# ISOLATION AND CHARACTERIZATION OF *SALMONELLA* BACTERIOPHAGES

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# ABSTRACT

## ISOLATION AND CHARACTERIZATION OF *SALMONELLA* BACTERIOPHAGES

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Numerous foodborne infections and outbreaks are associated with *Salmonella* which makes it a challenge in terms of human health and economy. Therefore, reducing the prevalence of *Salmonella* in food and food processing areas is of great importance. Antibiotics are the substances that are commonly used in various stages of food production in order to fight against *Salmonella*. However, concerns related with the antibiotic use like antibiotic resistance give rise to pursuit of safer methods to eliminate *Salmonella* from the environment. Bacteriophages (phages) are seen as promising tools for the control of bacteria as they are viruses that use bacterial cells as their hosts. Nonetheless, their characteristics must be well-defined in order to get GRAS status and be used in industry. Additionally, since the distribution of *Salmonella* serovars varies geographically, regional effects are also important in phage-based applications. For this reason, bacteriophage cocktails designed in other countries may not show sufficient effect against isolates in Turkey. Current study aims to isolate and characterize *Salmonella* bacteriophages from cattle-poultry feces coming from distinct regions in Turkey and from wastewater in order to find an alternative to antibiotics. In total 25 *Salmonella*

bacteriophages were isolated. The most abundant phages were against *S.* Enteritidis and other than that *S.* Typhimurium, *S.* Kentucky, *S.* Hadar, *S.* Telaviv and *S.* Anatum phages were obtained. In the next step, titers and host ranges of these bacteriophages were determined. Host range analysis revealed the differences in phage lysing capabilities of bacteriophages and showed that phages isolated from wastewater had broader host ranges compared to the phages obtained from feces. The phage with the broadest host range was defined as the phage whose indicator strain was *S.* Telaviv and it was capable of infecting 77.7% of the serovars partially or totally. Moreover, bacteriophages was subjected to single step growth curve experiments in order to determine their burst size and latent periods which are important parameters for their use in phage cocktails. Phage P1-224 exhibited the greater burst size (236 PFU/cell) with short latent period (15 min) among others. Bacteriophages were also subjected to Pulsed Field Gel Electrophoresis (PFGE) to determine their genome size. Isolated phages genome sizes were in the range of 33-124 kb. Besides, *Salmonella* isolation was performed from the collected samples. In total 4 *Salmonella* strains were isolated. Their subtyping was conducted by PFGE analysis. As a result, 2 *S.* Enteritidis and 2 *S.* Typhimurium were identified.

This study provided a better understanding of phage-host interactions and diversities in phages nature which possess significant importance for their use in food safety applications.

Keywords: *Salmonella,* antibiotic resistance*,* phage, host range, Pulsed Field Gel Electrophoresis (PFGE)

# ÖZ

## SALMONELLA BAKTERİYOFAJLARININ İZOLASYONU VE KARAKTERİZASYONU

Deniz, Aysu
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Dünyada çok sayıda gıda kaynaklı enfeksiyon ve salgın *Salmonella* ile ilişkilendirilmektedir. Bu da *Salmonella*'yı insan sağlığı ve ekonomik açıdan büyük bir problem haline getirmektedir. Bu nedenle *Salmonella* prevalansının gıdalarda ve gıda işleme alanlarında düşürülmesi büyük önem taşımaktadır. Antibiyotikler, *Salmonella* ile mücadele etmek amacıyla gıda üretiminin bir çok aşamasında yaygın olarak kullanılan maddelerdir. Ancak antibiyotik dirençlilik gibi antibiyotik kullanımının sebep olduğu endişeler, *Salmonella*'nın ortamdan eleminasyonu için daha güvenli yöntemlerin arayışına yol açmaktadır. Bakteriyofajlar, kısaca fajlar, bakterileri konakçı olarak kullanan virüsler olduğundan Salmonella kontrolü için ümit vaadeden araçlar olarak görülmektedir. Bununla birlikte GRAS statüsü alabilmek ve endüstride kullanılabilmeleri için özelliklerinin iyi tanımlanmış olması gerekmektedir. Ayrıca *Salmonella* serovarlarının dağılımı coğrafi olarak değişiklik gösterdiğinden, biyokontrol uygulamalarında da bölgesel etkiler önem taşımaktadır. Bu sebeple başka ülkelerde tasarlanan bakteriyofaj ürünleri Türkiye'deki izolatlara karşı yeterli etkiyi göstermeyebilir. Bu çalışma, antibiyotiklere alternatif olarak Türkiye'nin farklı bölgelerinden gelen sığır, kanatlı

dışkılarından ve atık sudan *Salmonella* bakteriyofajları izole ve karakterize etmeyi amaçlamaktadır. Bu amaçla, toplamda 25 *Salmonella* fajı izole edilmiştir. En çok sayıda elde edilen faj *S.* Enteritidis'e karşı olup *S.* Typhimurium, *S.* Kentucky, *S.* Hadar, *S.* Telaviv ve *S.* Anatum fajları da izole edilmiştir. Bir sonraki aşamada fajların titresi ve konakçı aralığı belirlenmiştir. Konakçı aralığı analizi, fajların farklı *Salmonella* izolatları üzerindeki etkinliğini ortaya çıkarmış olup atık sudan izole edilen fajların, dışkıdan elde edilenlere kıyasla daha geniş konakçı aralığına sahip olduğunu göstermiştir. En geniş konakçı aralığına sahip olan faj *S.* Telaviv fajı olarak tanımlanmıştır ve kullanılan serotiplerin %77.7 sinin üstünde kısmen veya tamamen etki göstermiştir. Bunlara ek olarak, fajların kokteyllerde kullanılmaları için önemli parametreler olan patlama boyutları ve latent periyotları, tek aşamalı büyüme eğrisi deneyleri ile belirlenmiştir. En yüksek patlatma boyutuyla birlikte (236 POB/hücre) en kısa latent periyodu (15 dk), P1-224 fajı göstermiştir. Bakteriyofajlar ayrıca genom boyutlarının belirlenmesi amacıyla Vuruşlu Alan Elektroforezine (PFGE) tabi tutulmuş olup genom boyutlarının 33-124 kb arasında yer aldığı saptanmıştır. Çalışmada, faj izolasyonundan ayrı olarak dışkı örnekleri ve atık sudan *Salmonella* izolasyonu da gerçekleştirilmiştir. Toplamda 4 *Salmonella* suşu izole edilmiş olup bu suşların serotipleri yine PFGE aracılığıyla belirlenmiştir. Bu çalışma, gıda güvenliğinde kullanılmak üzere büyük önem taşıyan bakteriyofajların, faj-konakçı ilişkilerinin belirlenmesi ve fajların yapısal farklılıklarının daha iyi anlaşılmasını sağlamıştır.

Anahtar Kelimeler: *Salmonella*, antibiyotik dirençlilik, bakteriyofaj, konakçı aralığı, Vuruşlu Alan Elektroforezi

To my sister,

and

To my parents.

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| | |
|---|---|
| **Abi** | Abortive Infection |
| **Amp** | Ampicillin |
| **Amc** | Amoxicillin-clavulanic acid |
| **Ce** | Competitive exclusion |
| **Cn** | Gentamicin |
| **Cro** | Ceftriaxone |
| **Eft** | Ceftiofur |
| **Fox** | Cefoxitin |
| **GRAS** | Generally Recognized As Safe |
| **K** | Kanamycin |
| **Kf** | Cephalothin |
| **MOI** | Multiplicity of infection |
| **N** | Nalidixic acid |
| **NTS** | Non-typhoidal *Salmonella* |
| **PCR** | Polymerase Chain Reaction |
| **PFGE** | Pulsed Field Gel Electrophoresis |
| **PFU** | Plaque forming unit |
| **S** | Streptomycin |
| **Sf** | Sulfisoxazole |
| **Sie** | Superinfection exclusion |
| **Sxt** | Sulfamethoxazole-trimethoprim |
| **T** | Tetracycline |

# CHAPTER 1

# INTRODUCTION

Foodborne illnesses have been a burden to public health for decades and immense numbers of people are getting affected every year. CDC estimates that 48 million people get sick and eventually 3,000 deaths occur per year due to the foodborne illnesses in the United States (CDC, 2020). The economic consequences of these illnesses cannot be underestimated, as well (Scharff, 2012). A large percentage of the diseases are caused by *Salmonella* and the infections occur due to the *Salmonella* are called salmonellosis. Various foods are linked with *Salmonella* outbreaks worldwide with an emphasis on poultry, pork and egg products (Antunes et al., 2016). However, many other foods such as beef, fresh produces, dairy products and even processed products are also involved in outbreaks. The risk of contamination is dependent on several factors and efforts are being made to prevent salmonellosis and reduce the risk in every aspect of food supply chain (Ngueng Feze et al., 2018). It is vital to implement strategies against *Salmonella* contamination in pre- and post-harvest periods of production (Ehuwa et al., 2021). Thus, interventions possess a critical importance in terms of elimination of this foodborne pathogen (Nayak et al., 2012).

Antibiotics are the substances that are widely used in order to fight against *Salmonella,* and they were not only employed to treat *Salmonella* infections but also took role in many steps of food production such as promoting the growth of animals (Kirchhelle, 2018). However, mis and overuse of these substances leads spread of antibiotic resistant bacteria through the food chain which brought some concerns in terms of population health (Ricke et al., 2015). Contamination of resistant bacteria to food may be through soil and water because animals are the shedders of *Salmonella* and resistant bacteria can remain in their gastrointestinal

systems. Also, there are other possible routes for resistant *Salmonella* to enter in food supply chain (Nair et al., 2018). The emergence and spread of antibiotic resistant bacteria have serious impacts on global health and economy. Therefore, implementing new strategies to control *Salmonella* in foods and production facilities gained importance (Ricke et al., 2015).

Bacteriophages are the viruses that infect bacterial cells. In other words, they are the predators of bacteria (Clokie et al., 2011). Their abundance in variety of environments makes them powerful tools to mitigate bacteria from the environment. Bacteriophages' specificity to their hosts is the most prominent attribute of them because high specificity offers the elimination of only target prokaryotic microorganisms (Kittler et al., 2017). In addition to those, bacteriophages do not require special conditions to reproduce. They are present with their hosts and they are self-replicating (Payne and Jansen, 2000). On the other hand, they are needed to be well characterized phenotypically and genotypically in order to take part in applications (Harada et al., 2018). Some parameters must be fulfilled by the phages to avoid complications. Their interactions with the host cells must be fully comprehended (Necel et al., 2020). Therefore, more effort should be made on bacteriophages and their possible applications in food industry.

In this study, bacteriophages infecting *Salmonella* were isolated from cattle-poultry feces and wastewater in order to offer an alternative method to antibiotics since antibiotic resistance became an important concern. The samples were supplied from several locations in Turkey. Since the distribution of *Salmonella* serovars is different in distinct regions, bacteriophages isolated in Turkey have a significant importance because they can be effective against local *Salmonella* serovars prevalent in Turkey. Besides that, *Salmonella* isolation and their genomic characterization was performed to provide a better understanding of *Salmonella* strains a in these samples.

Moreover, the efficacy of isolated bacteriophages was evaluated on various *Salmonella* strains and some of their characteristics were determined to identify them so that they can be employed as biocontrol agents to reduce the risk of *Salmonella* contamination in foods and food processing facilities. The identified bacteriophages will make a major contribution to the phage database.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 *Salmonella*

*Salmonella* is one of the most frequently encountered foodborne pathogens worldwide (Eng et al., 2015). *Salmonella* has been a serious concern since ancient times, and it continues to be a problem for public health and a global burden (Ellermeier and Slauch, 2006). According to the data published by CDC, in United States 1.35 million infections and 420 deaths per year occur due to *Salmonella* (CDC, 2021a)

*Salmonella* which is a member of Enterobacteriaceae family is rod shaped, Gram-negative and facultatively anaerobic bacterium. In general, it is known for being motile due to their flagella even though there are some exceptions (Wirtanen and Salo, 2016). Organisms under *Salmonella* genus have three different types of antigens which is used for identification or classification purposes (Todar, 2020). Those antigens are flagellar (H) antigen, somatic (O) antigen and Capsular (Vi) antigens (Baron, 1996). The White-Kauffmann-Le Minor scheme which shows the serovars of *Salmonella* was formed according to the O and H antigens that bacteria possess (Eng et al., 2015). Glycan units which comprised of sugars are the basis of O antigens. How these units are linked with each other as well as the type of linkage between O units defines the structure (Seif et al., 2019). Vi antigens are only found in some serovars of *Salmonella*.

Up to today, nomenclature of *Salmonella* has caused some confusions among scientists. Different classifications have been proposed. However, today CDC based nomenclature is used and this aims to prevent potential misunderstandings and provides uniformity. According to CDC classification, genus *Salmonella* is

divided into 2 different species as *Salmonella enterica* and *Salmonella bongori* which include different serotypes (Brenner et al., 2000). *Salmonella enterica* consists of 6 subspecies: *S. enterica* subsp. *enterica* (I)*, S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV), *S. enterica* subsp. *indica* (VI) (Löfström et al., 2015). *S. enterica* subsp. *enterica* is associated with the human and warm-blooded animal infections. On the other hand, other subspecies and *S. bongori* are linked to cold-blooded animals (Desai et al., 2013).

*S. enterica* subsp. *enterica* is known for being the major cause of *Salmonella* infections in humans and animals among other *Salmonella* subspecies (Cremonesi et al., 2020; Eng et al., 2015).

### 2.1.1 Salmonellosis

Disease caused by *Salmonella* is called salmonellosis and it is ranked as the second most common foodborne disease in humans (Andres and Davies, 2015). Generally, the infections are caused by consuming contaminated food (CDC, 2021a). Syptoms of salmonellosis usually occur in the form of gastrointestinal problems after 12-72 hours of infection (FDA, 2019). Severity and the course of the disease show difference among individuals. The condition of the person such as age, immune system is the factor that has an impact on the disease. Besides, the type of the *Salmonella* serovar causing the disease is important parameter in terms of severity. Even though most of the reported cases are mild, deaths may occur due to *Salmonella* infections (WHO, 2018). Each serotype of *Salmonella* may exhibit different symptoms in infected individuals so that certain problems may point out which microorganism is responsible of the situation (Crump, 2012).

Non-typhoidal salmonellosis can examined as non-invasive and invasive salmonellosis (Gal-Mor et al., 2014; Kurtz et al., 2017).

Non-typhoidal *Salmonella* are not necessarily restricted to humans (CFSPH, 2013).

According to Havelaar et al. only 1 case of salmonellosis out of 58 was reported in European Union which shows how the population is significantly affected by *Salmonella* infections (Havelaar et al., 2013). It is stated that 57,000 deaths per year occur globally due to non-typhoidal *Salmonella* (CDC, 2019). Particularly, animals are the main source of contamination for non-typhoidal salmonellosis. Consumption of contaminated animal products has the higher percentage as a cause of infection. Especially, raw or undercooked eggs are responsible for *Salmonella* infections all over the world (CDC, 2021a). Unpasteurized milk, meat products, poultry, fresh produces and are also linked to salmonellosis (Ngueng Feze et al., 2018).

Nevertheless, non-animal products can also carry the risk of contamination (EFSA, 2015). This may happen through the production when the poor sanitation procedures are followed. For instance, risk of contamination increases if meat or its juice is in contact with fresh vegetable (Berger et al., 2010).

Additionally, several outbreaks related with the cross-contamination have been reported up to today. Surfaces which are contaminated with *Salmonella* facilitate the spread of bacteria (FDA, 2019).

Enteric fever is another type of a disease caused by *Salmonella* serotypes which causes serious health problems in humans. *Salmonella* Typhi is causing typhoidal fever and Paratyphi A, B, C is responsible for paratyphoidal fever. Since both exhibits undifferentiable properties with each other, in general, they can be called as enteric fever (Connor and Schwartz, 2005). Humans are the shedders of *S.* Typhi and Paratyphi and the contamination occurs due to the fecal-oral route. Water and food can be contaminated by the microorganisms through the infected people or the carriers (Crump, 2012). The disease is especially seen in the under-developed countries where the clean water is not adequately accessible. Today, the risk of getting the disease is rather linked with the travelers for the developed countries (Gal-Mor et al., 2014).

According to the findings the presence of the Vi antigen plays a role in the pathogenicity of *S.* Typhi, and it allows the microorganism to endure the pH of the stomach (Robbins and Robbins, 1984). In order to be infected by *S.* Typhi, substantially the bacterium should invade the epithelial cells of intestines. After the invasion, it is spread through the other organs which makes it a systematic disease (Chowdhury et al., 2019). Symptoms of typhoid fever usually appears in 7 to 14 days after the ingestion of *S.* Typhi. Fever accompanied by unease starts to strike. When the disease progresses, fever accelerates. Other symptoms include abdominal cramps, nausea, chills. If the infection is not treated, complications take over (Parry et al., 2002).

### 2.1.2 Distribution of non-typhoidal *Salmonella* Serovars

Infections caused by NTS serovars make up a big part of foodborne diseases (Havelaar et al., 2015). Although this is a global problem, some regions are involved in outbreaks more than others. For example, number of cases are varied among Europe (Kirk et al., 2015). Furthermore, strains that are responsible for infections exhibit variation among countries. In some regions, some serotypes involved in infections dominate over others whereas in some regions, many serotypes are reported to be responsible for the cases. Besides, in different countries, different serovars are linked with the infections. For instance, *S.* Virchow is isolated from infected people in Australia more common than other countries. This distinction may be caused by several factors like animal population, farming or production practices and surveillance systems. However, still, some serotypes such as *S.* Enteritidis and *S.* Typhimurium are the dominant serovars that play a role in incidents (Cheng et al., 2019). One should note that under the certain serotypes, some strains might geographically cluster in different regions.

### 2.1.3 *Salmonella* Outbreaks

*Salmonella* outbreaks can be linked with foods and animals. An outbreak which affected 1722 people in 50 different states was reported in 2020 in United States. According to the investigation data the source was contact with the backyard poultry. When the animals are distributed across the country outbreaks became inevitable. *S.* Hadar was the related serovar (CDC, 2021b).

One of the biggest outbreaks took place in 2008-2009 in US because of the peanut butter. Nine people were dead and more than 700 people were infected. Related strain was *S.* Typhimurium. The responsible company declared bankruptcy (CDC, 2009).

Another outbreak due to *Salmonella* in history is the 2011 outbreak which was occurred due to ground turkey. Responsible strain was *S.* Heidelberg. One person was killed and 136 people were affected (Routh et al., 2015).

Eggs are one of the most outbreak related foods and in 2018 eggs were the causative agent for an outbreak in US. Contamination occurred due to *S.* Enteritidis and 44 people were affected. Thus, eggs were recalled (CDC, 2018).

### 2.1.4 Antibiotic Resistance in *Salmonella*

Antibiotics are used to fight against infections caused by bacteria. However, they also contribute to the spread of antibiotic resistance among bacteria which results in significant problems. Increased mortality rates and increased medical costs are some of the consequences of the antibiotic resistance in bacteria (WHO, 2021). Misuse of antibiotics is mainly associated with the emerging resistance. In total, 2.8 million infections with 35,000 deaths seen per year due to the resistant bacteria in the United States (CDC, 2020). According to CDC reports, there is a growing resistance in *Salmonella* and *Campylobacter* which are spreading via food and 212,500 infections occur each year due to drug-resistant nontyphoidal *Salmonella*

(CDC, 2019).

Today, 700,000 people die every year because of drug resistant diseases. By 2050, annual death numbers could rise to 10 million people due to antimicrobial resistance if no action is taken (WHO, 2019). Importantly, *Salmonella* species resistant to fluoroqinolone are among the high priority group (Castro-Vargas et al., 2020).

