike-2P protein + adjuvant CpG 1018)	Medigen Vaccine Biologics + Dynavax + National Institute of Allergy and Infectious Diseases (NIAID)
RNA based vaccine	"mRNA-1273.351. A lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine that encodes for a full-length, prefusion stabilized S protein of	Moderna + National Institute of Allergy and Infectious Diseases (NIAID)

CONCLUSION

Germ (Bacteria, yeast, virus, etc.) was exist in the past, it will exist in the future. Some viruses infect bacteria, some of them plant, and some of them humans. They cause infectious diseases. The disease may spread from person to person by blood transmission and other body fluids due to unsanitary conditions. Some viral infections can be deadly, some of them cause mild damage. The war against viruses and other germs continues by developing drugs and vaccines. While we fight against the germ, they continue to fight back by gaining resistance and changing their genome. Therefore COVID 19 is not the last, not the least virus humanity will challenge with, other pandemics will be in the future. Therefore, we all have to strengthen our research infrastructure, do planning and preparedness to ensure the capacities for pandemic response.

REFERENCES

Alfani, G., & Murphy, T. E. (2017). Plague and lethal epidemics in the pre-industrial world. The Journal of Economic History, 77(1), 314-343.

Ayalon, Y. (2015). Natural Disasters in the Ottoman Empire. Cambridge University Press.

Benedictow, O. J. (1992). Plague in the Late Medieval Nordic Countries. Oslo, Middlealderforlaget.

Bhattacharjee, M. K. (2016). Antibiotics That Inhibit Nucleic Acid Synthesis. In Chemistry of Antibiotics and Related Drugs (pp. 109-128). Springer, Cham.

Bregu, M., Draper, S. J., Hill, A. V., & Greenwood, B. M. (2011). Accelerating vaccine development and deployment: report of a Royal Society satellite meeting. Philosophical Transactions of the Royal Society B: Biological Sciences, 366(1579), 2841-2849.

De Haan, C. A., Kuo, L., Masters, P. S., Vennema, H., & Rottier, P. J. (1998). Coronavirus particle assembly: primary structure requirements of the membrane protein. Journal of Virology, 72(8), 6838-6850.

Deb, B., Shah, H., & Goel, S. (2020). Current global vaccine and drug efforts against COVID-19: Pros and cons of bypassing animal trials. Journal of Biosciences, 45(1), 1-10.

Dowling, A., O'Dwyer, J., & Adley, C. (2017). Antibiotics: mode of action and mechanisms of resistance. Antimicrobial research: Novel Bioknowledge and Educational Programs, 1, 536-545.

Dugassa, J., & Shukuri, N. (2017). Review on antibiotic resistance and its mechanism of development. Journal of Health, Medicine and Nursing, 1(3), 1-17.

Edwards, K., & Orenstein, W. (2021). COVID-19: Vaccines to prevent SARS-CoV-2 infection. U: UpToDate [Internet].

Epand, R. M., Walker, C., Epand, R. F., & Magarvey, N. A. (2016). Molecular mechanisms of membrane targeting antibiotics. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1858(5), 980-987.

Excler, J. L., Saville, M., Berkley, S., & Kim, J. H. (2021). Vaccine development for emerging infectious diseases. Nature Medicine, 27(4), 591-600.

Fitzgerald, D., & Haas, D. W. (2005). *Mycobacterium tuberculosis*. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 6th ed. Ed. New York: Elsevier/Churchill Livingstone, pp. 2852-2883.

Hafeez, A., Ahmad, S., Siddqui, S. A., Ahmad, M., & Mishra, S. (2020). A review of COVID-19 (Coronavirus Disease-2019) diagnosis, treatments, and prevention. EJMO, 4(2), 116-125.

Hinnebusch, B. J. (1997). Bubonic plague: a molecular genetic case history of the emergence of an infectious disease. Journal of Molecular Medicine, 75(9), 645-652.

Hobday, R. A., & Cason, J. W. (2009). The open-air treatment of pandemic influenza. American Journal of Public Health, 99(S2), S236-S242.

Huang, X., Wei, F., Hu, L., Wen, L., & Chen, K. (2020). Epidemiology and clinical characteristics of COVID-19. Archives of Iranian Medicine, 23(4), 268-271.

Humphries, M. O. (2013). The last plague: Spanish influenza and the politics of public health in Canada. University of Toronto Press.

Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. Journal of Anaesthesiology, Clinical Pharmacology, 33(3), 300.

Kausar, S., Said Khan, F., Ishaq Mujeeb Ur Rehman, M., Akram, M., Riaz, M., Rasool, G., ... & Malik, A. (2021). A review: Mechanism of action of antiviral drugs. International Journal of Immunopathology and Pharmacology, 35, 20587384211002621.

Klompas, M. (2021). Understanding Breakthrough Infections Following mRNA SARS-CoV-2 Vaccination. JAMA.

Kotra, L. P. (2007). Infectious Diseases. xPharm: The Comprehensive Pharmacology Reference, 1–2.

Kumar, D., Malviya, R., & Sharma, P. K. (2020). Corona virus: a review of COVID-19. EJMO, 4(1), 8-25.

Lauring, A. S., & Hodcroft, E. B. (2021). Genetic variants of SARS-CoV-2—what do they mean?. Jama, 325(6), 529-531.

Littman, R. J. (2009). The plague of Athens: epidemiology and paleopathology. Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine, 76(5), 456-467.

Littman, R. J., & Littman, M. L. (1973). Galen and the Antonine plague. The American Journal of Philology, 94(3), 243-255.

Liu, L., Wang, T., & Lu, J. (2016). The prevalence, origin, and prevention of six human coronaviruses. Virologica Sinica, 31(1), 94-99.

Liu, M. A. (2019). A comparison of plasmid DNA and mRNA as vaccine technologies. Vaccines, 7(2), 37.

Lofquist, J. M., Weimert, N. A., & Hayney, M. S. (2003). Smallpox: a review of clinical disease and vaccination. American Journal of Health-System Pharmacy, 60(8), 749-756.

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., ... & Tan, W. (2020). Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet, 395(10224), 565-574.

Marien, G. (2009). The Black Death in Early Ottoman Territories: 1347-1550 (Doctoral dissertation, Bilkent University).

Masters, P. S. (2006). The molecular biology of coronaviruses. Advances in Virus Research, 66, 193-292.

Mc Dermott, P. F., Walker, R. D., & White, D. G. (2003). Antimicrobials: modes of action and mechanisms of resistance. International Journal of Toxicology, 22(2), 135-143.

McCoy, L. S., Xie, Y., & Tor, Y. (2011). Antibiotics that target protein synthesis. Wiley Interdisciplinary Reviews: RNA, 2(2), 209-232.

Moule, M. G., & Cirillo, J. D. (2020). Mycobacterium tuberculosis dissemination plays a critical role in pathogenesis. Frontiers In Cellular and Infection Microbiology, 10, 65.

Mousavizadeh, L., & Ghasemi, S. (2021). Genotype and phenotype of COVID-19: Their roles in pathogenesis. Journal of Microbiology, Immunology and Infection, 54(2), 159-163.

Needham, J., & Lu, G. D. (2000). Science and Civilisation in China. Volume 6, Biology and Biological Technology. Part VI, Medicine (p. xviii261). Cambridge University Press.

Noh, J., & Danuser, G. (2021). Estimation of the fraction of COVID-19 infected people in US states and countries worldwide. PloS One, 16(2), e0246772.

Patterson, K. B., & Runge, T. (2002). Smallpox and the native Americans. The American Journal of The Medical Sciences, 323(4), 216-222.

Piret, J., & Boivin, G. (2020). Pandemics throughout history. Frontiers In Microbiology, 11.

Plotkin, S. A. (Ed.). (2011). History of vaccine development. Springer Science & Business Media.

Prasad, R., Shah, A. H., & Rawal, M. K. (2016). Antifungals: mechanism of action and drug resistance. Yeast Membrane Transport, 327-349.

Ragab, D., Salah Eldin, H., Taeimah, M., Khattab, R., & Salem, R. (2020). The COVID-19 cytokine storm; what we know so far. Frontiers In Immunology, 11, 1446.

Rampersad, S., & Tennant, P. (2018). Replication and expression strategies of viruses. Viruses, 55.

Shaw-Taylor, L. (2020). An introduction to the history of infectious diseases, epidemics and the early phases of the long-run decline in mortality. The Economic History Review, 73 (3), 1-19.

Shereen, M. A., Khan, S., Kazmi, A., Bashir, N., & Siddique, R. (2020). COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. Journal of Advanced Research, 24, 91.

Shimizu, M. (2019). Clinical features of cytokine storm syndrome. In Cytokine storm syndrome (pp. 31-41). Springer, Cham.

Siddell, S. G., Walker, P. J., Lefkowitz, E. J., Mushegian, A. R., Adams, M. J., Dutilh, B. E., ... & Davison, A. J. (2019). Additional changes to taxonomy

ratified in a special vote by the International Committee on Taxonomy of Viruses (October 2018). Archives of Virology, 164(3), 943-946.

Smith, I. (2003). Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. Clinical Microbiology Reviews, 16(3), 463-496.

Song, Y., Zhang, M., Yin, L., Wang, K., Zhou, Y., Zhou, M., & Lu, Y. (2020). COVID-19 treatment: close to a cure? –a rapid review of pharmacotherapies for the novel coronavirus. International Journal of Antimicrobial Agents, 106080.

Talbot, E. A., & Raffa, B. J. (2015). Mycobacterium tuberculosis. In Molecular medical microbiology (pp. 1637-1653). Academic Press.

Taylor, L. H., Latham, S. M., & Woolhouse, M. E. (2001). Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 356(1411), 983-989.

V'kovski, P., Kratzel, A., Steiner, S., Stalder, H., & Thiel, V. (2021). Coronavirus biology and replication: implications for SARS-CoV-2. Nature Reviews Microbiology, 19(3), 155-170.

van Riel, D., & de Wit, E. (2020). Next-generation vaccine platforms for COVID-19. Nature Materials, 19(8), 810-812.

Wagner, D. M., Klunk, J., Harbeck, M., Devault, A., Waglechner, N., Sahl, J. W., ... & Poinar, H. (2014). *Yersinia pestis* and the Plague of Justinian 541–543 AD: a genomic analysis. The Lancet Infectious Diseases, 14(4), 319-326.

Wang, M. Y., Zhao, R., Gao, L. J., Gao, X. F., Wang, D. P., & Cao, J. M. (2020). SARS-CoV-2: structure, biology, and structure-based therapeutics development. Frontiers In Cellular and Infection Microbiology, 10.

Wells, K., Morand, S., Wardeh, M., & Baylis, M. (2020). Distinct spread of DNA and RNA viruses among mammals amid prominent role of domestic species. Global Ecology and Biogeography, 29(3), 470-481.

Wheelis, M. (2002). Biological warfare at the 1346 siege of Caffa. Emerging Infectious Diseases, 8(9), 971.

Woo, P. C., Huang, Y., Lau, S. K., & Yuen, K. Y. (2010). Coronavirus genomics and bioinformatics analysis. Viruses, 2(8), 1804-1820.

Woo, P. C., Lau, S. K., Huang, Y., & Yuen, K. Y. (2009). Coronavirus diversity, phylogeny and interspecies jumping. Experimental Biology and Medicine, 234(10), 1117-1127.

Woolhouse, M. E., Brierley, L., McCaffery, C., & Lycett, S. (2016). Assessing the epidemic potential of RNA and DNA viruses. Emerging Infectious Diseases, 22(12), 2037.

Zaman, S. B., Hussain, M. A., Nye, R., Mehta, V., Mamun, K. T., & Hossain, N. (2017). A review on antibiotic resistance: alarm bells are ringing. Cureus, 9(6).

Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., ... & Shi, Z. L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 579(7798), 270-273.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., ... & Tan, W. (2020). A novel coronavirus from patients with pneumonia in China, 2019. New England Journal of Medicine.

CHAPTER 2

GENETIC INTERPLAY BETWEEN THE DAIRY *STREPTOCOCCUS THERMOPHILUS* AND ITS PHAGES

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INTRODUCTION

Streptococcus thermophilus has long been exploited as a milk clotting agent, starter, especially of yogurt manufacture, and recently, to a much lesser extent, in cheese production. This species has often been encountered in raw milk that constitutes its main natural source. It has generally been accepted that this species came to being very recently, as late as 30,000 years. Its genome has been considered at two levels of organization: the one includes genes indispensable for its survival, and the other, the remainder, may not be essential. Approaches employing computational prediction methods have evidenced that three main driving forces of evolution in prokaryotes, conjugation, competence, and transduction, have all played substantial roles in the shaping of its genome. These studies have also revealed 362 atypical genes of exogenous origin that could have been inserted into the genome of this new species through horizontal gene transfer (HGT) phenomena (Eng et al., 2011).

The production process of industrial yogurts involves the exploitation of two species of the lactic acid bacteria (LAB), *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus*. These starter species protocooperate by reciprocating their specific needs during the fermentation process of milk into yogurt (Fig. 1), and they appear to owe these precious attributes to the mutual events of horizontal gene transfer (Liu et al., 2009).

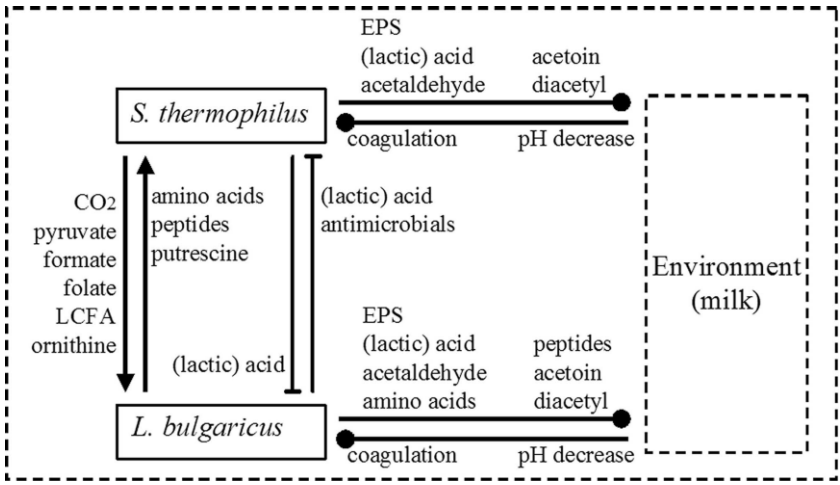

Figure 1. Schematic Illustration Of The Biochemical Interplay Taken Place Between The Two Yogurt Starters (Sieuwerts et al., 2008).

Genomes of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* have been unraveled for some time now, and this information has been used to identify the genetic basis, established through the genomewide horizontal gene transfer (HGT) events, of the biochemical interplay. HGT involves the interchange of DNA segments of chromosomal- and/or episomal origin between interspecies prokaryotes. It serves as a means of cohabitation, which is largely made possible by diverse biochemical activities of distantly related species (Nicolas et al., 2007; Liu et al., 2009).

In silico efforts, comparing genomes of three *S. thermophilus* strains, LMG 18311, CNRZ1066, and LMD9, have provided insightful clues on the HGT events, through which sets of genes, *cbs-cblB(cglB)-cysE*, for example, had been inserted into *S. thermophilus* (Table 1). This gene cluster has been found to be involved in the metabolism of amino acids, cysteine, and methionine. Data produced have also suggested

that this species has evolved together with *Lb. delbrueckii* subsp. *bulgaricus* (Liu et al., 2009).

Cysteine biosynthesis commences with the conversion of L-serine to *O*-acetylserine by the product of *cysE* gene, serine acetyltransferase, and the products of *cglB* and *cbs* convert homocysteine to cysteine (Fig. 2). It has been implied that these genes had originally resided in *Lb. delbrueckii* subsp. *bulgaricus* as a shorter, truncated form and that it had been disabled in the context of cysteine and methionine production in this organism (Liu et al., 2009).

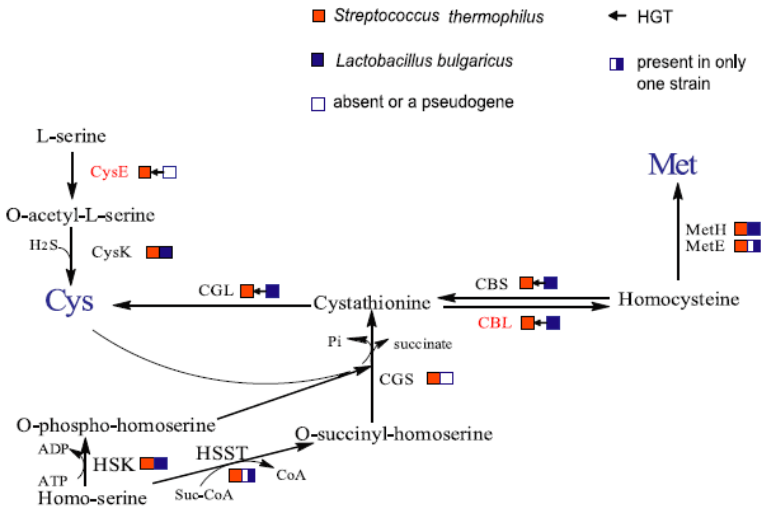

Figure 2: The Role Of HGT In The Synthesis Of Cysteine And Methionine In *S. thermophilus.*

Table 1: The Genes, epsIM and epsIL, Responsible For The Exopolysaccharide Synthesis Genes, On The Other Hand, Have Been Reported To Have Been Transferred From *S. thermophilus* Into *Lb. delbrueckii* subsp. bulgaricus And It Has Been Suggested That This Event Might Have Been Mediated Through Physical Contacts During Cocultivation Or In Yoghurt (Liu et al., 2009).

Gene cluster with	Gene ID(s) for strain:			GC content (%)	δ Value (10^3)	6 Plot position (%)	HGT mechanism-associated feature(s)	Function(s)
	LMG 18311	CNRZ1066	LMD9					
Low GC content								
S1	0098, 0099, 0100, 0102, 0103, 0108	0098, 0099, 0100, 0102, 0103	0131, 0133, 0134, 0135	30	64	75	Transposase, phage integrase	Lantibiotic/bacteriocin biosynthesis protein or exporter, phage integrase, and hypothetical proteins
S2			0141, 0142, 0143, 0144, 0145	36	90	98	Transposase	ABC-type peptide transport system
S3			0146, 0148, 0149, 0150	36	63	75	Transposase	Bacteriocin exporter, EPS-related protein
S4	0182, 0183	0182,[a] 0183		30	102	97		Transcriptional regulator, putative protein kinase
S5	0324, 0325, 0328	0324, 0325, 0328	1694	28	56	49	Transposase	ABC-type transporter, hypothetical protein
S6		0683, 0684, 0685, 0686, 0687, 0688, 0689, 0690		27	73	89	Transposase	Hypothetical proteins
S7	0706, 0707, 0709	0706, 0707, 0709		29	107	98	Phage	Hypothetical proteins
S8			0811, 0812, 0814, 0817	31	103	88	Transposase, phage	Hypothetical proteins
S9		0774, 0782		32	125	85	Phage	Hypothetical protein, phage-associated proteins
S10			1057, 1059, 1060, 1061, 1062, 1066	30	112	99.7	Transposase	EPS biosynthesis
S11	1041, 1042, 1043, 1044	1037, 1040, 1041, 1042, 1044		29	48	57	Transposase	UDP-*N*-acetylglucosamine enolpyruvyl transferase, regulator for MutR family, hypothetical protein, tyrosyl-tRNA synthetase
S12		1077, 1078, 1079, 1080, 1081, 1082		30	67	83	Transposase	EPS biosynthesis
S13	1091, 1092, 1093, 1094, 1095, 1096, 1097, 1098, 1099, 1100, 1102			30	84	99	Transposase	EPS biosynthesis
S14			1296, 1297, 1298, 1299, 1300, 1301	27	94	98		Macrolide efflux protein, peptidase F, regulator for MutR family, hydrolase, hypothetical proteins
S15			1328, 1329	29	68	30		UDP-*N*-acetylglucosamine 2-epimerase, hypothetical protein
S16	1393	1393	1351. 1352. 1355. 1356, 1358	30	71	90	Transposase	Multidrug efflux protein, regulator for MutR family, hypothetical proteins
S17		1479, 1480	1441, 1442, 1443	30	48	46		Glycosyltransferase involved in cell wall biogenesis and transcriptional activator *amrA*
S18	1481, 1484, 1486		1445	31	69	78		Hypothetical membrane proteins
S19			1474, 1475, 1476, 1477	31	66	89		CRISPR system-related proteins
S20	1512, 1514	1512, 1514		29	112	94		Hypothetical proteins
S21			1693, 1698	30	64	51	Transposase	Regulator for Xre family, abortive infection phage resistance protein
S22		1943, 1944	1915, 1916	27	89	87	Transposase	Bacteriocin-related proteins
S23	1947, 1948, 1949, 1950, 1951	1947, 1948, 1949, 1950, 1951	1919, 1920, 1921, 1922, 1924	28	88	99	Transposase	Regulator for MutR family and ABC transporter, putative protein kinase, hypothetical protein
S24	1976, 1977, 1978, 1983, 1989	1976, 1977, 1978, 1983, 1989	1955, 1955, 1960, 1966	29	70	49	tRNA	Conserved hypothetical proteins
High GC content								
S25	0040, 0041	0040, 0041	0058, 0059	49	75	67	Transposase	Purine metabolism
S26	0846, 0847, 0848	0846, 0847, 0848	0885, 0886, 0887	43	148	99.4	Transposase	Cys/Met metabolism
S27			1200, 1201	46	66	46	Transposase	Histidine synthesis
S28	1680, 1685	1685		48	161	83	Transposase	Putative bacteriocin

1. PHAGE GROUPS OF *STREPTOCOCCUS THERMOPHILUS*

Infection of a bacterium starts with phage adsorption on the surface of the host that necessitates the presence of a specific receptor and a phage ligand, sometimes called "anti-receptor". As in most of the other topics of molecular biology, insight gained in this area has been derived from studies on the infection of *E. coli* with its specific phages (Goldberg et al., 1994). Infections with T4 phage for example is largely mediated by the protein of *orf37* gene, gp37 (Wood et al., 1994). This protein constitutes the large subunit of the distal part of the T4 tail and performs the act of an anti-receptor via its C-terminus in a wide host range. Three of its known receptors are B lipopolysaccharide, OmpC, and OmpF (Te´tart et al., 1998). The phage λ anti-receptor, protein J, also interacts with its receptor through its carboxy-terminus. These two phage proteins, thus, appear to be the main determinants of the host specificity (Duplessis and Moineau, 2001).

Mostly *Siphoviridae* family of phages have been isolated from *S. thermophilus* cultures and they have been divided according to the ends and the packaging mode of their genome into two groups, cohesive or headful (*cos* and *pac*, respectively), (Le Marrec et al., 1997) (Fig. 3). Later on, a third group, known as 5093-like phages, have been included (Mills et al., 2011; Mahony et al., 2014). As could be seen below, so far, the number of groups increased to five.

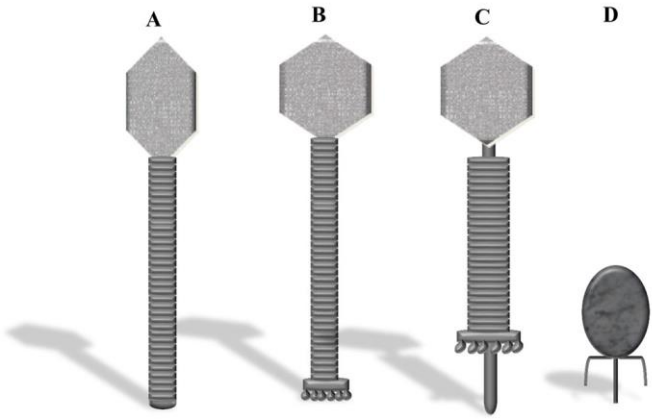

Figure 3: Morphotypes of Common LAB Phages. A: *Siphoviridae* (With Prolate-Head). B: *Siphoviridae* (Isometric-Head). C: *Myoviridae* Phages (With Long Contractile Tail). D: *Podoviridae* (With Short Non-Contractile Tails) (Mahony et al., 2014).

The first group, *cos*, has two major structural proteins (MSPs of 27 and 32 kDa). The latter, packaging, group has been characterized with three MSPs, having molecular weights of 15, 25, and 43 kDa). Computational analyses of the seven phage genomes have suggested that the ordering of the phage genes had a modular structure and that their evolution has followed a modular course (Figs. 4 and 5) (Duplessis and Moineau, 2001).

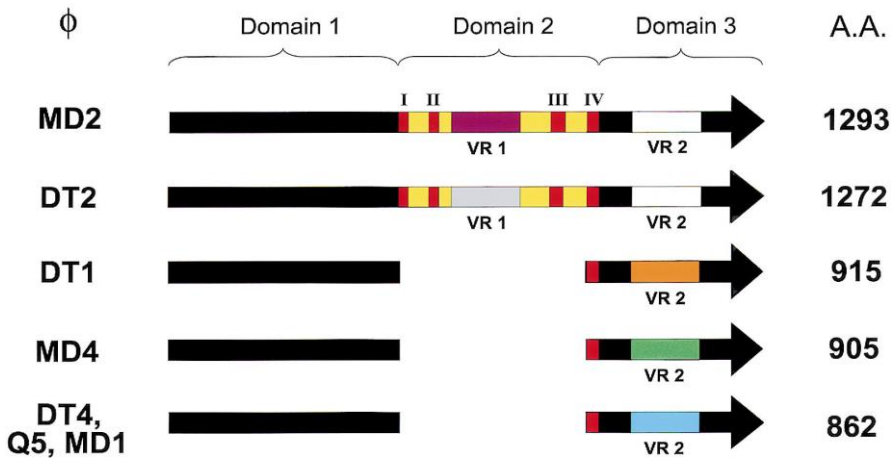

Figure 4: The Organisation Seven ORF18s. VR1, VR2: Variable Regions. Motifs I, II, III and IV: Collagen-Like Repeats.