Antibiotic resistance in *Salmonella* strains has been a great threat to human health and it has an impact on increased fatality rates of *Salmonella* infections. Both typhoidal and non-typhoidal serovars causing salmonellosis have been found to be linked with drug-resistance (Nair et al., 2018). According to Koutsoumanis et al., increase in the presence of resistant serovars to some antibiotics may demonstrate how the resistance spreads (Koutsoumanis et al., 2021). Increase in infections caused by resistant bacteria is a serious concern in Turkey, as well. Siriken et al. stated that *S.* Enteritidis is the most prevalent serotype in Turkey, and it is found to be resistant to six different antibiotics. Prominently, streptomycin is the one that *S.* Enteritidis has the highest resistance against. On the other hand, they reported that it is susceptible to ceftriaxone, cefotaxime and nalidixic acid. *S.* Typhimurium is also indicated to be sensitive to ceftriaxone, cefotaxime, nalidixic acid and gentamicin (Siriken et al., 2020). Another study conducted on determination of antibiotic resistance in *Salmonella* strains isolated in Turkey shows that 89.51 % of *Salmonella* strains used for investigation are resistant to at least 3 different antimicrobials. Trimethoprim, tetracycline, streptomycin is found to be the ones that the strains have the resistance against (Şahan et al., 2016).

Senses et al., reported that 49% of *Salmonella* isolates obtained from feces and blood samples of human in 2001 and 2004 showed resistance against ampicillin. It is the highest percentage among other antibiotics, and it is followed by tetracycline which 18.3% of the isolates have resistance against (Şenses et al., 2007).

Another study conducted in Turkey in order to identify antibiotic resistance in

*Salmonella* spp. isolated from different parts of raw chicken meat revealed that 82.85% of the isolated are resistant to tetracycline and nitrofurantoin while ampicillin resistance had been identified 57.15% of the isolates (Babacan and Karadeniz, 2019).

**Table 2.1** Resistance of *Salmonella* serotypes to antimicrobials (%) in Turkey (Gıda ve Kontrol Genel Müdürlüğü, 2018)

| | Ampicillin | Cefotaxime | Streptomycin | Tetracycline | Gentamicin | Chloramphenicol | Ciprofloxacin | Nalidixic acid | Trimethoprim | Sulphamethaxozole |
|---|---|---|---|---|---|---|---|---|---|---|
| *S.* Infantis | 15.6 | 0.6 | 53.8 | 43.1 | 4.6 | 9.2 | 7 | 85.9 | 52.4 | 93.8 |
| *S.* Kentucky | 67.9 | 7.4 | 47.1 | 53.7 | 29.7 | 16.5 | 21.5 | 90.1 | 23.1 | 96.7 |
| *S.* Enteritidis | 12.3 | 0 | 12.3 | 31.5 | 2.7 | 8.2 | 0 | 82.2 | 32.8 | 91.8 |
| *S.* Typhimurium | 5.9 | 0 | 17.6 | 35.3 | 0 | 0 | 0 | 64.7 | 17.6 | 82.3 |
| *S.* Mbandaka | 18.2 | 0 | 45.4 | 54.5 | 13.6 | 13.6 | 9.1 | 81.8 | 31.8 | 95.4 |
| *S.* Senftenberg | 17.6 | 0 | 23.5 | 35.3 | 0 | 11.7 | 5.9 | 82.3 | 35.3 | 94.1 |
| *S.* Hadar | 33.3 | 0 | 25 | 33.3 | 8.3 | 16.6 | 8.3 | 83.3 | 41.6 | 91.6 |

According to the EFSA, presence of resistance alters with regions. For example, even though *S.* Enteritidis has the lowest resistance to antimicrobial compounds, it has been reported that in Belgium *S.* Enteritidis has been associated with the higher resistance to ampicillin. Apart from that, higher resistance to ampicillin in *S.* Infantis has been identified in Italy compared to other countries in Europe (EFSA & ECDC, 2020).

Antibiotic resistance emerging in *Salmonella* spp. is a worldwide problem. A study published by CDC evaluated how resistance in non-typhoidal *Salmonella* spp. to antibiotics alters between 2004 to 2016 and compared the incidence rates in defined years. According to the scientists, resistance exhibits differences among serotypes while some serotypes are found to be more prone to acquire resistance. Also, results showed that infections associated with the resistance is 40% higher in

2015-2016 compared to 2004-2008 (Medalla et al., 2021). Furthermore, CDC 2019 report shows that drug-resistant NTS causes 212,500 deaths per year with 70 deaths whereas 5 deaths and 4,100 cases per year occur due to the drug-resistant *S.* Typhi. The collected data on the antibiotic resistance of both typhoidal and nontyphoidal *Salmonella* through the years illustrates the increasing trend in antibiotic resistance to certain antibiotics (CDC, 2019).

A study conducted by Brown et al. also reveals the data about *Salmonella* infections which were related to the resistant strains in the United States (Brown et al., 2017). According to the findings, 37 out of 176 (21%) outbreaks taking place between 2003 and 2012 were associated with the resistant bacteria. Prominently, children under 5 years make up the high portion of the patients who are affected by resistant strains of *Salmonella*. The study is also informative about the type of food products which are involved in the outbreaks and most common foods related to resistant *Salmonella* are defined as beef, chicken and turkey. *S.* Typhimurium, *S.* Heidelberg and *S.* Newport have been found as the serovars which are linked to resistance. The authors stated that resistant bacteria may spread through the food products which were originated from animals which are the carriers of resistant bacteria (Brown et al., 2017). Another study examined the salmonellosis outbreak which was caused by multidrug resistant *S.* Typhimurium and results showed that the presence of the antimicrobial genes which can be easily transferred may caused the multidrug resistance. Also, the authors added that bacteria causing that outbreak were more likely to derive from local strains. Thus, they emphasized the importance of the prevention strategies developed by the government (Xiang et al., 2020).

Antibiotic resistance in bacteria can develop with different mechanisms. For example, mutations and horizontal gene transfer play a role. Martinez et al., implies that antibiotic treatment may contribute to acquisition of antibiotic resistance by mutations taking place (Martínez et al., 2007). On the other hand, it has been

reported that by Nair et al. plasmid mediated horizontal gene transfer is the major route for transfer of resistance in *Salmonella enterica* species (Nair et al., 2018).

Antibiotics that are used in animal sector for several purposes can accelerate the spread and emergence of resistance among bacteria. Transmission of antibiotic resistant bacteria through the food environment has a significant impact on humans (Nair et al., 2018).

Extensive use of antibiotics has contributed the emergence of the resistance. When resistant bacteria encountered an antimicrobial compound and this compound does not affect the resistant bacteria so they can reproduce but the susceptible ones are being destroyed, resistant bacteria will dominate the environment and spread. Hence, reduction of antibiotic use is beneficial in terms of coping with antibiotic resistance (Read and Woods, 2014).

Among human clinical cases in 2016, *S.* Enteritidis was the most encountered seroytpe (70.5%) in Turkey. It is followed by *S.* Typhimurium (8.5%), *S.* Infantis (4.4%), *S.* Paratayphi B (3.2%) and *S.* Kentucky (2.7%). Also, *S.* Infantis had the highest prevalence in broilers (17.7%) followed by *S.* Kentucky (2.6%) and *S.* Enteritidis (1.5%) (Gıda ve Kontrol Genel Müdürlüğü, 2018). Acar et al. also investigated the *Salmonella* prevalence in Şanlıurfa which was selected as a pilot region (Acar et al., 2017). *Salmonella* prevalence showed variation depending on the isolation source. *S.* Infantis (25.8%) was the most encountered isolate in food samples and *S.* Kentucky (37.7%) had the highest prevalence among the animal isolates. Additionally, *S.* Kentucky and *S.* Typhimurium were isolated from all the sources including human, food and animal (Acar et al., 2017). Another study conducted in Marmara region showed the commonly isolated *Salmonella* serotypes. According to Cilo et al. *S.* Enteritidis (58.1%) was the mostly encountered serotype between 2002 and 2014. *S.* Typhimurium (9.4%), *S.* Infantis (4.9%), *S.* Newport (4.5%), *S.* Kentucky (4.1%) were also reported in the given percentages. The isolation sources included human feces, blood samples, wounds and urine samples (Dalyan Cilo et al., 2016). Moreover, Cesur et al. reported that

*Salmonella* isolated from raw chicken products contained mostly *S.* Infantis. From the isolated 19 strains 15 of them belong to *S.* Infantis and the rest was found to be *S.* Enteritidis (Cesur et al., 2019).

A comprehensive study conducted by Diker et al. in 9 districts of Turkey revealed the *Salmonella* occurrence in chicken farms (Diker et al., 2020). It was reported that *S.* Kentucky is the most abundant serotype in laying hens, and it was followed by *S.* Infantis and *S.* Enteritidis. Isolation frequency of *Salmonella* was indicated as 7.5% in feces and 11% in environmental samples during laying hen production. Thus, the authors stated that environmental samples and feces hold significant importance in terms of *Salmonella* contamination (Diker et al., 2020).

Besides, antibiotic susceptibility profiles of the *Salmonella* isolates were taken into consideration. *Salmonella* Control Program data from broilers revealed that some serotypes are resistant to some antibiotics such as sulfamethoxazole and nalidixic acid. While most of the serotypes showed low resistance to some of the other antibiotics (Gıda ve Kontrol Genel Müdürlüğü, 2018).

### 2.1.5 *Salmonella* Prevention Methods

*Salmonella* has been linked with several foods, but majorly contaminated meat products are the cause of salmonellosis (Al-Saigh et al., 2004). Similarly, fruits and vegetables contaminated through different routes possess a risk (Daş et al., 2006).

There are some strategies to reduce the colonization of *Salmonella* to ensure food safety. These strategies are implemented in several steps of food production (Stern et al., 2001). Controlling *Salmonella* in broiler meat in pre-harvest, on the harvest and post-harvest stages is crucial to avoid contamination and these stages include many parameters (Van Immerseel et al., 2009). For example, Stern et al. highlighted the importance of elimination of *Salmonella* in poultry before they arrived in the plant (Stern et al., 2001). Also, foremost prevention method has been indicated as avoiding from the introduction of *Salmonella* to the farm. Hence,

monitoring plays a significant role. Samples are collected regularly from several places that the animals are in contact. Secondly, vaccination procedures against *Salmonella* can be followed (MSD Animal Health, 2021). According to EFSA report, vaccination is successful to reduce *S*. Enteritidis and *S*. Typhimurium in flocks (EFSA, 2004).

Hygiene practices possess a substantial role to prevent *Salmonella* on farms as well as in processing plants. Cleaning and disinfections should be applied adequately. Feed and water decontamination has a significant importance on elimination of *Salmonella*. In drinking water, some organic acids and chlorine are used for decontamination while steam pelleting can be employed for feed decontamination although its efficacy is controversial (Van Immerseel et al., 2009). Berge & Wierup, summarized the heat treatment effects on feed decontamination (Berge and Wierup, 2012). Depending upon the initial loads of *Salmonella*, temperatures reaching up to 80-85°C for 1 minute can destroy the bacteria but still, equal distribution of heat should be applied (Berge and Wierup, 2012). Other strategies for feed include irradiation and again some organic acids (Van Immerseel et al., 2009). Also, Berge & Wierup mention the Lactic acid bacteria impacts on control of *Salmonella* through feed. Probiotics targeting the enhance the microflora of chicks to provide protection against pathogens are also employed. For that purpose, competitive exclusion (CE) cultures which can be defined as a form of probiotic culture is fed to the newborn chickens (Berge and Wierup, 2012). Antibiotics have been used as feed additives for many years but also brought some concerns such as emergence of antibiotic resistance (Williams and Tucker, 1975). Additionally, antibiotics are not always successful enough the eliminate bacteria from the animals. They cause reductions in load, but it is temporary thus resulting in misconclusions about their prevalence (Williams and Tucker, 1975). Bacteriophages are the entities that can be used to fight against bacteria. Even though they have some restrictions, they are promising agents for decontamination of feed. They can be employed in various steps of food production, as well (Van Immerseel et al., 2009). According to a study in 90's phages were given to

chickens orally and the results indicated that phages were successful on reducing *Salmonella* colonization in chiken's gut. Additionally, some other findings supported the efficacy of oral phage treatment by emphasizing that phages induced the lysis of host *Salmonella* serovars causing reductions in cell count (Żbikowska et al., 2020).

During processing, flocks that are *Salmonella* positive must be handled separately from others and equipment must be carefully sanitized (MSD Animal Health, 2021).

## 2.2 Bacteriophages

Bacteriophages are the viruses that infect bacteria, and they abundantly exist with their bacterial hosts in every environment, shedding light into the evolutionary steps of bacteria. Interaction of bacteriophages with their hosts plays a substantial role in terms of controlling the bacterial populations and hence affecting the bacterial evolution in many perspectives (Santos et al., 2014). Besides being the carriers of genetic information, phages effects on microbial population dynamics such as biodiversity and species distribution cannot be underestimated (Weinbauer, 2004).

Since bacteriophages can be found where their hosts are present, they can be isolated from many sources which include soil, wastewater, sea water, plants, animal farms and feces (Akhtar et al., 2014). Seawater is indicated as the environment where the phage diversity exists mostly. Again, agricultural areas can be shown as the places that phages are ubiquitously distributed (Breitbart, 2012; Williamson et al., 2003). Prevalence of phages in soil is affected by the type of land. Phages may be present in different morphologies depending on the soil properties (Williamson et al., 2005).

Many studies have been focusing on isolation of bacteriophages from wastewater, sewage and feces of animals. Study of Huang et al. includes isolation of

bacteriophages from environmental samples and majority of them were obtained from sewage (Huang et al., 2018). O'Flynn et al. points out the feces importance in terms of isolation source of bacteriophages. They could isolate *Salmonella* phages from pig feces and effluent of the pig farm (O'Flynn et al., 2006). Furthermore, Toribio-Avedillo et al. mentions the bacteriophages advantages in terms of detection of fecal pollution (Toribio-Avedillo et al., 2021). Muniesa et al. also addresses fecally contaminated waters as the source of *Myoviridae* and *Siphoviridae* (Muniesa et al., 1999).

Bacteriophages are also inhabitants of human intestines. It is known that gastrointestinal tract of humans contains a considerable number of bacterial cells as well as bacteriophages. Phages can influence the bacterial colonization in intestines through their lytic activity thus, having an impact on some metabolic activities (Łusiak-Szelachowska et al., 2017). Additionally, lysogenic cycle of phages may result in development of different factors in microflora like antibiotic resistance. According to Mc Grath & Sindeler, phages of *Salmonella*, *E. coli* and *Bacteroides* have been obtained from human stools (Mc Grath and van Sindeler, 2007).

Bacteriophage discovery dates back to 20$^{th}$ century. Even though Frederick Twort had studies on bacteriophages earlier in 1915, Felix d'Herelle is known for his discovery of phages in 1917 (Summers, 2016). During his experiments with a bacterium on agar, Herelle observed clear zones which meant bacteria could not grow there. However, the cause and significance of this situation could not be understood fully at once. Major advancement was made while he was working on dysentery. He again detected clear areas where the bacteria should be present, and he carried his studies forward. He discovered that those clear zones appeared because something killed the bacteria. Then, he named his discovery as bacteriophage which stands for virus infecting bacteria (Taylor, 2014). Thereby, it was understood that bacteriophages can be promising agents in order to destroy undesired bacteria.

Subsequently, nature of the bacteriophages was investigated, and some important

properties were revealed. Herelle led the formation of new terminology. He described the purification and titer of phages. Most importantly, he mentioned the host specificity of bacteriophages and added that they do not have the ability to grow in absence of their host cells (Taylor, 2014).

Examination of bacteriophages under electron microscope contributed remarkable advancements in terms of characteristics of phages. In 1930's, multiplication of phages was studied by German physicist Max Delbrück. That time, it was not known where the genetic information for replication located on a virus and how it is transmitted. Alfred Hershey was the one who showed that DNA was the answer to these questions; DNA has the information, and it enters the host cell. Later, several scientists including Hershey joined Delbrück and his team. With those studies Delbrück, Luria and Hershey won the Nobel Prize in 1969 (Vandamme and Mortelmans, 2019). Those developments provided a basis for molecular biology and further analysis on bacteriophages have profoundly revealed the characteristics and the nature of them. Ongoing research in this field help unfold the complexity of bacteriophages.

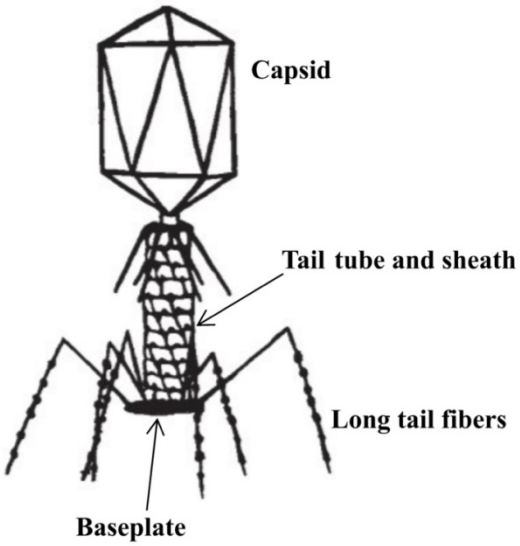



**Figure 2.1** Schematic representation of bacteriophage belonging to *Myoviridae* family [Adapted from: (Harada et al., 2018)].

### 2.2.1 Classification of Bacteriophages

Bacteriophage classification idea first built by Sir Macfarlane Burnet in 1930s. The proof of different sized phage existence leads to this classification. Later, H. Ruska discovered that phages exhibit diverse morphology which he ended up proposing a classification based on electron microscopy (Ackermann, 2011). Then, Holmes, classified bacteriophages according to their host relations. However, this method lost its validity soon (Ackermann, 2003). Significant progress was made by Lwoff, Horne and Tournier in 1962 and then by Lwoff, Tournier in 1966. Their system categorized the phages according to nucleic acid they contain, the symmetry of the nucleocapsid, presence of the envelope which surrounds the capsid and finally the number of capsomers-diameter of the nucleocapsid. If the phages consist of DNA, they are called deoxyviruses and similarly, if they contain RNA as a nucleic acid, they are riboviruses. Then, nucleocapsid types were defined as helical or cubic (Guttman, 2013; Lwoff and Tournier, 1966)

Since classification of bacteriophages is important and a controversial topic, International Committee for Taxonomy of Viruses (ICTV) have been founded in 1966. In its first report, six genera of phages were mentioned. However, changes occur as the new families or genera are introduced to the classification and the more is discovered about phages.

Today, viruses are classified depending on their properties. Those properties are their morphology, life cycle, pathogenicity, nucleic acids that they have and bacterial strains they bind (Żbikowska et al., 2020). Additionally, sequence similarities of phages play a substantial role for identification and differentiation of viruses (ICTV, 2020). Still, ICTV accepts proposals for virus taxonomy.

### 2.2.2 Structure

Phages consist of nucleic acid which defines their genetic material and a capsid.

Capsid is composed of proteins, and it is responsible for protecting the virus' genetic material by covering it. Proteins serve as building units as they are combined to form the capsid structure, yielding rather elastic and unbreakable protection layer for the genetic material. Genes have the information for the assembly of those proteins and since, the genomes of viruses are not sufficiently large to encode too much information, capsids are formed by generally the same type of proteins. The combination of capsid and genome establishes the nucleocapsid of the phage (Louten, 2016). Bacteriophage capsids are generally found in two major shapes. They can be in either icosahedral or helix form and icosahedral shape is widespread among phages. Helical form is more commonly found in filamentous types (Louten, 2016).

Apart from those, some viruses contain membranes which consist of lipids. These membranes are called envelopes. While enveloped structure is commonly encountered in human or archaeal viruses, most of the bacteriophages do not have envelopes (Mäntynen et al., 2019). Presence of the envelope impacts the interaction of the virus with its host because proteins playing a role in attachment varies according to the structure of the virus (Maginnis, 2018).

Other constituent of the phages is the tail and most of the phages (more than 90%) which belong the order of *Caudovirales* have tails. Genetic material is transmitted through the tails from bacteriophage to the bacteria that they infect and that bacteria is recognized by the receptor binding proteins which are found on the baseplate of the phage. Baseplate's link with the capsid is provided by contractile sheath and baseplate is responsible for controlling the interaction between the bacteria and its phage (Harada et al., 2018; Huang and Xiang, 2020). Six long tail fibers are organized by the baseplate, and they detect their hosts. As they bind to the host, short tail fibers are induced and attachment occurs (Rossmann et al., 2005).

Bacteriophage classification is a bit complex process and still there are many unknowns about the phages. However, as mentioned before there are some

parameters that ICTV considers to group bacteriophages. One of the parameters is their genetic material: single stranded DNA, double stranded DNA, single stranded RNA, double stranded DNA where the majority is comprised of dsDNA phages (Harada et al., 2018; Sharma et al., 2017).

Other than their genetic material type, phages are classified by considering their morphology. The tailed ones hold the majority, and they are *Caudovirales*. *Caudovirales* are non-enveloped with linear double stranded DNAs. *Myoviridae*, *Siphoviridae*, *Podoviridae* represents the tailed families that are under *Caudovirales* order. Three families differ from each other based on their tail morphology. *Myoviridae* is the family whose members have long contractile tails and according to Leiman & Shneider, phages possessing contractile tails are lytic ones (Leiman and Shneider, 2012). *Siphoviridae* are with long and non-contractile tails and *Podoviridae* family has short non-contractile tails. Other families exhibit varieties in terms of their shape, nucleic acids, and major characteristics.

**Table 2.2** Overview of phage families (Ackermann, 2007)

| Shape | Nucleic Acid | Virus Group | Particulars | Example |
|---|---|---|---|---|
| Tailed | dsDNA, L | *Myoviridae* | Tail contractile | T4 |
| | | *Siphoviridae* | Tail long, noncontractile | λ |
| | | *Podoviridae* | Tail short | T7 |
| Polyhedral | ssDNA, C | *Microviridae* | Conspicuous capsomers | ϕX174 |
| | dsDNA, C, S | *Corticoviridae* | Complex capsid,lipids | PM2 |
| | dsDNA, L | *Tectiviridae* | Inner lipid vesicle, pseudotail | PRD1 |
| | ssRNA, L | *Leviviridae* | Poliovirus-like | MS2 |
| | dsRNA, L,seg | *Cystoviridae* | Envelope, lipids | ϕ6 |
| Filamentous | ssDNA, C | *Inoviridae* | a) Long filament | fd |
| | | | b) Short rods | MVL1 |
| | dsDNA, L | *Lipothrixviridae* | Envelope, lipids | TTV1 |
| | dsDNA, L | Rudiviridae | TMV-like | SIRV-1 |
| Pleomorphic | dsDNA, C, S | *Plasmaviridae* | Envelop,lipids,no capsid | L2 |
| | dsDNA, C, S | *Fuselloviridae* | Same, lemon-shaped | SSV1 |
| | dsDNA, L, S | *Salterprovirus* | Same, lemon-shaped | His1 |
| | dsDNA, C, S | *Guttaviridae* | Droplet-shaped | SNDV |

C: Circular; L: Linear; S: Superhelical; seg: segmented;

### 2.2.3 Receptors-Phage Relation

Infection mechanism of phages varies according to the host cell structure and bacteriophage group. Bacteria that are going to be infected by its phage have receptors on their surface. These receptors resemble to a lock on a door, and they can be in different forms such as lipopolysaccharides and some proteins, so the nature of the bacteria has a great influence on how the phage binds (Maginnis, 2018). Gram-positive differs from Gram-negative bacteria in terms of many properties and hence the receptors vary accordingly.

Gram negative bacteria includes outer and inner membranes. Outer membrane proteins and lipopolysaccharide molecules located at the outer membrane serve as receptors of bacteria. Thus, affecting the relation between phage and host (Bertani and Ruiz, 2018). For instance, T5 bacteriophage is known for infecting *E. coli*. Long tail fibers found on T5 are responsible for sensing the host cell. Therefore, they target the O-antigen of LPS. This first attachment is referred as reversible binding (Mc Grath and van Sinderen, 2007). Then, irreversible binding takes place as the tail proteins interact with the proteins found on outer membrane of host cell (Bertozzi Silva et al., 2016).

On the other hand, Gram positive bacteria do not have an outer membrane which can act as a barrier. Instead, their peptidoglycan layer is thicker, and it includes teichoic acids which have several roles. Prominently, acting as receptors for phage attachment one of their functions (Rajagopal and Walker, 2017).

### 2.2.4 Host Range of Bacteriophages

Phages' binding capability to bind bacteria depends on the structure of the receptor binding proteins (RBPs) found on phages. Some phages are able to infect wider range of bacterial strains compared to others and this variation is related to how they bind to their host cells. Phages infecting narrow strains are known as monovalent phages. They can attach to a one receptor. Thus, they cannot infect as many strains as polyvalent phages do. Generally, phages are monovalent so that there are limited bacterial strains that phages can infect even if the strains belong to same species (Abdelsattar et al., 2021a).

These alterations in viruses bring advantages in terms of their use as biocontrol agents. Especially, phage therapy relies on host range determination. In some cases, narrow host range is preferable whereas sometimes broad host ranged phages are beneficial. For instance, the undesired bacteria may be infected by its phage without destroying other microorganisms in the environment (Abdelsattar et al.,

2021a). On the other hand, phages infecting multiple strains can be selected in phage therapy applications in order to be used instead of antibiotics (Ross et al., 2016).

### 2.2.5 Infection Mechanism of Bacteriophages

Communication between tail fibers and the baseplate occurs when the tail fibers attach to their target cell because the alignment of the tail fibers alter. Harada et al. stated that the alteration on the baseplate is also associated with the ions present in the environment (Harada et al., 2018). The changes taking place in the orientation of baseplate leads some variations in the shape of the phage. As the capsid gets closer through the host cell, the movement of the tail tube through the host cell paves the way for the transmission of the genetic material. However, the contractions occurring in the tail does not necessarily mean that DNA is going to be ejected from the phage. It is thought that some molecules who act as receptors are required on the host cells cytoplasm for the translocation of phage's genetic material (Harada et al., 2018). As in the example of T5 phage, first tail fibers are involved, and they reversibly attach to the host and when the capsid proteins are included in the process irreversible binding occurs. The barriers that are passed through by the phage for infection are depending on the type of the bacteria (Huang and Xiang, 2020). Bacteriophage's ability to degrade host cells parts like cell wall or membranes is crucial. They can achieve this degradation via their enzymes and the enzymes involved show variety because the enzymatic activity depends on the type of barrier they encounter (Harada et al., 2018).

The idea about the DNA injection requires some components is supported by Lettelier et al. with the explanation of DNA transfer from phage to host. Injection of viral DNA could be explained by different phenomena. The idea of the DNA transmission via the electrochemical gradient was put forward and thought membrane potential is the key. However, it was then understood that the membranes which are not energized also involved in the DNA transmission. Most

appropriate explanation was shown as the DNA binding proteins (Letellier et al., 1999). Even though there is no certainty about which factor drives phage DNA out of the tail tube, another assumption about the host cell protein presence could help was proposed by Xu and Xiang (Xu and Xiang, 2017). In the example of T4 phage, the tail tube cannot pass through cytoplasmic membrane. There should be another possible way for entering into that membrane. Their approach was some proteins are required for pore formation and the presence of host cell proteins are playing a role in penetration process (Xu and Xiang, 2017).

### 2.2.6 Life Cycle

Since bacteriophages lack vital particles for protein synthesis, they strictly require their hosts, and they can multiply as long as they use their hosts (Guttman, 2013). Prominently, bacteriophages are indigenous to their hosts. This specificity can be in species level or even it can be in strain level. Once a bacteriophage binds its hosts and injects its genetic material, they may end up with burst of the host cell, without bursting integrate their genomes to the host cell chromosome or form new phages and free them by budding (Verheust et al., 2010).

In fact, that life cycle determines how the bacteriophage is going to affect its host (Campbell, 1961).

Virulent phages are the ones that lyses their hosts in order to reproduce and these are called lytic bacteriophages. On the other hand, temperate phages follow lysogenic strategy which does not immediately end up with bursting of host cell. Instead, temperate phages incorporate their genetic material into host bacterial cell (Clokie et al., 2011).

#### 2.2.6.1 Lytic Cycle

When a virulent bacteriophage finds its host and injects its genetic material, it uses

the host cells mechanism to replicate itself (Ofir and Sorek, 2018). First, early proteins are generated. If the genetic material of the phage is DNA, it transcribes the DNA into messenger RNA (mRNA). Because mRNAs are responsible for bringing codes of DNA to the ribosomes and by doing that phage allows the synthesis of polypeptides which ruins the host DNA. Thereby, phage DNA takes over (Pierce and Scott, 2019). While some early proteins are for destroying the host genome, some of them are used in the replication of phage genome so that phage can replicates itself and make copies. Afterwards, late proteins are expressed. Capsids and tails are built from those proteins (Steward, 2018).

In detail, procapsids lead the formation of capsids and procapsids form in the presence of some components. Scaffolding proteins are the ones that forms the core inside the procapsid aiding the unity of coat proteins while portal proteins are related with the DNA movements When these structures interacted, assembly of the procapsid is initiated (Aksyuk and Rossmann, 2011; Prevelige and Cortines, 2018).

Head assembly is followed by DNA packaging which requires ATP hydrolysis (Aksyuk and Rossmann, 2011). ATP included reactions may show differences depending on the type of the bacteriophage (Fujisawa and Morita, 1997). Portal complex is involved in DNA entrance into the procapsid. It is considered that the DNA transport into the procapsid is due to the portal-terminase complex (Hendrix, 1998). Terminase -also called packaging enzyme- is the key factor playing a role in DNA packaging and it has several functions. Small subunit (TerS) of the terminase phage knows its own viral DNA and as it interacts with the DNA they form a complex: TerS:DNA. Then, the large subunit become involved and cuts off the DNA and together they locate into the procapsid until all the genome is transferred (Dedeo et al., 2019; Hendrix, 1998). Maturation also takes place as the DNA is packed and scaffolding proteins leave the capsid. Changes in the prohead are seen and its stability increases (Aksyuk and Rossmann, 2011). Subsequent step is the tail assembly. Variations regarding to formation of tails are seen among phages

belonging to different families.

Newly formed phages possess enzymes that degrade host cell's certain structures as they do to enter the cell in order to be liberated. These are basically holin and endolysin. Due to the activity of them, the unity of the cell is corrupted (Fernandes and São-José, 2018).

#### 2.2.6.2 Lysogenic Cycle

Other cycle that bacteriophages may undergo is lysogeny. Unlike lytic phages, temperate phages' actions do not result in the burst of the host cell. Instead, genetic material of the phages becomes integrated with the host genome (Chen et al., 2020). In that respect, temperate phages' effect on bacterial population dynamics is significant. Because temperate phages act as vectors variations in the bacterial host genome is inevitable. Host cell can gain resistance against the same bacteriophages as a result of lysogeny. Furthermore, virulence factors may be transmitted through lysogenic phages (Chen et al., 2020). Herold et al. explains this by emphasizing the presence of Shiga toxin (Stx) genes in lambdoid phages and how phages shape the transmission of the genes (Herold et al., 2004).

Importantly, lysogenic cycle does not necessarily mean that phages cannot switch their mechanisms. It is possible for a phage to follow lysis after lysogeny. The condition of the host cell affects this alteration. When nutrients are consumed and cell is exhausted phages are more likely to be lysogenic (Howard-Varona et al., 2017). Moreover, lysogeny is found to be related with the multiplicity of infection. When the phage number becomes higher compared to host cells, phages are at risk which means this situation is not in favor of bacteriophages. Thus, lysogenic mode is activated in order to prevent lytic cycle (Abdelsattar et al., 2021a).

Besides, pH of the environment, UV are some other contributors that may create stress on phages thus, resulting in shift from lysogeny to lysis (Abdelsattar et al., 2021a).

Additionally, lysogeny is affected by the presence of some genes in phages. For instance, bacteriophage λ is known for its mechanism to go under lysogeny or lysis. The development of these two modes is depending on the presence and the expression of the repressor proteins (Schubert et al., 2007).

### 2.2.7 Cyclic Replication of Phages

Single step growth curve represents a single step of a bacteriophage replication, and it provides information about the life cycle of bacteriophages. Ellis and Delbrück are known for being the first to perform experiments to demonstrate a single step growth curve. The growth curve is definitive in terms of characteristics of a phage and several features such as burst size and latent period can be determined accordingly (Kutter and Sulakvelidze, 2005). As mentioned previously, its life cycle involves various steps for formation and infection. Therefore, one cycle of phage growth helps understand the nature of phages.

Latent period is the required time for phage particles to reproduce in the host cell so that they appear in the medium (Sinha et al, 2018). As the lysis occurs, latent period is finished and at the end of the latent period, number of plaques increases due to the release of newly formed phage particles. As the new phage particles are released, they infect their host bacteria resulting in more plaques to form (Petsong et al., 2019). Latent period is dependent on both the host degrading enzymes that phage possess and the host cell condition (Abedon et al., 2001).

Calculation of a phage's burst size helps understand how many phage particles are liberated from a one infected cell. The ratio of released phage particles to the infected cells at the latent period gives the burst size which is a characteristic of a phage (Petsong et al., 2019).

Burst size, latent period of a bacteriophage are related characteristics and they take role in bacteriophage's decision mechanism for its survival. Keeping lysis timing

and burst size at optimum levels becomes significantly important for phages. Wang et al, stated that intermediate latent period provides better fitness to bacteriophages although it is not easy to define the intermediate time (Wang, 2006). Abedon et al. mentions a trade-off associated with the burst size and latent period. Because as the progeny is released from a host, the host who is required for a phage particle to generate is being destroyed. They stated that as the host density increases, latent period becomes shorter due to the availability of host bacteria (Abedon et al., 2003).

Multiplicity of infection (MOI) plays an important role during single step growth curve experiments. MOI, here, is calculated by taking the ratio of virus particles to bacterial host cells. If MOI value is adjusted as 1, it is considered that one phage particle infects one host cell (Sinha et al., 2018). However, still it might not be that accurate because for example, one host cell may adsorb more than one phage. Also, all host cells present in the medium may not be get infected by the phages (Sinha et al., 2018).

### 2.2.8 Host Resistance Against Bacteriophage

The ability of bacteria to resist their bacteriophages is a concern that cannot be underestimated regarding to the phage usage as a biocontrol tool. Bacteria can adopt different strategies against their viruses and these interactions hold importance in terms of bacterial population dynamics (Oechslin, 2018).

One strategy is related with adsorption of bacteriophages by hosts. First step of the infection process is the adsorption, and it is known that bacterial cells develop a mechanism to protect themselves (Labrie et al., 2010). Adsorption of a phage occurs when the phage receptors are recognized by the host surface. Alterations on the surface receptors of bacteria enables bacteria to defend itself. Proteins produced by host cells are effective structure against phages and these proteins play a role in inhibition of phages. Production of lipoproteins by some strains of *E. coli* may

change or mask the receptors that are recognized by *E. coli* phages (Labrie et al., 2010).

As mentioned previously, O-antigens found in *Salmonella* lipopolysaccharide are acting as receptors for *Salmonella* infecting phage P22. For P22 to bind the cell wall, first it overcomes these O-antigens LPS by degrading them. However, alterations in the LPS structure of *Salmonella* limit phage adsorption (Wang et al., 2019).

Another strategy used by bacterial cells to block phages is the prevention of phages' DNA injection. Again, proteins are playing role and they are called as superinfection exclusion (Sie) systems. Indeed, these systems are encoded by prophages. The activities and mechanisms of these systems are not exactly clear for all phages. Sie proteins found in bacteriophages are important in terms of bacterial populations. For example, T4 phage has Sie systems, and it blocks other bacteriophage infections. Another example can be given as the *S.* Typhimurium phage P22. When P22 infects its host, it prevents other phage infections by means of Sie proteins (Labrie et al., 2010; Seed, 2015).

Phages may be adsorbed and transfers its genetic material into the bacteria, but this does not mean all the barriers are passed for a phage. Still, phage needs to replicate itself and one obstacle that it may face is the restriction-modification (R-M) system. R-M system is generally present among prokaryotes. This system's activity involves annihilating the predator's DNA as a defense (Labrie et al., 2010; Sitaraman, 2016). Restriction Endonucleases (RE) and methyltransferases (MTase) are some of the components of R-M system. These components recognize the same sequence but have distinct activities on it. Normally, MTase is responsible for modifying its own DNA and phage DNA remains unmethylated. When RE detects this unmethylated sequence, it degrades it by cutting at the specific sites (Sitaraman, 2016). On the other hand, if methylation occurs, phage can continue its lytic cycle and degrading enzyme does not affect it. Therefore, main determinant here is the activity of RE and MTase. However, bacteriophages can tackle this

problem. The R-M efficiency is related to the recognition sites present on the DNA of the phage and due to the mutations taking place in their genetic material, they lack endonuclease recognition sites (Labrie et al., 2010).

Another strategy for a bacterium to escape from its predator is CRISPR-Cas system that it harbors. This system allows bacteria to know the foreign nucleic acids and destroys them. If bacterium faces with a foreign genetic material, it takes small part from the foreign DNA into the CRISPR array which makes is immunized against its virus (Pawluk et al., 2018). The virus' DNA sequence is stored in array as spacers. When the spacer is transcribed and processed to yield crRNAs and these crRNAs are recognized by a complementary DNA sequence (protospacer), target DNA is cleaved. The immunity of bacteria occurs due this process (Bourgeois et al., 2020).

Bacteria have also the ability of preventing newly synthesized virions to go outside of the cell by a process called abortive infection (Abi). Thus, this process targets to protect nearby populations and results in the death of the infected cell. But still, how the Abi systems are initiated, and their mechanisms are not fully comprehended. Abi system in *Lactococcus lactis* has been studied and identified because this bacterium is widely used in cheese sector, and it is exposed to phage attacks. Different Abi proteins play role in different steps of phage infection. For example, new virions cannot be fully assembled and released due to the AbiZ activity (Seed, 2015). Hence, it is critical in reducing the burst size of the phage. Additionally, AbiZ takes part in the premature lysis of the infected cell for the sake of bacterial population (Rostøl and Marraffini, 2019).

### 2.2.9 Phages Overcome the Bacterial Defense

Since the bacteriophages and bacterial cells evolve together, phages also can overcome the resistance developed by bacteria against themselves. Even though the host cells adopt mechanisms to avoid invasion, still phages can detect and

recognize changes taking place in the host cell (Abdelsattar et al., 2021a).

As mentioned in the previous section, mutations occurring in the phage genome allows phage to lose restriction sites which enables them to escape from the endonuclease activity of bacteria. Presence of methylated genomes in bacteriophages helps avoid bacterial resistance, as well (Abdelsattar et al., 2021a).

When phage infects the host cell, lytic cycle is triggered which results in bursting of the host. However, there are still some bacterial cells that persist. Importantly, this survivor cells will have additional repeat and new spacer which is coming from the genome of bacteriophage causing the phage resistance in the bacteria that survived. Even though these bacteria are resistant to phages carrying identical spacer, bacteria are still susceptible to the phages that does not contain this specific proto- spacer. So, point mutations taking place in phage proto spacer help phage avoid the resistance in bacteria (Labrie et al., 2010).

Bacteriophages also possess anti-CRISPR (Acr) proteins as well as anti-Abi systems that help them defeat bacterial immunity (Pawluk et al., 2018). Acr proteins which are very diverse are basically inhibit the CRISPR-Cas system in bacteria (Harrington et al., 2017).

## 2.3 Phage Therapy for Pathogens

Bacteriophage usage to control foodborne pathogens dates back 100 years in Russia. Today, due to their unique nature, they are used in various areas (Grose and Casjens, 2014). Phages application as therapeutic agents is possible in various steps of food chain. However, for this purpose identification and characterization of bacteriophages should be done in order to obtain efficient results (Carey-Smith et al., 2006). Phages possessing definite properties become prominent in terms of phage therapy (Skurnik et al., 2007). Therefore, the authors emphasize the necessity of a database containing information about numerous phages based on their characteristics and sequences (Skurnik et al., 2007).