One of the well-characterized *S. thermophilus* phages, DT1, consists of an isometric head, 60nm in diameter, and a 260nm long, non-contractile tail with 8 nm diameter. Its genome is 34 820 bp linear DNA with cohesive ends. It has two major structural proteins with molecular masses of 26 and 32 kDa. The genome is poor in guanine and cytosine, approximately 39%. So far 46 open reading frames (ORFs) have been mapped, and 20 of these putative functions have been allotted. The protein encoded by *orf18* could be acting as the anti-receptor (Tremblay and Moineau, 1999) because the weight and location of its gene appear to be the same as the gpJ and J gene in Lambda phage. The two proteins also have the same isoelectric (pI) points. Some evolutionary features of *orf18* also has resemblances to those of the other phages in terms of the distribution of conserved regions (Te´ tart et al., 1996; 1998; Lucchini et al., 2000). Furthermore, ORF18 of DT1 has similar amino acid composition at its N-terminus to those of seven other phages, including DT4, MD1,

MD2, and Q5, in which coding sequences for collagen-like repeats has been mapped. The carboxy-terminal region of ORF18 also included collagen-like repeats and a variable region (VR2, 145 amino acids) (Duplessis and Moineau, 2001).

Genomic sequencing studies and following computational analyses have shown that the genome of an *S. thermophilus* phage is organised into four large modules: (1) containing genes responsible for the packaging of DNA and tail genes, with *cos* configuration (Sfi21 type) or with *pac* configuration (Sfi11 type) (Lucchini et al., 1999); (2) spanning structural tail genes, and the genes of lysis and lysogeny; (3) often a Sfi21-type replication module, divided into two different gene clusters (Desiere et al., 1997), and (4) a 5kb long module, constituting the 3´-end of the genome (Bru¨ssow and Desiere, 2001).

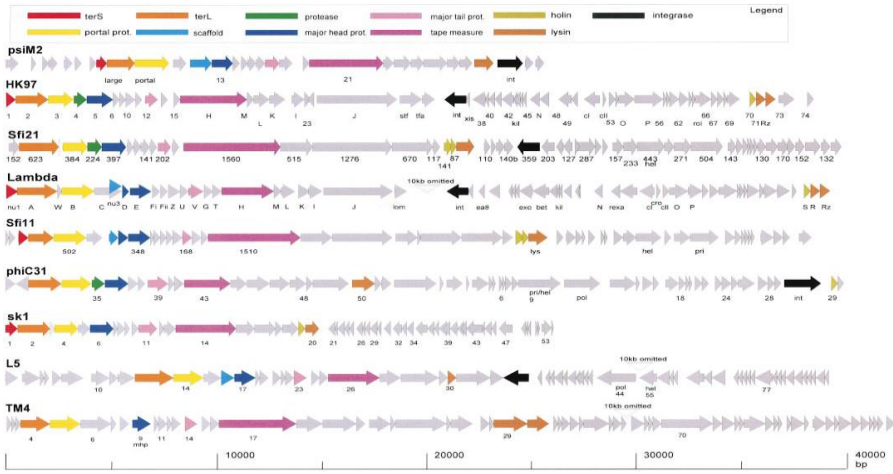

Figure 5: Alignment of The *Siphoviridae* Genomes (Bru¨ssow and Desiere, 2001).

In 2016, a third phage type, forming the group 987 with four members, has been reported. The members appeared to have been the products of hybrid genomes of those phages (Fig. 6.) that have been shown to be able to adsorb onto dairy strains of *S. thermophilus* as well as *Lactococcus lactis.* Thus this discovery has added another dimension to the vulnerability of dairy industries regarding the capability of infectious agents and to the possibilities of countermeasures that should be taken to challenge these devious entities (McDonnell et al., 2016).

Hybrid genomes of the 987 group seemed to have been dependent, within the confines of the dairy industry, on horizontal gene transfer cases, involving the replication- and structural modules of *S. thermophilus* and *L. lactis*, respectively. This group has been morphologically related to those of the *L. lactis* phages. This could be expected but one other striking feature of the new group has been that the genomes of 987 group harbored significant divergence from within, as opposed to the previous data obtained from other groups of the *S. thermophilus* phages. The highly mosaic and diverse nature of the novel hybrid genomes calls to mind both the production of bacteriophage-insensitive mutant (BIM) strains of *S. thermophilus* and the implementation of related rotation programs to alleviate the problem of inadvertent hybrid phage damage in fermentation plants (McDonnell et al., 2016).

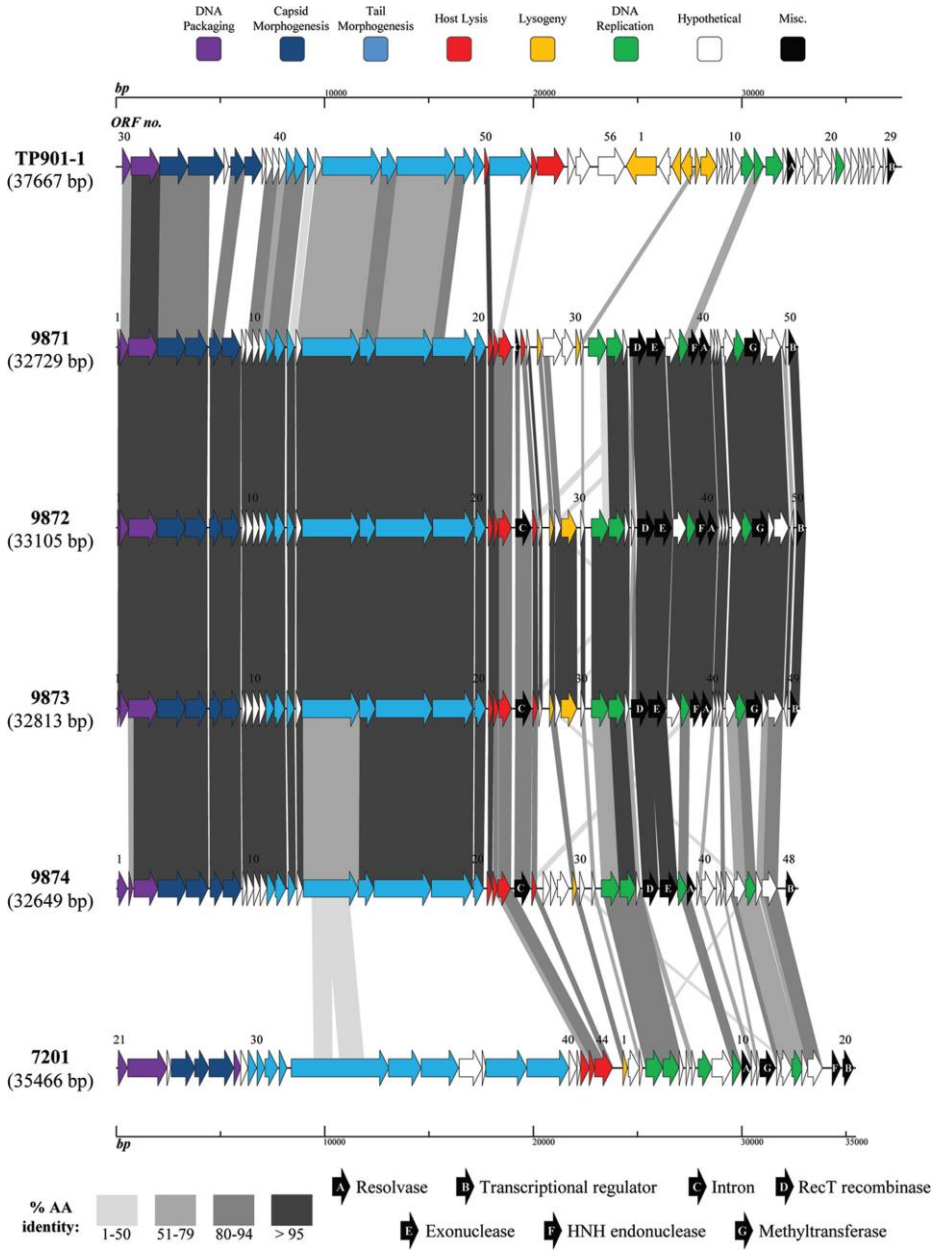

Figure 6: Genomic Organization of Four 987 Phages With Respect To P335 And P7201 (McDonnell et al., 2016).

Arrows: predicted ORFs (their assignments have been indicated with the colored boxes, above). Gene products have been indicated with grey and dark shades (McDonnell et al., 2016).

A fourth type phage assigned for S. thermophilus has been 5093, found in a cheese starter, CSK939. Its genome contained 37,184 bp DNA, with a rather dissimilar sequence composition to those of either *cos* or *pac* group. Surprisingly, however, it has shown a significant similarity to the genomes of other streptococci, suggesting that this phage could have a rather large streptococcal host range and that it has experienced substantial interspecies DNA exchange phenomena by HGT or recombination mechanisms (Mills et al., 2011).

2. PHAGE GENOMIC DIVERSITY

Cos group contains the most comprehensively investigated infectious entities and it has so far been the most recurrent and most diverse group in the dairy industry (Fig. 7). These concordant findings could imply that they hold the longest evolutionary history among the *S. thermophilus* phages. It appears, on the other hand, that such a high diversity in nucleotide sequence has not changed the ordering of the genes (Lavelle et al., 2018).

Pac group also allows a highly significant sequence diversity among its isolated members (Fig. 7). One extremely important feature of this group was that the nucleotide sequence encoding the "the anti-receptor" device displayed very high percentages of dissimilarity. Similarly, gene clusters forming both the modules of lysogeny and

replication seem to have been highly diverged. These findings have also been in concordance with the rather wide host range of the *pac* phages (Lavelle et al., 2018).

Hybrid phage genomes, of 5093 and 987, appear to display lesser diversity from within than those of either *cos* or *pac* phages (Fig. 7). This could be accounted for by the fact that the hybrid phages are much newer entities with respect to the other groups. Nucleotide comparison studies have also revealed that hybrid phages divergence had been generated by the small insertion and deletions (indels) rather than shuffling of the large DNA fragments (Lavelle et al., 2018).

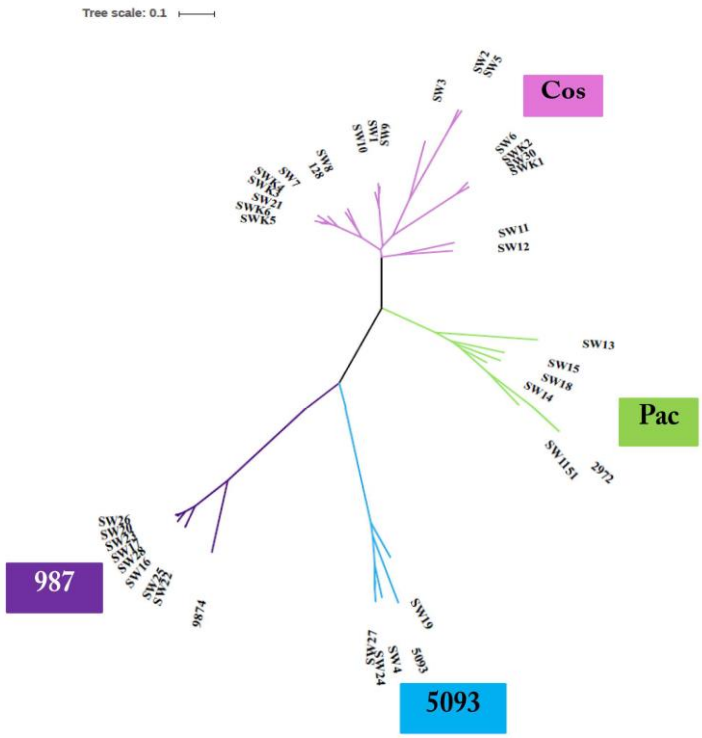

Figure 7: Genomic Diversity of *S. thermophilus* Phage Groups (Lavelle et al., 2018).

Proteomic data, built in a phylogenetic tree (Fig. 8) at first glance seems to differentiate *cos* group into a single phylogenetic entity and that the three of the remaining four groups were evolutionarily closer to the *cos* proteome than that of the *pac* group (Philippe et al., 2020).

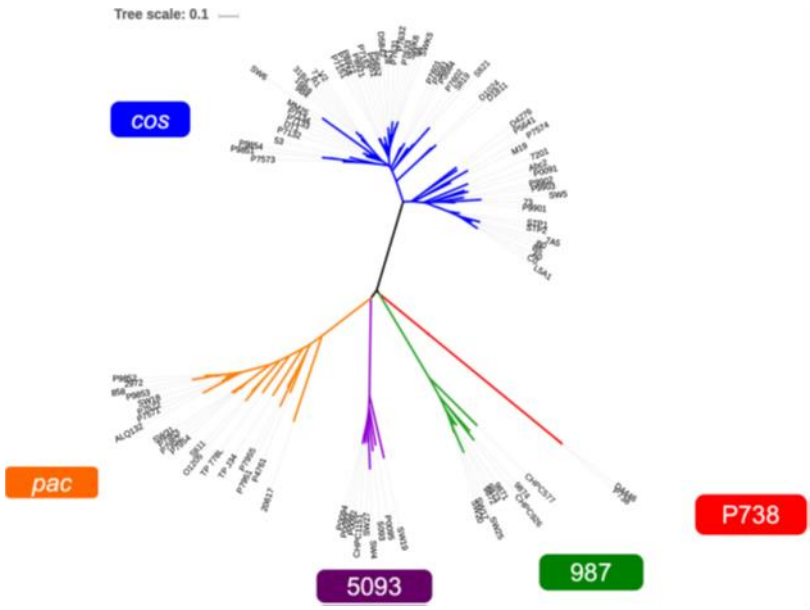

Figure 8: Phylogenetic tree reflecting the proteomic data.

Amino acid comparison studies on the carbohydrate-binding domain of receptor-binding proteins (RBPs) have suggested that these host-specifying devices have evolved independently from the remaining genomic modules in a highly diverse range of phages (Fig. 9). This recalls the possibility to mind that phage modules are not necessarily to have co-evolved. The "independent" evolution could be driven by the gene shuffling mechanism. Hence, phages seem to obtain carbohydrate-binding modules in order to be able to diversify their

host range. Here, the members of *Brussowvirus* and *Moineauvirus* (BVs and MVs, Fig. 10) appear to have served as good donors for the genes of RBP device (Hanemaaijer et al., 2021).

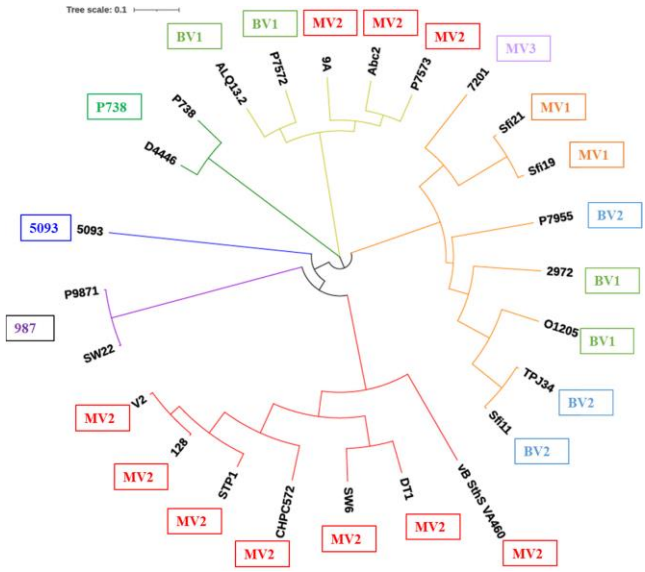

Figure 9: Phylogenetic Tree of Partial RBP Amino Acid Sequence Involved In Carbohydrate Binding.

3. CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS, CRISPR, OF *S. THERMOPHILUS*

Genome projects have mainly aimed at deciphering the genes and their annotations. Later on, it has become clear that spacer DNA residing between the genes loci has been coding functional molecules, other than proteins, named noncoding RNA (ncRNA). One of these chromosomal elements, having the widest distribution and a very diverse loci organization, has been identified in prokaryotes in 1987 and called clustered regularly interspaced short palindromic repeat CRISPR (Ishino et al., 1987; Deveau et al., 2010).

A CRISPR locus is devoid of open reading frames and includes a fixed range, between 2 and 375, of direct repeats with lengths between 21 and 48 bp. The repeats are interrupted by non-repeating spacers, ranging from 26 to 72 bp (Fig. 10). Approximately one-third of the repeats appear to have been shortened. The final repeat at the rightmost end has often been found to be less preserved with respect to the others. The locus is flanked by an A+T rich leader sequence and a promoter at its upstream end. The CRISPR locus is further specified with some functionally related, 4 to 20 genes within its neighborhood, located up- or downstream, that are named *cas* (CRISPR-associated genes). The genes encode for a series of Cas proteins which form Cas endonucleases. These two entities build together a CRISPR/Cas system. As many as 20 and diverse CRISPRs, making up approximately 1% of the genome and can exist as different loci along the prokaryote chromosome. They can also be mapped in plasmid DNA (Deveau et al., 2010).

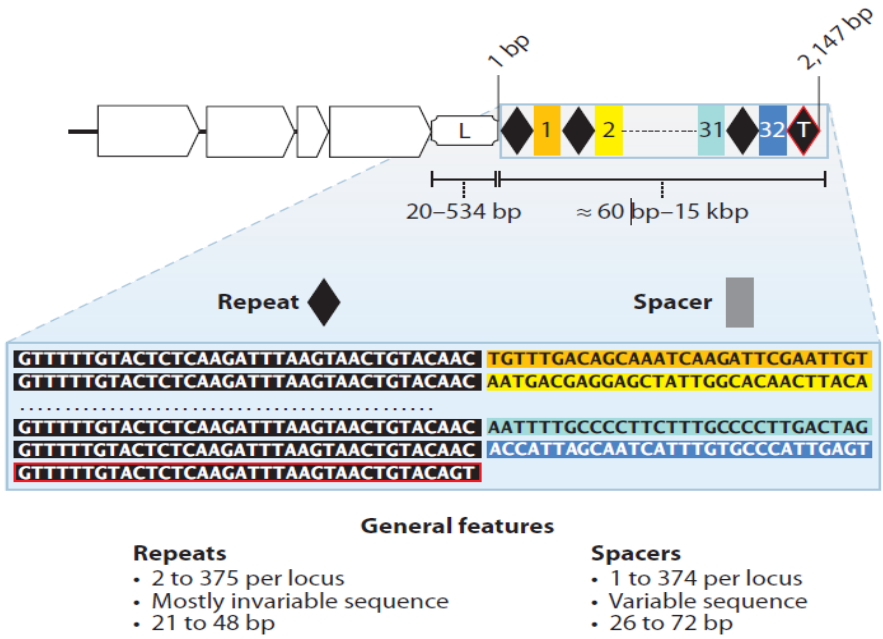

Figure 10: Physical Constitution Of A CRISPR Unit.

CRISPR/Cas loci may constitute a substantial part of the prokaryote immune system against horizontal DNA transfer affected by phage infections and plasmid transformations. This protective system seems to operate via RNA-interference of protein translation (Fig. 11). Palindromic nature of CRISPR enables its transcripts to assume highly firm stem-loop structures, as evidenced for other functional RNA species (Deveau et al., 2010).

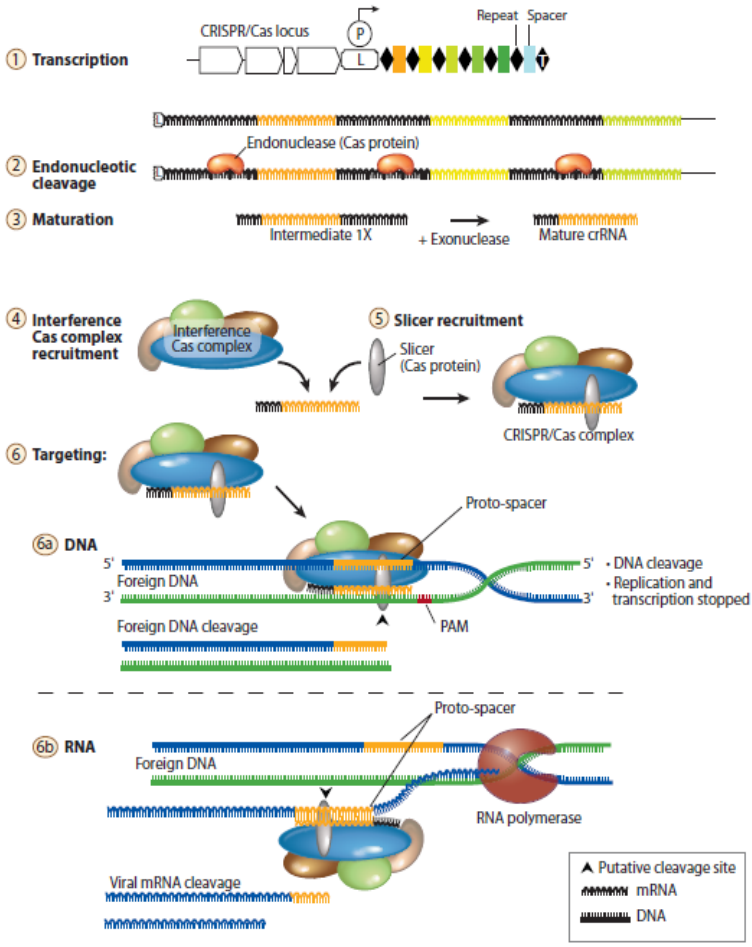

Figure 11: Interference of Translation Through CRISPR RNA.

Unraveling of CRISPR/Cas nucleotide structure and function has prompted some intensive research on *S. thermophilus* to identify or generate bacteriophage-insensitive strains or mutants (BIMs). It has been expected that a "natural", adaptive, immune strain should have experienced phage infections before its isolation. And it has been evidenced that phage resistance could be made possible by the addition of one single repeat 5′ to the CRISPR loci in *S. thermophilus*.

It turns out that a resistant bacterial strain can be infected by a mutant phage. These two findings set a scenario where an interplay might be taking place between the host CRISPRs and phage genome (Fig. 12) (Deveau et al., 2010).

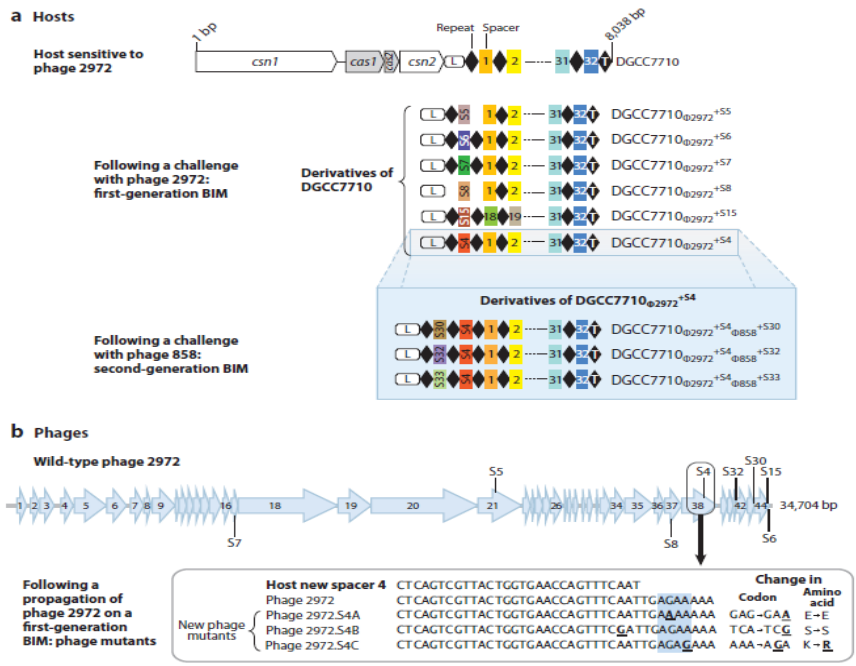

Figure 12: CRISPR Can Mediate A Genetic Interplay Between Dairy *S. Thermophilus* and Its Phages.

Cas1 and Cas2 are expressed in cells harboring CRISPR loci and their abundance appears to be an indicator for the resistance activity of *S. thermophilus* LMD-9 (He et al. 2013; Goh et al. 2011). On the basis of the types of Cas proteins produced, the CRISPR/Cas systems are organized into three subtypes. The presence of Cas3, Cas9, and Cas10, specify the three subtypes I, II, and III, respectively, and they are these are the most frequently found subtypes in *S. thermophilus*. Of the 27

isolates of *S. thermophilus*, more than 96% contained CRISPR/Cas loci (Hu et al., 2020).

Genomic sequencing studies have implied that CRISPR1 (Fig. 13) could be the oldest CRISPR as it has been mapped in 78% of *S. thermophilus* studied and it harboured the highest number of direct repeats (DRs) (Hu et al., 2020).