Since many bacteriophages are specific to their hosts, their host-range examinations should be done properly, and the undesired bacteria should be diagnosed for a successful treatment. Bacteriophages having a broad range in terms of infectivity seem more desirable for a phage therapy due to the diversities in bacterial species (Skurnik et al., 2007). Although bacteriophages which display high specificity to the hosts and have narrow host ranges, it is still possible to use them as therapeutic agents due the phage cocktails which contains other phages to increase the effectiveness (Loc-Carrillo and Abedon, 2011). Besides, narrow host range of bacteriophages can be advantageous because unlike antibiotics phages does not affect beneficial bacteria such as inhabitants of human gut. Additionally, since phages' major components are nucleic acids and proteins, their breakdown is not harmful and considered as a process that is naturally occurring (Carlton et al., 2005).

Furthermore, phages can be preferable than antibiotics. Extensive use of antibiotics is related with the emergence of antibiotic resistance and phages can infect antibiotic resistant bacteria (Loc-Carrillo and Abedon, 2011).

There are some requirements that a phage must fulfill in order to be used in therapy. Being lytic and non-transducing are the minimum requirements regarding to safety concerns (Skurnik et al., 2007). For example, bacteriophages that infect *Listeria* species are commonly temperate which means they can carry other bacterial genes to the infected one (Hodgson, 2000). Additionally, Loc-Carillo and Abedon, states that phages with low virulence should not be employed for therapeutic purposes. Because the risk of poor adsorption makes those phages undesirable (Loc-Carrillo and Abedon, 2011).

According to Carlton et al., selection of virulent bacteriophages for applications yields desired results and, he offers to pick phages who have different lysis capabilities or phages who is effective on broader range of bacteria (Carlton et al., 2005).

### 2.3.1 Application of Bacteriophages in Food Industry

Food is associated with variety of disease and microorganisms can be transmitted through food (Oliver et al., 2005). According to the CDC, diseases transmitted through food fall into two groups. The first is traceable diseases caused by pathogens. The second is food-borne diseases of unknown origin due to lack of data. Disease caused by pathogens constitutes a significant portion and is of great importance in terms of both health and economy. CDC estimates that around 228,000 hospitalizations and 2,600 deaths occur because of the foodborne pathogens and nontyphoidal *Salmonella* is taking its part in top 5 pathogens that results in both hospitalization and death (CDC, 2018).

Bacteriophages applicability as additives to preserve food is assessed by FDA and EFSA and in the current situation SalmoFresh™ against *Salmonella* as well as Listshield™ against *L. monocytogenes* and Ecoshield™ against *E. coli* is approved by FDA (Fernández et al., 2018). Also, *Salmonella* phages called PhageGuard S are approved by FDA whereas it is not being used in Europe (PhageGuard, 2019). Still, studies continue.

Up to today, many studies have been conducted to control *Salmonella* prevalence in foods by using bacteriophages. Many studies including biocontrol with *Salmonella* phage examine the efficacy of phages in poultry (Coffey et al., 2010). Han et al., evaluated one of the most encountered serotypes of *Salmonella*, *S.* Enteritidis phages activity in milk, liquid whole egg and chicken breasts (Han et al., 2017; Zhang et al., 2014). The contaminated milk samples with *S.* Enteritidis were treated with a phage cocktail consisting of seven bacteriophages that are lytic on S. Enteritidis. Authors reported that even though *S.* Enteritidis could grow to $10^7$ to $10^9$ CFU/mL in the milk samples which had been previously inoculated with *S.* Enteritidis, in samples treated with phage cocktail, *Salmonella* count was very low in every food sample they used (Han et al., 2017).

Abdelsattar et al., assessed the activity of *Salmonella* phage in milk. For this

purpose, they isolated *Salmonella* bacteriophage ZCSE6 from milk and used it on artificially contaminated milk sample. Significant reduction in *Salmonella* load was observed. Thereby, they concluded that ZCSE6 is an efficient tool in terms of biocontrol besides contributing to extension of shelf life of the milk (Abdelsattar et al., 2021b).

Another study conducted by Huang et al. investigates the activity of *Salmonella* bacteriophages in ready to eat (RTE) products. They isolated bacteriophages first and evaluated their properties to determine their potential as biocontrol agent. Then, bacteriophage LPSE1's applicability was tested on milk, sausage and lettuce. The findings support the bactericidal activity of phages on tested products (Huang et al., 2018).

Since chicken breasts are closely related to *Salmonella* infections in humans, Kim et al. evaluated activity of bacteriophages specific to the *S.* Enteritidis. In order to achieve this, they used phage cocktail consisting of four bacteriophages in their experiments. The results established the efficacy of phages targeting *S.* Enteritidis because reduction in *Salmonella* load was considerable (Kim et al., 2020).

Similarly, Atterbury et al. evaluated how bacteriophages against Salmonella are effective on chicken broilers. They isolated many bacteriophages, but they selected the phages according to host ranges and the ones with broader host ranges against *S.* Enteritidis, *S.* Hadar and *S.* Typhimurium were used. Chicken broilers are contaminated with specific *Salmonella* serovars under lab conditions. *S.* Typhimurium and *S.* Enteritidis loads were reduced by phage application whereas S. Hadar did not get affected that much from the phage (Atterbury et al., 2007).

Bacteriophage studies also conducted on sprout seeds. Pao et al. reported that application of bacteriophages on *Salmonella* contaminated broccoli and mustard seeds suppressed the growth of *Salmonella* at a certain level although there are differences in reduction efficacy on each type of seed. Nevertheless, authors remark the potential of phage applications despite the fact that they concluded as

more research are required in this area (Pao et al., 2004).

The study of Modi et al. investigates the applicability of phages on both a product and its raw material which is a good illustration of phage application in food industry. Two types of milk -raw and pasteurized- which is then further processed is inoculated with *S.* Enteritidis and with bacteriophage. Bacteria load was measured to understand whether phage is mitigating the growth of *Salmonella* in cheese and milks. According to the findings, cheese made from raw milk without the addition of bacteriophage was found to include high load of *S.* Enteritidis whereas when phage is applied *Salmonella* was not present after 89 days. Results were in the same direction as previous findings of phage application (Modi et al., 2001).

Thung et al. contributed to the investigation of *Salmonella* phage uses in food industry by trials on artificially *S.* Enteritidis contaminated food samples including fruit juice, eggs, beef and chicken meat. Samples were treated by a single phage. The results again revealed that phages are promising for fighting with foodborne pathogens. Especially, reduction in *Salmonella* prevalence by phages helps strengthen the knowledge about bacteriophage usage (Thung et al., 2017).

A comparative study has been conducted by Hungaro et al. to identify the bacteriophage and chemical agents' activity in reducing *Salmonella* spp. (Hungaro et al., 2013). The isolation source of phages used in the study was chicken feces. After the phages characterized by RFLP and host ranges were determined, cocktail consisting of five bacteriophages were applied to the chicken skin. Also, contaminated chicken skin was treated with chemical sanitizers which are used in industry. The authors reported that both agents resulted in similar log reductions as each other which supports the applicability of bacteriophages as an alternative to conventional chemical sanitizers (Hungaro et al., 2013).

One of the most attributed articles published by Bigwood et al. sheds light to the bacteriophage usage for foodborne pathogen inactivation (Bigwood et al., 2008).

The effect of phages was evaluated both raw and cooked meat and different temperatures which mimic the room and refrigeration conditions were tested. Although the phage efficiency varied with changing conditions, significant reduction of the viable cells was observed. Thus, the authors concluded that phages can be used in controlling the pathogens present in foods (Bigwood et al., 2008).

Leverentz et al. reported that *Salmonella* has the ability to survive on fresh cut melons and apples up to a certain load depending on the temperature (Leverentz et al., 2001). As the temperature is raised, *Salmonella*'s survival ability on food is increasing. On melons the load of *Salmonella* was reduced with the application of phage cocktail. On the other hand, the load was not affected significantly on apple slices independent of temperature. The study points out that phage activity may be dependent on pH of the food but still, phages are promising biocontrol agents (Leverentz et al., 2001).

Many of the foodborne outbreaks are linked with seafood and Le et al. evaluated the outcomes of phage therapy on edible oysters (Le et al., 2018). Bacteriophages were isolated from sewage water and used on artificially *E. coli* and *S.* Enterica contaminated oysters. Decrease in host numbers were detected which indicates the use of bacteriophages can be advantageous (Le et al., 2018).

Besides being tested on foods, research has been conducted on the potential use of bacteriophages in many areas of food production. Bacteriophages can be also employed for the food processing facilities in order to eliminate the pathogenic bacteria from the contact surfaces (Moye et al., 2018). Surfaces that the food are being handled can be the source of cross-contamination due to the *Salmonella* presence (Woolston et al., 2013). Kusumaningrum et al. established the survival of some foodborne pathogens including *S.* Enteritidis on surfaces and how they transmit to the food. According to their study, pathogens may be present on stainless steel which possess a risk in terms of food safety (Kusumaningrum et al., 2003).

Methods for disinfection usually involve heat, UV light treatments or chemicals which have some undesired side effects in both foods and surfaces (Woolston et al., 2013). Chemical residues may remain, or the corrosive effects of the substances can be seen on the equipment. Woolston et al. investigated how bacteriophages can reduce the surface contamination on glass and steel and for that purpose they used commercially available SalmoFresh™. Efficiency of SalmoFresh™ was found to be dependent on the *Salmonella* strains. *S.* Kentucky and *S.* Brandenburg load was reduced whereas *S.* Paratyphi B did not get affected. Nevertheless, when the composition of SalmoFresh™ was changed with other lytic bacteriophages that infect *S.* Paratyphi B, obtained results were promising. Also, it is revealed that contents of phage cocktails can be selected based upon the strains causing the problem (Woolston et al., 2013).

Bacteriophages are also applicable in agriculture. Studies conducted on plants date back to 1920's. Phage cocktails targeting the pathogens which cause disease in plants were tried on different plants (Dy et al., 2018). Holtappels et al. made a comprehensive review to provide a better understanding of phage biocontrol on various crops. They highlight the importance of identification of the pathogens playing role in the infection of crops in order to develop a successful phage cocktail (Holtappels et al., 2021).

Midani and Choi assessed the bacteriophage application on melon plants in order to prevent a disease called bacterial fruit blotch. First, plant leaves were treated with bacteria and then its bacteriophage is given into the soil to test whether phage is transported through the plant. Results showed that bacteriophage was present on the leaf after 24 hours and significant reduction in the severity of disease was seen (Rahimi-Midani and Choi, 2020).

### 2.3.2 Application of Bacteriophages for Human Infections

Host specificity of bacteriophages provide many advantages in terms of their application in humans, as well. Due to the emergence of antibiotic resistance in bacteria and limited discoveries in antibiotic, phages become prominent agents. However, the main challenge for phage application is the criteria that should be met by phages (Furfaro et al., 2018). Studies have been focusing on the treatment of specific cases by phages such as skin ulcers, gastrointestinal infections or burns. The trials associated with the treatment of dysentery with bacteriophages dates to D'Herelles's bacteriophage discovery. Later, successful results were obtained in Georgia phage therapy against dysentery. For *Salmonella* infection treatments in humans, although the course of the disease was not affected much, the duration of the infection was shortened as phages were introduced to the patients (Abedon et al., 2011). There are ongoing studies on children to assess the efficacy of phages to treat diarrhea caused by ETEC and EPEC. Bacteriophages were given orally to the children suffering from diarrhea. According to the authors' interpretation, bacteriophages were not successful in amplification in intestines, and this may be because of the inadequate dose of given bacteriophages. Thus, they concluded that more investigations should be done to understand phage and bacteria interactions in vivo (Sarker et al., 2016).

### 2.3.3 Aim of the Study

This study aims to find an alternative method to antibiotic usage in order to prevent the emergence and spread of antibiotic resistance in *Salmonella* through food supply chain while ensuring safety of food products. Because treatment of infections become harder as the strains acquire resistance which will eventually cause an increase in death numbers. Furthermore, since the distribution of *Salmonella* serovars shows variation from region to region, the effectiveness of phage cocktails developed in other countries will not be high on the serovars in

Turkey. Therefore, there is a need for phage cocktails which are effective against *Salmonella* serovars which are frequently encountered in Turkey. For that purpose, the foremost thing is to isolate bacteriophages in Turkey and define their characteristics in order to use them in phage-based applications. Furthermore, this study will contribute to the phage database and provide information about the phages which are commonly isolated from this region. Besides, isolation of *Salmonella* allows the assessment of the most prevalent serotypes in Turkey and helps demonstrate how phages coexist with their hosts.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Chemicals and Materials

The analytical grade of all the substances used in the studies was carefully selected. A list of chemicals, materials and the commercial manufacturers of those materials are presented in the Table C.1.

## 3.2 Sampling

Cattle, poultry feces and wastewater are the source of bacteriophage isolation. Fecal samples were provided from different farms located in Turkey and wastewater samples were collected from METU wastewater facility. Locations and which type of sampling was used in the study can be seen in Table 3.1 in detail.

**Table 3.1** Sampling scheme

| Location | Date | Source |
|---|---|---|
| Antalya (n=2) | 26.01.2021 | Cattle Farm (n=1) |
| | | Goat Farm (n=1) |
| Şanlıurfa (n=5) | 01.02.2021 | Cattle Farm (n=3) |
| | | Poultry Farm (n=2) |
| Adıyaman (n=5) | 09.02.2021 | Cattle Farm (n=3) |
| | | Poultry Farm (n=2) |
| Ankara (n=1) | 03.03.2021 | Wastewater facility |
| Şanlıurfa (n=5) | 06.04.2021 | Cattle Farm (n=3) |
| | | Poultry Farm (n=2) |
| Adıyaman (n=5) | 06.04.2021 | Cattle Farm (n=3) |
| | | Poultry Farm (n=2) |
| Sakarya (n=1) | 07.04.2021 | Cattle Farm (n=1) |
| Bilecik (n=9) | 20.04.2021 | Cattle Farm (n=9) |

## 3.3 Isolation of *Salmonella*

Wastewater samples from METU wastewater facility and fecal samples from cattle, goat and poultry were collected and stored at 4°C until the isolation of *Salmonella* and bacteriophages. Isolation was performed according to ISO 6579:2002. Each fecal sample were weighed as 10 g and mixed with 90 mL of Buffered Peptone Water (BPW) in stomacher bags for 60 seconds. Stomacher bags were incubated at 37°C for 18 hours. This step was followed by selective enrichment which included the transfer of 100 µL sample to the 9 mL Rappaport Vassiliadis Soy (RVS) Broth. Broths were incubated at 42°C overnight. Afterwards, 10 µl was taken from the sample by pipette and transferred to Xylose Lysine Desoxcholate (XLD) Agar. Inoculum was plated on the agar by spread plate method and plates were left for overnight incubation at 37°C. Suspicious colonies forming black dots were taken by inoculating loop and streaked to Brain Heart Infusion (BHI) agars in duplicates. Plates were again incubated at 37°C for 24 hours.

### 3.3.1 Molecular Confirmation of *Salmonella* Isolates by *InvA* Gene

Colonies grow on BHI agars were confirmed by Polymerase Chain Reaction (PCR). The amplified gene was the *invA* gene which is a specific region for *Salmonella* (Rahn et al., 1992). The procedure started with DNA isolation from the samples. For that purpose, single colony was picked and transferred to newly prepared BHI agar and incubated overnight. Also, MET S1-657 was streaked onto BHI agar as a positive control for the experiment. Afterwards, suspicious colonies from BHI agars were transferred to 0.2 mL PCR tubes containing 95 µL of double deionized water ($ddH_2O$) which had been autoclaved beforehand. 0.2 mL PCR tubes were put into microwave oven for 30 secs at 90 V for the cells to be lysed. Next, master mix was prepared in an Eppendorf tube and 24 µL from it was pipetted to new 0.2 mL PCR tubes. The components of master mix can be seen in Table 3.2

**Table 3.2** Master mix components for PCR of the *invA* gene

| **Reagent** | **Primer Sequence 5'-3'** | **Volume (µl)** |
|---|---|---|
| $ddH_2O$ | - | 17.5 |
| 5xMyTaq Reaction Buffer | - | 5 |
| *invA*- F | GAATCCTCAGTTTTTCAACGTTTC | 0.5 |
| *invA*- R | TAGCCGTAACAACCAATACAAATG | 0.5 |
| MyTaq Red DNA Polymerase | - | 0.5 |

Volumes of the components of master mix was adhusted according to the number of samples. Then, 1 µL of the lysed cells was added to each PCR tubes containing 24 µL of master mix. Tubes were placed into the T100™ Thermal Cycler (Bio-Rad). Conditions of the thermal cycler for *invA* gene is indicated in Table 3.3.

**Table 3.3** PCR conditions for *invA*

| Temperature (°C) | Time | Cycles |
|---|---|---|
| 94 | 8 min | x 1 |
| 94 | 30 sec | |
| 60 | 30 sec | x 35 |
| 72 | 30 sec | |
| 72 | 5 min | x 1 |
| 4 | ∞ | x 1 |

PCR products were taken out from the Thermal Cycler and loaded onto the gel which had been prepared by using 1.5% agarose and 0.5 x Tris Borate EDTA (TBE) buffer. PCR products were loaded into the wells as 5 μL and the ladder was loaded as 3 μL for the optimum run. The gel was taken into the electrophoresis tank and conditions were adjusted as 110 V, 400 mA and 50 min. After run was finished, agarose gel was put into staining solution Et-Br [0.002 mg EtBr/ml $dH_2O$] for 5 min which was followed by destaining step for 30 min. $ddH_2O$ was used as destaining medium. Results were obtained by using UV light (Biorad-Gel Doc XR Documantation System, USA).

### 3.3.2 Pulsed Field Gel Electrophoresis (PFGE) for *Salmonella* Isolates

BHI agars were inoculated with the target bacteria and incubated overnight prior to the experiment. Then, colonies were taken by sterile cotton swab and suspended in 4 mL Cell Suspension Buffer (CSB). Optical Density for 1.3 mL suspensions were measured at 610 nm ($OD_{610}$) by spectrophotometer. Suspensions which had the OD at 610 nm as 1.3-1.4 were replaced in ice to be used in the experiment. 400 μL from each suspension were transferred into Eppendorf tubes and incubated at 37°C for 10 min. By that time, agarose/SDS solution was prepared. After 10 min, 20 μL of Pro K solution was added to each Eppendorf tube. For the following step 400 μL of agarose/SDS solution was added to the samples and mixed well by pipetting.

Mixture was transferred to the plug molds and left for cooling at room temperature.

Cell Lysis Buffer (CLB) and Protein K were distributed as 5 mL and 25µL, with respectly to the Falcon tubes which had been previously labeled with the sample names. Formed plugs were transferred to those falcon tubes and incubated at 54°C and 170 rpm for 2 hours. Next step involved washing the plugs. Steps were applied as stated in Table 3.4. For that purpose, Tris-EDTA (TE) [1M Tris-HCl; 0.5M EDTA; $ddH_2O$] buffer and sterile deionized water were replaced in water bath at 50°C.

Initially, CLB-Pro K solution in Falcon tubes were poured after 2 hours of incubation. Plug washing started with sterile deionized water for the first 2 steps and followed by TE buffer for the last 4 steps. Between the steps, there was an incubation period in shaking incubator at 50°C.

**Table 3.4** Plug washing steps

| **Step Number** | **Reagent** | **Amount (mL)** | **Incubation Time (min)** |
|---|---|---|---|
| 1 | Sterile $ddH_2O$ | 10 | 10 |
| 2 | Sterile $ddH_2O$ | 10 | 10 |
| 3 | TE buffer | 10 | 15 |
| 4 | TE buffer | 10 | 15 |
| 5 | TE buffer | 10 | 15 |
| 6 | TE buffer | 10 | 15 |

After the $6^{th}$ step, TE buffer was poured, and 5 ml of fresh TE buffer was added to Falcon tubes allowing plugs to store for months in 4°C.

Plugs were cut into 2 mm slices and transferred to Eppendorf tubes. Then, H buffer solution was prepared and distributed to each Eppendorf tube containing plugs as 200 µl. Tubes were incubated at 37°C for 10 min. By that time Xba1 enzyme solution was prepared (175 µl $ddH_2O$; 20 µl H buffer; 5 µl Xba1) for restriction of the DNA and when the tubes were taken out of the incubator and H buffer was

removed from all the tubes, Xba1 enzyme solution was distributed to each tube as 200 µl. After it was made sure that all the slices stayed in the solution, they were placed into the incubator at 37°C for 4 hours.

SKG Agarose gel consisting of 1.5 g SKG agarose, 7.5 mL 10 x TBE and 142.5 mL dd$H_2O$ was prepared by microwaving and cooling at water bath held at 55°C. Meanwhile, running buffer was prepared and running conditions of PFGE were adjusted according to the conditions listed in Table 3.5. When the agarose gel was ready to be loaded and the slices were out of the incubator, enzyme solution was removed, and slices were placed to the wells carefully in the gel. Sealing agarose which was prepared beforehand was poured onto the wells allowing it to cover each well on the gel. Then, gel was replaced into the tank and thiourea was added onto the gel. System was started.

**Table 3.5** Pulsed Field Gel Electrophoresis Conditions

| DNA size interval | 30 kb – 700 kb |
|---|---|
| % agarose | 1% |
| Voltage | 6.0 v/cm |
| Run time | 19 h |
| Temperature set | 14°C |
| Included angle | 120° |
| Initial switch time | 2.2 s |
| Final switch time | 63.8 s |
| Pump seed | 70 (1 L/min) |

After the running time finished, gel was held at staining solution for 45 minutes which was followed by holding at water for 30 minutes. Finally, image was taken under UV light by Biorad-Gel Doc XR Documantation System.

## 3.4 Isolation of Bacteriophages

### 3.4.1 *Salmonella* Isolates

In this study, 8 different *S. enterica* serovars were selected and used as host strains in isolation of bacteriophages as indicated in Table 3.6. *Salmonella* strains used for phage isolation in this study represents the most frequently encountered *Salmonella* serotypes in Turkey. These serovars are *S.* Enteritidis, Typhimurium, Infantis, Kentucky, Montevideo, Hadar, Telaviv and Anatum. All isolates which had already been isolated and stored at -80°C were obtained from METU Food Safety Laboratory at Food Engineering Department. The isolation source of *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis and *S.* Kentucky are chicken meat and all of them were isolated in 2005. *S.* Montevideo was isolated from ground meat. *S.* Telaviv was isolated from Offal during TUBITAK Project No: 111O192 in 2012. *S.* Hadar was isolated from cheese coming from Urfa district and *S.* Anatum was isolated from sheep ground beef during TUBITAK Project No: 111O192 in 2012. The isolates were given an ID Code and frozen with glycerol kept at -80°C. Detailed information can be seen in Table 3.6.

**Table 3.6** Serotypes used in bacteriophage isolation

| **Isolate ID Code** | **Genus** | **Species** | **Serotype** | **Source of Isolation** | **Year of Isolation** | **City of Isolation** |
|---|---|---|---|---|---|---|
| MET S1-001 | *Salmonella* | *enterica* | Enteritidis | Chicken meat | 2005 | Ankara |
| MET S1-002 | *Salmonella* | *enterica* | Typhimurium | Chicken meat | 2005 | Ankara |
| MET S1-006 | *Salmonella* | *enterica* | Infantis | Chicken meat | 2005 | Ankara |
| MET S1-007 | Salmonella | *enterica* | Kentucky | Chicken meat | 2005 | Ankara |
| MET S1-015 | *Salmonella* | *enterica* | Montevideo | Ground meat | 2005 | Ankara |
| MET S1-063 | *Salmonella* | *enterica* | Telaviv | Offal | 2012 | Şanlıurfa |
| MET S1-163 | *Salmonella* | *enterica* | Hadar | Cheese | 2012 | Şanlıurfa |
| MET S1-248 | *Salmonella* | *enterica* | Anatum | Sheep ground meat | 2012 | Şanlıurfa |

Working stocks of *Salmonella* isolates were prepared on BHI agar and they are kept at 4°C. Liquid cultures of *Salmonella* strains were prepared from these agars by transferring a colony to 10 mL BHI broths and keeping broths at 37°C incubator for 16-18 hours before the experiments.

All the serovars showed in Table 3.6 were used in phage isolation from wastewater whereas only *S.* Enteritidis, *S.* Typhimurium and *S.* Infantis were used in phage isolation from cattle and poultry feces. Since wastewater and sewage are defined as the rich source of bacteriophage isolation, host serovar diversity was increased while isolating phages from wastewater (Akhtar et al., 2014). All the serovars selected represented the most prevalent serovars in Turkey (Gıda ve Kontrol Genel Müdürlüğü, 2018).

### 3.4.2 Fecal Samples

Fecal samples were weighed precisely as 10 grams with sterile spoons and for each sample weighing tools were used separately in order to prevent contamination.

Each weighed sample was transferred into stomacher bags and 90 mL Saline Magnesium (SM) Buffer was added. Mixture was put into homogenizer and mixed for 120 seconds straight. Stomacher bags were taken and put into shaking incubator which was adjusted to 37°C- 150 rpm beforehand and held for 2 hours. After 2 hours, 30 mL of the sample was transferred to Falcon tubes which had been already labeled as the sample names by glass pipettes.

Falcon Tubes were centrifuged at 9,000 rpm for 10 minutes at 4°C in order to get rid of the pellet and be ready for filtration. Supernatant was collected and filtered through 0.45 μm pore-size cellulose acetate membrane filters to new Falcon Tubes. 5 mL double strength TSB was added to each filtered supernatant. Selected Salmonella serovar hosts also added to the mixture in 100 μl. *Salmonella* serovars were grouped and determined which ones were going to be used together. For this purpose, *S.* Enteritidis, *S.* Typhimurium and *S.* Infantis was incubated together in one Falcon tube containing filtered supernatant and double strength TSB (2xTSB). After overnight incubation at 37°C, Falcon tubes are collected and centrifuged again at 9,000 rpm for 10 minutes at 4°C. Supernatant was filtered through 0.22 μm pore-size filters without disturbing the pellet. After filtration, bacteriophage suspensions are ready to use for subsequent experiments and they are stored at 4 °C.

### 3.4.3 Wastewater Samples

Since the samples collected from METU wastewater facility are in liquid form, they were directly put into Falcon tubes as 50 mL and centrifuged at 9,000 rpm – 4°C for 10 minutes and supernatant was filtered through 0.22 μm pore-size cellulose acetate membrane filters. 5 mL from each supernatant were transferred to new Falcon tubes and 5 mL of double strength TSB was added to each of them. Here, *Salmonella* isolates were grouped and determined which ones were going to

be used together. *Salmonella* hosts were transferred to each Falcon tube in 100 µL. Table 3.7 indicates the components of the Falcon tubes.

**Table 3.7** Host strains and components used for incubation of wastewater samples

| Falcon Tube | Components |
|---|---|
| 1 | • 5mL supernatant<br>• 5 mL 2xTSB<br>• 100 µl- S1-001<br>• 100 µl- S1-002 |
| 2 | • 5mL supernatant<br>• 5 mL 2xTSB<br>• 100 µl- S1-006<br>• 100 µl- S1-007<br>• 100 µl- S1-015 |
| 3 | • 5mL supernatant<br>• 5 mL 2xTSB<br>• 100 µl- S1-063<br>• 100 µl- S1-163<br>• 100 µl-S1-248 |

After an overnight incubation at 37°C, mixtures were spun at 9,000 rpm – 4°C for 10 minutes. Supernatant was filtered through 0.22 µm pore-size cellulose acetate membrane filters without disturbing the pellet. After filtration, bacteriophage suspensions are ready to use for subsequent experiments and they are stored at 4°C.

Double plaque assay is the most common technic for bacteriophage enumeration and results are reported in plaque forming units (PFU/mL) (Ács et al., 2020).

For double agar overlay, 100 µL of indicator host strain was vortexed with 100 µL filtrate containing phages for about 10 seconds in 4 mL of 0.6% Luria-Bertani (LB) top agar. Then, it was poured onto solid 1.5% LB agar and left for solidification at room temperature. Afterwards, all plates are collected and put into incubator at 37°C for 24 hours for observing clear zones. Double plaque overlay was applied to

all the phage suspensions.

### 3.4.4 Phage Purification

After the plaques are visual on petri plates, one plaque was selected and took by pipette tip. It was made sure that other plaques were not disturbed. Plaque taken was transferred into Eppendorf Tubes (EP) containing 900 µL of 0.9% NaCl solution. Serial dilutions were prepared up to $10^{-9}$. From $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$ and $10^{-9}$ dilutions, double plaque assay was applied. This procedure was repeated 3 times to make sure to observe same plaque morphology at all the steps. After the 3rd step, single plaque was taken with pipette tip and suspended in Eppendorf tube containing 900 µL 0.9% NaCl solution. From that EP tube, double plaque assay was performed directly and incubated at 37°C overnight.

### 3.4.5 Phage Freezing

Plaques were collected by pouring Saline Magnesium (SM) Buffer (1 M Tris-HCl pH: 7.5; 0.1 M NaCl; 0.01 M $MgSO_4.7H_2O$ and %2 gelatin) onto petri plate. It was waited for 30 minutes at room temperature and every 5 minutes plates were shaken gently. Buffer was collected in the Falcon Tubes and centrifuged at 4,000 rpm for 30 minutes. Supernatant was filtered with 0.22 µm pore-size filters. Filtered lysate was transferred 1 mL to eppendorf tubes and stored at 4°C. Also, lysates were prepared to be stored at -80°C. Therefore, 850 µL of filtered phage lysate was mixed with 150 µL glycerol in cryotubes and put into -80°C. These steps were applied to all the samples and each phage stock was prepared in triplicates. All the phage stocks were given a METU ID Code. Given numbers were assigned according to the isolation order. Also, details belonging to the stocks were entered to the database under their ID Codes.

### 3.4.6 Phage Titer Determination

For titer determination, 100 μL was taken from phage stock which was stored at 4°C and transferred into Eppendorf tube containing 900 μL 0.85% NaCl solution. Serial dilutions were prepared up to $10^{-9}$. From the last 5 dilution, double plaque assay was performed, and plates were incubated for 24 hours at 37°C. After 24 hours, plaques were counted and reported in plaque forming units (PFU/mL).

### 3.4.7 Phage Host Range Determination

For the host range determination 36 *S. enterica* isolates were used from METU Food Safety Laboratory. First, it was made sure that titer of all the phages were at least $10^8$ PFU/mL. Then, host range determination was applied by performing spot test. Petri plates were divided into 8 equal parts and all the parts were labeled with the phage ID and bacterial ID. Then, 100μL of the host bacteria was put into 4 mL semi-solid (0.6%) LB broth and slowly poured onto solid LB agar covering everywhere. It was allowed to dry at room temperature for 15-30 minutes. Afterwards, 5μL of phage stock was taken and spotted onto the bacterial lawn. Different phage suspensions were spotted onto each divided part. Petri plates were allowed to dry again at room temperature and then placed into the incubator at 37°C carefully. While choosing *Salmonella* serovars which were used in host range determination, their isolation source and PFGE types were considered. For example, even though there were 5 *S*. Enteritidis tested, their isolation sources and PFGE types were different from each other. Main reason was to detect if any variations are occurring with the same phage suspension due to those properties.

**Table 3.8** *Salmonella* isolates used in host range determination

| METU ID | *Salmonella* serotypes | Phenotypic AMR Profile | PFGE Type | Source of isolation |
|---|---|---|---|---|
| MET S1-003 | Virchow | Susceptible | NA | Chicken meat |
| MET S1-008 | Thompson | KSTAmpKfSfSxtCn | NA | Chicken meat |
| MET S1-010 | Senftenberg | STSfN | NA | Chicken meat |
| MET S1-011 | Agona | KSTSfN | NA | Chicken meat |
| MET S1-050 | Infantis | KSTAmpSfN | PT08 | Chicken meat |
| MET S1-065 | Montevideo | SfSxtNT | PT25 | Offal |
| MET S1-074 | Telaviv | SfSxtNT | PT33 | Offal |
| MET S1-087 | Othmarchen | Susceptible | PT27 | Sheep ground meat |
| MET S1-163 | Hadar | AmpKfN | PT41 | Cheese |
| MET S1-166 | Newport | Sf | PT39 | Cattle |
| MET S1-170 | Montevideo | Susceptible | PT44 | Cattle |
| MET S1-172 | Montevideo | Sf | PT31 | Cattle |
| MET S1-184 | Paratyphi B | Susceptible | PT15 | Human |
| MET S1-185 | Typhimurium | Sf | PT15 | Human |
| MET S1-217 | Enteritidis | Susceptible | PT04 | Human |
| MET S1-220 | Typhi | Sf | PT23 | Human |
| MET S1-221 | Enteritidis | Susceptible | PT05 | Human |
| MET S1-223 | Typhimurium | TAmp | PT23 | Human |
| MET S1-240 | Kentucky | Susceptible | PT10 | Human |
| MET S1-411 | Enteritidis | Susceptible | PT51 | Red pepper |
| MET S1-530 | Telaviv | Susceptible | PT34 | Cheese |
| MET S1-542 | Kentucky | Sf | PT03 | Sheep |
| MET S1-548 | Anatum | Susceptible | PT42 | Sheep ground meat |
| MET S1-579 | Anatum | Susceptible | PT42 | Cow ground meat |
| MET S1-657 | Typhimurium | STAmpAmcSfCn | PT14 | Sheep |
| MET S1-663 | Typhimurium | TAmpKf | PT13 | Sheep |
| MET S1-713 | Braenderup | NA | PFGE Ref. | Unspecified |
| MET S1-742 | Enteritidis | NI | PT06 | Chicken meat |
| MET S1-807 | Infantis | CroEftSfSxtCKSAmpAmcTeFoxKf | NI | Unspecified |
| MET S1-857 | Infantis | NI | PT73 | Sludge |
| MET S1-864 | Mbandaka | SxtSfAmpAzmPef | PT65 | Sludge |
| MET A2-003 | Typhimurium | Susceptible | PT59 | Sludge |
| MET A2-012 | Enteritidis | Susceptible | PT55 | Sludge |
| MET A2-072 | Kentucky | KfSfAmpNAzmPef | PT72 | Sludge |
| MET A2-088 | Typhimurium DT104 | NI | NI | Unspecified |
| MET A2-099 | Liverpool | Susceptible | PT54 | Chicken meat |

### 3.4.8 Single Step Growth Curves

For the single step growth curves method described by Clokie et al. with

modifications was used (Clokie et al., 2018). Single step growth curves were obtained for bacteriophages and with its indicator bacterial strains. Fresh liquid culture of *S.* Enteritidis was prepared in BHI broth and incubated for 16 h at 37°C. Additionally, since the host cells should be in mid-log phase, BHI broth was inoculated with *S.* Enteritidis and kept at 37°C incubator for 8 h. Afterwards, optical density was adjusted to 1.03 – 1.05 at 600 nm ($OD_{600}$) by the addition of 0.85% NaCl and this culture was transferred in 9.9 mL in a tube and left for incubation for 5 min. Next, 0.1 mL from the previously prepared phage solution ($10^6$ PFU/mL) was added to the bacterial culture letting MOI value to become 0.01 and allowed for 5 min bacteria to adsorb phages. After 5 min 0.1 mL of culture was removed from this tube and added into the tube containing 9.9 mL LB broth for lowering the phage titer. Also, 1.0 mL was taken from this tube and transferred into a flask containing chloroform which was used as adsorption control. Afterwards, 1 mL of culture was taken again from tube containing 9.9 mL culture and added to 9 mL LB broth. Another dilution was prepared from this tube by transferring 1 mL of mixture to 9 mL LB broth. Last tube's phage titer reached to $10^1$ PFU/mL.

After these preparations, in every 6 min for 90 min double plaque assay was applied from dilutions. Plates were incubated at 37°C for 24 h plaque counting. Burst size, latent period of bacteriophages were determined accordingly.

As Abedon et al. stated growth curves are built based on general assumptions that host cells that are infected do not divide, phages are not infecting a host who is already infected (Abedon et al., 2001).

### 3.4.9 Genome Size Determination of *Salmonella* Bacteriophages

Genome sizes of bacteriophages were also determined by Pulsed Field Gel Electrophoresis by the methods described by Lingohr et al. (Lingohr et al., 2009). In order to perform PFGE to *Salmonella* phages, first double plaque assay was conducted, and plates were incubated prior to the experiment so that phages could

be collected by SM buffer. For that purpose, 10 mL of SM buffer was poured onto the plates which were completely lysed by bacteriophages. Plates with SM buffer were held for 30 min in room temperature, and they were transferred to mini centrifuge tubes for centrifugation at 9,000 rpm for 15 minutes. Then, the solution was filtered through 0.22 μL filters to new Eppendorf tubes. 1.2% plug agarose was prepared and held in water bath at 50°C. Next, 400 μL of molten plug agarose was taken and put into phage solutions and mixed well by pipetting. Plug casting mold was filled with 250 μL from the agarose-phage mix. Plugs were allowed to solidify at room temperature. Meanwhile, 100 mL Phage Lysis (PL) buffer (50 mM Tris; 50 mM EDTA; 1% SDS) was prepared and distributed as 5 mL to Falcon tubes which were labeled beforehand. After the time was up for plugs, they were removed from the mold by spatula and put into labeled falcon tubes. Tubes were held at incubator adjusted to 54°C for 2 hours. The next step was washing the plugs. Thus, TE was replaced into water bath at 54°C. Solution in falcon tubes was poured ensuring that the plugs were still present in the tubes. Following steps for washing were same as the conditions indicated in table 3.4. Plugs were stored at 4°C until next day.

Agarose gel (1% SeaKem Gold Agarose; 0.5 x TBE) was prepared and allowed to solidify. Plugs were sliced as 2 mm and loaded into the wells on the gel. Wells were covered by sealing agarose. The gel was replaced into the buffer present in the PFGE tank.

Staining and rinsing of the gel was done as described previously in the PFGE for bacterial isolates section. Results were obtained under UV light and images were examined by using Biorad-Gel Doc XR Documantation System.

### 3.4.10 PFGE Gel Analysis of Bacteriophages and *Salmonella* Isolates

All obtained PFGE gel images were uploaded to BioNumerics Software (Applied Maths, Belgium) and those images were processed and normalized. Obtained data

were transferred to database where all the previous data belonging to the isolates of METU Food Safety Laboratory. After all the bands were defined and normalization was applied to the gels to get accurate results cluster analysis was conducted by using comparison table. Cluster analysis was conducted via Dice Coefficient. Optimization and band matching tolerance were chosen as 1.5%.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Isolation of *Salmonella*

In total, 4 *Salmonella* strains were able to be isolated from the collected samples. After the isolates were confirmed by PCR, they were stored in -80°C as triplicates in Food Safety Laboratory. Suspicious colonies appearing as black dots on XLD agars were transferred to BHI agars to perform PCR in order to confirm that they are *Salmonella* strains. PCR master mix contained the primers for *invA* gene found in *Salmonella*.