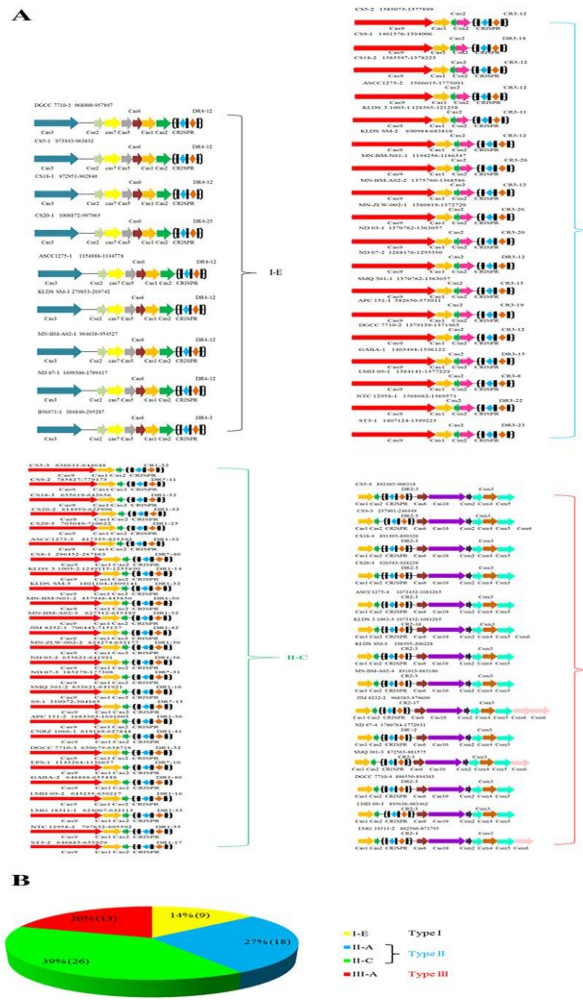

Figure 13: CRISPR Diversity In *S. Thermophilus.*

Seven direct repeats have been mapped in *S. thermophilus* and four of them were most frequently encountered (DR1, DR2, DR3, and DR4), and the remaining three with much fewer frequencies (Fig. 14) (Hu et al., 2020).

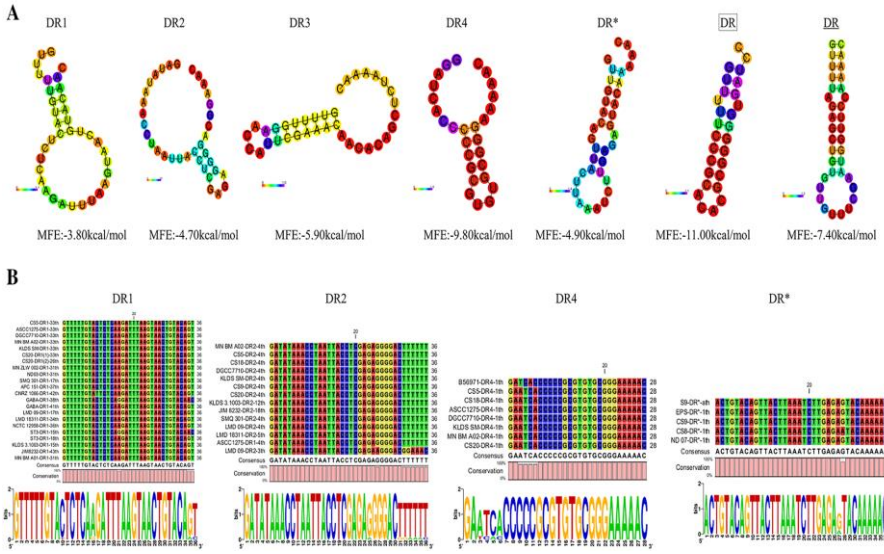

Figure 14: Minimum Free Energy, MFE, Based Secondary Structures of *S.thermophilus* CRISPR RNA.

In the strains of *S. thermophilus*, CNRZ1066, JIM8232, and KLDS 3.1003, the number of spacers differed but their length remained rather similar, between-33 and 35 bp. Again CRISPR1 also appeared to possess the highest number of spacers, while their number was similar in CRISPR3 and CRISPR4 and much lesser in CRISPR2.

Similarity search between CRISPR spacers and available database sequences has revealed that a spacer DNA could be homologous to that of the several *S. thermophilus* phages. The CRISPR-Cas system interferes with phage infections through cutting the phage DNA: upon

invasion by a phage, small pieces of phage DNA are inserted into the host genome as CRISPR spacers. These new spacers could be detected in CRISPR1 and CRISPR3. Then follows transcription of the CRISPR locus and the single-stranded crRNA is produced. This RNA molecule complexes with Cas through its secondary structure, and forms CRISPR nucleoprotein complex (crRNP). The crRNP assumes the role of an endonuclease and is guided by its crRNA to specifically cleave invading phage DNA (Hao et al., 2018).

CONCLUSION

The chapter was prepared with the intention to provide a concise information on the genetics of dairy *S. thermophilus* in relation to its phages. The text was extricated from key references with relevant Figures. In essence, it was tried to make a collection of the key findings on the subject and to reemphasize them together.

It appears that the starter performance of dairy *S. thermophilus* is indispensably dependent on the presence of *Lactobacillus delbrueckii* subsp. *bulgaricus* in the making of yogurt. Evolutionary studies have indicated that the basis of such genetic acquirements is the horizontal gene transfer. Five groups of *S. thermophilus* phages have been established. A *cos* phage, DT1, poses to be the oldest and widest infective agent for dairy *S. thermophilus*. An anti-receptor phage protein, ORF18, has been suggested on the basis of comparative genomic sequence analyses. Such studies have also shed a substantial amount of light as to the physical structures of the phages of dairy *S. thermophilus*. The phage genomes have been found to show

modularity in functional as well as evolutionary senses. Later phage groups seem to possess hybrid genomes that have been established within the confines of dairy industry and involved the infection of both *S. thermophilus* and *Lactococcus lactis*. *Cos* phages form the most diverse and widest group of infectious entities. Nucleotide data have indicated that *cos* and *pac* groups are closer to one another than the other three groups. In the proteomic comparison, on the other hand, *cos* forms one distinct entity, and the remaining four seem to be clustered together in the opposite direction. An even more striking finding has been that the receptor-binding module appeared to have been independent of the remaining three genomic modules in its evolution. Details of the physical features of *S. thermophilus* CRISPR were also reemphasized and text was reinforced with relevant Figures. Here it would be worth reinstating that immune dairy *S. thermophilus* strains could be reinfected with a mutant phage. Three CRISPR subtypes have been suggested for dairy *S. thermophilus* and CRISPR1 appeared to be the oldest. Some secondary structures for CRISPR RNA presented have been generated on the basis of minimum free energy. A schematic representation was also included to show how the CRISPR RNA interfere, through its secondary structure, with the function of translational machinery.

REFERENCES

Bru¨ssow, H., and F. Desiere. (2001). Comparative phage genomics and the evolution of Siphoviridae: insights from dairy phages, *Mol. Microbiol.*, 39, 213–223.

Desiere, F., Lucchini, S., Bruttin, A., Zwahlen, M.-C., and BruÈssow, H. (1997). A highly conserved DNA replication module from *Streptococcus thermophilus* phages is similar in sequence and topology to a module from *Lactococcus lactis* phages, *Virology*, 234, 372-382.

Deveau, H., Garneau, J.E., and Moineau, S. (2010). CRISPR/Cas system and its role in phage-bacteria interactions, *Annu. Rev. Microbiol.*, 64, 475–493.

Duplessis, M. and S. Moineau. (2001). Identification of a genetic determinant responsible for host specificity in *Streptococcus thermophilus* bacteriophages, *Mol. Microbiol.*, 41, 325–336.

Eng, C., Thibessard, A., Danielsen, M., Rasmussen, T.B., Mari, J.F., Leblond, P. (2011). In silico prediction of horizontal gene transfer in *Streptococcus thermophilus*, *Arch. Microbiol.*, 193, 287–297.

Goldberg, E., Grinius, L., and Letellier, L. (1994). Recognition attachment and injection. In Molecular Biology of Bacteriophage T4", Karam, J.D. (ed.). Washington, DC: American Society for Microbiology Press, 347 – 356.

Hanemaaijer, L., Kelleher, P., Neve, H., Franz, C. M. A. P., de Waal, P.P., van Peij, N. N. M. E., van Sinderen, D., Mahony, J. (2021). Biodiversity of Phages Infecting the Dairy Bacterium *Streptococcus thermophilus*, Microorganisms, 9, 1822.

Hao, M., Cui, Y., Qu, X., (2018). Analysis of CRISPR-Cas system in *Streptococcus thermophilus* and its application, Front. Microbiol., 9, 257.

Hu, T., Cui, Y., and Qu, X. (2020). Characterization and comparison of CRISPR Loci in *Streptococcus thermophilus*, Arch. Microbiol., 202, 695–710.

Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., Nakata, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase

isozyme conversion in *Escherichia coli*, and identification of the gene product, J. Bacteriol., 169, 5429–33.

Lavelle, K., Martinez, I., Neve, H., Lugli, G., Franz, C., Ventura, M., et al. (2018). Biodiversity of *Streptococcus thermophilus* Phages in Global Dairy Fermentations, Viruses, 10, 57730.

Le Marrec, C., van Sinderen, D., Walsh, L., Stanley, E., Vlegels, E., Moineau, S., Heinze, P., Fitzgerald, G., Fayard, B. (1997). Two groups of bacteriophages infecting *Streptococcus thermophilus* can be distinguished on the basis of mode of packaging and genetic determinants for major structural proteins, Appl. Environ. Microbiol., 63, 3246-3253.

Liu, M., Siezen, R. J., Nauta, A. (2009). In silico prediction of horizontal gene transfer events in *Lactobacillus bulgaricus* and *Streptococcus thermophilus* reveals protocooperation in yogurt manufacturing, *Ap*pl. Environ. Microbiol., 75, 4120–9.

Lucchini, S., Desiere, F., and BruÈssow, H. (1999). Comparative genomics of *Streptococcus thermophilus* phage species supports a modular evolution theory, J. Virol., 73, 8647-8656.

Lucchini, S., Sidoti, J., and Bru¨ssow, H. (2000). Broad-range bacteriophage resistance in *Streptococcus thermophilus* by insertional mutagenesis, Virology, 275, 267–277.

Mahony, J., Bottacini, F., van Sinderen, D., Fitzgerald, G. F. (2014). Progress in lactic acid bacterial phage research, Microb. Cell. Fact., 13, S1.

McDonnell, B., Mahonym, J., Neve, H., Hanemaaijer, L., Noben, J.P., Kouwen, T., van Sinderena, D. (2016). Identification and analysis of a novel group of bacteriophages infecting the lactic acid bacterium *Streptococcus thermophilus*, Appl. Environ. Microbiol., 82, 5153-5165.

Mills, S., Griffin, C., O'Sullivan, O., Coffey, A., McAuliffe, O. E., Meijer, W. C., Serrano, L. M., Ross, R. P. (2011). A new phage on the 'Mozzarella' block: Bacteriophage 5093 shares a low level of homology with other *Streptococcus thermophilus* phages, Int. Dairy J., 21, 963-969.

Nicolas, P., Bessieres, P., Ehrlich, S. D., Maguin, E., van de Guchte, M. (2007). Extensive horizontal transfer of core genome genes between two *Lactobacillus* species found in the gastrointestinal tract, BMC Evol. Biol., 7, 141.

Philippe, C., Levesque, S., Dion, M. B., Tremblay, D. M., Horvath, P., Lüth, N., et al. (2020). Novel genus of phages infecting *Streptococcus thermophilus*: genomic and morphological characterization, Appl. Environ. Microbiol., 86, 1–40.

Sieuwerts, S., de Bok, F. A., Hugenholtz, J., van Hylckama Vlieg, J. E. (2008). Unraveling microbial interactions in food fermentations: from classical to genomics approaches, Appl. Environ. Microbiol., 74, 4997–5007.

Te´ tart, F., Repolla, F., Monod, C., and Krisch, H.M. (1996). Bacteriophage T4 host range is expanded by duplication of a small domain of the tail fiber adhesin, J. Mol. Biol., 258, 726–731.

Te´tart, F., Desplats, C., and Krisch, H.M. (1998). Genome plasticity in the distal tail fiber locus of the T-even bacteriophage: recombination between conserved motifs swaps adhesin specificity, J. Mol. Biol., 282, 543 – 556.

Tremblay, D.M., and Moineau, S. (1999). Complete genomic sequence of the lytic bacteriophage DT1 of *Streptococcus thermophilus*, Virology, 255, 63–76.

Wood, W.B., Eiserling, F.A., and Crowther, R.A. (1994). Long tail fibers: genes, proteins, structure and assembly. In Molecular Biology of Bacteriophage T4, Karam, J.D. (ed.). Washington, DC: American Society for Microbiology *Press*, 347 – 356.

CHAPTER 3

CURRENT APPROACHES FOR THE DETECTION OF MRSA

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INTRODUCTION

Staphylococcus aureus is a particularly significant microbiological agent for public health due to its capacity to produce a wide range of infectious diseases and its ability to adapt to a variety of environmental situations (Grema et al., 2015). These bacteria cause many diseases such as food poisoning, pneumonia, wound infections, and hospital infections in humans (Lee et al., 2004). *S.aureus* infections are treated with a variety of antibiotics, including penicillin, beta-lactam, and non-beta-lactam antibiotics. However, some strains of *S. aureus* have developed resistance to these antibiotics (Rayner and Munckhof, 2005). Currently, most strains of *S. aureus* are resistant to the penicillin derivative. These are called methicillin-resistant *Staphylococcus aureus* (MRSA) (Grema et al., 2015). It has been reported in many studies that MRSA causes more than four times more morbidity and mortality than antibiotic-susceptible *Staphylococcus* strains (Guk et al., 2017). Methicillin resistance of *S. aureus* is encoded by the mecA or mecC genes on the staphylococcal cassette chromosome (SCC), which is a mobile genetic element (Bayraktar et al., 2021). Due to the horizontal transfer of a mobile genetic element (mecA), MRSA can produce extra penicillin-binding proteins (PBPs) with very low binding to β-lactam group antibiotics compared to normal *S. aureus* PBPs (Xu et al., 2016, Turner et al., 2019). The PBP2a protein is an important MRSA biomarker produced by the mecA gene, facilitating the action of transpeptidase, which changes the bacterial cell wall structure, thus preventing antibiotics

from disrupting cell wall synthesis (IWG-SCC, 2009). Since Staphylococci colonize the skin and mucous membranes of humans and warm-blooded animals, they can infect other humans and animals from these regions, as well as contaminate many food and food processing environments, especially meat and dairy products (Seguin et al., 1999).

As a result of inappropriate and excessive use of antibiotics (especially the beta-lactam group), multi-antibiotic resistant bacterial strains such as MRSA emerge in humans and animals, and they spread in the community, especially from the hands and noses of humans (Askari et al., 2012). In addition, MRSA can be colonized in domestic animals such as cats, dogs, horses, fish, animals living in nature, animal food products such as meat, milk and its products, food processing facilities such as slaughterhouses, hospital environments, and spread to the society from there (Grema et al., 2015). Hospital-acquired MRSA infection (HA-MRSA) and Community-acquired MRSA (CA-MRSA) infections have been reported in many studies, in recent years, livestock-associated MRSA (LA-MRSA) cases have been reported (Khairullah et al., 2020).

The purpose of this book chapter is to review all current methods used in the diagnosis of MRSA and to form a vision for researchers who will work in this field.

1. METHODS FOR THE DETECTION OF MRSA

1.1. Biological (Culture-based) Detection Methods

MRSA isolates of clinical or non-clinical origin can be cultured in basic and selective media with the most conventional method after sampling from the source to be isolated. After growing on basic selective media, resistance to antibiotics such as methicillin and oxacillin can be determined. Today, cefoxitin and cephamycin antibiotics are preferred because they induce penicillin-binding protein 2a (PBP2a) and provide a more sensitive selection for MRSA (Hulme, 2017; Hassoun et al., 2017). MRSA identification with conventional culture methods takes approximately 48-72 hours, but today's commercial media that provide specific MRSA growth with chromogenic color changes shorten this time considerably. Xu et al. reviewed these chromogenic media, Oxoid Brilliance MRSA, CHROMagar MRSA, BBL CHROMagar MRSA, MRSASelect, and chromID MRSA in their article (Xu et al., 2016) (Figure 1). There is also similar commercial media HardyCHROM MRSA selective and differential agar (Spratt et al., 2020). The color and shape of colonies formed by MRSA isolates on commercial media are shown in Figure 1.

Cell culturing by diluted in nutrient broth and cultured on chromogenic agar is the gold standard for MRSA detecting from all types of the specimen (Ermenlieva et al., 2016; Gill, 2020).

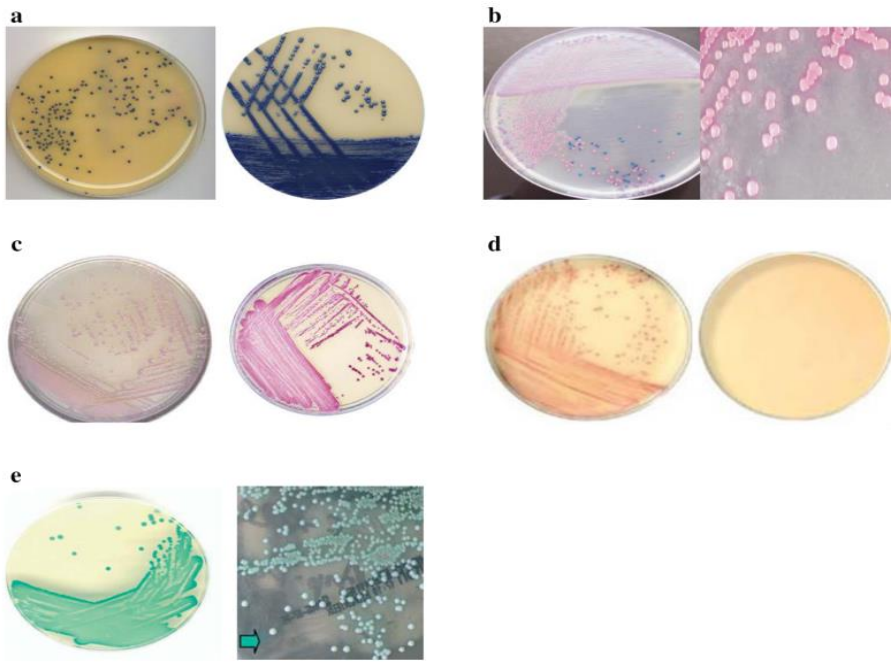

Figure 1. The Color and Morphology Of MRSA Colonies İn Different Chromogenic Media (a. Oxoid Brilliance MRSA, b. CHROMagar MRSA, c. BBL CHROMagar MRSA d. MRSASelect, e. chromID MRSA)

In a study conducted in 2020, Celik et al. reported that RCE agar medium containing peptone, beef extract, NaCl, agar, 4 µg/mL cefoxitin, and red cabbage extract, is significantly suitable for the diagnosis of MRSA and it could be a good alternative to expensive commercial chromogenic media (Celik et al., 2020).

The development of devices that apply automated culture techniques has provided a serious convenience especially for hospitals that need to run a high number of patient specimens in a short period of time. Devices such as Isoplater (Vista Technology Edmonton, Alberta, Canada), Isola 8 (bioMerieux), walk away processor unit (WASP), BD Kiestra TLA system can automatically process patient samples

taken with swabs or specimen containers and inoculate by streaking on agar plates (Karlowsky, 2016; Hulme, 2017).

However, culture-based methods can be expensive in situations such as surgery. In this case, point-of-care (POC)/point-of-need (PON) testing based on molecular-based or various sensor technologies may be a good solution for MRSA detection. The first article stating the advantages of POC tests was published in 2013, and since then, this subject has been studied in many articles by many researchers (Hulme, 2017; van Belkum and Rochas, 2018; Becherer et al., 2020).

1.2. Biochemical Detection Methods

Biochemical methods include several traditional approaches to providing clues in bacterial species identification. Chemical characteristics such as catalase test, oxidase reaction, growth in 10 - 15% sodium chloride, mannitol fermentation, DNase and coagulase tests indole production test, methyl red, triple sugar iron agar, Voges-Proskauer, citrate use, starch hydrolysis, carbohydrate fermentation, nitrate reduction test are used for MRSA detection (Saravanan et al., 2021; Rahimi and Bouzari, 2015). For typing MRSA isolates, easy identification kits are available on the market, such as PhP-RF plates (PhPlate AB, Stockholm, Sweden), API strips, and the BBL Crystal MRSA ID System (Becton Dickinson Microbiology Systems, Maryland, USA) in which various parts of bacterial metabolism are tested by biochemical reactivity (Arbique et al., 2001; Rahimi and Bouzari, 2015; van Belkum and Rochas, 2018).

1.3. Nucleic Acid Amplification-Based Methods

There are many molecular methods developed over the years for the identification of MRSA. These methods involve the isolation of one of the nucleic acids (DNA or RNA) of MRSA (in some cases, molecular tests can be performed from the colony without isolation) and then the amplification (PCR) of specific gene regions (nuc, SCCmec cassette chromosome genes, sodA, PVL, etc.) (Gill et al., 2019). Very small amounts of MRSA DNA can be amplified and detected using diverse technical tools, such as polymerase chain reaction (PCR), real-time PCR, loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), strand displacement amplification (SDA), helicase dependent amplification (HDA), transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), and other similar methods (Yan et al., 2014; van Belkum and Rochas, 2018; Becherer et al., 2020). Another alternative PCR method is multiplex-PCR, which targets amplification of multiple MRSA gene regions and has recently been used to differentiate between MRSA and methicillin-resistant coagulase-negative staphylococci (MR-CoNS) (Gill et al., 2019).

The technology, which is thought to make the newest and most important contributions to the diagnosis and characterization of many microorganisms, especially MRSA, is the new generation sequencing (NGS) technique, which provides the illumination of all primary structures of bacterial chromosome sequences. Whole genome sequencing (WGS) is a method developed with NGS technologies, has

commercial forms accessible to the diagnostic laboratory, and is thought to play an important role in the diagnosis and identification of pathogens and the rapid detection of their epidemiological characteristics from clinical and non-clinical sources without culturing processes (Rossen et al., 2018).

Apart from these methods, DNA hybridization and DNA microarray methods, which have been developed for gene expression analysis of microorganisms isolated from environmental and clinical environments in recent years, have also made a significant contribution to the diagnosis of microorganisms. In addition, These methods are useful not just for detecting pathogens, but also for conducting epidemiological studies that will determine the origin of contamination by genotyping (Saravanan et al., 2021).

In 2017, Guk et al. applied a practical, sensitive and rapid detection method for MRSA using the antibody-like dCas9/sgRNA complex in the CRISPR-mediated DNA FISH method. They also suggested that this approach may be applied for quick identification of all bacteria, not just MRSA (Guk et al., 2017).

1.4. Immunological Methods

MRSA can be diagnosed using immunological approaches depending on the specific binding of antigen and antibody to each other. For the detection of MRSA cells or enterotoxins (such as SE-A, SE-B), there are competitive, direct, or sandwich-based enzyme-linked immunosorbent assay (ELISA) methods. ELFA is an immunologically

based method that is more sensitive to biochemical tests than ELISA. Among various immunoassay techniques such as immunofluorescence, immunoelectrophoresis, radioimmunoassay, and immunodiffusion, ELISA is the most practical approach in diagnosis (Saravanan et al. 2021).

Methicillin resistance in *Staphylococcus* species is mediated by the mecA gene, which encodes penicillin-binding protein 2a (PBP2a). Latex agglutination test, an immunochromatographic method, has been developed as a rapid and inexpensive test for the diagnosis of MRSA. This test uses a monoclonal antibody against the mecA gene product, the PBP2a protein. In the presence of the PBP2a protein, there is a visible agglutination of the antibody-bound latex particles. The latex agglutination test has very high sensitivity and specificity to accurately identify MRSA and differentiate MRSA from MSSA. BinaxNOW and Clearview Exact PBP2a tests are also immunochromatographic methods with similar characteristics (Hassoun et al., 2017).

1.5. Mass Spectrometry Methods

It has been reported in many studies that matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a method that can compete with the gold standard methods for the rapid and reliable identification of many bacteria, especially MRSA. However, since this method requires pure culture, as in most molecular diagnostic methods, a minimum of 24-48 hours is required

for culture. So being a time-consuming method makes MALDI-TOF MS not suitable for point of care testing.

Mass spectrometry (MS) methods, including gas chromatography MS (GC-MS), high-resolution tandem MS (LC-MS/MS), and liquid chromatography-MS (LC-MS), have been used recently for the identification of bacteria. Ionization techniques such as matrix-assisted laser desorption ionization (MALDI-TOF) and electrospray ionization (ESI)-MS easily perform the analysis of various molecules, cells, proteins, and DNA. MALDI-TOF is a solid phase carrier, on the contrary, ESI uses a liquid phase, which makes ESI more compatible with PCR and other amplification methods. In addition, the advantages of ESI include the ability to identify directly from samples without subculture (Hulme, 2017).

1.6. Biosensor Methods

Biosensors are devices that combine the biological recognition element and biological materials with a physical transducer to analyze the biological response by converting it into a measurable signal. The fact that they are very fast compared to alternative methods has put biosensor-based methods ahead of traditional diagnostic methods in POC studies. Due to their extraordinary sensitivity and specificity, biosensors have attracted attention in many scientific industries (Ye et al. 2019).

Biorecognition elements can be enzymes, nucleic acids, or biological molecules such as antibodies. For example, DNA biosensors work by

detecting and processing the signal resulting from nucleic acid hybridization on the sensor surface. It also typically consists of a molecular probe immobilized on the sensor surface, a short DNA sequence that detects the target genetic segment and enables specific biomolecular interaction (Oueslati et al., 2021). Specially prepared nanomaterials provide better conductivity, optical and mechanical properties by providing more surface area, increasing efficiency and compatibility with biomolecules. These properties make nanomaterials an excellent bioreceptor and transducer for biomaterials. A wide variety of biosensors have been developed for the rapid detection of MRSA and many other pathogenic bacteria, for example, optical biosensors, electrochemical biosensors, luminescence-based biosensors. Atal Gill has summarized this subject quite well in his article from his doctoral thesis. (Gill et al., 2019). In Figure 2, the table that compares the advantages and disadvantages of bacterial diagnostic methods prepared by Saravanan et al., which includes various biosensor techniques, is given (Saravanan et al., 2021).

S. no	Detection method	Advantages	Disadvantages
1	Culture-dependent method	Ability to detect single bacterial strain Recognition of viable cells Appropriate toward suitable media	Risk for contamination Intense requirement of time and resource
2	Culture-independent method	Accurate and multiplex detection	High-level skill is required in obtaining results
3	Piezoelectric biosensor	High amplitude and frequency range	Depends on temperature Less energy efficiency
4	Bioluminescence biosensor	Simplicity, inexpensiveness and high intensity	Depends on the process of emission of light
5	Electrochemical biosensor	Portable system, faster response and high stability	Performance is influenced by temperature and ph
6	Optical biosensor	Faster optimization and easy design	Only optimization and no quantification
7	Spectroscopic techniques	Sensitive toward the surface of the molecule	Interference of fluorescence and time-consuming
8	Gas chromatography–mass spectrometry (GC–MS)	Volatile and nonvolatile compounds can be analyzed Accuracy and low running cost	Sample preparation is difficult
9	Enzyme-linked immunosorbent assay (ELISA)	Quick and easy to carry out Highly specific	Difficulty is faced in measuring the enzyme activity
10	Enzyme-linked fluorescent assay (ELFA)	Much sensitive toward biochemical tests	Depends on the emission of fluorescence
11	Immunomagnetic separation (IMS)	Effective isolation of cells from fluids	Preparation of beads is required in the case of magnetic beads separation
12	DNA microarray	High throughput Analysis of multiple genes	Confusing to first time users Results are not reproducible
13	DNA hybridization	Reduced cost, rapid detection	Based on DNA assay
14	Multiplex PCR	Less time and effort consumption	Optimization is difficult and increased cost
15	Real-time PCR	Simple and rapid data analysis	Emission spectra overlapping and nonspecific binding

GC–MS gas chromatography–mass spectrometry, *ELISA* enzyme-linked immunosorbent assay, *ELFA* enzyme-linked fluorescent assay, *IMS* immunomagnetic separation, *DNA* deoxyribonucleic acid, *PCR* polymerase chain reaction

Figure 2. Advantages and Disadvantages of MRSA Detection Methods (Saravanan et al., 2021)

CONCLUSION

MRSA is a pathogen that has been a serious public health problem for decades. Many studies are being carried out to prevent the spread of MRSA in society and to reduce the burden of the difficulties in the treatment, diseases, and deaths caused by this bacterium on the economy. Although there are many effective, sensitive, and practical

methods in the diagnosis of MRSA, many of these methods require qualified technical personnel who can work effectively in the microbiology laboratory and use specific instruments. Although PCR-based diagnostic techniques are highly specific, many require culturing the bacteria to be tested, which is time-consuming. Although PCR-based diagnostic techniques are highly specific and considered the gold standard, they are time-consuming as they require culturing the bacteria to be tested. Although MALDI-TOF, GC-MS, and immunological methods are easy to apply and give results in a short time compared to other applications, it is a fact that they require complex and expensive devices. Biosensors have become very important tools for point of care diagnosis, as they can detect MRSA quickly without the need for culture, are easily applicable and portable. The integration of biosensors with other fields, such as nanotechnology, is an area open to improvement in terms of sensing possibilities for many biological molecules. As a result, the economic situation of places such as research laboratories and hospitals where MRSA diagnosis will be made, the sensitivity of the diagnosis, and the need for timing are effective in determining the diagnostic method to be used.

REFERENCES

Arbique, J., Forward, K., Haldane, D., & Davidson, R. (2001). Comparison of the Velogene Rapid MRSA identification assay, Denka MRSA-Screen assay, and BBL Crystal MRSA ID system for rapid identification of methicillin-resistant *Staphylococcus aureus*. Diagnostic Microbiology and Infectious Disease, 40(1-2), 5-10.

Askari, E., Soleymani, F., Arianpoor, A., Tabatabai, S. M., Amini, A., & NaderiNasab, M. (2012). Epidemiology of mecA-methicillin resistant *Staphylococcus aureus* (MRSA) in Iran: a systematic review and meta-analysis. Iranian Journal of Basic Medical Sciences, 15(5), 1010.

Bayraktar, B., Gündüz, A., Oryaçin, E., Erdemir, D., Teke, L., Aktaş, E., ... & Bozdoğan, B. (2021). Early Detection and Control of Methicillin resistant *Staphylococcus aureus* Outbreak in an Intensive Care Unit. Balkan Medical Journal, *38*(1).

Becherer, L., Borst, N., Bakheit, M., Frischmann, S., Zengerle, R., & von Stetten, F. (2020). Loop-mediated isothermal amplification (LAMP)–review and classification of methods for sequence-specific detection. Analytical Methods, 12(6), 717-746.

Boucher, H., Miller, L. G., & Razonable, R. R. (2010). Serious infections caused by methicillin-resistant *Staphylococcus aureus*. Clinical Infectious Diseases, 51(Supplement_2), S183-S197.

Celik, C., Ildiz, N., Sagiroglu, P., Atalay, M. A., Yazici, C., & Ocsoy, I. (2020). Preparation of nature inspired indicator based agar for detection and identification of MRSA and MRSE. Talanta, 219, 121292.

Diekema, D. J. (2001). SENTRY Partcipants Group: Surver of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis, 32(2), 114-32.

Ermenlieva, N. M., Todorova, T. T., Tsankova, G. S., Popova, T. K., & Georgieva, E. P. (2016). Effectiveness of MRSA detection methods in the laboratory practice–a brief review. Journal of IMAB–Annual Proceeding Scientific Papers, 22(2), 1157-1159.

Gill, A. A. S. (2020). *Synthesis, characterization and application of novel composite nano-materials for the electrochemical detection of MRSA and related drugs* (Doctoral dissertation).

Grema, H. A., Geidam, Y. A., Gadzama, G. B., Ameh, J. A., & Suleiman, A. (2015). Methicillin resistant *Staphylococcus aureus* (MRSA): a review. Adv Anim Vet Sci, *3*(2), 79-98.

Guk, K., Keem, J. O., Hwang, S. G., Kim, H., Kang, T., Lim, E. K., & Jung, J. (2017). A facile, rapid and sensitive detection of MRSA using a CRISPR-mediated DNA FISH method, antibody-like dCas9/sgRNA complex. Biosensors and Bioelectronics, 95, 67-71.

Hassoun, A., Linden, P. K., & Friedman, B. (2017). Incidence, prevalence, and management of MRSA bacteremia across patient populations—a review of recent developments in MRSA management and treatment. Critical Care, 21(1), 1-10.

Hulme, J. (2017). Recent advances in the detection of methicillin resistant *Staphylococcus aureus* (MRSA). BioChip Journal, 11(2), 89-100.

International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). (2009). Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. Antimicrobial Agents and Chemotherapy, 53(12), 4961-4967.

Karlowsky, J. A. (2016). Clinical Microbiology In Vitro Diagnostic Devices in Canada: Regulatory Requirements and Product Information. Manual of Commercial Methods in Clinical Microbiology: International Edition, 491-493.

Khairullah, A. R., Sudjarwo, S. A., Effendi, M. H., Harijani, N., Tyasningsih, W., Rahmahani, J., ... & Riwu, K. H. P. (2020). A Review of Methicillin-

Resistant *Staphylococcus aureus* (MRSA) on Milk and Milk Products: Public Health Importance. Sys Rev Pharm, 11 (8): 59, 69.

Lee, J. H., Jeong, J. M., Park, Y. H., Choi, S. S., Kim, Y. H., Chae, J. S., ... & Eo, S. K. (2004). Evaluation of the methicillin-resistant *Staphylococcus aureus* (MRSA)-screen latex agglutination test for detection of MRSA of animal origin. Journal of Clinical Microbiology, 42(6), 2780-2782.

Oueslati, R., Jiang, Y., Chen, J., & Wu, J. (2021). Rapid and Sensitive Point of Care Detection of MRSA Genomic DNA by Nanoelectrokinetic Sensors. Chemosensors, 9(5), 97.

Rahimi, F., & Bouzari, M. (2015). Biochemical fingerprinting of methicillin-resistant *Staphylococcus aureus* isolated from sewage and hospital in Iran. Jundishapur Journal of Microbiology, 8(7).

Rayner, C., & Munckhof, W. J. (2005). Antibiotics currently used in the treatment of infections caused by *Staphylococcus aureus*. Internal Medicine Journal, *35*, S3-16.

Rossen, J. W., Friedrich, A. W., & Moran-Gilad, J. (2018). Practical issues in implementing whole-genome-sequencing in routine diagnostic microbiology. Clinical Microbiology and Infection, 24(4), 355-360.

Saravanan, A., Kumar, P. S., Hemavathy, R. V., Jeevanantham, S., Kamalesh, R., Sneha, S., & Yaashikaa, P. R. (2021). Methods of detection of food-borne pathogens: a review. Environmental Chemistry Letters, 19(1), 189-207.

Seguin, J. C., Walker, R. D., Caron, J. P., Kloos, W. E., George, C. G., Hollis, R. J., ... & Pfaller, M. A. (1999). Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. Journal of Clinical Microbiology, 37(5), 1459-1463.

Spratt, H., Levine, D., Hanks, J., Price, G., Gentner, K., Brunton, L., ... & Ledbetter, J. (2020). Colonization of Environmental Methicillin-Resistant Staphylococcus Aureus in a Newly Constructed Children's Outpatient Clinic. American Journal of Infection Control, 48(8), S53.

Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., Eichenberger, E. M., Shah, P. P., Carugati, M., ... & Fowler, V. G. (2019). Methicillin-resistant

Staphylococcus aureus: an overview of basic and clinical research. Nature Reviews Microbiology, *17*(4), 203-218.

Välimaa, A. L., Tilsala-Timisjärvi, A., & Virtanen, E. (2015). Rapid detection and identification methods for *Listeria monocytogenes* in the food chain–a review. Food Control, 55, 103-114.

Van Belkum, A., & Rochas, O. (2018). Laboratory-based and point-of-care testing for MSSA/MRSA detection in the age of whole genome sequencing. Frontiers in Microbiology, 9, 1437.

Xu, Z., Hou, Y., Peters, B. M., Chen, D., Li, B., Li, L., & Shirtliff, M. E. (2016). Chromogenic media for MRSA diagnostics. Molecular Biology Reports, 43(11), 1205-1212.

Yan, L., Zhou, J., Zheng, Y., Gamson, A. S., Roembke, B. T., Nakayama, S., & Sintim, H. O. (2014). Isothermal amplified detection of DNA and RNA. Molecular BioSystems, 10(5), 970-1003.

Ye, Y., Guo, H., & Sun, X. (2019). Recent progress on cell-based biosensors for analysis of food safety and quality control. Biosensors and Bioelectronics, *126*, 389-404.

CHAPTER 4

THE SHORT NOTES ON THE BIOLOGY OF HARVESTMEN (OPILIONES, ARACHNIDA) AND TAXONOMIC APPROACHES TO TURKISH HARVESTMEN

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INTRODUCTION

The Opiliones are one of the most populous and most cosmopolitan groups of the Arachnids. Opiliones are found in almost all ecosystems of the world. Most Opiliones live in agro-ecosystems of tropical and temperate regions. They are known as predators and indicators of some insects harmful to agriculture. For this reason, these creatures have been the subject of many studies around the World (Cokendolpher, 1990; 1993; Docherty, 1993; Sunderland and Sutton, 1980; Adams, 1984). Opiliones are active in spring, summer and autumn.

1. SHORT NOTES BIOLOGY OF OPILIONES

Opiliones are represented by about 6,650 species in the world, in 4 suborders, namely Cyphophthalmi, Laniatores, Dyspnoi, and Eupnoi. When looking at the external morphology of Opiliones in general, it is seen that the body is oval or diagonal. In Opiliones, the body consists of two parts, the prosoma, and the opistosoma, as in all other arachnids. Unlike spiders, Opiliones do not have a pedicel that connects the prosoma and opistosoma. Prosoma and opistosoma are fused in Opiliones (Şekil 1).

Figure 1: Opiliones Habitus (Photo was taken by İ. Çorak Öcal)

The first of these parts, the prosoma, consists of six segments and a pair of members emerge from each segment. The first pair of these six pairs of members emerging from the prosoma form the chelicera, the second pair forms the pedipalps, and the remaining four pairs form the walking legs. Prosomal segments fused with each other. However, the fifth and sixth segments show a more distinct segmentation. The pedipalp shows a six-segmented structure. These segments are the coxa (coxa), trochanter (trochanter), femur, patella, tibia, and tarsus, respectively. Their main function is sensory. In addition, the maxillary lobe (gnathobase) of the coxa aids in the ingestion and assimilation of food. Opiliones, like other arachnids clean mouthparts with pedipalps or first walking legs. In Opiliones, the structure of each walking leg consists of seven segments. These are the coxa, trochanter, femur, patella, tibia, metatarsus, and tarsus, respectively. The tubercles on the

leg segments vary according to the species. The complex structure of legs in Opiliones has added an important taxonomic character to them and even made them get their name from this structure. In fact, the famous arachnologist Savory (1938) stated “The study of Opiliones is the study of legs”.

Opiliones move slowly due to the very long second walking legs. For example, they move slower in comparison to spiders and ants on rough, terrain such as tall grasses, lawns, or stream beds. However, they can run very fast in case of danger. In reality, Opiliones use their first, third, and fourth pair of legs in order to walk. The second pair of legs act as the attachment and sensory. Opiliones use their second pair of legs when choosing a habitat. For instance, they track water with their legs.

It has been discovered that Opiliones are severely affected when they lose one of their second legs. (Cloudsley and Thompson, 1958; Sankey and Savory, 1974; Guffey, 1998). When caught by the Opiliones’ predator by their long leg, they can leave the metatarsus and tarsus segments to escape. They are not capable of regeneration.

Opiliones have a pair of eyes, and the eyes are located either in the middle of the prosoma or near the front, in an area called the "ocular area". However, in the Trogulidae family, the eyes are on a pair of projections located in front of the prosoma and extend forward. The spines and teeth on the ocular area are distinctive characteristics in taxonomy. Some species living inside caves do not have eyes (Çorak,

2004). The dark markings on the prosoma often give the appearance of a saddle. These patterns and shapes are used to define the species. However, the degree of pigmentation allows the saddle to show great variation, making it an unreliable taxonomic characteristic.

The ventral surface of the opistosoma is usually devoid of tubercles. However, in species belonging to the *Sabacon* genus, the ventrum is completely covered with hairs. Male and female reproductive organs are normally covered by the genital operculum. Genital operculum consists of 2nd and 3rd sternitis. In Opiliones, the genital opening in both genders is located below the opisthosomal extension extending between the last pair of legs. In females, the genital opening turns into a long egg-laying tube (ovipositor) after fertilization.

Opiliones are omnivores. This situation is known as 'coxi-sternal feedin' and is mainly seen in scorpions. In Opiliones, based on the secretory system, a pair of scent gland openings called 'odoriferous glands' are located at the sides of the prosoma. When Opiliones encounter their predators, it releases a foul-smelling substance from their scent glands to chase them away or gain some time to escape. However, it has also been determined that Opiliones use these glands for copulation by emitting a pleasant smell to attract the opposite individual (Hillyard and Sankey, 1989).

Opiliones cannot move rapidly like other arachnids. Because their long and moreover even longer legs cause them a disadvantage when passing through weed or grass. In Opiliones, the hatched nymphs

resemble the mother. On average, they molt 5-7 times (Martens, 1978; Hilyard and Sankey, 1989). Sexual dimorphism is observed in herbivores. Opiliones are especially active in spring, summer, and autumn.

Opiliones generally prefer moist places. They are frequently encountered in fields, grassland, vineyard, garden, and forest ecosystems. (Figure 2).

Figure 2: Opiliones Habitus, Ilgaz Mountain National Park (Photo was taken by İ. Çorak Öcal)

Opiliones can form large populations, especially in the pasture (Jennings, 1983). Opiliones are found in the hollows of trees in forests, in the rich humus layer on the ground, and sometimes on trees (Figure 3). Opiliones are also found in steppe ecosystems.

Herein they usually live either on leaves, in clumps of grass, or among the leaves of plants such as *Astragalus* and *Verbascum.* The dense grasses located by the rivers or lakes are the environments where Opiliones are most concentrated. They can also be seen under stones in such environments. Opiliones are also found in mountain ecosystems (Ljovuschkin and Starobogatov, 1963). Many species have been recorded in the higher parts of the Alps, the Carpathians, and the Caucasus (Maclde, 1970).

Figure 3: Opiliones Habitus, Ilgaz Mountain National Park (Photo was taken by İ. Çorak Öcal)

Opiliones have been described as omnivorous creatures in nature. Researchers who made the first studies of Opiliones such as Menge (1850) and Henking (1888) claimed that they ate nothing but plants or

dead insects. However, Simon (1879b), O. Pickard-Cambridge (1890b), and Banks (1901) stated in their studies that Opiliones mostly feed on live insects. Rühm (1926) stated that she observed a Phalangium eating a live wasp. Juberthie (1964), Martens (1978b), and Moritz (1993) saw Opiliones primarily as predators, while Gnaspini (1996) described them as omnivores. This shows that the dietary habits of Opiliones differ according to the taxa they belong to. There are 3 subordoes in Turkey, namely Cyphophthalmi, Dyspnoi, and Eupnoi. The dietary habits of the families distributed in Turkey are given in Table 1.

Table 1: Nutritional Habits of Opiliones Families in Turkey

	Food	**References**
Cyphophthalmi		
Sironidae	Dipterans (Calliphora) and beef, and ant pupae.	Juberthie,1964, 1964 joseph1868b,
Eupnoi		
Phalangiidae	Homopterans and dipterans, spiders, eartworms, gastropods ans juices and mushroom.	Silhavy 1942, Adams 1984
Sclerosomatidae	Eartworms, Small lepidopterans, dead ants, spiders, bits, eartworms and gastropods, algae and lichens, acarid mites	Halaj & Cady 2000, Bishop 1949b, sankey 1949b, Parisot 1962, adams 1984
Dyspnoi		
Dicranolasmatidae	Drosophila, lepidopteran larva	Gruber 1993
Ischyropsalididae	Dastropods, dşpterans and dead insect, isopods	Martens 1965, 1978b, 1975c
Nemastomatidae	Collembolans, Homopterans, dipterans isopods, spiders, eartworms, gastropods	Mitov 1988, Sunderland & Sutton 1980, Adams 1984
Trogulidae	Gastropods, dead insect, earthworms	Pabst 1953

Studies have shown that Opiliones mostly feed on soft-bodied small arthropods and other invertebrates. (Edgar, 1971). Their menu includes many insects such as small snails and worms, baby centipedes, spiders, earth mites, jump-tailed, aphids, small hemipters, and homopters (Phillipson, 1960a; 1960; Adams, 1984b). Spiders top the predator list of Opiliones. In fact, some spider species have only Opiliones on their diet (Douglass, 2001). Scorpions, centipedes, and many coleopteras also feed on Opiliones (Blumberg and Crossley, 1983). According to Adams (1984), frogs, some lizards, pointed-nosed mice, badgers, foxes, hedgehogs and fish are also predators of Opiliones.

2. TAXONOMIC APPROACHES TO OPILINES OF TURKEY

To date, 105 Opiliones species belonging to 7 families have been identified from Turkey. However, considering the zoogeographic position of Turkey, it is thought that the diversity of the species is much higher.

2.1. Sironidae Simon, 1879

They are small Opiliones. The body is usually oval shaped. Bodies can be seen in yellow, orange, red, or blackish colors. Their body is around 1-2.5 mm. This group is also known as cave Opiliones. The eyes have been disappeared. It is not a large group in terms of the number of species. It is distributed along Europe and North America.

2.2. Phalangiidae Latraille, 1802

It is the most common family in the world and Turkey. It is the family that almost characterizes Opiliones the best. Their body is about 2.2-12 mm. Their body colors are usually grey or it ranges between light and brown. Prosoma and opisthosoma have a saddle of dark pigments dorsally. The body is soft. The eyes are on the ocular area. The tarsus of the pedipalpus is longer than the tibia. The legs are long: The first and third legs are significantly longer than the body length while the second and fourth legs are at least 1.5 times the body length. The tubercle-like structures on the abdomen and extremities are well developed. There is usually a trident at the anterior margin of the prosoma. In Phalangiidae species, there are odoriferous gland openings that can be seen easily on the anterior sides of the prosoma that can secrete pleasant or bad odor (odoriferous glands). Ovipositor is long and segmented with a bifurcated tip. It has adapted to almost all ecosystems in the world. Southern and southeastern Asia, Middle Asia, Africa, North America, Australia, New Zealand (Pinto-da-Rocha et al., 2007)

2.3. Sclerosomatidae Simon, 1879

It is a small and delicate group. They are approximately 2mm in length. Some neotropical species can be 10mm. The body is oval and often bulged. The ocular area is covered with large or small granules and spins. The ventral apophysis in the chelicera is blunt and curved. The pedipalp is thin and long. The species have all kinds of colors,

from light to dark, and metallic-colored patches, stripes, or spots that are very in size and color on dorsal and ventral surfaces are also present. (Roewer 1953, Martens 1973, Pinto-da-Rocha et.all. 2007). They are distributed along South America, Central and North America, Europe, Africa, and Asia (Pinto-da-Rocha et al., 2007).

2.4. Dicranolasmatidae Simon, 1879

In this group, the body length is between 3.0-6.4 mm. Legs are short. The body flattens towards the dorsal side. Towards the tip of the prosoma, there are two curved projections on a head-like structure. The eyes are right in the middle of these curved projections. The odoriferous gland openings cannot be detected by the eyes. Dorsum is covered with a structure called “scutum magnum”. Opisthosoma is larger than prosoma. There are no salient sequential tubercles. Pedipalps are long and weak in adults. Pedipalps are half the body length. Pedipalps are nailless and there are simple spines on them. The body color is brownish black. Due to a special substance secreted by the body, the external surface is extensively covered with soil particles. They are distributed in Mediterranean countries, the Southern Alps, Carpathians, Eastward to the Caucasus, and Iraq (Pinto-da-Rocha et al., 2007).

2.5. Ischyropsalididae Simon, 1879

They are Opiliones with very long, strong, and large chelicerae. Their length is between 3.8-8.5 mm on average. Chelicerae are usually armed with tubercles and spines. The body is covered with various tubercles. Their legs are of medium length compared to their body.

The pedipalp is thin and long. Ovipositor is unsegmented. Some species live in caves. Members of this genus are restricted to Europe, with many species found in the mountains (Pyrenees, Alps, Carpathian Mountains and Dinaric Mountains). They reach into the Netherlands, northwestern Germany and Poland in the north and to Calabria in the South (Pinto-da-Rocha et al., 2007).

2.6. Nemastomatidae Simon, 1872

The dorsum of Opiliones in this family has a strong and arched structure. Their length is between 1.2-5.6 mm on average. The body has a hard-shelled exoskeleton called the skutum. The ocular area is near the anterior of the scutum and is protruding in some species. In some species belonging to this family, the odoriferous glands are invisible to the eyes. The ovipositor is unsegmented. Their exoskeletons contain fine or thick granules and large or different-shaped structural elements. There are low segmental humps in this area (Cokendolpher, 1984). Occurring in nearly all of Europe, Scandinavia, and Iceland, Urals and Caucasus, North Africa, Asia, Nort America.

2.7. Trogulidae Sundevall, 1833

The body of Opiliones in this family is flattened. Their length is between 2-22 mm on average. The legs are usually short and blunt, and they walk as if they were crawling on the ground or the object they are standing on. In addition, this group of animals is called "slimy Opiliones" because they live in damp grass or under leaves.

They mainly feed on mollusks. The length is approximately 10 mm. The cuticle that covers the body secretes a sticky fluid. This liquid keeps the soil particles attached to the body surface. Therefore, it is difficult to detect many of the species when they are dormant. These species provide camouflage with these features. Unlike the species in the other family, the eyes are not located on semicircular mounds. The structures that are at the front side of the head can be seen as adjacent, separate or crescent shaped. These structures are called "cucullus".

The tubercle-like structures on the abdomen and extremities are not well developed. They live in the soil zone under stones and logs, as well as among moss, grass, and leaves. It is distributed along Europe, Mediterranen, North Africa and Southwest Asia (Pinto-da-Rocha et al., 2007).