**Table 4.1.** Information of Isolated *Salmonella* strains

| METU ID | Genus | Serovar | Source | Sample Collection Date | City |
|---|---|---|---|---|---|
| MET A2-230 | *Salmonella* | Enteritidis | Cattle Farm | 01.02.2021 | Şanlıurfa |
| MET A2-231 | *Salmonella* | Enteritidis | Cattle Farm | 01.02.2021 | Şanlıurfa |
| MET A2-232 | *Salmonella* | Enteritidis | Cattle Farm | 01.02.2021 | Şanlıurfa |
| MET A2-233 | *Salmonella* | Enteritidis | Cattle Farm | 01.02.2021 | Şanlıurfa |
| MET A2-234 | *Salmonella* | Enteritidis | Cattle Farm | 01.02.2021 | Şanlıurfa |
| MET A2-235 | *Salmonella* | Enteritidis | Cattle Farm | 01.02.2021 | Şanlıurfa |
| MET A2-236 | *Salmonella* | Typhimurium | Poultry Farm | 01.02.2021 | Şanlıurfa |
| MET A2-237 | *Salmonella* | Typhimurium | Poultry Farm | 01.02.2021 | Şanlıurfa |
| MET A2-238 | *Salmonella* | Typhimurium | Poultry Farm | 01.02.2021 | Şanlıurfa |
| MET A2-239 | *Salmonella* | Typhimurium | Poultry Farm | 01.02.2021 | Şanlıurfa |
| MET A2-240 | *Salmonella* | Typhimurium | Poultry Farm | 01.02.2021 | Şanlıurfa |
| MET A2-241 | *Salmonella* | Typhimurium | Poultry Farm | 01.02.2021 | Şanlıurfa |

100 bp ladder

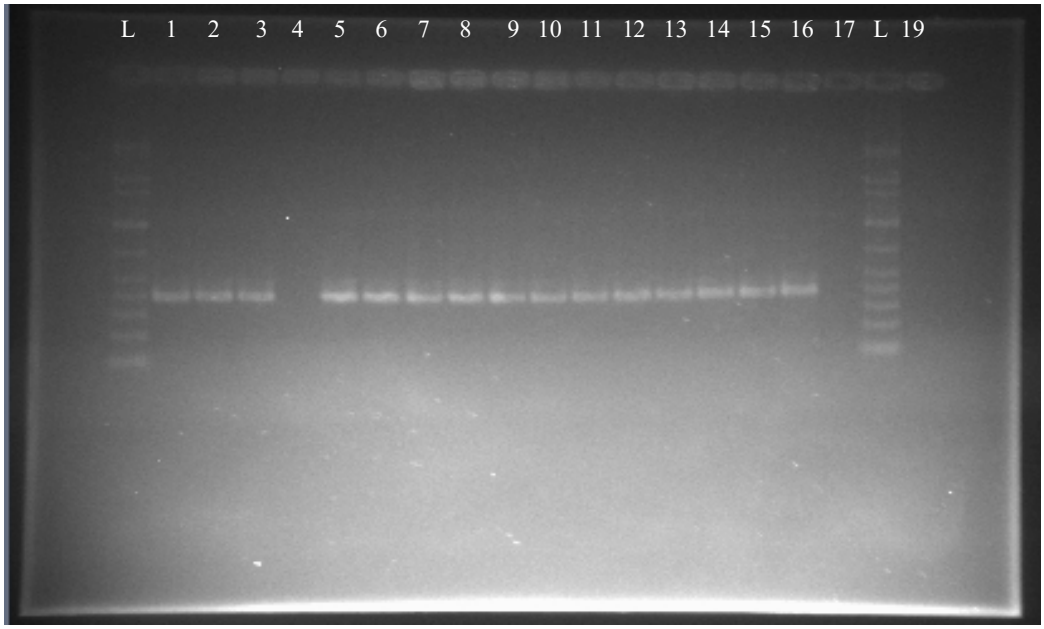
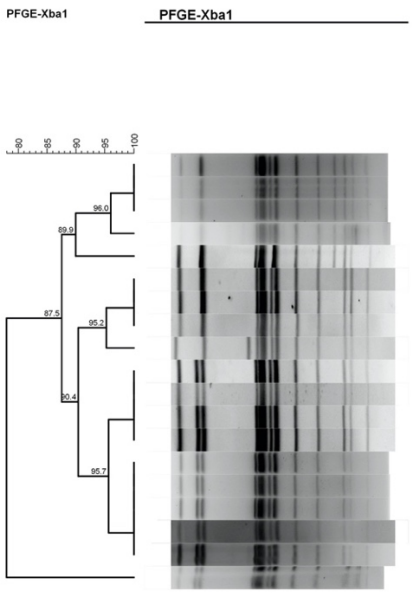



**Figure 4.1** PCR gel electrophoresis image for *Salmonella* isolates. L: DNA ladder; 1: MET S1-001; 2: MET S1-657; 3: MET S1-679; 4,17: Negative control; 5,6,7: MET A2-230; 8,9,10: MET A2-233; 11,12,13: MET A2-236; 14,15,16: MET A2-239

Isolates gave the same band size (389 bp) as the strains which were defined as the control. All the *Salmonella* strains were isolated from samples supplied from different farms in Sanliurfa in February. Half of the strains were obtained from cattle feces and other half from poultry feces. Any *Salmonella* were not able to be isolated from other samples collected during this study. Although suspicious colonies were seen on XLD agars, when PCR was performed with them there were no bands on the gel.

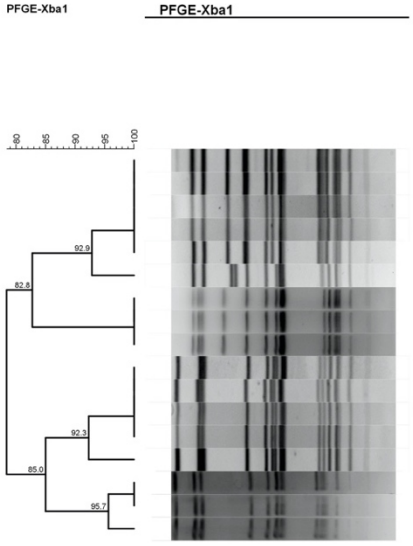
Further analysis to the isolates was performed by PFGE which was defined as the gold standard for bacteria subtyping (Neoh et al., 2019). Serotypes of the 4 isolates were determined by cluster analysis. MET A2- 230 and MET A2-233 share the same patterns as *S.* Enteritidis isolates in the database (MET A2-031, MET A2-032 and MET A2-033) which had been isolated from sludge during 2016. Thus, their

serotypes were identified as *S.* Enteritidis. The remaining isolates (MET A2-236 and MET A2-239) found to be representing *S.* Typhimurium according to their PFGE results. In order to compare the isolates obtained in this study, other serovars of *Salmonella* were provided from METU Food Safety Laboratory Database.




| Key | PFGE Type | Genus | Serovar | Source | Location | Year |
|---|---|---|---|---|---|---|
| MET S1-909 | PT53 | Salmonella | Enteritidis | Sludge | Ankara | 2015 |
| MET S1-910 | PT53 | Salmonella | Enteritidis | Sludge | Ankara | 2015 |
| MET S1-911 | PT53 | Salmonella | Enteritidis | Sludge | Ankara | 2015 |
| MET A2-014 | PT53 | Salmonella | Enteritidis | Sludge | Ankara | 2015 |
| MET S1-411 | PT51 | Salmonella | Enteritidis | Food | Ankara | 2012 |
| MET S1-221 | PT05 | Salmonella | Enteritidis | Human | Şanlıurfa | 2011 |
| MET S1-768 | PT05 | Salmonella | Enteritidis | Food | Ankara | 2015 |
| MET S1-771 | PT05 | Salmonella | Enteritidis | Food | Ankara | 2015 |
| MET S1-217 | PT04 | Salmonella | Enteritidis | Human | Şanlıurfa | 2011 |
| MET S1-660 | PT06 | Salmonella | Enteritidis | Sheep | Şanlıurfa | 2012 |
| MET S1-742 | PT06 | Salmonella | Enteritidis | Food | Ankara | 2013 |
| MET S1-756 | PT06 | Salmonella | Enteritidis | Food | Ankara | 2015 |
| MET S1-762 | PT06 | Salmonella | Enteritidis | Food | Ankara | 2015 |
| MET A2-031 | PT06 | Salmonella | Enteritidis | Sludge | Ankara | 2016 |
| MET A2-032 | PT06 | Salmonella | Enteritidis | Sludge | Ankara | 2016 |
| MET A2-033 | PT06 | Salmonella | Enteritidis | Sludge | Ankara | 2016 |
| MET A2-230 | PT06 | Salmonella | Enteritidis | Cattle feces | Sanliurfa | 2020 |
| MET A2-233 | PT06 | Salmonella | Enteritidis | Cattle feces | Sanliurfa | 2021 |
| MET A2-113 | PT06 | Salmonella | Enteritidis | Chicken | Ankara | 2019 |

**Figure 4.2** Cluster analysis of MET A2-230 and MET A2-233




| Key | PFGE Type | Genus | Serovar | Source | Location | Year |
|---|---|---|---|---|---|---|
| MET S1-211 | PT13 | Salmonella | Typhimurium | Human | Şanlıurfa | 2010 |
| MET S1-223 | PT13 | Salmonella | Typhimurium | Human | Şanlıurfa | 2011 |
| MET S1-625 | PT13 | Salmonella | Typhimurium | Food | Şanlıurfa | 2012 |
| MET S1-653 | PT13 | Salmonella | Typhimurium | Cattle | Şanlıurfa | 2012 |
| MET S1-663 | PT13 | Salmonella | Typhimurium | Sheep | Şanlıurfa | 2012 |
| MET S1-657 | PT14 | Salmonella | Typhimurium | Sheep | Şanlıurfa | 2012 |
| MET A2-002 | PT59 | Salmonella | Typhimurium | Sludge | Kayseri | 2015 |
| MET A2-003 | PT59 | Salmonella | Typhimurium | Sludge | Eskişehir | 2015 |
| MET A2-004 | PT59 | Salmonella | Typhimurium | Sludge | Eskişehir | 2015 |
| MET S1-185 | PT35 | Salmonella | Typhimurium | Human | Şanlıurfa | 2012 |
| MET S1-204 | PT15 | Salmonella | Typhimurium | Human | Şanlıurfa | 2010 |
| MET S1-233 | PT35 | Salmonella | Typhimurium | Human | Şanlıurfa | 2012 |
| MET S1-236 | PT35 | Salmonella | Typhimurium | Human | Şanlıurfa | 2012 |
| MET S1-207 | PT35 | Salmonella | Typhimurium | Human | Şanlıurfa | 2010 |
| MET A2-215 | | Salmonella | Typhimurium | Turkey feces | Sanliurfa | 2020 |
| MET A2-236 | PT94 | Salmonella | Typhimurium | Poultry feces | Sanliurfa | 2021 |
| MET A2-239 | PT94 | Salmonella | Typhimurium | Poultry feces | Sanliurfa | 2021 |

**Figure 4.3** Cluster analysis of MET A2-236 and MET A2-239

*S.* Enteritidis serovars were isolated from cattle feces and *S.* Typhimurium were isolated from poultry feces collected from Sanliurfa. Isolated serovars are the most common types in Turkey. Additionally, bacteriophages against *S.* Enteritidis (MET P1-206) were isolated from the same source used in *S.* Enteritidis isolation (MET A2-230 and 233). The same situation was also valid for *S.* Typhimurium and its bacteriophages i.e both *S.* Typhimurium and its phage was isolated from same Sanliurfa poultry feces.

## 4.2 Isolation and Titer Determination of *Salmonella* Bacteriophages

From 33 samples collected from cattle and poultry farms and wastewater facility in Turkey, 25 *Salmonella* phages were isolated and purified. *S.* Enteritidis was found to be the most dominant serotype as host for phage isolation and yielded 21 phage isolates. The other 4 phages infecting different serotypes include *S.* Kentucky, *S.* Hadar, *S.* Anatum and *S.* Telaviv. 6 phages were isolated from 8 samples of poultry feces, 13 phages from 22 sample of cattle feces and 6 phages from 1 sample of wastewater. Isolated bacteriophages were given a METU ID Code and stored as three replicates in 4°C and in -80°C.

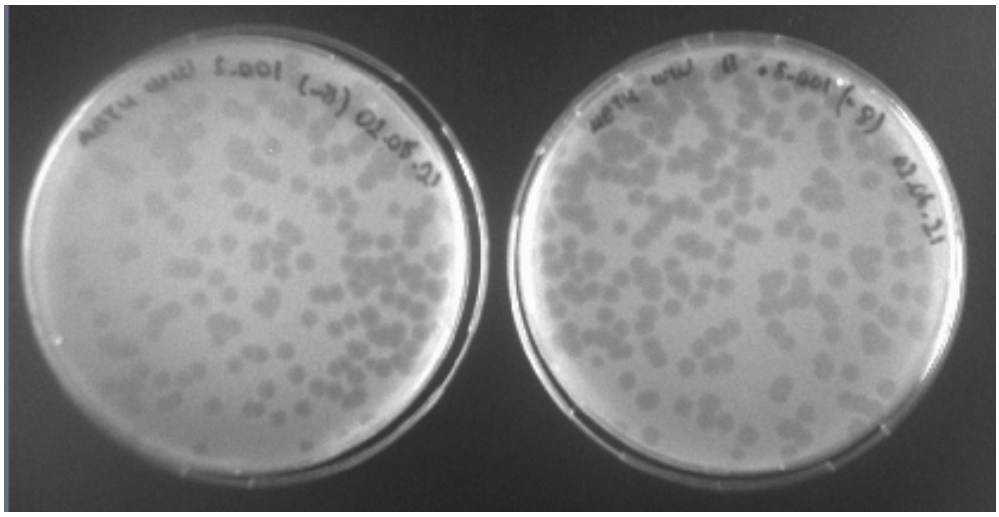


**Figure 4.4** Double plaque assay results from 8$^{th}$ dilution of MET P1-224

**Table 4.2** Detailed Information of isolated *Salmonella* bacteriophages

| PhageID | Genus | Target Serotype | Titer (PFU/mL) | Source | Date | City |
|---|---|---|---|---|---|---|
| MET P1-206 | *Salmonella* | Enteritidis, Typhimurium | $2*10^9$ | Cattle Farm | February | Şanlıurfa |
| MET P1-207 | *Salmonella* | Enteritidis, Typhimurium | $2*10^9$ | Cattle Farm | February | Şanlıurfa |
| MET P1-208 | *Salmonella* | Enteritidis, Typhimurium | $2*10^9$ | Cattle Farm | February | Şanlıurfa |
| MET P1-209 | *Salmonella* | Enteritidis, Typhimurium | $1.5*10^9$ | Cattle Farm | February | Şanlıurfa |
| MET P1-210 | *Salmonella* | Enteritidis, Typhimurium | $1.5*10^9$ | Cattle Farm | February | Şanlıurfa |
| MET P1-211 | *Salmonella* | Enteritidis, Typhimurium | $1.5*10^9$ | Cattle Farm | February | Şanlıurfa |
| MET P1-212 | *Salmonella* | Enteritidis | $2*10^9$ | Cattle Farm | February | Şanlıurfa |
| MET P1-213 | *Salmonella* | Enteritidis | $2*10^9$ | Cattle Farm | February | Şanlıurfa |
| MET P1-214 | *Salmonella* | Enteritidis | $2*10^9$ | Cattle Farm | February | Şanlıurfa |
| MET P1-215 | *Salmonella* | Enteritidis, Typhimurium | $2*10^9$ | Poultry Farm | February | Şanlıurfa |
| MET P1-216 | *Salmonella* | Enteritidis, Typhimurium | $2*10^9$ | Poultry Farm | February | Şanlıurfa |
| MET P1-217 | *Salmonella* | Enteritidis, Typhimurium | $2*10^9$ | Poultry Farm | February | Şanlıurfa |
| MET P1-218 | *Salmonella* | Enteritidis, Typhimurium | $1*10^9$ | Poultry Farm | February | Şanlıurfa |
| MET P1-219 | *Salmonella* | Enteritidis, Typhimurium | $1*10^9$ | Poultry Farm | February | Şanlıurfa |
| MET P1-220 | *Salmonella* | Enteritidis, Typhimurium | $1*10^9$ | Poultry Farm | February | Şanlıurfa |
| MET P1-221 | *Salmonella* | Enteritidis | $8*10^8$ | Cattle Farm | February | Adıyaman |
| MET P1-222 | *Salmonella* | Enteritidis | $8*10^8$ | Cattle Farm | February | Adıyaman |
| MET P1-223 | *Salmonella* | Enteritidis | $8*10^8$ | Cattle Farm | February | Adıyaman |
| MET P1-224 | *Salmonella* | Enteritidis | $2.2*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-225 | *Salmonella* | Enteritidis | $2.2*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-226 | *Salmonella* | Enteritidis | $2.2*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-227 | *Salmonella* | Enteritidis | $1*10^8$ | Wastewater Facility | March | Ankara |
| MET P1-228 | *Salmonella* | Enteritidis | $1*10^8$ | Wastewater Facility | March | Ankara |
| MET P1-229 | *Salmonella* | Enteritidis | $1*10^8$ | Wastewater Facility | March | Ankara |
| MET P1-230 | *Salmonella* | Kentucky | $1.2*10^9$ | Wastewater Facility | March | Ankara |

Table 4.2 (continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| MET P1-231 | *Salmonella* | Kentucky | $1.2*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-232 | *Salmonella* | Kentucky | $1.2*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-233 | *Salmonella* | Hadar | $3*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-234 | *Salmonella* | Hadar | $3*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-235 | *Salmonella* | Hadar | $3*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-236 | *Salmonella* | Telaviv | $4*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-237 | *Salmonella* | Telaviv | $4*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-238 | *Salmonella* | Telaviv | $4*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-239 | *Salmonella* | Anatum | $3.310^9$ | Wastewater Facility | March | Ankara |
| MET P1-240 | *Salmonella* | Anatum | $3.3*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-241 | *Salmonella* | Anatum | $3.3*10^9$ | Wastewater Facility | March | Ankara |
| MET P1-242 | *Salmonella* | Enteritidis | $3.2*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-243 | *Salmonella* | Enteritidis | $3.2*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-244 | *Salmonella* | Enteritidis | $3.2*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-245 | *Salmonella* | Enteritidis | $1*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-246 | *Salmonella* | Enteritidis | $1*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-247 | *Salmonella* | Enteritidis | $1*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-248 | *Salmonella* | Enteritidis, Typhimurium | $5*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-249 | *Salmonella* | Enteritidis, Typhimurium | $5*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-250 | *Salmonella* | Enteritidis, Typhimurium | $5*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-251 | *Salmonella* | Enteritidis, Typhimurium | $2.8*10^9$ | Poultry Farm | April | Şanlıurfa |
| MET P1-252 | *Salmonella* | Enteritidis, Typhimurium | $2.8*10^9$ | Poultry Farm | April | Şanlıurfa |
| MET P1-253 | *Salmonella* | Enteritidis, Typhimurium | $2.8*10^9$ | Poultry Farm | April | Şanlıurfa |
| MET P1-254 | *Salmonella* | Enteritidis | $3*10^9$ | Poultry Farm | April | Şanlıurfa |
| MET P1-255 | *Salmonella* | Enteritidis | $3*10^9$ | Poultry Farm | April | Şanlıurfa |
| MET P1-256 | *Salmonella* | Enteritidis | $3*10^9$ | Poultry Farm | April | Şanlıurfa |

Table 4.2 (continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| MET P1-257 | *Salmonella* | Enteritidis | $2.6*10^9$ | Poultry Farm | April | Adıyaman |
| MET P1-258 | *Salmonella* | Enteritidis | $2.6*10^9$ | Poultry Farm | April | Adıyaman |
| MET P1-259 | *Salmonella* | Enteritidis | $2.6*10^9$ | Poultry Farm | April | Adıyaman |
| MET P1-260 | *Salmonella* | Enteritidis, Typhimurium | $5*10^9$ | Poultry Farm | April | Adıyaman |
| MET P1-261 | *Salmonella* | Enteritidis, Typhimurium | $5*10^9$ | Poultry Farm | April | Adıyaman |
| MET P1-262 | *Salmonella* | Enteritidis, Typhimurium | $5*10^9$ | Poultry Farm | April | Adıyaman |
| MET P1-263 | *Salmonella* | Enteritidis | $3*10^9$ | Cattle Farm | April | Şanlıurfa |
| MET P1-264 | *Salmonella* | Enteritidis | $3*10^9$ | Cattle Farm | April | Şanlıurfa |
| MET P1-265 | *Salmonella* | Enteritidis | $3*10^9$ | Cattle Farm | April | Şanlıurfa |
| MET P1-266 | *Salmonella* | Enteritidis | $2*10^9$ | Cattle Farm | April | Şanlıurfa |
| MET P1-267 | *Salmonella* | Enteritidis | $2*10^9$ | Cattle Farm | April | Şanlıurfa |
| MET P1-268 | *Salmonella* | Enteritidis | $2*10^9$ | Cattle Farm | April | Şanlıurfa |
| MET P1-269 | *Salmonella* | Enteritidis | $1*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-270 | *Salmonella* | Enteritidis | $1*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-271 | *Salmonella* | Enteritidis | $1*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-272 | *Salmonella* | Enteritidis | $4*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-273 | *Salmonella* | Enteritidis | $4*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-274 | *Salmonella* | Enteritidis | $4*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-275 | *Salmonella* | Enteritidis | $2.6*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-276 | *Salmonella* | Enteritidis | $2.6*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-277 | *Salmonella* | Enteritidis | $2.6*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-278 | *Salmonella* | Enteritidis | $3*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-279 | *Salmonella* | Enteritidis | $3*10^9$ | Cattle Farm | April | Bilecik |
| MET P1-280 | *Salmonella* | Enteritidis | $3*10^9$ | Cattle Farm | April | Bilecik |

Distribution of bacteriophages depends on their host prevalence since they require their hosts to continue their life. This indicates that phages are abundant where their host microorganisms are (Clokie et al., 2011). Thus, animal feces and

wastewater samples are shown as a source of *Salmonella* bacteriophage isolation (Carey-Smith et al., 2006; O'Flynn et al., 2006). Muniesa et al. reported that they were able to isolate coliphages from sewage (Muniesa et al., 1999).