RESULTS

There are 6,650 Opiliones species in the world. They commonly prefer moist places. They are frequently encountered in the field, grassland, vineyard, garden, steppe, mountain, and forest ecosystems. Opiliones can form large populations, especially in pastures (Jennings, 1983). Opiliones can be found in the hollows of trees in the forests, in the rich humus layer on the ground, on the trees, among the dense grass near the streams or lakes, under the stones (Ljovuschkin and Starobogatov, 1963). Many species have been recorded in the higher reaches of the Alps, the Carpathians, and the Caucasus (Maclde, 1970). Since they have a significant place among the indicator species and in the ecological chain, studies with Opiliones maintain their

importance. (Sunderland and Sutton, 1980; Adams, 1984; Corak et al., 2014). Studies with opilines have focused especially on systematics and ecology. The used criteria in the identification of Opiliones species are: Location of the teeth, spines, and tubercles found in almost every part of the body, body length, type and color, existence of ocular area, the color of the saddle, pattern shape, and volume, whether it has tergites, leg structures, soil and sand coverage on body surfaces, whether the chelicerae show sexual dimorphism, existence of a false segment in the tarsus segment, presence of a nail at the tip of the pedipalps. However, the most reliable characteristic in the identification of the species is the morphology of the genital organs. The diversity of Opiliones species in Turkey is very low compared to rest of the world. However, considering geographical factors such as Turkey's location being a transition bridge between continents, presence of four seasons, and vegetation, the diversity of species is expected to be much higher. In short, the diversity of Opiliones species in Turkey has not been fully determined yet.

As with many arthropods, European scientists made the first studies on Opiliones. Identification of Opiliones species in Europe coincides with the beginning of the 18th century. As with spiders and scorpions, the first description and classification of Opiliones belong to Linnaeus (1767). Linnaeus named many species and published them in her work "Sytema Nature". After Linnaeus, famous arachnologists such as Fabricius, Degeer, Herbst, Hermann, Kraepelin, Meade, Koch, Thorell added many species to the European Opiliones list. Gruber published

many works on Opiliones in Central Europe, and also by evaluating some specimens collected from Turkey, she recorded a new species (1969).

The first studies by domestic researchers were made as a master's thesis. (Corak, 2004; Kurt, 2004). These studies were followed by systematic, electromicroscopic, and molecular studies. However, despite these studies, Turkey's Opiliones species list has not been completed yet.

REFERENCES

Adams, J. (1984). The habitat and feeding ecology of woodland harvestmen (Opiliones) in England. Oikos, 361-370.

Banks, N. (1901). Synopses of North-American invertebrates. XVI. The Phalangida. The American Naturalist, *35*(416), 669-679.

Bishop, S.C. (1949). *The Phalangida (Opiliones) of New York: With Special Reference to the Species of the Edmund Niles Huyck Preserve, Rensselaerville, New York* (Vol. 9, No. 3). The Academy.

Blumberg, A.Y., & Crossley Jr, D.A. (1983). Comparison of soil surface arthropod populations in conventional tillage, no-tillage and old field systems. Agro-ecosystems, *8*(3-4), 247-253.

Cloudsley-Thompson, J. L. (1958). *Spiders, scorpions, centipedes and mites, the ecology and natural history of woodlice, myriapods and arachnids* (No. 595.4 C56).

Cokendolpher, J.C. (1984). Homonyms of American and European Leiobunum (Opiliones, Palpatores, Leiobuninae). Journal of Arachnology, 12(1): 118–119.

Çorak, İ. (2004). *Anadolu'dan toplanmış otbiçenlerin sistematiği ve biyoekolojisi (Arachnida: Opilionida)* (Master's thesis, Kırıkkale Üniversitesi).

Çorak, Ö.İ., Kayhan, Y.N., Bayram, A. (2014). Biyoindikatör Olarak Otbiçenler, Kırıkkale Üniversitesi Bilimde Gelişmeler Dergisi, 3(1): 57-59.

Douglass, H. (2001). Morse short communication harvestmen as commensals of crab spıders. Journal of Arachnology, *29*, 273-275.

Edgar, A.L. (1971). Studies on the biology and ecology of Michigan Phalangida (Opiliones). Misc. Publs Mus. Zool. 144: 1-64

Gnaspino, P. (1996). Population ecology ofGoniosoma spelaeum, a cavernicolous harvestman from south-eastern Brazil (Arachnida: Opiliones: Gonyleptidae). *Journal of Zoology*, *239*(3), 417-435.

Gruber, J. (1990). Beobachtungen zur Ökologie und Biologie von Dicranolasma scabrum (Herbst)(Arachnida: Opiliones). Teil I. *Annalen des*

Naturhistorischen Museums in Wien. Serie B für Botanik und Zoologie, 393-426.

Gruber, J. (1969). Weberknechte der Familien Sironidae und Trogulidae aus der Turkei.(Opiliones, Arachnida).(Ergebnisse der osterreichisch-turkischen Anatolien-Expeditionen 9). Rev. Fac. Sci. Univ. ‘'Istanbul,(B), 34, 75-88.

Guffey, C. (1998). Leg autotomy and its potential fitness costs for two species of harvestmen (Arachnida, Opiliones). Journal of Arachnology, 296-302.

Halaj, J., & Cady, A.B. (2000). Diet composition and significance of earthworms as food of harvestmen (Arachnida: Opiliones). American Midland Naturalist, 487-491.

Henking, H. (1888). Biologische Beobachtungen an Phalangiden. Zoologischen Jahrbüchern, Systematik, *3*(319), 35.

Hillyard, P.D., & Sankey, J.H. (1989). *Harvestmen: keys and notes for the identification of the species* (Vol. 4). Brill Archive.

Jennings, A.L. (1983). Biogeographical variation in the harvestman Mitopus morio (Opiliones, Arachnida). Journal of Zoology, 200(3), 367-380.

Joseph, G. (1868). Nachtrag zur Beschreibung von Cyphophthalmus duricorius S. 241 seq. Berliner Entomologische Zeitschrift, 12(2), 269-272.

Juberthie, C. (1964). Recherches sur la biologie des Opiliones. *Ann. Spel.*, *19*, 1-238.

Kurt, K. (2014). Updated checklist of harvestmen (Arachnida: Opiliones) in Turkey. Archives of Biological Sciences, 66(4), 1617-1631.

Kurt, K. (2005). Niğde ili ve çevresinde yayılış gösteren Opiliones (Otbiçen)’in (Familya: Gagrellidae, Phalangiidae, Ischyropsalididae) sistematiği (Doctoral dissertation, Yüksek Lisans Tezi, Niğde Üniversitesi Fen Bilimleri Enstitüsü, Niğde).

Linne, C.A. (1767). Systema Naturae, sive Regna tria Naturae systematicae proposita per Classes, Ordines, Genera, Species cum Characteribus & Differentiis. 12 th edit., vol. 1, n 2.

Ljovuschkin, S.I., & Starobogatov, J.I. (1963). Biospeologica sovietica, Cave spiders from Krim and Caucasus. Bulletinof Moscow Society of Nature Evlorers. 68: 41- 51.

Maclde, D.W. (1970). Notes on the distribution of British harvestmen. Bulletin of the British Arachnological Society, 1, 84.

Martens, J. (1973). Opiliones aus dem Nepal-Himalaya. II. Phalangiidae und Sclerosomatidae (Arachnida).

Martens, J. (1978). Weberknechte, Opiliones. *Die Tierwelt Deutschlands. 64 Teil., 464*.

Menge, A. (1850). Über die Lebensweise der Afterspinnen. *Schr. Naturforsch. Ges. Danzig*.

Moritz, M. (1993). 11. Ordnung Opiliones, Weberknechte, Kanker. S. 402–421. *Lehrbuch der Speziellen Zoologie. Begründet von A. Kaestner*, *4*.

Parisot, C. (1962). Étude de quelques opilions de Lorraine. Vie et Milieu, 179-198.

Phillipson, J. (1960). A contribution to the feeding biology of Mitopus morio (F) (Phalangida). The Journal of Animal Ecology, 35-43.

Phillipson, J. (1960). The food consumption of different instars of Mitopus morio (F.) (Phalangida) under natural conditions. The Journal of Animal Ecology, 299-307.

Pinto-da-Rocha, R., Machado,G., & Giribet, G.(2007). "Harvestmen: The Biology of Opiliones". (Harvard University Press: Cambridge, MA.) pp. 597

Pickard-Cambridge, O. 1890b. On the British species of Phalangidae, or Harvestmen. Proc. Dorset. Nat. Hist. Field Club, 11:163-216

Roewer, C. F. (1953). Neotropische Gagrellinae (Opiliones, Arachnidae). *Mitteilungen aus dem Museum für Naturkunde in Berlin. Zoologisches Museum und Institut für Spezielle Zoologie (Berlin)*, *29*(1), 180-264.

Rühm, J. (1926). Über die Nahrung von Phalangium L. Zoologischer Anzeiger, *68*, 154-158.

Sankey, J.H.P. (1949). Observations on the food, enemies and parasites of British harvest-spiders (Arachnida, Opiliones). Entom. Mon. Mag., *85*, 246-247.

Savory, T.H. (1938). Notes on the biology of harvestmen. J. Queckett Microsc. Club, *1*, 89-94.

Sankey, J. H., & Savory, T. H. (1974). British harvestmen. Synopsis of the British fauna, No. 4.

Silhavy, V. (1942). De Aranees family Thomisidae in Balcans Occidental viventibus. *Sbornik Klubu prirodovedeckeho v Brne za rok*, 90-95.

Simon, E. L. (1874). *Les arachnides de France* (Vol. 1). Librarie Encyclopedique de Roter.

CHAPTER 5

THE EXCRETORY SYSTEM OF ORTHOPTERA

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INTRODUCTION

The structures responsible for the excretory system in insects are called Malpighian tubules. The main functions of the Malpighian tubules are removal of nitrogenous waste products from the body, transport, metabolism, and detoxification of organic dissolved substances, regulation and osmoregulation of the ionic composition of the hemolymph (Maddrell, 1969; Jarial, 1990; Gullan and Cranston, 2012; Pal and Kumar, 2012; Chapman, 2013; Gautam and Tapadia, 2014; Rigoni and Conte, 2014; Amutkan et al., 2015). The Malpighian tubules, which are homologous to the nephrons of vertebrates, and the hindgut work together to remove waste products from metabolism in the body (Ramsay, 1955; Savage, 1956; Pal and Kumar, 2012; Gautam and Tapadia, 2014). The renal system of the human kidney, which has the same developmental principles as the Malpighian tubules of insects, filters the blood in the body and ensures the removal of waste products and osmoregulation. Insects, on the other hand, do not have glomerular filtration in their excretory systems. Instead, secretion and absorption for the filtration of extracellular fluid is done by epithelial transport mechanisms (Maddrell, 1969; Gautam and Tapadia, 2014).

The Malpighian tubules do not only function in the filtration and removal of waste products from the hemolymph resulting from metabolism, but also in the metabolism of some harmful or toxic organic substances taken from plants by nutrition. For example, the substance called salicylate found in some plants protects the plant

against phytophagous insects. The Malpighian tubules of some insects such as *Acheta domesticus* Linnaeus, 1758 (Orthoptera: Gryllidae), *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae), *Tenebrio moliter* L. (Coleoptera: Tenebrionidae) provide insect protection by removing dietary salicylate from the hemolymph (Ruiz-Sanchez et al., 2007).

In order to perform all these tasks, the extracellular fluid is first filtered. This filtration is accomplished by a relatively non-selective barrier. The filtrate formed as a result of filtration does not contain large molecules such as proteins, but smaller molecules such as harmful and waste metabolism products. This filtrate travels through the lumen of the Malpighian tubule towards the proximal end (to the point where the Malpighian tubule attaches to digestive tract) (Wigglesworth, 1965; Maddrell, 1981; Bradley, 1985; Chapman, 1998). During this progression, some substances in the filtrate are secreted back into the hemolymph by the Malpighian tubule. Thus, the homeostasis of the body is maintained by controlling the water and ion balance. In addition, in some insects, the cold tolerance of the insect is adjusted by providing ion homeostasis (Martini et al., 2007; Pacheco et al., 2014; Dow et al., 2021; Overgaard et al., 2021).

The Malpighian tubules are also responsible for the accumulation of various ions within spherocrysts in their epithelial cells. Accumulated ions are important elements in sustaining many vital activities. For example, metal ion concentrations have an effect on the metamorphosis of some insects. Environmental pollution also has an

effect on metal accumulation in insects. The fact that metal ion accumulations reach a level higher than the optimum level has a negative effect. This accumulation effects not only the insect, but also its predators through the food chain (Lindqvist and Block, 1994; Lindqvist et al., 2015).

1. THE MALPIGHIAN TUBULES IN ORTHOPTERA

The excretory system of many insects consists of numerous tubules which are free in the hemolymph. The free ends (distal) of the Malpighian tubules are closed and the other side (proximal) of the Malpighian tubules are attached to the the midgut-hindgut region of the alimentary canal (Beams et al., 1955; Maddrell, 1969, 1971; Jarial, 1990; Martini et al., 2007; Delakorda et al., 2009; Gautam and Tapadia, 2014). The Malpighian tubules, which vary greatly in structure and number among various insect taxa, often lie freely in the body cavity, but in some species they are in contact with some parts of the digestive tract (Wigglesworth, 1965; Bradley, 1985; Chapman, 1998; Denholm and Skaer, 2005). In some insect species such as *Rhodnius prolixus* Stål, 1859 (Heteroptera), the Malpighian tubules can connect directly to the digestive tract from the rectum (Denholm and Skaer, 2005; Martini et al., 2007; Acar, 2009; Chapman, 2013; Pacheco et al., 2014). However, the Malpighian tubules connect to the alimentary canal from the midgut-hindgut junction and do not show any connection with other parts of the digestive tract in many Orthoptera species, as well as *Poecilimon cervus* Karabag, 1950

(Tettigoniidae), *Troglophilus neglectus* Krauss, 1879 (Rhaphidophoridae), *Gryllus campestris* (Linnaeus, 1758) (Gryllidae), *Leptophyes albovittata* (Kollar, 1833) (Tettigoniidae), *Conocephalus (Xiphidion) fuscus fuscus* (Fabricius, 1793) (Tettigoniidae) (Wigglesworth and Salpeter, 1972; Bradley, 1985; Delakorda et al., 2009; Polat et al., 2017; Amutkan Mutlu et al., 2021; Polat, 2021).

The number of the Malpighian tubules, as well as the regions where they attach to the digestive tract, varies among different insect groups. The quantity of the Malpighian tubules is generally higher common in species of the Orthoptera order than other insect orders. The presence of approximately 250 Malpighian tubules in an adult grasshopper has been reported in previous studies (Savage, 1956; Schmidt, 1979; Bradley et al., 1982; Jarial, 1990; Yea and Yu, 1992; Chapman, 1998; Acar, 2009; Gullan and Cranston, 2012; Pacheco et al., 2014; Amutkan et al., 2015). The number of the Malpighian tubules is 100-150 in *M. desertus*, 200-300 in *Melanoplus differentialis differentialis* (C.Thomas, 1865) (Orthoptera: Acrididae), approximately 250 in *Schistocerca gregaria* (Forskål, 1775) (Orthoptera, Acrididae), 90-150 in *G. campestris*, 50-60 in *T. neglectus*. There are numerous Malpighian tubules have also been reported in *C. fuscus fuscus*, *L. albovittata,* and *P. cervus* (Beams et al., 1955; Savage, 1956; Acar, 2009; Delakorda et al., 2009; Polat, 2016, 2021; Polat et al., 2017; Amutkan Mutlu et al., 2021).

In the insects' excretory system, the tubular secretion is the only way of passage of water and dissolved substances into the lumen of the

Malpighian tubule. Secretion and absorption of extracellular fluid in the hemolymph are carried out by epithelial transport mechanisms. The Malpighian tubules generally are composed of two segments, distal and proximal. The distal segment of the Malpighian tubule, away from the alimentary canal, is responsible for urine secretion and hemolymph filtration. Other low molecular weight molecules in the hemolymph diffuse into the tubule lumen according to their concentration differences. The epithelial cells in this region allow water, organic solids, and ions such as Na^+, K^+, Cl^- to pass into the lumen from the hemolymph, thus, iso-osmotic fluid is accumulated in the lumen. This transition is initiated by the stimulation of insect diuretic hormone. The proximal segment of the Malpighian tubule, close to the digestive tract, is responsible for reabsorption. It reabsorbs ions such as K^+ and Cl^- from the fluid in the lumen formed by the distal segment and sends them back to the hemolymph, thereby modifying the fluid in the lumen. However, water reabsorption does not occur in the proximal tubule. This stage of reabsorption is completed by the hindgut region of the digestive tract (Ramsay, 1953, 1958; Beams et al., 1955; Maddrell, 1969, 1971; Jarial, 1990; Martini et al., 2007; Gautam and Tapadia, 2014). When we look at the external morphology of the Malpighian tubules in some inscet orders, it is seen that the proximal and distal segments show different structural features. In some Orthoptera species such as *C. fuscus fuscus, Pseudochorthippus parallelus parallelus* (Zetterstedt, 1821) (Acrididae), *G. campestris, Bolua turkiyae* Ünal, 1999 (Tettigoniidae), *Poecilimon ataturki* Ünal, 1999 (Tettigoniidae), and *P. cervus,* the

Malpighian tubules are unsegmented (Polat, 2016, 2021; Polat et al., 2017, 2020; Amutkan Mutlu et al., 2018, 2020).

Control of water balance and ion transport is a very crucial factor in maintaining insect homeostasis (Martini et al., 2007). The Malpighian tubules provide the electrolyte and water balance of the internal environment by filtering soluble substances such as mineral salts and water through the absorption of the hemolymph (Gullan and Cranston, 2012). Waste material is discharged to the last intestine (Maddrell, 1971; Delakorda et al., 2009). Ion homeostasis in the body is also of vital importance for chill-susceptible species living in cold regions. It has been reported in various studies that the cold tolerance of insects varies depending on the ion concentration difference between the hemolymph and hindgut. When the temperature of the air falls below the critical thermal minimum of the insect, slowdowns occur in enzyme-dependent transport mechanisms. In this case, although water and Na^+ ions will be reabsorbed in the hindgut, these substances escape back to the hindgut in an insect in a chill coma. Changes in not only Na^+ but also K^+ ion homeostasis affect the vital activities of the insect by causing deterioration in neuromuscular function. If this situation lasts for a long time, it first causes death at the cellular level, and then results in the death of the insect. The Malpighian tubules act a critical role in establishing this ion and water balance (Andersen et al., 2013; Coello Alvarado et al., 2015; Des Marteaux and Sinclair, 2016; Des Marteaux et al., 2018; Lebenzon et al., 2020).

The Malpighian tubule consists of a single layer of cuboidal or pyramidal epithelial cells lining the lumen. Thanks to this structure, liquids and solutes are easily transported between the hemolymph and tubule lumen. Multiple folds are usually seen in the basal plasma membrane of epithelial cells. On the apical plasma membrane of the cells, on the other hand, there are microvilli of varying lengths, and they scatter tight or sparse. In addition, cells are connected to each other by large desmosomes in their lateral membranes. All these structures cause cells to display a polar structure. The outermost part of the cells is muscle tissue. Muscle tissue, on the other hand, allows the tubule to contract and transport substances from the hemolymph to the tubule lumen and then to the hindgut (Beams et al., 1955; Maddrell, 1969, 1971; Jarial, 1990; Martini et al., 2007; Delakorda et al., 2009). Uniform epithelial cells were seen in the Malpighian tubules of *G. campestris*, *P. cervus,* while *L. albovittata* individuals have 2 kinds of epithelial cells according to their electron density, but no difference at organelle level. In *C. fuscus fuscus* nymphs, the Malpighian tubules have got 3 types of cells (Polat, 2016, 2021; Polat et al., 2017; Amutkan Mutlu et al., 2021).

Microvilli and basal membrane folds in epithelial cells are considered as indicators of active transport mechanisms. In addition, these structures regulate osmotic transport and the passage of small materials from the hemolymph to the tubule lumen (Delakorda et al., 2009; Pal and Kumar, 2012, 2013). Numerous short basal membrane folds and many mitochondria between them have been described in

Malpighian tubule epithelial cells of *P. cervus, T. neglectus, G. campestris, Grylloblatta compodeiformis* (Walker) (Orthoptera: Grylloblattidae), *L. albovittata, C. fuscus fuscus,* and *M. differentialis differentialis* (Beams et al., 1955; Jarial, 1990; Delakorda et al., 2009; Polat, 2016, 2021; Polat et al., 2017; Amutkan Mutlu et al., 2021). Mitochondria are frequently observed not only in the region of basal membrane folds, but also in the middle of cells or especially on the side of microvilli. A large number of mitochondria between microvilli and their basal regions meet their energy needs. In Orthoptera species such as *L. migratoria, S. gregaria, P. cervus,* there are many long and scattered microvilli on the apical side of the epithelial cells of the Malpighian tubules and mitochondria in these regions (Maddrell, 1971; Prado et al., 1992; Da Cunha et al., 2012; Amutkan et al., 2015, Polat, 2016).

In many species belonging to the Orthoptera order, most epithelial cells in the Malpighian tubules appear to have cytoplasmic structures called spherocrystals, which are arranged in concentric lamellae with different electron densities. Spherocrystals are considered as accumulation sites of toxic and non-toxic waste products. In addition, spherocrystals provide functions such as the accumulation of minerals and organic materials necessary for cells and the regulation of mineral composition in insect tissues. Studies from different species have reported that spherocrystals contain Ca, C, K, Fe, Mg, Ba, Cl, Cu, N, O, Na, P, S, Ni, Si, and etc. These ion accumulations in insects are very important structurally and functionally for the survival of the

insect. For instance, strengthening of the mandible occurs in the presence of Zn in herbivorous species of Orthoptera order. Thus, the insect gains the cutting force of plant parts more easily. In addition, Zn performs a functional task by participating in the structure of various enzymes in many insects (Lindqvist and Block, 1994). Secretory vesicles in cells are believed to be played a role in the storage or secretion of proteinaceous substances (Sohal and Lamb, 1979; Wessing and Zierold, 1992; Delakorda et al., 2009; Chapman, 2013; Pal and Kumar, 2013, 2014; Zhong et al., 2015; Amutkan Mutlu et al., 2021; Tibbett et al., 2021). Epithelial cells of the Malpighian tubules in Orthoptera species such as *P. cervus, T. neglectus,* and *L. albovittata* contain many secretory vesicles and spherocrysts of various sizes (Delakorda et al., 2009; Polat, 2016; Amutkan Mutlu et al., 2021).

REFERENCES

Acar, G. (2009). *Melanogryllus desertus* (Pallas, 1771) (Orthoptera: Gryllidae)'ta Malpighi tüpçüklerinin morfoloji ve histolojisi. Master thesis, Ege University, Institute of Science, İzmir.

Amutkan Mutlu, D., Polat, I., & Suludere, Z. (2021). Histomorphology of the Malpighian tubules and the chemical composition of the spherocrystals in the tubule epithelial cells of adult *Leptophyes albovittata* (Kollar, 1833) (Orthoptera, Tettigoniidae). Microscopy and Microanalysis, 27(5), 1174-1182.

Amutkan Mutlu, D., Polat, I., Ünal, M., & Suludere, Z. (2018). Morphology and histology of the Malpighian tubules in *Pseudochorthippus parallelus parallelus* (Orthoptera, Acrididae). Ecology 2018 International Symposium, 19-23 June 2018, Kastamonu, Turkey.

Amutkan Mutlu, D., Polat, I., Ünal, M., & Suludere, Z. (2020). Morphology and ultrastructure of the Malpighian tubules in *Bolua turkiyae* Ünal, 1999 (Orthoptera, Tettigoniidae). 2nd International Eurasian Conference on Science, Engineering and Technology, 07-09 October 2020, Turkey.

Amutkan, D., Suludere, Z., & Candan, S. (2015). Ultrastructure of digestive canal of *Graphosoma lineatum* (Linneaus, 1778) (Heteroptera: Pentatomidae). Journal of the Entomological Research Society, 17(3), 75-96.

Andersen, J. L., Findsen, A., & Overgaard, J. (2013). Feeding impairs chill coma recovery in the migratory locust (*Locusta migratoria*). Journal of Insect Physiology, 59(10), 1041-1048.

Beams, H. W., Tahmisian, T. N., & Devine, R. L. (1955). Electron microscope studies on the cells of the Malpighian tubules of the grasshopper (Orthoptera, Acrididae). The Journal of Biophysical and Biochemical Cytology, 1(3), 197-212.

Bradley, T. J. (1985). The excretory system, structure and physiology. In G.A. Kerkut and L. I. Gilbert (Eds.), *Comprehensive Insect Physiology*,

Biochemistry and Pharmacology. First Edition. London: Pergamon Press, 421-465.

Bradley, T. J., Stuart, A. M., & Satir, P. (1982). The ultrastructure of the larval Malpighian tubules of a saline-water mosquito. *Tissue and Cell, 14*, 759-773.

Chapman, R. F. (1998). *The insects: Structure and function.* (Fourth Edition). London: Cambridge University Press, 770.

Chapman, R. F. (2013). The excretory system: Structure and physiology. In G. A. Kerkut and L. I. Gilbert (Eds.), *Comprehensive insect physiology biochemistry and pharmacology, regulation: Digestion, nutrition, excretion.* London: Pergamon Press, 421-466.

Coello Alvarado, L. E., MacMillan, H. A., & Sinclair, B. J. (2015). Chill-tolerant *Gryllus* crickets maintain ion balanca at low temparatures. Journal of Insect Physiology, 77, 15-25.

Da Cunha, F. M., Caetano, F. H., Wanderley-Teixeira, V., Torres, J. B., Teixeira, A. A., & Alves, L. C. (2012). Ultra-structure and histochemistry of digestive cells of *Podisus nigrispinus* (Hemiptera: Pentatomidae) fed with prey reared on bt-cotton. Micron, 43(2), 245-250.

Delakorda, S. L., Letofsky-Papst, I., Novak, T., Hofer, F., & Pabst, M. A. (2009). Structure of the Malpighian tubule cells and annual changes in the structure and chemical composition of their spherites in the cave cricket *Troglophilus neglectus* Krauss, 1878 (Raphidophoridae, Saltatoria). Arthropod Structure and Development, 38, 315-327.

Denholm, B., & Skaer, H. (2005). Development of the Malpighian tubules in insects. In L. I. Gilbert, K. Iatrou, S. S. Gill (Eds.) *Comprehensive molecular insect science.* UK: University of Cambridge, 291-314.

Des Marteaux, L. E., & Sinclair, B. J. (2016). Ion and water balance in *Gryllus* crickets during the first twelve hours of cold exposure. Journal of Insect Physiology, 89, 19-27.

Des Marteaux, L. E., Khazraeenia, S., Yerushalmi, G. Y., Donini, A., Li, N. G., & Sinclair, B. J. (2018). The effect of cold acclimation on active ion transport

in cricket ionoregulatory tissues. Comparative Biochemistry and Physiology, Part *A*, 216, 28-33.

Dow, J. A. T., Krause, S. A., & Herzyk, P. (2021). Updates on ion and water transport by the Malpighian tubule. Current Opinion in Insect Science, 47, 31-37.

Gautam, N. M., & Tapadia, M. G. (2014). Overview of Malpighian tubules development and function in *Drosophila melanogaster*. Journal of Scientific Research,*58,* 87-98.

Gullan, P. J., & Cranston, P. S. (2012). *Böcekler: Entomolojinin ana hatları* (Çev. A. Gök). Ankara: Nobel Yayınevi, 83.

Jarial, M. S. (1990). Fine structure of Malpighian tubules *Grylloblatta compodeiformis* (Orthoptera: Grylloblattidae). Transactions of the American Microscopical Society, 109(4), 329-341.

Lebenzon, J. E., Des Marteaux, L. E., & Sinclair, B. J. (2020). Reversing sodium differentials between the hemolymph and hindgut speeds chill coma recovery but reduces survival in the fall field crickets, *Gryllus pennsylvanicus*. Comparative Biochemistry and Physiology, Part A, 244, 110699.

Lindqvist, L., & Block, M. (1994). Excretion of cadmium and zinc during moulting in the grasshopper *Omocestus viridulus* (Orthoptera). Environmental Toxicology and Chemistry, 13(10), 1669-1672.

Lindqvist, L., Block, M., & Tjälve, H. (1995). Distribution and excretion of Cd, Hg, methyl-Hg and Zn in the predatory beetle *Pterostichus niger* (Coleoptera: Carabidae). Environmental Toxicology and Chemistry, 14(7), 1195-1201.

Maddrell, S. H. P. (1969). Secretion by the Malpighian tubules of *Rhodnius.* The movements of ions and water. The Journal of Experimental Biology, 51, 71-97.

Maddrell, S. H. P. (1971). Fluid secretion by the Malpighian tubules of insects. *Philosophical Transactions of the Royal Society of London B, 262,* 197-207.

Maddrell, S. H. P. (1981). The functional design of the insect excretory system. The Journal of Experimental Biology, 90, 1-15.

Martini, S. V., Nascimento, S. B., & Morales, M. M. (2007). *Rhodnius prolixus* Malpighian tubules and control of diuresis by neurohormones. Annals of the Brazilian Academy of Science, 79(1), 87-95.

Overgaard, J., Gerber, L., & Andersen, M. K. (2021). Osmoregulatory capacity at low temperature is critical for insect cold tolerance. Current Opinion in Insect Science, 47, 38-45.

Pacheco, C. A., Alevi, K. C. C., Ravazi, A., & Azeredo Oliveira, M. T. V. (2014). Review: Malpighian tubules, an essential organ for insects. Entomology, Ornithology and Herpetology: Current Research, 3(3), 1-3.

Pal, R., & Kumar, K. (2012). Ultrastructural features of the larval Malpighian tubules of the flesh fly *Sarcophaga ruficornis* (Diptera: Sarcophagidae). International Journal of Tropical Insect Science, 32(3), 166-172.

Pal, R., & Kumar, K. (2013). Malpighian tubules of adult flesh fly, *Sarcophaga ruficornis* Fab. (Diptera: Sarcophagidae): An ultrastructural study. Tissue and Cell, 45(5), 312-317.

Pal, R., & Kumar, K. (2014). Malpighian tubules of pharate adult during pupal-adult development in flesh fly, *Sarcophaga ruficornis* Fab. (Diptera: Sarcophagidae). The Journal of Basic and Applied Zoology, 67(1), 10-12.

Polat, I. (2016). *Poecilimon cervus* Karabag, 1950'un sindirim, boşaltım, dişi ve erkek üreme sisteminin ultrastrüktürel özellikleri. PhD thesis, Gazi University, Institute of Science, Ankara.

Polat, I. (2021). Morphology and ultrastructure of the nymphal Malpighian tubules of *Conocephalus fuscus fuscus* (Fabricius, 1793) (Orthoptera, Tettigoniidae). Gazi University Journal of Science, 1-1.

Polat, I., Amutkan Mutlu, D., Ünal, M., & Suludere, Z. (2020). Histology and morphology of the Malpighian tubules in *Poecilimon ataturki* Ünal, 1999 (Orthoptera, Tettigoniidae). 3rd International Eurasian Conference on Biological and Chemical Sciences, 19-20 March, 2020, Ankara, Turkey.

Polat, I., Özyurt, N., Amutkan, D., Suludere, Z., & Candan, S. (2017). Fine structure of the Malpighian tubules in *Gryllus campestris* (Linnaeus, 1758) (Orthoptera, Gryllidae). Turkish Journal of Zoology, 41(3), 379-389.

Prado, M. A., Montuenga, L. M., Villaro, A. C., Etayo, J. C., Polak, J. M., & Sesma, M. P. (1992). A novel granular cell type of locust Malpighian tubules: Ultrastructural and immunocytochemical study. Cell and Tissue Research, 268(1), 123-130.

Ramsay, J. A. (1953). Active transport of water by the Malpighian tubules of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae). Journal of Experimental Biology, 31(1), 104-113.

Ramsay, J. A. (1955). The excretory system of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae). Journal of Experimental Biology, 32, 183-199.

Ramsay, J. A. (1958). Excretion by the Malpighian tubules of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae): Amino acids, sugars and urea. The Journal of Experimental Biology, 35, 871-891.

Rigoni, G. M., & Conte, H. (2014). Malpighian tubules in larvae of *Diatraea saccharalis* (Lepidoptera: Crambidae): A morphological comparison between non-parasitized and parasitized by *Cotesia flavipes* (Hymenoptera: Braconidae). Advances in Entomology, 2(4), 202-210.

Ruiz-Sanchez, E., Van Walderveen, M. C., Livingston, A., & O'Donnell, M. J. (2007). Transepitelial transport of salicylate by the Malpighian tubules of insect from different orders. Journal of Insect Physiology, 53, 1034-1045.

Savage, A. A. (1956). The development of the Malpighian tubules of *Schistocerca gregaria* (Orthoptera). Querterly Journal of Microscopical Science, 97(4), 599-615.

Schmidt, B. A. (1979). Ultrastructure of differentiated Malpighian tubules from cockroach lymphs during the moltind cycle. Journal of Morphology, 162, 361-388.

Sohal, R. S., & Lamb, R. E. (1979). Storage-excretion of metallic cations in the adult housefly, *Musca domestica*. Journal of Insect Physiology, 25(2), 119-124.

Tibbett, M., Green, I., Rate, A., De Oliveira, V. H., & Whitaker, J. (2021). The transfer of trace metals in the soil-plant-arthropod system. Science of the Total Environment, 146260.

Wessing, A., & Zierold, K. (1992). Metal-salt feeding causes alterations in concretions in *Drosophila* larval Malpighian tubules as revealed by x-ray microanalysis. Journal of Insect Physiology, 38(8), 623-632.

Wigglesworth, V. B. (1965). The principles of insect physiology. London: Methuen and Co, 546.

Wigglesworth, V. B., & Salpeter, M. M. (1972). Histology of the Malpighian tubules in *Rhodnius prolixus* Stål (Hemiptera). Journal of Insect Physiology, *8*, 299-307.

Yea, S. Y., & Yu, C. H. (1992). Morphology of the Malpighian tubules in the German cockroach, *Blattella germanica* L. Bulletin of the Institute of Basic Scienc*e*, Inha University, *13*, 97-105.

Zhong, H., Zhang, Y., & Wei, C. (2015). Morphology and ultrastructure of the Malpighian tubules in *Kolla paulala* (Hemiptera: Cicadellidae). Zoologischer Anzeiger, 257, 22-28.

CHAPTER 6

BETWEEN INTACT AND URBAN FRESHWATER: COMPARISON OF MORPHOMETRIC SCALE ON *HYDROCHARA CARABOIDES* (LINNAEUS, 1758) BY SUPPORT OF MOLECULAR IDENTIFICATION

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INTRODUCTION

Environmental manipulations and human activities are major causes of stress on natural ecosystems (Cooper, 1993). While the human population of the earth continues to grow, the resulting expansion of population will affect aquatic resources (Brown et al., 2005). Therefore, human activities negatively impact ecosystems (Wen et al., 2017). Wetlands are the most important part of the environment (Yalçınkaya, 2006), freshwater systems are vitally important ecosystem either (Xu et al., 2020).

A wide range of research has been done in the aquatic ecosystem area around the world and in Turkey too (Wooldridge,1978; Smetana, 1980; Hansen, 1987, 1991; Schödl, 1991, 1993; Gentili, 1995, 2000; Löbl and Smetana, 2004; Vafei et al., 2007; Incekara et al., 2016; Bektaş et al., 2019). Both intact freshwater ecosystems provide many economically valuable commodities (Baron and Poff, 2004) and urban freshwater ecosystems offer an important opportunity to educate on natural systems (Hassall, 2014). Consequently, as for intact aquatic areas Zökün floating island far from the settlement has been selected; as for urban aquatic areas, Erzurum Wetlands near to Erzurum city has been preferred.

Coleoptera ordo incorporates varied climate-conditions and multifarious biotops (Bektaş, 2015). In the space of Coleoptera ordo, Hydrophilidae Latreille, 1802 family is a leading group of water beetles with about 3000 described species classified in 6 subfamilies

and 12 tribes (Short and Fikáček 2013). This family, also known as water scavenger beetles (Taşar et al., 2014), is a large family. *Hydrochara* Berthold, 1875 genus is huge species (Bektas, 2015). *Hydrochara caraboides* (Linnaeus, 1758), known belong to Hydrophilidae family. It flies strongly and could easily move (Guest 1996); additionally, climate change stands to have a tremendous impact on the world's freshwater ecosystems (Picazo et al., 2020), Silver Water Beetle (H. caraboides) is being listed as endangered in the Red Data Book of Hexapods (Guest, 1996), so this species was selected in our study.

Besides, the importance of alterations in body shape have important consequences for hydrodynamics and swimming performance (Abet et al., 2020). The objectives of this manuscript are to contribute to knowledge and evaluate whether there are any different of morphological scale in different (Ferreira et al., 2006) region where intact wetland and wetland that thought to be contaminated and close to the city. Environmental factors can either directly influence body proportions or indirectly affect morphometric variation (Esin and Fedosov, 2016). Tukaj et al. (1998) empathized that morphometric analysis was used to study changes by oil pollution. Because scanning electron microscope (SEM) has shown great potential for some analysis as it can provide high-resolution images at very high magnifications as well as elemental analysis (Hayes et al., 2019), the difference in measurements of the same both species and body's structures has been examined in two different regions (in intact and

urban freshwaters).

Additionally, species-level identification is often not feasible with morphology based approaches. DNA metabarcoding represents an alternative approach for studying multiple stressor interactions at the species level (Werner et al., 2021). Environmental DNA (eDNA) has been reported to capture species diversity in much greater detail than traditional techniques (Leese et al., 2020). eDNA has attracted attention as a method for investigating aquatic organisms (Akatsuka et al., 2020). In some studies, environmental DNA (eDNA) analysis has been demonstrated to be an effective instrument to monitor species. It enables the prediction of the presence/absence (Ficetola et al., 2008), DNA analysis has varied advantages over traditional survey methods: it has a reduced cost (Evans et al., 2017), it can detect scarce species (Goldberg et al., 2011).

This is the first comparative study of the measurement of body structures between urban and intact regions. The model study provides the opportunity to relate the state of an ecosystem to environmental changes and simultaneously provide an understanding of the underlying ecological interactions. Consequently, models may be used to predict the effects of management interventions on ecosystem states in space and time and thus provide valuable tools for water management (Vries et al., 2021). In this study, we aimed to ensure verification between morphological-molecular identification, examine pollution's effect in morphological and morphometrically of the aquatic insects. This manuscript reviews to reveal the difference

of morphometric measurements between urban and intact freshwater ecosystems with molecular support.

1. AIM OF STUDY

Worldwide, wetlands are vital areas, which have critically important for biodiversity. Hence, there is a need to understand and different studies on how to protect to sustainability of these regions. In this sense; while the intact and urbanized region has opted for researching area, *Hydrochara caraboides* (Linnaeus, 1758) insect (hexapod) species have been selected for samples, decreasing in number due to reasons such as polluting. Due to the fact that effective results can be obtained to morphometric measurements of ten male insects from each region, belonging to the same species and all male, were collected from both areas. It was found that the scales in wetlands near the city were statistically smaller. It is concluded that possible pollution adversely affects the biological development of insects. Measured body structures are following; body length, antenna, maxillary palp, legs, elytra, and aedegophor (male sexual organ). Furthermore, this study has striven to show the efficacy of the use of molecular techniques as an alternative method for fast and accurate data collection. In this manner, this work will be a methodological reference source for future animal systematic studies and ecological research. In this way, by under our data, it was understood that links of pollution-body structures. Consequently, our outcomes will bring a new perspective on the protection of wetlands.

2. METHODS

2.1. Study area

Adults stage of *Hydrochara caraboides* (Coleoptera: Hydrophilidae) were collected from their natural habitat in Zökün floating lake (Tortum) and Erzurum Wetland (Yakutiye) by sieves that having 3,15 x 1 mm pores in summer seasons on Erzurum provinces, Turkey (Fig. 1. A., B., C., D., E.). The materials (hexapods) have been vividly stored in small bottles and live individuals were brought to the laboratory and maintained at 25 ºC under a special container. Scales of temperature and pH were registered in close time to each other.

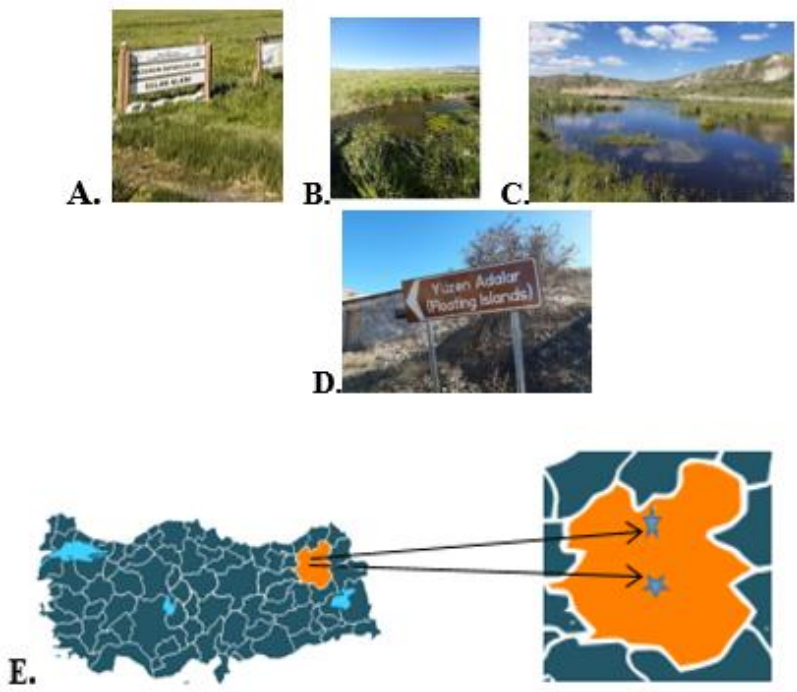


Figure 1. Research Area of Urban Wetlands (**A, B**), Intact Wetlands (**C**, **D**), and Map (**E**) (Anonymous, 2021).

2.2. Morphological study

Samples (hexapods) were cleaned with a brush before identification, and then aedeagophor of the hexapod was dissected under a stereo microscope in the laboratory. Then, dissected under a stereo microscope in the laboratory, photographs of the main diagnostic characters were taken using a microscope. At morphological identification, Bektaş (2015) were been used. Collection data were explained in Tab.1. In order that non-contamination to be taken samples for molecular studies, with a view to results of our study are more reliable, it has been studied very carefully in the laboratory (especially in laminar air flow cabinet) and environments. In order to avoid and prevent contamination, it was quite a sensibility attended in every stage of research by collogues. Ten adult hexapods (*H. caraboides*) were taken from both areas. Body structures (body length, antenna, maxillary palp, legs, elytra, and aedegophor) were measured, using a stereo microscope capable of measuring on millimeters (mm).

2.3. Molecular identification

DNA isolation from samples was performed using the "Quick-DNA Fecal / Soil Microbe Miniprep Kit". The amount of isolated DNA was determined fluorometrically with Qubit 3.0. In the PCR study, the mitochondrial cytochrome oxidase subunit 1 (COI) gene region of approximately 700bp was replicated using the primers LCO1490 - HCO2198. Primer sequences used and PCR conditions are given below;

LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3'
HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

95°C 10 min. – initial denaturation
35 circulation:
95 °C 30 seconds – denaturation
50-55 °C 30 seconds – annealing (gradient from PCR 10. cyc +0.2 plus)
72 °C 15 seconds – extension

The temperature was diminished to 4 ° C and PCR was completed. Samples were sequenced with the ABI 3730XL Sanger sequencer and BigDye Terminator v3.1 Cycle Sequencing Kit.

3. RESULTS AND DISCUSSION

3.1. Morphological studies

All species indicated broader thermal ranges at the global level (Markovic et al., 2021), this level has a wide range from terrestrials to wetlands. Wetlands have an important place among other ecosystems in terms of protecting natural balance and biological diversity. Today, wetland conservation projects and studies are of great national/international importance (Fauziah, 2009; Çoşkun et al., 2018). Freshwater ecosystems are dynamic region, so humanity need to maintain these natural dynamics for it. In wetlands, the most invasive ordo is coleopteran, in this ordo includes many species (Saruhan and Tuncer, 2010). That is why the effect of pollution on a morphometric scale was investigated, and measurements were made on the same *H. caraboides* species, decreasing in number due to reasons such as polluting, climate change etc., collected from both

regions. To collect samples has given as a table (Tab. 1). The scale data were evaluated statistically.

On morphological (traditional) identification, body scales of *H. caraboides* (Linnaeus, 1758) species body length is 15.0-18.0 mm in length and 7.0-9.0 mm in width. Dorsal surfaces are black color and shiny. The structure of the ventral media is exactly the third pair of legs; the last three segments of antennas are very dark in color. Maxillary palps are dark brown, but the tip of the last segment is lighter. Femur and tibia on legs are dark light (Fig. 2.A) and aedeagofor is 3.1-3.5 mm long, longitudinally on the dorsal surface of the middle lobe is a thin line and tip of parameters are curved (Fig. 2.B) (Bektaş, 2015).

Table. 1. Information of PH, Temperature, Location On Time of Collection.

Species / Collected number / Sex	**Coordinates**	**Altitude (meter)**	**Lake's PH**	**Lake's Temperature (^{0}C)**	**Time/hour of Collection**	**Location name**
H. caraboides 10 individuals / Male	$40^0 15^{11} 55^1$ N (latitude) $41^0 34^{11} 07^1$ E (longtude)	1953	7,53	23,5	13.07.2020 / 14:30 p.m	Zökün Floating lake, Tortum, Erzurum, Turkey
H. caraboides 10 individuals / Male	$40^0 38^{11} 37^1$ N (latitude) $41^0 27^{11} 36^1$ E (longtude)	1756	7,02	22,8	14.07.2020 /14:10 p.m	Erzurum Wetlands, Yakutiye, Erzurum, Turkey

Figure. 2. Hexapod: *Hydrochara caraboides*; **A.** Dorsal, **B.** Ventral, **C.** Audogophor (Male Sexual Organ) (Scale: 1 Mm). (Photographs: M. Bektas).

Morphometric characters are continuous characters depicting features of body shape (Abet et al., 2020), therefore a morphometric scale of structure of the same hexapod species was collected from close to urbanized freshwater and intact freshwater. Adult samples have been examined under a stereo microscope.

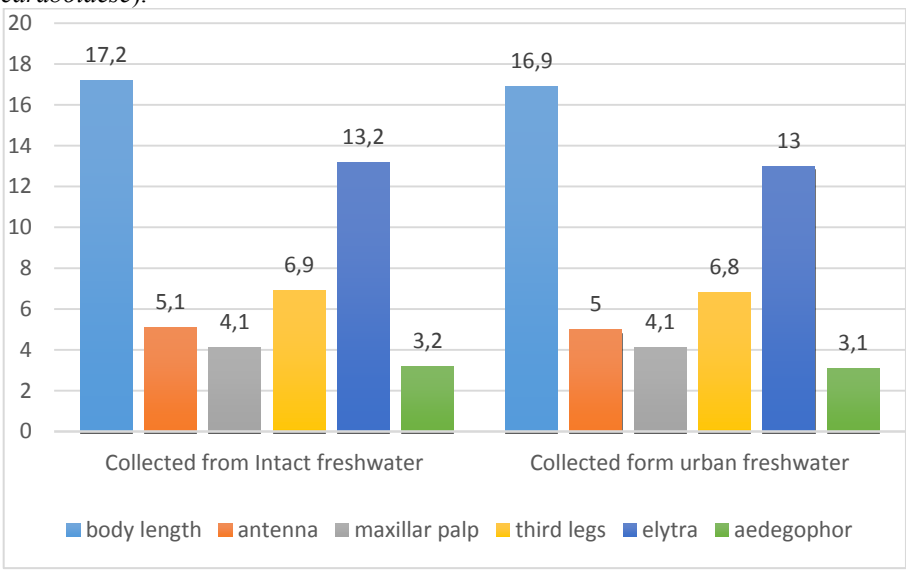
Table 2. Averages Length Of Morphometric Scales, Standard Deviation and Divergence: On Hexapods Structure (*H. caraboides*) Between Two Research Areas.

H. caraboides	**Body length**(mm)	**Antenna** (mm)	**Maxillar palp** (mm)	**Third legs** (mm)	**Elytra** (mm)	**Aedegophor** (mm)
Sample I*	17,2 ± 0,02	5,1±0,01	4,1±0,01	6,9±0,02	13,2±0,01	3,2±0,01
Sample II**	16,9 ± 0,02	5,0±0,01	4,1±0,01	6,8±0,02	13,0±0,01	3,1±0,01
Divergence	**+0,3**	**+0,2**	**+0**	**+0,1**	**+0,2**	**+0,1**

***** (Sample I: Collected from Intact freshwater, Zökün floating island)
****** (Sample II: Collected form urban freshwater, Erzurum Wetland)

Unlike intact wetlands (the floating island) in wetland near to city (Erzurum wetland), there is little difference in morphometric scales. This discrepancy is thought to have a negative effect on the hexapod development due to pollution factor depend on heavy metal accumulation. Other than above mention measurements given in Tab. 2., a general difference was not observed according to Smetana (1980) and Hansen (1987).

Table 3. Graphic: Averages length of morphometric on samples (Hexapod: *H. caraboidese*).



The results of this work (Tab. 3) lead us to count on use of these methodologies in the study of new advantage will facilitate the description to the sustainability of the wetlands where close to urbanized regions. On account of collected insects are not thought that climatic differences will be a determinant. It has been concluded that statistical differences in the morphometric scale will have pollution effects. We believe that this study will attract the attention of scientists who study insect systematics and polluting effects on body structures of aquatic creatures.