O'Flynn et al. reported that *Salmonella* phages that have a lysing capability on wider range of *Salmonella* strains were isolated from effluent rather than poultry production unit and cattle mart (O'Flynn et al., 2006). Also, a study on isolation of *E. coli* O157:H7 phages concluded that sewage was the richest source, and it was followed by slaughterhouse wastewaters (Sakin Şahin et al., 2020). Additionally, Huang et al. pointed out that phages isolated by using *S.* Enteritidis were obtained in greater amounts in sewage (Huang et al., 2018). Akhtar et al. compared the fecal samples and wastewater as an isolation source and indicated that wastewater yielded in more phages that were active against wider serotypes of *Salmonella.* The authors explained the reason for this by stating that phages in sewage may face with various hosts than they do in feces (Akhtar et al., 2014).

In *Salmonella* phage isolation, *Salmonella* serovar diversity was increased while performing experiment with wastewater samples. In addition to the serovars used in fecal samples, *S.* Kentucky, *S.* Anatum, *S.* Hadar, *S.* Montevideo and *S.* Telaviv were used as indicator serovars due to outcomes of the mentioned studies.

The results showed that bacteriophages that were isolated from wastewater were active against *S.* Enteritidis, *S.* Hadar, *S.* Anatum, *S.* Telaviv and *S.* Kentucky whereas any phages against *S*. Montevideo, *S.* Typhimurium and *S.* Infantis were not able to be isolated. However, in fecal samples of cattle and poultry only *S.* Enteritidis and *S.* Typhimurium phages were obtained.

In Turkey, *S.* Enteritidis possess a significant importance since it was the most isolated one in human clinical samples (Gıda ve Kontrol Genel Müdürlüğü, 2018). In this regard, bacteriophages isolated by *S*. Enteritidis may expected to be high in number among other serovars.

Any bacteriophages from fecal samples from Antalya which were arrived in January were not able to be isolated. Used samples belonged to the goat and cattle. On the other hand, February samples coming from Şanlıurfa and Adıyaman resulted in isolation of 5 bacteriophages by using *S.* Enteritidis. A total of 3 out of 6 cattle farms and 2 out of 4 poultry farms from both cities, bacteriophages were isolated. From the samples that arrived in April, in total 6 bacteriophages were collected. Again, all phages were obtained by using *S.* Enteritidis as a host. Cattle feces supplied from another city, Bilecik, resulted in isolation of 7 bacteriophages out of 9 samples, all against *S.* Enteritidis. Also, plaque morphologies of all samples of Bilecik showed similarities with each other where some of them formed halo around the clear zones on bacterial lawn. In general, plaques that formed by *S.* Enteritidis and Typhimurium reached up to 3 mm in diameter whereas other bacteriophages isolated by using serovars other than *S.* Enteritidis generated comparatively small sized plaques and no halo formation was observed.

Since all isolated bacteriophages had sufficient titer ($10^8$ PFU/mL), all of them were used in host range determination with two exceptions (Petsong et al., 2019). MET P1-227 and MET P1-257 and their replicates were eliminated due to their lysogeny capabilities meaning that 23 bacteriophages were tested at the end. Nevertheless, their titers were determined again prior to the experiments to see if any decrease in titers occurred during storage.

## 4.3 Host Range Determination of Bacteriophages

Host range determination provided a basis for lysis profiles of bacteriophages which will be essential for cocktail preparation. 23 bacteriophages were tested with 36 *Salmonella* isolates. These serovars represented the most frequently encountered ones in Turkey and their isolation source was also taken into consideration to see if there is any variation occurring due to the source. Furthermore, *S.* Paratyphi B and *S.* Typhi were included in the host range determination since they are responsible for causing typhoid fever in humans so that efficacy of isolated bacteriophages was

evaluated on them.

Lysis capability of bacteriophages showed variation. Bacteriophages isolated from wastewater samples have the broadest host range. MET P1-230, MET P1-233, MET P1-236 and MET P1-239 have found to be effective on 23, 19, 28 and 22 of the isolates, respectively. MET P1-230, MET P1-236 MET P1-239 are the ones that mostly formed fully or partially clear zones on bacterial lawn. These bacteriophages are effective on nearly same isolates proposing that they identify similar receptors of hosts. These findings support the Akhtar et al. conclusion since the phages having the broader host range were isolated from wastewater samples. As they explained, reason for this maybe the bacteriophages encounter diverse hosts than they do in feces (Akhtar et al., 2014). Furthermore, as stated by Parmar et al. diverse microbial community drives the diverse bacteriophage presence suggesting that wastewaters harbor phages with broader host range since changes in bacterial population occur frequently (Parmar et al., 2018).

Interestingly, even though MET P1-230 was isolated by using *S.* Kentucky (MET S1-007) did not infect *S.* Kentucky (MET A2-072) isolated from sludge whereas it lysed MET S1-240 and MET S1- 542 whose isolation sources were human and animal, respectively. This situation may be due to the receptors that phages bind. Same situation also occurred with some *S.* Enteritidis phages. MET P1-224, MET P1-242, MET P1-245, MET P1-248, MET P1-251 and MET P1-254 did not infect *S.* Enteritidis MET S1- 217 which had been isolated from human. Additionally, all the *S.* Enteritidis phages did not totally but partially lysed MET S1- 217 and MET S1- 221 isolates. Both serovars were isolated from human. On the other hand, all phages were effective against *S.* Enteritidis whose source was food and sludge. These findings will be taken into consideration while determining phage cocktail components depending on the purpose of the cocktail.

It is noteworthy to state that 83.3% of the tested *S.* Typhimurium is partially and totally lysed by all the bacteriophages independent of their target strain which is a promising result since *S.* Typhimurium is related to the outbreaks in Turkey. 80%

of the tested *S.* Enteritidis was again partially and totally lysed by all bacteriophages. This result also reveals the susceptibility of the tested *S.* Enteritidis to phages. According to data from *Salmonella* Control Program, *S.* Enteritidis was the major causative agent in outbreaks took place in 2016 (Gıda ve Kontrol Genel Müdürlüğü, 2018).

*S.* Infantis was not affected by any of the bacteriophages independent of their target serovars. *S.* Montevideo is again did not get infected by bacteriophages, only MET P1-236 formed turbid plaques on two *S.* Montevideo isolates. *S.* Paratyphi B was strongly lysed by all the bacteriophages whereas *S.* Typhi was resistant to all of them.

Bacteriophages isolated from farms by *S.* Enteritidis were able to infect 30.5-50% of the isolates while phages from wastewater were effective against 33.3-77.7% of them.

Phages obtained from poultry farms were partially or totally effective on 36.1-50% isolates and 36.1-50% of the isolates were found to be susceptible to the phages from cattle farms. On average, phages isolated from farms were able to infect 41.7% and phages from wastewater were effective on 57.8% of the isolates. These results showed that phages isolated from wastewater were more effective on tested isolates compared to the ones from cattle and poultry farms.

The lysis capability of bacteriophages may exhibit differences with the studies conducted in other countries (Petsong et al., 2019). The serovars linked with the outbreaks show variation among countries thus isolated phages can be expected to be show variation.

All in all, isolated phages were observed to have lysing capabilities of different serovars which enables them to use in phage applications. Still, they need to fulfill other parameters in order to be used for therapeutic purposes thus further investigations are required.

In host range experiments, formed turbid zones do not necessarily mean that bacteriophages are lysogenic. Therefore, further experiments should be carried out.

**Table 4.3** Susceptibilities of *Salmonella* isolates used in host range analysis with isolated bacteriophages

| | **Bacteriophages** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **206** | **209** | **212** | **215** | **218** | **221** | **224** | **230** |
| Host of isolation | S1-001 | S1-001 | S1-001 | S1-001 | S1-001 | S1-001 | S1-001 | S1-007 |
| Source of isolation | C.F | C.F | C.F | P.F | P.F | P.F | WW | WW |
| | **Susceptibility (%)** | | | | | | | |
| Enteritidis | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 |
| Typhimurium | 100 | 100 | 83.3 | 83.3 | 83.3 | 83.3 | 83.3 | 100 |
| Infantis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Kentucky | 0 | 33.3 | 0 | 0 | 0 | 0 | 0 | 66.7 |
| Montevideo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anatum | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| Hadar | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| Telaviv | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 100 |
| | **233** | **236** | **239** | **242** | **245** | **248** | **251** | **254** |
| Host of isolation | S1- 163 | S1-063 | S1-248 | S1-001 | S1-001 | S1-001 | S1-001 | S1-001 |
| Source of isolation | WW | WW | WW | C.F | C.F | C.F | P.F | P.F |
| | **Susceptibility (%)** | | | | | | | |
| Enteritidis | 100 | 100 | 100 | 80 | 80 | 80 | 80 | 80 |
| Typhimurium | 83.3 | 83.3 | 100 | 83.3 | 83.3 | 83.3 | 83.3 | 83.3 |
| Infantis | 0 | 33.3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Kentucky | 66.7 | 100 | 66.7 | 0 | 0 | 0 | 0 | 0 |
| Montevideo | 0 | 66.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anatum | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 |
| Hadar | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 |
| Telaviv | 100 | 100 | 100 | 0 | 50 | 50 | 50 | 0 |

Table 4.3 (continued)

| | **260** | **263** | **266** | **269** | **272** | **275** | **278** |
|---|---|---|---|---|---|---|---|
| Host of isolation | S1-001 | S1-001 | S1-001 | S1-001 | S1-001 | S1-001 | S1-001 |
| Source of isolation | P.F | C.F | C.F | C.F | C.F | C.F | C.F |
| | | | **Susceptibility (%)** | | | | |
| Enteritidis | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Typhimurium | 100 | 83.3 | 83.3 | 83.3 | 100 | 100 | 100 |
| Infantis | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Kentucky | 33.3 | 0 | 0 | 0 | 0 | 33.3 | 0 |
| Montevideo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anatum | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hadar | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Telaviv | 100 | 0 | 0 | 0 | 100 | 100 | 100 |

C.F: Cattle Farm, P.F: Poultry Farm, WW: Wastewater

## 4.4 Single Step Growth Curves

Latent period and burst size of each bacteriophage were determined from the single step growth curves. In conducted experiments, isolated bacteriophages and their target serovars were used to construct growth curves. Each bacteriophage was tested with the corresponding target strains. Bacteriophage P1-236 was excluded from the experiments because of the presence of lysogenic phages. Since clear plaques become uncountable as the lysogenic phages present on agar, the results are unreliable.

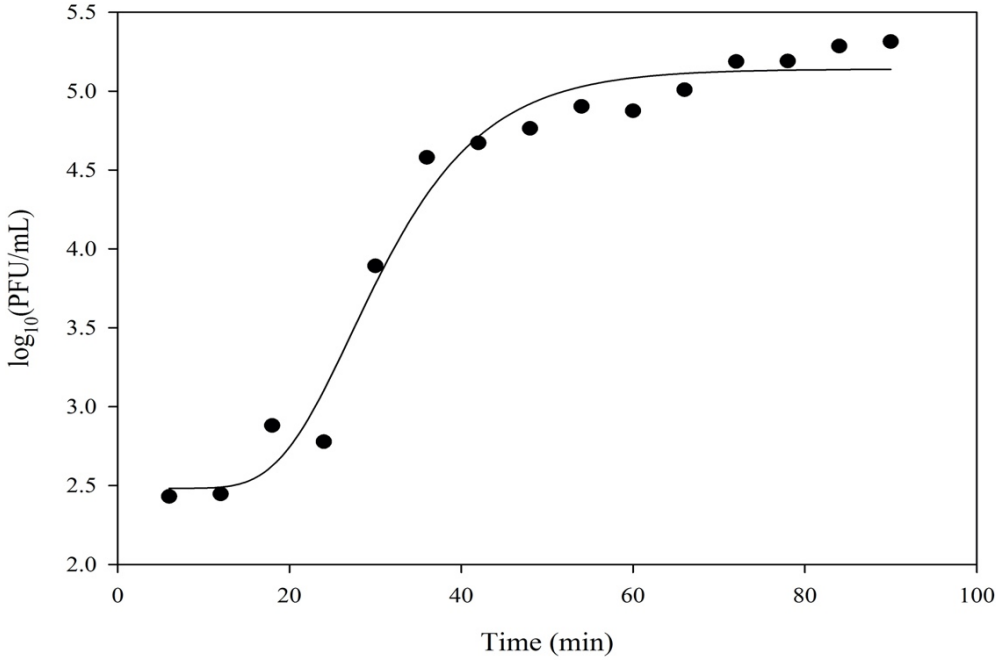



**Figure 4.5** Single step growth curve of P1-224 with its host S1-001

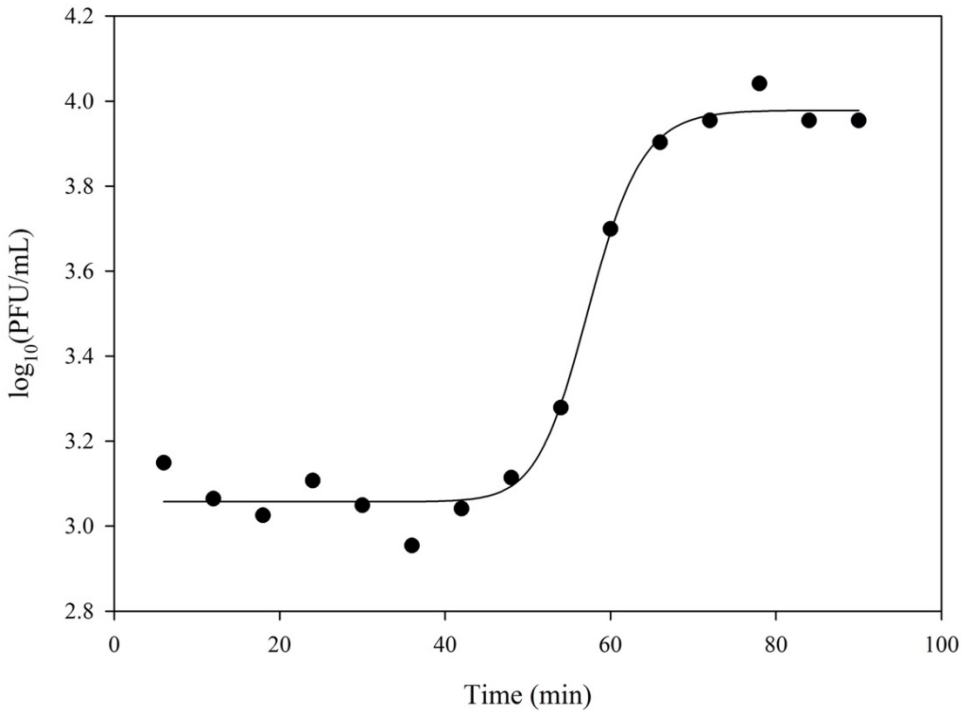



**Figure 4.6** Single step growth curve of P1-230 with its host S1-007

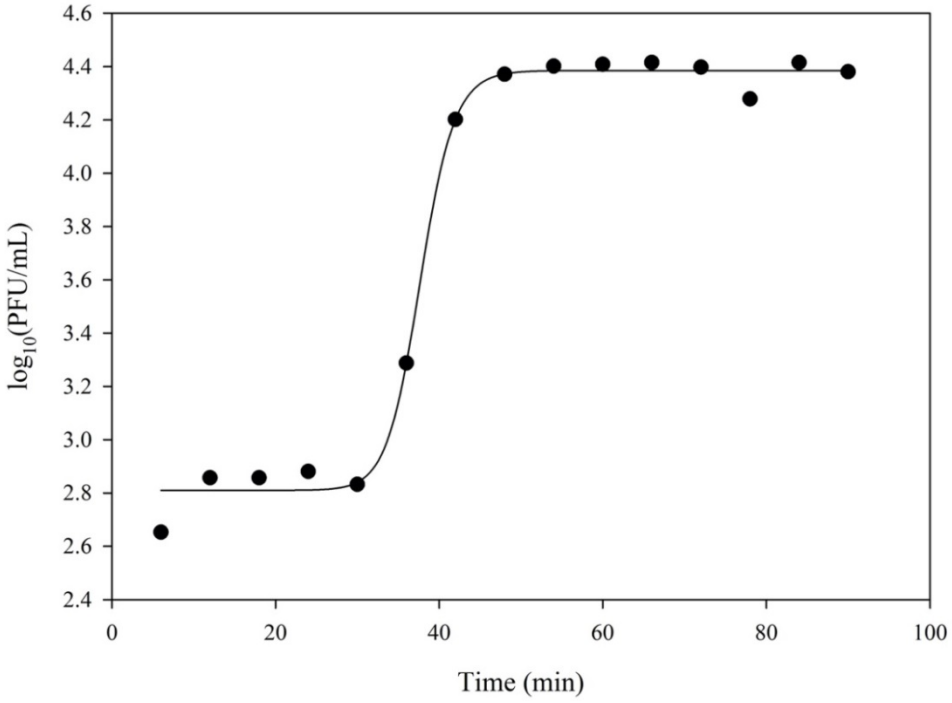



**Figure 4.7** Single step growth curve of P1-233 with its host S1-163

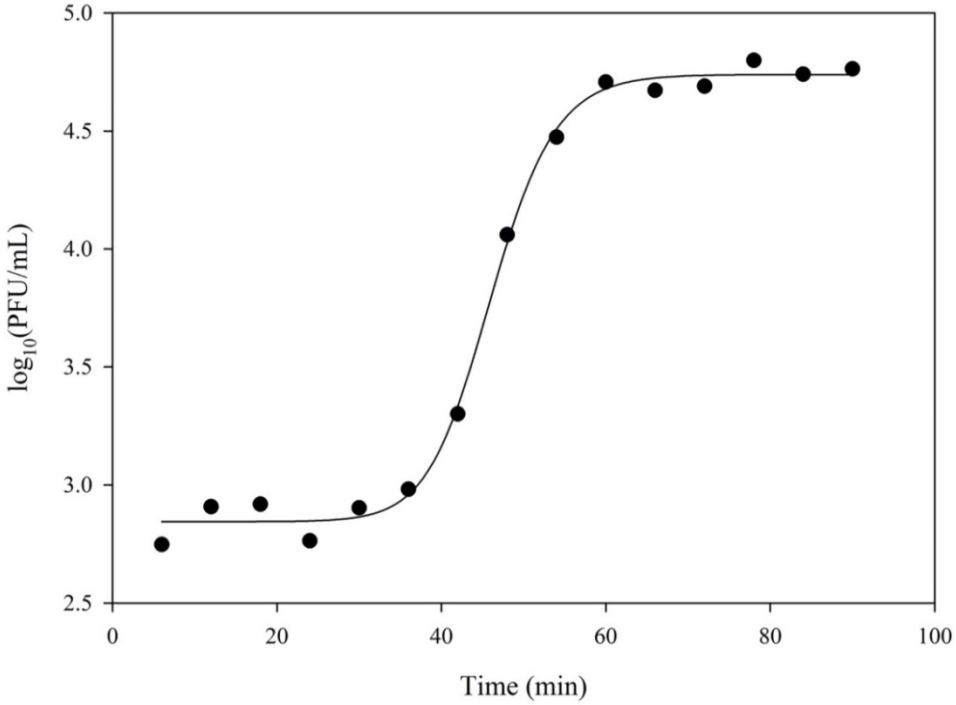



**Figure 4.8** Single step growth curve of P1-239 with its host S1-248

Latent periods and burst sizes showed variation among bacteriophages. Phage P1-224 demonstrated a latency period of 15 min and its burst size was calculated as 236 PFU/cell. Latent period of P1-230 was found as 42 min and burst size was 8 PFU/cell. Another phage P1-233 had a latent time of 40 min and burst size of 35 PFU/cell. Lastly, latent period of P1-239 was found to be 30 min with 76 PFU/cell burst size.