3.2. Molecular Identification

DNA technique has dramatically facilitated investigations of the distribution and abundance of macroorganisms in the natural environment (Doi et al., 2021). The species determination has been

prepared according to the closest species on NCBI. Analysis of sequence, such as;

1) LCO1490 forward primer Sanger sequence

GAACGGTGATCTTTGGACATGAGCTGGATAGTAGGACATCACTAAGAATT
CTAATTCGAGCTGAATTAGGGAACCCTGGAACCCTAATTGGTGATGATCA
AATTTATAATGTTATTGTAACAGCACATGCTTCATCATAATTTTCTTTATAGT
TATACCTATTATAATTGGTGGATTTGGTAATTGATTAGTTCCACTAATACTAG
GAGCTCCTGATATAGCTTTCCCACGAATAAACAATATAAGATTCTGGCTTC
TCCCACCTCTTTAACTTTACTTTTGATAAGAAGAATAGTTGAAAATGGGGC
CGGAACTGGTTGAACAGTTTATCCTCCCCTTTCTTCCAATATTGCCCATGG
TGGTGCATCTGTAGATTTAGCTATTTTCAGATTACATCTAGCAGGGATTTCA
TCAATTTTAGGTGCAGTTAATTTTATTACTACAGTAATCAATATACGATCAG
GGGGTTTAACATATGATCGAATACCTTTATTTGTATGATCTGTAGCTATTAC
AGCATTACTTTTACTTTTATCATTACCAGTTTTAGCTGGAGCTATTACTATAC
TTTTAACAGATCGAAATTTAATACATCATTCTTTGATCCAGCAGGTGGAGG
TGACCCAATTCTTACACATTTATTTGATTTTTTGTCACCTGGAAGTTACATA
TCTTTATGATTGGTGACCATCTTTATTGTTTGGGGACCACACAACGTGGGT
GGCACCGCCTCACCTGA

NCBI Query Coverage: 85%
NCBI Identity Percent: 95.04%
NCBI (the nearest similar species): *Hydrochara caraboides* voucher ZFMK-TIS-1261

2) HCO2198 reverse primer Sanger sequence

ACTGATCGCTAGATGTGTCACTCCACCTGCTGGATCAAGAATGATGTATTT
AAATTTCGATCTGTTAAAAGTATAGTAATAGCTCCAGCTAAAACTGGTAAT
GATAAAAGTAAAAGTAATGCTGTAATAGCTACAGATCATACAAATAAAGGT
ATTCGATCATATGTTAAATTTGTTGATCGTATATTGATTACTGTAGTAATAAA
ATTAACTGCACCTAAAATTGATGAAATCCCTGCTAGATGTAATCTGAAAAT
AGCTAAATCTACAGATGCACCACCATGGGCAATATTGGAGAAAGGGGAGG
ATAAACTGTTCAACCAGTTCCGGCCCCATTTTCAACTATTCTTCTTATCAA
AAGTAAAGTTAAAGATGGTGGGAGAAGCCAGAATCTTATATTGTTTATTCG
TGGGAAAGCTATATCAGGAGCTCCTAGTATTAGTGGAACTAATCAATTACC
AAGTCCACCAATTATAATAGGTATAACTATAAAGAAAATTATGATGAAAGC
ATGTGCTGTTACAATAACATTATAAATTTGATCATCACCAATTAGGGTTCCA
GGGTTCTCTAATTCAGCTCGAATTAGAATTCTTAGTGATGTTCCTACTATTC
CAGCTCATGCTCCAAGATAAAGTATAAAGTACCAATATCTTTATGATTTGTT
GACCACTCATGAAGTTTATGATTTTTCGTCGCCCCC

NCBI Query Coverage: 89%

NCBI Identity Percent: 96.21%
NCBI (the nearest similar species): *Hydrochara caraboides* voucher ZFMK-TIS-1261

It is known that there are 2932 species of the Hydrophilidae family, belonging to 169 genera that have been identified to date. Hydrophilidae are spread all over the world except Antarctica (Archangelsky et al., 2016). As taxonomy is based only on morphological principles, it reduces the reliability of the correct identification of species. Therefore, it is important to use molecular methods in the diagnosis of species (Gholam Zadeh, 2017). At sample hexapod, molecular verification has also been fulfilled for this purpose. The aquatic coleopteran fauna and its biodiversity in freshwater (intact and near urbanized region) will be demonstrated by detailed field studies for the first time using PCR-based molecular techniques in two different areas.

CONCLUSIONS

Our results showed that environmental divergence in morphometric measurements between *H. caraboides* species taken from the wetland of near the city and the intact species were discussed. In particular, pollution effects on the same species have become noticeable with our results. We hope that more knowledge achieves to in this study, mainly about pollution effect, molecular support, morphometric differences. It has been concluded that it is a very important pioneering study in terms of sustainability in the way of affecting the diversity of living things, length of antenna-leg-length-elytra. New

trends on molecular support and uncovering morphometric differences in molecular studies will reveal the results of pollution effects and will have positive impacts on the sustainability of wetlands. In addition, one aim of our study is to estimate to reveal the biological developments of pollution and human populations on aquatic insects with molecular support.

REFERENCES

Anonymous 2021. https://www.idefix.com/kataloglar_/Turkiye-Ne-Okuyor-Erzurum/50033.

Abed, J. A, Jawad, I. A., & Ibán˜ez. A. I. (2020). Morphometric and meristic variations of Photopectoralis bindus (Valenciennes, 1835) in the waters Iraq: implications for stock identification and management. Bollettino del Museo Civico di Storia Naturale di Verona, 44, Botanica Zoologia, 61-74.

Archangelsky, M., Beutel, R. G., & Komarek, A. (2016). Coleoptera, Beetles Volume 1: Morphology and Systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim) 2nd edition: Chapter 12, Hydrophiloidea Latreille, 1802. Walter de Gruyter GMBH, 231-272, 561pp, Berlin/Boston, Germany.

Baron, J. S., & Poff, N. L. (2004). Sustaining Healthy Freshwater Ecosystems. Universities council on water resources. Water resources update, issue 127, pages 52-58.

Bektaş, M. (2015). Gaziantep, Hatay, Kahramanmaraş, Kilis ve Osmaniye illeri Helophoridae, Hydrochidae ve Hydrophilidae (Coleoptera) Faunası. Doktora Tezi. Fen Bilimleri Enstitüsü, Atatürk Üniversitesi, Erzurum, Turkey.

Bektaş, M., Taşar, G. E., Incekara, U., & Polat, A. (2019). A Faunistic Study on Aquatic Coleoptera of the Eastern Mediterranean Region of Turkey. Munis Entomology and Zoology, cilt.14, ss.478-488.

Bektas, M., Orhan, F., Erman, O. K., & Barıs, O. (2020). Bacterial microbiota on digestive structure of Cybister lateralimarginalis torquatus (Fischer von Waldheim, 1829) (Dytiscidae: Coleoptera). Archives of Microbiology, 203:635–641 (Springer).

Brown, L. R., Gray, R. H., Hughes, R. M., & Meador, M. R. (2005). Introduction to Effects of Urbanization on Stream Ecosystems. American Fisheries Society Symposium, 47: 1–8.

Cooper, C. M. (1993). Biological Effects of Agriculturally Derived Surface Water

Pollutants on Aquatic Systems-A Review. Journal of Environmental Quality. Symposium Paper.

Coşkun, B., Ak, D., & Yıldırım, P. Ç. (2018). Mevzuatta Son Dönemlerde Meydana Gelen Gelişmeler Bağlamında Çevresel Açıdan Korunan Alan Yönetiminin Değerlendirilmesi. Aksaray üniversitesi iktisadi ve idari bilimler fakültesi dergisi, Cilt: 10 Sayı: 4, 29-44.

Doi, H., Minamoto, T., Takahara, T., Tsuji, S., Uchii, K., Yamamoto, S., Katano, I., & Yamanaka, H. (2021). Compilation of real-time PCR conditions toward the standardization of environmental DNA methods. Ecological Research, 36: 379–388.

Esin, E. V., & Fedosov, A. E. (2016). The effect of chronic volcanic pollution on the morphometric characteristics of juvenile Dolly Varden (Salvelinus malma W.) on the Kamchatka peninsula. Hydrobiologia, 783: 295–307.

Evans, N. T., Olds, B. P., Renshaw, M. A., Turner, C. R, Li, Y., Jerde, C. L., Mahon, A. R., Pfrender, M. E., Lamberti, G. A., & Lodge, D. M. (2016). Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. Molecular Ecology Resources, 16 (1): 29–41.

Fauziah, A. (2009). Diversity and New records of coleopteran water beetles (Dytiscidae, Hydrophilidae) in Kenyir water Catchment of Terengganu, Malaysia, International J. Zoological, Res. 5: 1-8.

Ferreira, P. S. F., Pires, E. M., Guedes, R. N. C., Mendes, M., & Coelho, L. A. (2006). Seasonal abundance and sexual variation in morphometric traits of Oxelytrum discicolle (Brulle, 1840) (Coleoptera: Silphidae) in a Brazilian Atlantic Forest. Biota Neotrop. [online], Vol.6, n.2, ISSN 1676-0611.

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. Biology Letters, 4(4): 423–425.

Gentili, E. (1995). Hydrophilidae: 3. The Genus Laccobius Erichson in China and neighboring areas (Coleoptera), In Jach: Water Beetles of China, 1, Pp: 41.

Gentili, E. (2000). Distibuzione del genere Laccobius (Coleoptera: Hydrophilidae)

in Anatolia e problemi relativi, Biogeographia, XXI, 173-214.

Gholam Zadeh, S. (2017). Türkiye'de yayılış gösteren bazı Helophoridae, Hydrochidae ve Hydrophilidae Türlerinin Mitokondriyal Sitokrom Oksidaz C genine dayanarak, moleküler karakterizasyonu. Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, Doktora tezi.

Guest, J. (1996). Survey of the Lesser Silver Water beetle Hydrochara caraboides in Cheshire. English Nature Research Reports. No:248. https://aquadocs.org/handle/1834/26922.

Hansen, M. (1987). The Hydrophilidae (Coleoptera) of Fennoscandia and Denmark, Fauna Ent. Scand., 18, 1-253.

Hansen, M. (1991). The Hydrophiloid Beetles. Phylogeny, Classification and A revision of the genera (Coleoptera: Hydrophiloidea), Biologiske Skrifter 40, The Royal Danish Academy of Science and Letters, Pp: 368.

Hassall, C. (2014). The ecology and biodiversity of urban ponds. Wires Water, 1:187–206.

Incekara, U., Bektas, M., Taşar, G. E., & Polat, A. (2016). A New Record for The Turkish Fauna (Coleoptera: Hydrophilidae), with Further Notes on The Laccobius sinuatus Motschulsky, 1849 and Coelostoma transcaspicum Reitter, 1906. Turkish Journal of Science Technology, 11, (2): 21-23.

Leese, F., Sander, M., Buchner, D., Elbrecht, V., Haase, P., & Zizka, V. M. A. (2020). Improved freshwater macroinvertebrate detection from environmental DNA through minimized no target amplification. Environmental DNA, 00: 1–16.

Markovic, D., Freyhof, J., & Kärcher, O. (2021). Continental vs. Global Niche-Based Modelling of Freshwater Species' Distributions: How Big Are the Differences in the Estimated Climate Change Effects? Water, 13, 816.

Picazo, F., Vilmi, A., Aalto, J., Soininen, J., Casamayor, E. O., Liu, Y., Wu, Q., Ren, L., Zhou, J., Shen, J., & Wang, J. (2020). Predicted shifts in microbial patterns along mountain streams under climate change. https://assets.researchsquare.com/files/rs-102078/v1/e93dac3b-0b3d-4e70-b57d-7177a6a6f819.pdf?c=1631859451 (17/09/202).

Saruhan, I., & Tuncer, C. (2010). Kültürel Entomoloji. Anadolu Tarım Bilim. Derg.,

25(1):21-27.

Schödl, S. (1991). Revision der Gattung Berosus Leach 1. Teil: Die palearktischen Arten der Untergattung Enoplurus (Coleoptera: Hydrophilidae). Koleopt. Rundsch., 61, 111–135.

Schödl, S. (1993). Revision der Gattung Berosus Leach 3. Teil: Die palaarktischen und orientalischen Arten der Untergattung Berosus s. str. (Coleoptera: Hydrophilidae). Koleopt. Rundsch., 63, 189-233.

Short, A. E. Z., & Fikáček, M. (2013). Molecular phylogeny, evolution, and classification of the Hydrophilidae (Coleoptera). Systematic Entomology, 38 (4): 723–752.

Smetana, A. (1980). Revision of the genus Hydrochara Berth. (Coleoptera: Hydrophilidae). Mem. Ent. Soc. Can., 111: 1-100.

Taşar, G. E., Polat, A., Incekara, U., & Bektaş, M. (2014). Kuyucuk Gölü Ramsar Alanının Hydrophilidae ve Helophoridae (Coleoptera: Polyphaga) Faunası. Biyoloji Bilimleri Araştırma Dergisi, 7(1): 11-13. ISSN: 1308-3961, E-ISSN: 1308-0261, www.nobel.gen.tr.

Tukaj, Z., Bohdanowicz, J., & Aksmann, A. (1998). A morphometric and stereological analysis of ultrastructural changes in two Scenedesmus (Chlorococcales, Chlorophyta) strains subjected to diesel fuel oil pollution, Phycologia, 37:5, 388-393.

Vries, J. D., Kraak, M. H. S., Skeffington, R. A., Wade, A. J., Piet, F. M., & Verdonschot, P. F. M. (2021). A Bayesian network to simulate macroinvertebrate responses to multiple stressors in lowland streams, Water Research, 194, 116952.

Wen, Y., Schoups, G., & Giesen, N. (2017). Organic pollution of rivers: Combined threats of urbanization, livestock farming and global climate change. Scientific Reports, 7:43289.

Werner, M. T., Beermann, A. J., Elbrecht, V., Zizka, V. M. A., & Leese, F. (2021). Analyzing multiple stressor effects on EPT taxa in a mesocosm experiment with DNA metabarcoding. Arpha Conference Abstracts, 4: e64752.

Wooldridge, D. P. (1978). Paracymus of the palearctic faunal region (Coleoptera:

Hydrophilidae). J. Kans. Entomol. Soc., 51 (1), 123-130.

Xu, Y., Chan, F. K. S., He, J., Johnson, M., Gibbins, C., Kay, P., Stanton, T., Xu, Y., Li, G., Feng, M., Paramor, O., Yu, X., & Zhu, Y. G. (2020). A critical review of micro plastic pollution in urban freshwater environments and legislative progress in China: Recommendations and insights. Critical Reviews in Environmental Science and Technology, 1-44. https://doi.org/10.1080/10643389.2020.1801308.

Yalçınkaya, O. (2006). "Liberal Su Pazarları ve Çok uluslu Şirketler", Karizma Dergisi-Beyaz Petrol: Su Özel Sayısı, Yıl. 7, Sayı: 27, Temmuz-Ağustos-Eylül 2006, İstanbul Entegre Matbaacılık.

CHAPTER 7

THE APPLICATION AREAS OF FISH CELL CULTURES

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INTRODUCTION

Cell culture means growing prokaryotic cells or eukaryotic cells under sterile conditions in a laboratory environment (Seferitz and Vallier 2017). Among eukaryotic cells, the cell culture is made from different tissues of a wide variety of organisms such as fish (Castaño and Becerril, 2004; Connolly et al., 2015), rats (Heller et al., 2021), and humans (Rho et al., 2009; Adey et al., 2013; Bartomel et al., 2021).
Fish, one of the vertebrate groups, can live in a wide variety of habitats ranging from freshwaters to salty waters, from cold waters in polar regions to warm waters in tropical regions, from shallow waters to deep waters (Powers, 1989; Bolis et al., 2001). For this reason, it is used as a model organism either *in vivo* or *in vitro* in many multidisciplinary studies in the field of biology such as environmental toxicology, cancer genetics, aquaculture, endocrinology, and developmental biology (Bolis et al., 2001).

Cell cultures are divided into two parts according to the way the cells are obtained: primary cell cultures and cell lines. Primary cell cultures are based on the process of enzymatically obtaining cells from tissues taken from organisms and reproducing these cells *in vitro*. Cell lines are immortal cells created by spontaneous or by chemical mutation occurring in primary cell cultures (Ark ve ark., 2017). Therefore, this chapter aimed to summarize the application areas of primary cell cultures and cell lines as *in vitro* models of fish.

1. PRIMARY FISH CELL CULTURES

Primary cell cultures are the first cell culture isolated from a fresh tissue by enzymatic methods (such as trypsin, collagen). In this cell culture, the structure of the cells is similar to the cells found in the tissue from which they are isolated. Although they vary according to the cell type, they generally retain their characteristics for 9-10 passages. Therefore, studies with primary cell cultures give closer and/or similar results to studies with *in vivo* studies. Thus, both *in vivo* and *in vitro* characteristics among *in vitro* studies indicate that these cell culture strains are a good model (Allahverdiyev, 2018). However, the high risk of contamination, short life span, and the fact that cells stop proliferating after a while are the limitations of primary cell cultures in *in vitro* studies (Ark ve ark., 2017; Allahverdiyev, 2018).

Primary cell cultures from fish are generally obtained from gill (Minghetti et al., 2014) (Figure 1), liver (Pesonen et al., 2000) (Figure 2), and kidney (Ribas et al., 2014) organs. Fish gill tissue is an organ with many functions such as gas exchange, osmoregulation, acid-base exchange, removal of nitrogenous wastes from the body. Also, damage to this tissue can lead to direct death of the fish (Minghetti et al., 2014). Fish liver tissue has similar metabolic functions in the liver of mammals. It is a place where metabolic processing and storage of nutrients, enzyme synthesis, bile secretion, xenobiotic substance metabolism occur (Wolf and Wolfe, 2005). Fish kidney tissue is another organ involved in osmoregulation (Davidson, 2014).

Primary gill cell cultures are *in vitro* systems used to study the effects of various aquatic pollutants on organisms (Minghetti et al., 2014; Arslan et al., 2021). It is especially used in biological monitoring studies based on the analysis of pollutants in aquatic systems including river, streams (Minghetti et al., 2014; Schnell et al., 2015). Primary gill (Stott et al., 2015) and kidney (Ribas et al., 2014) cell cultures are used in pharmacological studies investigating the mechanisms of action of drugs. Primary liver or hepatocyte cells can be used to investigate the effects of various pollutants in ecotoxicology (Pesonen et al., 2000; Arslan et al., 2021), as well as in studies examining the expression of factors such as IGF-I by molecular studies (Schmid et al., 2000).

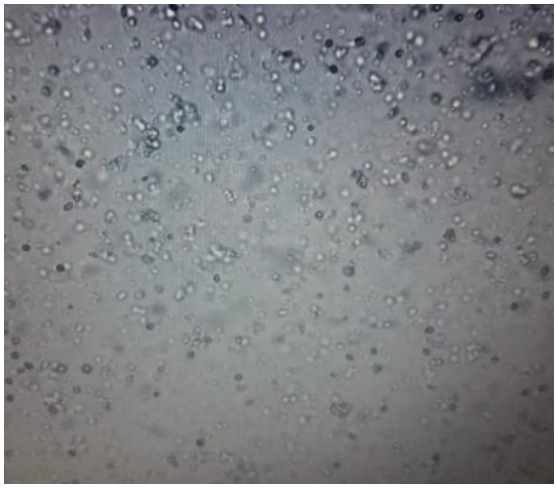

Figure 1. Primary Gill Cell Culture Derived From Carp *Cyprinus carpio* (X10, taken by Pınar Arslan)

Figure 2. Primary Hepatocyte Cell Culture Derived From Tench *Tinca tinca* (X10, taken by Pınar Arslan)

2. FISH CELL CULTURES/LINES

The first fish cell line was rainbow trout gonadal (RTG-2) cell line derived from gonad tissue of *Salmo gairdneri*. In the following years, fish cell lines have been made from many freshwater and marine fish species. In general, when fish cell lines are examined, it is observed that they are obtained from tissues of organs such as skin (Figure 3), fins, gills, heart, kidneys, brain, gonads (Figure 4), and liver (Lakra et al., 2011). In Cellusaurus, the information of cell bank, the updated fish cell lines count is 800 (Bairoch, 2021). There are fish cell cultures in many cell banks, including the American Type Cell Culture (ATCC) collection, which is the most well-known cell bank in the world (Lakra et al., 2011). In our country, there are 5 fish cell cultures/lines in Republic of Turkey Ministry of Agriculture ŞAP

Institute (Anonymous, 2021) (Table 1). Some of the studies with some fish cell lines are summarized in Table 2 according to their usage areas.

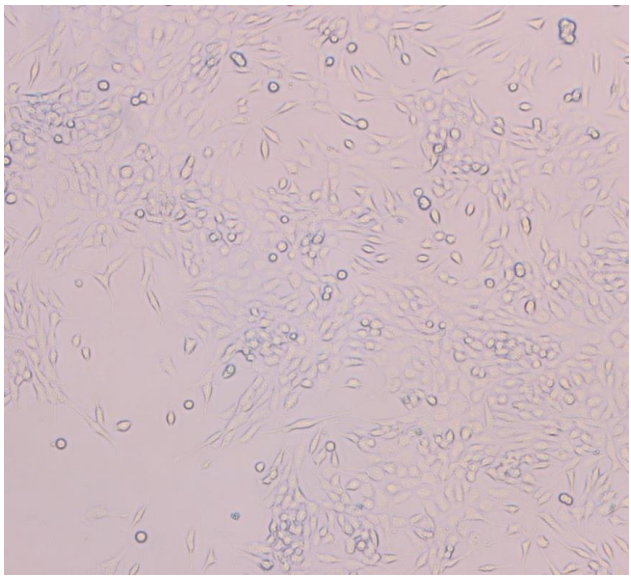

Figure 3. EPC Cell Line (X10, taken by Pınar Arslan)

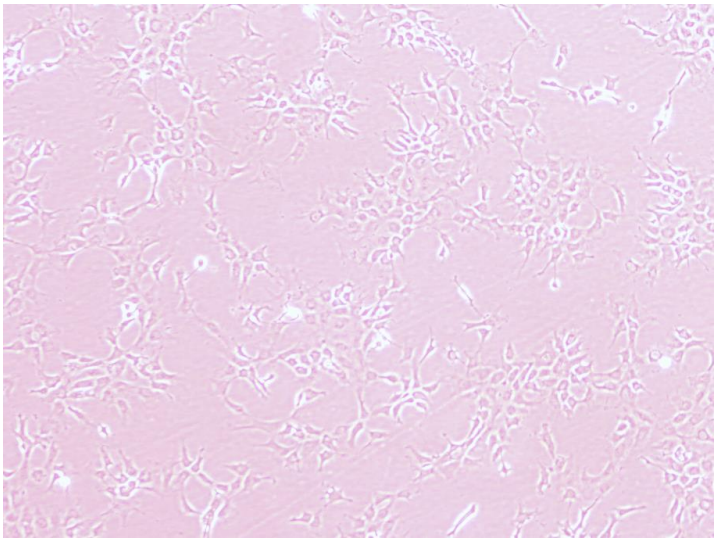

Figure 4. RTG-2 Cell Line (X10, taken by Pınar Arslan)

Table 1. Some of the Fish Cell Line and Their Information

Fish species	Short name	Catalog number	Cell type/morphology	Tissue origin
Ictalurus nebulosus brown bullhead	BB	95121807[a] CCL-59[b]	Fibroblast	Fish posterior trunk
Lepomis macrochirus bluegill	BF-2	96020201 CCL-91	Fibroblast	Fish caudal trunk
Oncorhynchus tshawytscha chinook salmon	CHSE-2K	95122203 CRL-1681	Mixed	Embryo
Cyprinus carpio carp	EPC	95122202 CRL-2872	Epithelial	Epithelioma papullosum
Oncorhynchus mykiss rainbow trout	RTG-2	95121808 CCL-55	Fibroblast	Testis and ovary (mixed)

[a]The catalog number of Republic of Turkey Ministry of Agriculture ŞAP Institute
[b]The catalog number of American Type Culture Collection

Table 2. Some of the Application Areas of Some Fish Cell Lines

Application area	Fish species	Cell line	Cell type/morphology	Tissue origin	References
Virology	*Channa* (*Ophicephalus*) *striatus* sea bass	SSN-1	Fibroblast	Whole fry tissue	Iwamoto et al., 2000
	Sparus aurata gilthead seabream	SaB-1	Neuron-like	Brain	Ruiz-Palacios et al., 2020a
Virology and immunology	*Micropterus salmoides* largemouth bass	MSH	Fibroblast	Heart	Zeng et al., 2022
Virology, immunology and toxicology	*Fundulus heteroclitus* mummichog	FuB-1	Epithelial	Brain	Ruiz-Palacios et al., 2020b
Toxicology	*Poeciliopsis lucida*	PLHC-1	Epithelial	Liver	Caminada et al., 2006
	Oncorhynchus mykiss rainbow trout	RTG-2	Fibroblast	Testis and ovary (mixed)	
	Poeciliopsis lucida	PLHC-1	Epithelial	Liver	Zurita et al., 2007

Table 2. Some of the Application Areas of Some Fish Cell Lines (Continue)

Application area	Fish species	Cell line	Cell type/morphology	Tissue origin	References
Toxicology	*Lepomis macrochirus* bluegill	BF-2	Fibroblast	Fish caudal trunk	Babich et al., 1986
	O. mykiss rainbow trout	RTG-2	Fibroblast	Testis and ovary (mixed)	Babin and Tarazona, 2005
		RTL-W1	Epithelial	Liver	
	Cyprinus carpio carp	EPC	Epithelial	Epithelioma papullosum	Jos et al., 2009
	L. macrochirus bluegill	BF-2	Fibroblast	Fish caudal trunk	
Toxicology and aquatic biomonitoring studies	*O. mykiss* rainbow trout	RTG-2	Fibroblast	Testis and ovary (mixed)	Kosmehl et al., 2004
		RTL-W1	Epithelial	Liver	
	O. mykiss rainbow trout	RTG-2	Fibroblast	Testis and ovary (mixed)	Yurdakök Dikmen et al., 2020
	Poeciliopsis lucida	PLHC-1	Epithelial	Liver	

CONCLUSION

In this study, primary cell cultures and cell lines obtained from fish are briefly summarized. Although primary cell lines are generally used in toxicology and environmental monitoring studies because they have *in vitro* and *in vivo* characteristics, the usage areas of fish cell lines include different fields such as virology, immunology, toxicology, environmental monitoring.

REFERENCES

Adey, A., Burton, J., Kitzman, J., Hiatt, J.B., Lewis, A.P., Martin, B.K., Qui, R., Lee, C., Shendure, J. (2013). The Haplotype-Resolved Genome and Epigenome of the Aneuploid HeLa Cancer Cell Line. Nature, 500, pp 207–211.

Allahverdiyev, A.M. (2018). Somatik ve Kök Hücre Kültür Sistemlerinin Temel İlkeleri. Nobel Tıp Kitapevleri. İstanbul.

Anonymous (2021). The catalog of cell lines of Republic of Turkey Ministry of Agriculture ŞAP Institute. Accessed date 18.11.2021. https://vetkontrol.tarimorman.gov.tr/sap/Belgeler/HUKUKhucrekatalog.pdf

Ark, M., Özdemir, A., Şimay, Y.D., & İbişoğlu, B. (2017). Hücre Kültürü ve Temel Moleküler Biyoloji Protokolleri. Ankara Nobel Tıp Kitapevleri, Ankara.

Arslan, P., Yurdakok-Dikmen, B., Ozeren, S.C., Kuzukiran, O., & Filazi, A. (2021). In vitro Effects of Erythromycin and Florfenicol on Primary Cell Lines of *Unio crassus* and *Cyprinus carpio*. Environmental Science and Pollution Research, 28(35) pp 48408-48416.

Babich, H., Shopsis, C., & Borenfreund, E. (1986). In vitro Cytotoxicity Testing of Aquatic Pollutants (cadmium, copper, zinc, nickel) Using Established Fish Cell Lines. Ecotoxicology and Environmental Safety, 11(1), pp 91-99.

Babín, M.M., & Tarazona, J.V. (2005). In vitro Toxicity of Selected Pesticides on RTG-2 and RTL-W1 Fish Cell Lines. Environmental Pollution, 135(2), pp 267-274.

Bairoch, A. (2021). The Cellosaurus: A Cell Line Knowledge Resource. https://web.expasy.org/cellosaurus/.

Bartolomei, M., Bollati, C., Bellumori, M., Cecchi, L., Cruz-Chamorro, I., Santos-Sánchez, G., Ranaldi, G., Ferruzza, S., Sambuy, Y., Arnoldi, A., Mulinacci, N., & Lammi, C. (2021) Extra Virgin Olive Oil Phenolic Extract on Human Hepatic HepG2 and Intestinal Caco-2 Cells: Assessment of the Antioxidant Activity and Intestinal Trans-Epithelial Transport. Antioxidants, 10(1), pp 118.

Bolis, C. L., Piccolella, M., Dalla Valle, A. Z., & Rankin, J. C. (2001). Fish as Model in Pharmacological and Biological Research. Pharmacological Research, 44(4), pp 265–280.

Caminada, D., Escher, C., & Fent, K. (2006). Cytotoxicity of Pharmaceuticals Found in Aquatic Systems: Comparison of PLHC-1 and RTG-2 Fish Cell Lines. Aquatic Toxicology, 79(2), pp 114-123.

Castaño, A., & Becerril, C. (2004) In vitro Assessment of DNA Damage After Short- and Long-term Exposure to Benzo(a)pyrene Using RAPD and the RTG-2 Fish Cell Line. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 552(1–2), pp 141-151.

Connolly, M., Fernandez-Cruz, M.-L., Quesada-Garcia, A., Alte, L., Segner, H., & Navas, J.M. (2015). Comparative Cytotoxicity Study of Silver Nanoparticles (AgNPs) in a Variety of Rainbow Trout Cell Lines (RTL-W1, RTH-149, RTG-2) and Primary Hepatocytes. International Journal of Environmental Research and Public Health, 12(5), pp 5386-5405.

Davidson A.J. (2014). Kidney Regeneration in Fish. Nephron Experimental Nephrology, 126, pp 45-49.

Heller, H., Pisarevskaja, A., Bölicke, N., Barkleit, A., Bok, F., & Wober, J. (2021). The Effect of Four Lanthanides Onto A Rat Kidney Cell Line (NRK-52E) Is Dependent On The Composition Of The Cell Culture Medium. Toxicology, 456, 152771.

Iwamoto, T., Nakai, T, Mori, K., Arimoto, M, & Furusawa, I. (2000). Cloning of The Fish Cell Line SSN-1 For Piscine Nodaviruses. Diseases of Aquatic Organisms, 43, pp 81-89.

Jos, A., Cameán, A.M., Pflugmacher, S., & Segner, H. (2009). The Antioxidant Glutathione In The Fish Cell Lines EPC And BCF-2: Response To Model Pro-Oxidants As Measured By Three Different Fluorescent Dyes, Toxicology in Vitro, 23(3), pp 546-553.

Kosmehl, T., Krebs, F., Manz, W., Erdinger, L., Braunbeck, T., & Hollert, H. (2004). Comparative Genotoxicity Testing of Rhine River Sediment Extracts Using the Comet Assay with Permanent Fish Cell Lines (RTG-2

and RTL-W1) and The Ames Test*. Journal of Soils and Sediments, 4, 84.

Lakra, W.S., Swaminathan, T.R., Joy, K.P. (2011). Development, characterization, conservation and storage of fish cell lines: a review. Fish Physiology and Biochemistry, 37, pp 1–20.

Minghetti, M., Schnell, S., Chadwick, M.A., Hogstrand, C., & Bury, N.R. (2014). A Primary Fish Gill Cell System (FIGCS) for Environmental Monitoring of River Waters. Aquatic Toxicology, 154, pp 184-192.

Pesonen, M., Teivainen, P., Lundström, J., Jakobsson, E., & Norrgen, L. (2000). Biochemical Responses of Fish Sac Fry and a Primary Cell Culture of Fish Hepatocytes Exposed to Polychlorinated Naphthalenes. Archives of Environmental Contamination and Toxicology, 38, pp 52–58.

Powers, D.A. (1989). Fish as Model System. Science, 246, pp 352–358.

Rho, J.K., Choi, Y.J., Lee, J.K., Ryoo, B.-Y., Na, I.I., Yang, S.H., Kim, C.H., & Lee, J.C. (2009). Epithelial to Mesenchymal Transition Derived from Repeated Exposure to Gefitinib Determines the Sensitivity To EGFR Inhibitors In A549, A Non-Small Cell Lung Cancer Cell Line. Lung Cancer, 63 (2), pp 219-226.

Ribas, J.L.C., da Silva, C.A., de Andrade, L., Galvan, G.L., Cestari, M.M., Trindade, E.S., Zampronio, A.R., & de Assis, H.C.S. (2014). Effects of Anti-Inflammatory Drugs in Primary Kidney Cell Culture of A Freshwater Fish. Fish & Shellfish Immunology, 40(1), pp 296-303.

Ruiz-Palacios, M., Almeida, M., Martins, M.A., Oliveira, M., Esteban, M.A., & Cuesta, A. (2020b). Establishment of a Brain Cell Line (Fub-1) From Mummichog (*Fundulus heteroclitus*) and Its Application to Fish Virology, Immunity and Nanoplastics Toxicology. Science of The Total Environment, 708, 134821.

Ruiz-Palacios, M., Esteban, M.A., & Cuesta, A. (2020a). Establishment of a Brain Cell Line (Sab-1) From Gilthead Seabream and Its Application to Fish Virology. Fish & Shellfish Immunology, 106, pp 161-166.

Schmidd, A.C., Reinecke, M., & Kloas, W. (2000). Primary Cultured Hepatocytes of the Bony Fish, *Oreochromis mossambicus*, the Tilapia: A Valid Tool for

Physiological Studies On IGF-I Expression in Liver. Journal of Endocrinology, 166, pp 265–273.

Schnell, S., Bawa-Allah, K., Otitoloju, A., Hogstrand, C., Miller, T.H., Barron, L.P., & Bury, N.R. (2015). Environmental Monitoring of Urban Streams Using A Primary Fish Gill Cell Culture System (FIGCS), Ecotoxicology and Environmental Safety, 120, pp 279-285.

Segeritz, C.P., & Vallier, L. (2017). Cell Culture: Growing Cells as Model Systems In Vitro. Basic Science Methods for Clinical Researchers, 151–172.

Stott, L.C., Schnell, S., Hogstrand, C., Owen, S.F., & Bury, N.R. (2015). A Primary Fish Gill Cell Culture Model to Assess Pharmaceutical Uptake And Efflux: Evidence For Passive And Facilitated Transport. Aquatic Toxicology, 159, pp 127-137.

Wolf, J.C., & Wolfe, M.J. (2005). A Brief Overview of Nonneoplastic Hepatic Toxicity in Fish. Toxicologic Pathology, 33(1), pp 75-85.

Yurdakök Dikmen, B., Aydın, F.G., Tutun, H., & Sevin, S. (2020). Evaluation of Antropogenic Aquatic Toxicity by and Alternative and New Approach; using PLHC-1 and RTG-2 cell lines and CYP1A1 Biomarker. KSU Journal of Agriculture and Nature, 23(1), pp 247-258.

Zeng, W., Dong, H., Chen, X., Bergmann, S.M., Yang, Y., Wei, X., Tong, G., Li, H., Yu, H., & Chen, Y. (2022). Establishment and Characterization of a Permanent Heart Cell Line from Largemouth Bass *Micropterus salmoides* and Its Application to Fish Virology and Immunology, Aquaculture, 547, 737427.

Zurita, J.L., Jos, A., del Peso, A., Salguero, M., Cameán, A.M., López-Artíguez, M., & Repetto, G. (2007). Toxicological assessment of indium nitrate on aquatic organisms and investigation of the effects on the PLHC-1 fish cell line, Science of The Total Environment, 387(1–3), pp 155-165.

CHAPTER 8

CHECKLIST OF TURKISH CRIOCERINAE (CHRYSOMELIDAE)

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INTRODUCTION

Turkey is one of the countries with the richest biological diversity in Europe and the Middle East and ranks ninth in terms of biological diversity on the European continent. Our country, which is a dec point between north and south; west and east, contains 3 different regions important for biological diversity, including Europe-Siberia, Iran Turan and the Mediterranean from 37 flora regions of the world. Turkey, where three different regions meet in an area that covers a very small part of the world, shows the characteristics of a small continent in terms of biological diversity. In addition to the presence of species belonging to three different flora, the transitional areas between these regions constitute very rich areas in terms of endemic and hybrid species (Özhatay et al., 2003)

Criocerinae is represented by about 1400 species in the world and 13 species in Turkey (Jolivet ve Hawkeswood, 1995; Özdikmen & Özbek, 2014).

Turkish Criocerinae, which includes a total of 13 species, is quite common in Turkey. The highest number of species is represented in the Eastern Anatolia Region. It is followed by the Central Anatolia Region. Aegean Region, Mediterranean Region, Black Sea Region, and Marmara Region are represented by a significant number of species. In other words, the Southeastern Anatolia Region is now represented by a single species. (Özdikmen & Özbek, 2014).

1.SUBFAMILY CRIOCERINAE Latreille, 1804

1.1. Genus *Crioceris* Geoffroy, 1762

***C. asparagi* (Linnaeus, 1758);** Records in Turkey: **TR-A:** Aksaray, Amasya, Antalya, Isparta, İzmir, Kayseri, Karabük, Kars, Osmaniye (Gül-Zümreoglu 1972; Tomov & Gruev ˘ 1975; Gruev & Tomov 1984; Turanlı et al. 2002; Gök & Çilbiroglu˘ 2003; Warchałowski 2003; Özdikmen & Turgut 2008b; Aslan et al. 2009; Özdikmen & Aslan 2009; Ekiz et al., 2013; Özdikmen & Özbek, 2014).

***C. bicruciata* (Sahlberg, 1823);** Records in Turkey: **TR-A:** Aydın, Iğdır, Isparta, İzmir, Kars, Manisa (Sahlberg 1913; Tomov & Gruev 1975; Gruev & Tomov 1984; Aslan 2000; Turanlı et al. 2002; Warchałowski 2003; Gruev 2005a; Özdikmen & Turgut 2008; Sen & Gök 2009; Ekiz et al., 2013; Özdikmen & Özbek, 2014)

***C. duodecimpunctata* (Linnaeus, 1758)**; Records in Turkey: **TR-A:** Aksaray, Amasya, Ankara, **ÇANKIRI,** Erzurum, İzmir, Konya, Kars, Niğde, Samsun – **TR-E** (Medvedev 1970; Gül-Zümreoglu 1972; Tomov & Gruev 1975; Gruev & Tomov 1984; Aslan 2000; Turanlı et al. 2002; Gruev 2005a; Özdikmen & Turgut 2008; Ekiz et al., 2013; Özdikmen & Özbek, 2014; Bal et al., 2018).

***C. paracenthesis* (Linnaeus, 1767)**; Records in Turkey: **TR-A:** Isparta, Kars – TR-E: İstanbul (Aslan 2000a; Gök & Çilbiroglu 2003; Gruev 2005b; Ekiz et al., 2013; Özdikmen & Özbek, 2014).

***C. quatuordecimpunctata* (Scopoli, 1763)**; Records in Turkey: **TR-A:** Kars (Aslan 2000; Schöller 2002; Ekiz et al., 2013; Özdikmen & Özbek, 2014).

***C. sokolowi* Jakobson, 1894**; Records in Turkey: **TR-A:** Ankara, Konya, Niğde (Özdikmen & Turgut, 2008; Ekiz et al., 2013; Özdikmen & Özbek, 2014).

1.2.Genus *Lema* Fabricius, 1798

1.2.1. Subgenus *Lema* Fabricius, 1798

***L. cyanella* (Linnaeus, 1758)**; Records in Turkey: **TR-A:** Erzurum (Aslan 2000; Ekiz et al., 2013; Özdikmen & Özbek, 2014).

1.3. Genus *Lilioceris* Reitter, 1913

***L. faldermanni* (Guérin-Méneville, 1844);** Records in Turkey: **TR-A:** Ankara, Antalya, Bayburt, Erzurum, Isparta, Kahramanmaraş – **TR-E** (Aslan 2000; Warchałowski 2003; Gruev 2005a; Özdikmen &Turgut 2008; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Özbek, 2014).

***L. lilii* (Scopoli, 1763);** Records in Turkey: **TR-A:** İzmir (Gül-Zümreoglu 1972; Turanlı et al. 2002; Özdikmen & Turgut 2008; Ekiz et al., 2013; Özdikmen & Özbek, 2014).

***L. merdigera* (Linnaeus, 1758);** Records in Turkey: **TR-A: ERZİNCAN,** İzmir, Kahramanmaraş, Kütahya (Gül-Zümreoglu 1972; Turanlı et al. 2002; Özdikmen & Turgut 2008; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Özbek, 2014; Aslan et al., 2020).

1.4. Genus *Oulema* Gozis, 1886

***O. duftschmidi* (Redtenbacher, 1874);** Records in Turkey: **TR-A:** Adana, Aksaray, Ankara, Afyon, Bolu, Çankırı, Düzce, Eskişehir, Karaman, Kayseri, Konya, Kırşehir, Mersin, Samsun (Özdikmen & Turgut 2008; Ekiz et al., 2013; Özdikmen & Özbek, 2014).

***O. gallaeciana* (Heyden, 1870);** Records in Turkey: **TR-A:** Bayburt, Bolu, Erzurum, Kayseri, Karabük, Kars (Gruev & Tomov 1979; Aslan 2000; Özdikmen & Turgut 2008; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Özbek, 2014).

***O. melanopus* (Linnaeus, 1758);** Records in Turkey: **TR-A**: Adana, Afyon, Aksaray, Ankara, Antalya, Ardahan, Aydın, Çanakkale, **ÇANKIRI,** Çorum, Diyarbakır, Eskişehir, Erzincan, Erzurum, Gaziantep, Hatay, Iğdır, İzmir, Kahramanmaraş, Karaman, Kastamonu, Kayseri, Konya, Karabük, Kars, Kütahya, Manisa, Muğla, Osmaniye, Samsun, Siirt, **TOKAT**– TR-E: Edirne, Kırklareli, Tekirdağ (Medvedev 1970; Gül-Zümreoglu 1972; Tomov & Gruev 1975; Gruev & Tomov 1984; Campobasso et al. 1999; Aslan 2000; Turanlı et al. 2002; Gök & Çilbiroglu˘ 2003; Gök & Gürbüz 2004; Gruev 2005a; Özdikmen & Turgut 2008; Aslan et al. 2009; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Özbek, 2014; Medvedev, 2015; Bal et al., 2018, Özdikmen et al., 2020).

***O. obscura* (Stephens, 1831);** Records in Turkey: **TR-A: ARTVİN,** Bayburt, Bolu, Karabük, Kayseri, Erzurum, Kars (Ekiz et al., 2013; Gök & Turantepe, 2019).

***O. rufocyanea* (Suffrian, 1847);** Records in Turkey: **TR-A: AFYON**, Ankara (Bezdek & Baselga, 2015; Gök &Bostan, 2020).

2. RESULTS

As a result of research, one new record from Çankırı province for the genus *Crioceris*, one new record from Erzurum province for the genus *Lilioceris*, one new record from Afyonkarahisar, Artvin, Çankırı, and Tokat provinces were given for the genus *Oulema*. The species and the provinces in which they have just been recorded are given below.

SPECIES/SUBSPECIES	STATUS OF THE SPECIES
Crioceris duodecimpunctata (Linnaeus, 1758)	It is new record for **Çankırı** province and hereby **Western Black Sea region** of Turkey.
Oulema obscura (Stephens, 1831)	It is recorded for the first time from **Artvin** province
Lilioceris merdigera (Linnaeus, 1758)	It is recorded for the first time from **Erzincan** province.
Oulema melanopus (Linnaeus, 1758)	It is recorded for the first time **Çankırı** and **Tokat** provinces.
Oulema rufocyanea (Suffrian, 1847)	It is recorded for the first time from 26 Ağustos Nature Park (**Afyonkarahisar**).

Along with these data, *Oulema rufocyanea* (Suffrian, 1847) was found only in Ankara province, while it was recorded from Afyonkarahisar province as the second record for Turkey. *Lilioceris merdigera* (Linnaeus, 1758) was recorded from Erzincan province as the fourth Record for Turkey after Izmir, Kahramanmaraş and Kütahya provinces.

REFERENCES

Aslan et al. (2009). Aslan EG, Gök A, Gürbüz MF, Ayvaz Y. 2009. Species composition of Chrysomelidae (Coleoptera) in Saklıkent vicinity (Antalya, Turkey) with observations on potential host plants. J Entomol Res Soc. 11(3):7–18.

Aslan, E.G., Kaya, Ö. D. & Ünal, E. (2020). Contributions to the Knowledge of Leaf Beetle (Coleoptera: Chrysomelidae) Fauna in Elazığ, Erzincan and Tunceli Provinces, Turkey, Mehmet Akif Ersoy Üniversitesi Fen Bilimleri Enstitüsü Dergisi 11(Ek Sayı 1): 273-280.

Aslan, İ. (2000); Doğu Anadolu'da Criocerinae (Coleoptera :Chrysomelidae) altfamilyası üzerinde faunistik ve sistematik çalışma. Türkiye 4. Entomoloji Kongresi, 12-15 Eylül 2000, Aydın, 511-522.

Bal, N., Özdikmen, H. & Kıyak, S. (2018). Thirty new leaf beetles for the fauna of Çankırı province in Turkey (Chrysomelidae). Munis Entomology & Zoology. 13(2): 507-518.

Bezdek, J. & Baselga, A. (2015). Revision of western Palaearctic species of the *Oulema melanopus* group, with description of two new species from Europe (Coleoptera: Chrysomelidae: Criocerinae), Acta Entomologica Musei Natıonalis Pragae, Volume 55(1), pp. 273–304.

Campobasso G, Colonnelli E, Knutson L, Terragitti G., Cristofaro M & editors. (1999). Wild plants and their associated insects in the palearctic region, primarily Europe and the Middle East. Rome (Italy): United States. Department of Agriculture, Agricultural Research Service; 249 p.

Ekiz, A.N., Sen, I., Aslan, E.G., & Gök, A. (2013), 'Checklist of Leaf Beetles (Coleoptera: Chrysomelidae) of Turkey, excluding Bruchinae', Journal of Natural History, 47, 2213.

Gök, A. & Çilbiroglu, E. G. (2003). The Chrysomelidae fauna of Kovada Stream Arboretum (Eğirdir– Isparta,Turkey). Nouv Revue Ent (NS). 20:61–73.

Gök, A. & Gürbüz, M. F. (2004). The Chrysomelidae fauna of the Islands of Beyşehir Lake in Turkey. Nouv Revue Ent (NS). 21:43–48.

Gök, A. & Turantepe, E. (2019). Additions to the fauna of Chrysomelidae (Coleoptera) from Hatila Valley National Park (Artvin, Turkey), with notes on host plant preferences and zoogeographic evaluations, Caucasian Entomological Bulletin, 15(1): 135–146.

Gök, A.& Bostan, K. (2020). The First Faunistic Data on the Leaf Beetles (Coleoptera: Chrysomelidae) of 26 Ağustos Nature Park, Afyonkarahisar, Turkey, J. Entomol. Res. Soc.,22(1): 83-99, 2020.

Gruev B & Tomov, V. (1984). Fauna Bulgarica 13, Coleoptera, Chrysomelidae, Part I, Orsodacninae, Zeugophorinae, Donaciinae, Criocerinae, Clytrinae, Cyrptocephalinae, Lamprosomatinae, Eumolpinae. Sofia: In Aedibus Academie Scientiarum Bulgaricae. 220 p.

Gruev B, Tomov, V. (1979). Zur Kenntnis einiger in der Türkei, Jugoslawien und Griechenlve vorkommender Arten der Familie Chrysomelidae (Coleoptera) aus der Zoologischen Staatssammlung München. Spixiana. 2(3):259–267.

Gruev B. (2005b). The mediterranean leaf beetles in Bulgaria (Insecta: Coleoptera: Chrysomelidae). Paper presented at: Proceedings of the Balkan Scientific Conference of Biology; Plovdiv, Bulgaria.

Gruev, B. (2005a). A comparative list of the leaf beetles of the balkan countries (Coleoptera: Chrysomelidae). Trav Sci Univ Plovdiv Animalia. 41:23–46.

Gül-Zümreoglu S. (1972). Izmir Bölge Zirai Mücadele Araştırma Enstitüsü Böcek ve Genel Zararlılar Katalogu, 1928–1969, 1.Kısım. İzmir: T.C. Tarım Bakanlığı Zirai Mücadele ve Zirai Karantina Genel Müdürlügü Yayınları; p. 48–52.

Jolivet, P. & Hawkeswood, T. (1995). Host-plants of Chrysomelidae of the world. Backhuys Publ., Leiden, 120-180.

Medvedev, L. N. (1970). A List of Chrysomelidae Collected by Dr. W. Wittmer in Turkey (Coleoptera). Rev Suisse Zool. 77(2):309–319.

Medvedev, L. N. (2015). To the knowledge of leaf beetles (Coleoptera: Chrysomelidae) from Turkey, Caucasıan Entomological Bullettin, 11(2): 391–394.

Özdikmen, H. & Özbek, H. (2014). Chorotype identification for Turkish Chrysomeloidea (Coleoptera) Part IV – Chrysomelidae: Donaciinae and Criocerinae. Munis Entomology and Zoology, 9(1), 161-169.

Özdikmen, H. & Turgut, S. (2008). The subfamily Criocerinae of Turkey (Coleoptera: Chrysomelidae) with two new records and zoogeographical remarks. Munis Entomology & Zoology 3 (1): 239-250.

Özdikmen, H. & Turgut, S. (2008b). The subfamily Criocerinae of Turkey (Coleoptera: Chrysomelidae) with two new records and zoogeographical remarks. Mun Ent Zool. 3;1:239–250.

Özdikmen, H. (2011). A comprehensive contribution for leaf beetles of Turkey with a zoogeographical evaluation for all Turkish fauna (Coleoptera: Chrysomelidae). Mun Ent Zool. 6(2):540–638.

Özdikmen, H., & Aslan, K. (2009). First records of some leaf beetles for Mediterranean region in Turkey and south Turkey (Coleoptera: Chrysomelidae). Mun Ent Zool. 4;1:276–279.

Özdikmen, H., Bal, N. & Coral Şahin, D. (2020). A contribution to the knowledge of leaf-beetles (Coleoptera: Chrysomelidae) in Turkey using data of specimens in Nazife Tuatay Plant Protection Museum (Turkey, Ankara). Munis Entomology & Zoology. 15(1): 269-297.

Özhatay, N., Byfield, A. & Atay, S. (2003). Türkiye'nin Önemli Bitki Alanları. WWF Türkiye, İstanbul, 17-19.

Sahlberg, J. (1913). Coleoptera Mediterranea Orientalia, Quae in Aegypto, Palaestina, Syria, Caramania atque in Anatolia Occidentali anno 1904. Öfversigt af Finska Vatenskaps– Societetens Förhandlingar. 55 A;19:1–281.

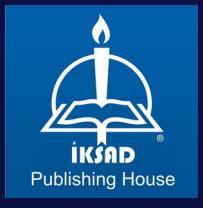
Schöller M. (2002). Taxonomy of Cryptocephalus Geoffroy: what do we know? (Coleoptera: Chrysomelidae: Cryptocephalinae). Mit Int Entomol Verein. 27:59–76.

Şen, İ & Gök A. (2009). Leaf beetle communities (Coleoptera: Chrysomelidae) of two mixed forest ecosystems dominated by pine–oak–hawthorn in Isparta province, Turkey. Ann Zool Fenn. 46:217–232.

Tomov, V. & Gruev, B. (1975). Chrysomelidae (Coleoptera) collected by K. M. Guichard in Turkey, Greece and Yugoslavia. Trav Sci Univ Plovdiv, Bulgaria. Biology. 13(4):133–151.

Turanlı, F. & Kısmalı, S. (2002). *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)'nın larva dönemlerine uygulanan *Bacillus thurinqiensis* var. tenebrionis'in, sindirim sistemi üzerindeki etkileri. Türk Entomol Derg. 26(3):209–227.

Warchałowski, A. (2003). Chrysomelidae: the leaf beetles of Europe and the mediterranean Area. Warszawa: Natura Optima dux Foundation; 600 p.



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