**Table 4.4** Burst size and latent period of the bacteriophages

| **Phage ID** | **Burst Size (PFU/cell)** | **Latent Period (min)** |
|---|---|---|
| MET P1-224 | 236 | 15 |
| MET P1-230 | 8 | 42 |
| MET P1-233 | 35 | 40 |
| MET P1-239 | 76 | 30 |

As it is clearly seen that all bacteriophages exhibited different characteristics in terms of latent period and burst size. Also, there are many studies that had been focusing on these characteristics of phages. For example, Park et al. reported that a phage that can lyse *S.* Typhimurium and *E. coli* O157:H7 had a latent period as 25 min in *S.* Typhimurium and its burst size was above 200 PFU/cell. The authors emphasized the lytic activity of that phage due to its shorter latent period and high burst size among other phages belonging to *Myoviridae* family (Park et al., 2012). Because it is known that phage latent period is associated with the burst sizes. Abedon et al. stated that longer latent periods are linked with greater burst sizes but at the same time shorter latent period means shorter times are required for phages to generate (Abedon et al., 2001). Wang et al. also explains that longer latent periods are resulting in larger burst sizes because number of released progenies will increase as more time is taken. On the other hand, with shorter latent periods, less progeny will be released but this will assist the phages to undergo more life cycles. Thus, it is suggested that intermediate time can be optimum for latency (Wang,

2006). On the other hand, bacteriophages with shorter latent period and larger burst sizes are defined to be the prominent ones in phage applications suggesting that they work with high efficiency (Li et al., 2021; Petsong et al., 2019). Petsong et al. reported that *S.* Enteritidis phages that they isolated had a 97.7 PFU/cell burst size and *S.* Typhimurium had 173.7 PFU/cell with short latent periods (Petsong et al., 2019). *Salmonella* phage isolated by Li et al. had a burst size of 163 PFU/cell with 10 min of latent period and the authors bring out its speed to kill host microorganisms (Li et al., 2021). In the line with these findings P1-224 exhibited a high burst size and short latent period which makes it a strong candidate to take part in pathogen control practices even though there are many other parameters to be evaluated. Besides, this bacteriophage distinctly from other phages go through a second life cycle during growth curve experiments which may be related to the short latent period.

A *Salmonella* bacteriophage isolated in a study by Zhang et al. had a burst size of 34 PFU/cell with 20 min of latent period and authors stated that this phage can be convenient as biocontrol agent due to the short latent time indicating that its lytic activity may be high (Zhang et al., 2021). P1-233 isolated in this study could be also suitable for pathogen control. Its burst size is favorable, but its latent period is longer compared to the phages reported in literature. For example, Bao et al. their phages against *S.* Pullorum had a burst size of 77.5-86 PFU/cell with a less than 20 min of latent periods and they described these burst sizes as large. They also emphasized this condition, large burst size with short latent period, is the required combination for efficient release of new phages which is a result of fast replication (Bao et al., 2011). With these findings one can say that there is a significant difference in the latent period of P1-233 and other phages reported, P1-233 might not be desirable for therapeutic purposes. However, this long latent period may still be beneficial for a phage. It does not necessarily mean that, delay in the lysis occurred due to the lack of resources in the host. Thereby, phage in the host cell may reproduce more as it takes more time resulting in release of more progenies into the environment (Wang, 2006).

Nevertheless, bacteriophages with shorter latent time and larger burst size are identified as favorable ones in applications. Thus, P1-239 isolated in this study becomes prominent in terms of growth characteristics due to its intermediate latent period and larger burst size. P1-230 showed a very small burst size with long latent period among other bacteriophages which makes it not a strong option for phage applications.

These differences between bacteriophages may be due to their holin-endolysin systems because lysis timing is managed by holins and lots of parameters are dependent on holin activity (Young, 2002). As the holin functions, endolysins damage the membrane of host cell resulting in lysis (Wang, 2006). Therefore, diversities in holins may be one of the reasons for distinct latent periods among bacteriophages.

In addition to those, host density in the environment is another parameter for the display of phage characteristics and it may be crucial for phage selection for application.

Abedon et al. stated that latent period may vary according to the host cell numbers due to the abundance of susceptible bacteria (Abedon et al., 2003). In the presence of high density of host, phages may find cells rapidly resulting in relatively shorter latent periods. When bacteria density is less, phages will have to take its time to find bacteria that they infect so bacteria will be more precious for phages. Then they want to benefit from the bacteria as much as they can which means more progeny is produced. In that case, burst size is increased although the period for lysis is increased. Therefore, at lower host densities phages with longer latent periods may be preferable whereas at high densities, short latent period work better (Abedon et al., 2003).

All these factors and parameters are contributing to the phages' applicability as biocontrol agents. As far as the bacteriophages meet the desired characteristics,

they can be involved in phage-based practices. Still, further investigations like whole genome sequencing are required for determining other characteristics.

## 4.5 Bacteriophage Genome Size Determination

Some of the isolated *Salmonella* bacteriophages' genome sizes were determined by PFGE. Also, their relations were assessed by cluster analysis. Other *Salmonella* phages present in METU Food Safety Laboratory database were also included in cluster analysis. This allowed to extend the list and provided a wider data to compare. All gel images were evaluated by BioNumerics, and phages' genome sizes were determined. Figure 4.9 shows the PFGE gel image of some isolated bacteriophages.

In total, 24 *Salmonella* bacteriophages (7 from this study, 17 from METU Food Safety Lab. database) were analyzed by PFGE and dendogram was created based on their genome size. However, since cluster analysis was performed on the basis of a single band, provided data is insufficient to make a conclusion about the genetic relations of bacteriophages.

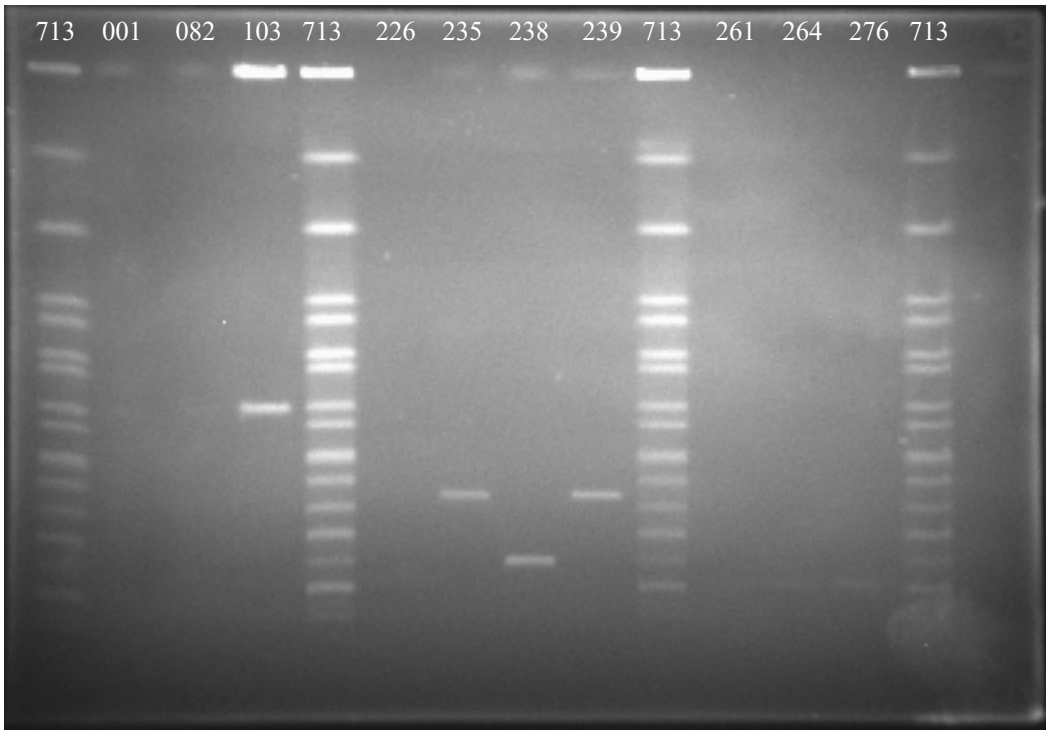
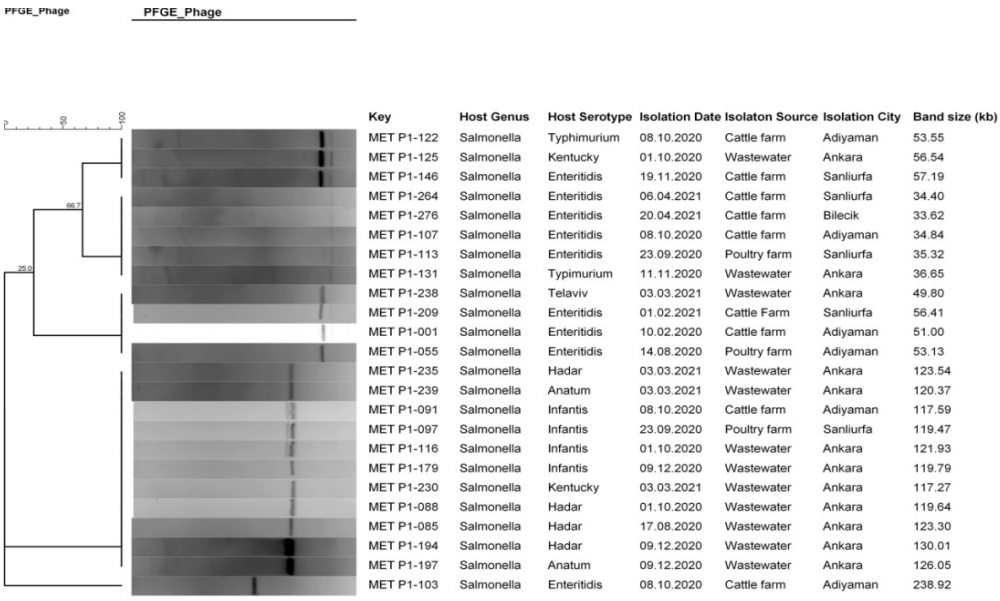



**Figure 4.9** PFGE gel image. 713: *S.* Braenderup as reference serovar.




| Key | Host Genus | Host Serotype | Isolation Date | Isolaton Source | Isolation City | Band size (kb) |
|---|---|---|---|---|---|---|
| MET P1-122 | Salmonella | Typhimurium | 08.10.2020 | Cattle farm | Adiyaman | 53.55 |
| MET P1-125 | Salmonella | Kentucky | 01.10.2020 | Wastewater | Ankara | 56.54 |
| MET P1-146 | Salmonella | Enteritidis | 19.11.2020 | Cattle farm | Sanliurfa | 57.19 |
| MET P1-264 | Salmonella | Enteritidis | 06.04.2021 | Cattle farm | Sanliurfa | 34.40 |
| MET P1-276 | Salmonella | Enteritidis | 20.04.2021 | Cattle farm | Bilecik | 33.62 |
| MET P1-107 | Salmonella | Enteritidis | 08.10.2020 | Cattle farm | Adiyaman | 34.84 |
| MET P1-113 | Salmonella | Enteritidis | 23.09.2020 | Poultry farm | Sanliurfa | 35.32 |
| MET P1-131 | Salmonella | Typimurium | 11.11.2020 | Wastewater | Ankara | 36.65 |
| MET P1-238 | Salmonella | Telaviv | 03.03.2021 | Wastewater | Ankara | 49.80 |
| MET P1-209 | Salmonella | Enteritidis | 01.02.2021 | Cattle Farm | Sanliurfa | 56.41 |
| MET P1-001 | Salmonella | Enteritidis | 10.02.2020 | Cattle farm | Adiyaman | 51.00 |
| MET P1-055 | Salmonella | Enteritidis | 14.08.2020 | Poultry farm | Adiyaman | 53.13 |
| MET P1-235 | Salmonella | Hadar | 03.03.2021 | Wastewater | Ankara | 123.54 |
| MET P1-239 | Salmonella | Anatum | 03.03.2021 | Wastewater | Ankara | 120.37 |
| MET P1-091 | Salmonella | Infantis | 08.10.2020 | Cattle farm | Adiyaman | 117.59 |
| MET P1-097 | Salmonella | Infantis | 23.09.2020 | Poultry farm | Sanliurfa | 119.47 |
| MET P1-116 | Salmonella | Infantis | 01.10.2020 | Wastewater | Ankara | 121.93 |
| MET P1-179 | Salmonella | Infantis | 09.12.2020 | Wastewater | Ankara | 119.79 |
| MET P1-230 | Salmonella | Kentucky | 03.03.2021 | Wastewater | Ankara | 117.27 |
| MET P1-088 | Salmonella | Hadar | 01.10.2020 | Wastewater | Ankara | 119.64 |
| MET P1-085 | Salmonella | Hadar | 17.08.2020 | Wastewater | Ankara | 123.30 |
| MET P1-194 | Salmonella | Hadar | 09.12.2020 | Wastewater | Ankara | 130.01 |
| MET P1-197 | Salmonella | Anatum | 09.12.2020 | Wastewater | Ankara | 126.05 |
| MET P1-103 | Salmonella | Enteritidis | 08.10.2020 | Cattle farm | Adiyaman | 238.92 |

**Figure 4.10** Cluster Anlaysis of *Salmonella* bacteriophages by Bionumerics. Phages P1-206, P1-209, P1-230, P1-233, P1-235, P1-238 and P1-276 belong to this study.

Genome size of the bacteriophages were assessed by PFGE in this study, for that purpose bacteriophages isolated from different serovars of *Salmonella* were primarily used. Gao et al. evaluated the genome sizes of 142 bacteriophages which are infecting *Salmonella enterica subsp. enterica* and they reported that there was an extensive variation in their genome sizes (6.4 to 358.7 kb) where the majority have 30-50 kb genomes (Gao et al., 2020). Genome sizes of phages isolated in this study were in the range of 33-124 kb. Genome sizes of 7 bacteriophages out of 25 were able to be found. 18 phages whose genome sizes could not be determined were targeting *S.* Enteritidis. This may be due to the smaller sized genomes that they possess. For further analysis, PFGE conditions can be changed such as electrophoresis time can be increased, or different ladder can be used. For the analyzed phages, although in general the results showed that phages using same serovar as host have similar band sizes, still there are variations in the band sizes of bacteriophages isolated by using same strains. Phages isolated in this study were compared with the ones that are already found in METU phage database. In total, 9 *S.* Enteritidis phages were compared and 5 of them had the same genome size as each other, other 3 were clustered together meaning that their genome sizes were found to be similar. On the other hand, MET P1-103 which is isolated against *S.* Enteritidis had the largest genome size ~238 kb among all other analyzed phages. The reason for these diversifications seen among phages infecting the same serovars might be due to this: even though it is considered that only one type of bacteriophage was present in obtained phage suspension, in fact there can be different types of phages in that phage suspension. Again, from the Figure 4.10 it can be clearly seen that 2 of the *S.* Kentucky phages' genome sizes are different from each other and they are found in different clusters. At the same time, all *S.* Hadar bacteriophages fall within the same cluster having the band size ~ 120 kb. Additionally, *S.* Anatum phages are in the same cluster with ~ 120 kb band size.

No relation was found between the produced band size and isolation source of bacteriophages.

**Table 4.5** Band sizes of bacteriophages

| **METU ID** | **Host serotype** | **Isolation source** | **Band size (kb)** |
|---|---|---|---|
| MET P1-209 | *S*. Enteritidis | Cattle farm | 56.41 |
| MET P1-230 | *S*. Kentucky | Wastewater | 117.27 |
| MET P1-235 | *S*. Hadar | Wastewater | 123.54 |
| MET P1-238 | *S.* Telaviv | Wastewater | 49.80 |
| MET P1-239 | *S*. Anatum | Wastewater | 120.37 |
| MET P1-264 | *S*. Enteritidis | Cattle farm | 34.40 |
| MET P1-276 | *S*. Enteritidis | Cattle farm | 33.62 |

As a result, it is important to remember that cluster analysis was only based on a single band, and it is not definitive in terms of phage genomic relations. Further analyses are required for the identification of phage similarities.

# CHAPTER 5

# CONCLUSION

The ability of *Salmonella* to persist in different types of foods and environment has been a serious concern worldwide. The actions taken to control *Salmonella* play a crucial role in terms of food safety and there are several strategies to fight against it. Antibiotics are employed in various steps of food production in order to eliminate *Salmonella*. Infections are treated with antibiotics, as well. However, misuse of these substances results in emergence and spread of antibiotic resistance. There has always been a seeking for developing methods without any side-effects to mitigate *Salmonella* from foods and food production areas. Bacteriophages are the promising biological entities in that regard, and they can be harbored in applications to control *Salmonella*. In order to use bacteriophages in foods as biocontrol agents, there are some requirements to be fulfilled. Investigation of the traits of bacteriophages poses significant importance. Since bacterial clustering differs from region to region, prevalence of dominant *Salmonella* serotypes in distinct regions also shows variation. Isolating and characterizing bacteriophages in Turkey provides information about the frequency and distribution of bacteriophages in this area.

In this study, *Salmonella* bacteriophages (n=25) and *Salmonella* strains from various regions in Turkey were isolated. Isolation sources included cattle-poultry farms and wastewater. Isolated *Salmonella* serotypes were determined as *S.* Enteritidis and *S.* Typhimurium by cluster analysis. Also, *S.* Enteritidis was the indicator serovar for most of the bacteriophages (n=21). In other words, 21 bacteriophages were able to be isolated by using *S.* Enteritidis. Remaining bacteriophages (n=4) are against *S.* Hadar, *S.* Anatum, *S.* Kentucky, *S.* Telaviv. Bacteriophages obtained from wastewater were able to infect more *Salmonella*

serovars than those obtained from farms.

Isolated bacteriophages' titers were determined and all of them were used in host range determination. 36 different isolates related to foodborne illnesses were selected. While selecting those isolates, their prevalence in Turkey was taken into account by considering their isolation sources. This allows to see if bacteriophages displayed any differences on infecting the same serovars isolated from variety of sources. Components of phage cocktails for phage applications will be determined based on this information provided by host range experiments. According to the results, bacteriophage which displayed the broadest host range was isolated from wastewater and its indicator serovar was *S.* Telaviv. It was partially or totally infective on 28 serovars. However, it cannot be defined as promising in phage applications due to the presence of lysogenic phages in the phage lysate. Besides, the source of other phages which are infective on more serovars were wastewater again.

Another investigated parameter was the latent period and burst size of the isolated phages and these characteristics were obtained from single step growth curves of phages. Phages exhibited different latent periods and burst sizes. Phage named P1-224 was found to have the shortest latent period as 15 min with largest burst size as 236 PFU/cell among others. Genomic sizes of bacteriophages were also evaluated and dendogram was created based on their genome size. In total, 5 clusters were seen in phage dendogram.

This study forms a basis for the recognition of bacteriophages isolated in Turkey and helps undertstand some of their characteristics to prepare effective phage cocktails against frequently encountered *Salmonella* strains in Turkey.

Still, further investigations are required to understand phage-host interactions, virulent characteristics of phages and their behaviour in different environment for their applicability in food safety. Their characteristics must be well defined to avoid complications and achieve successful phage-based applications.

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# APPENDICES

## A. PFGE GEL IMAGES

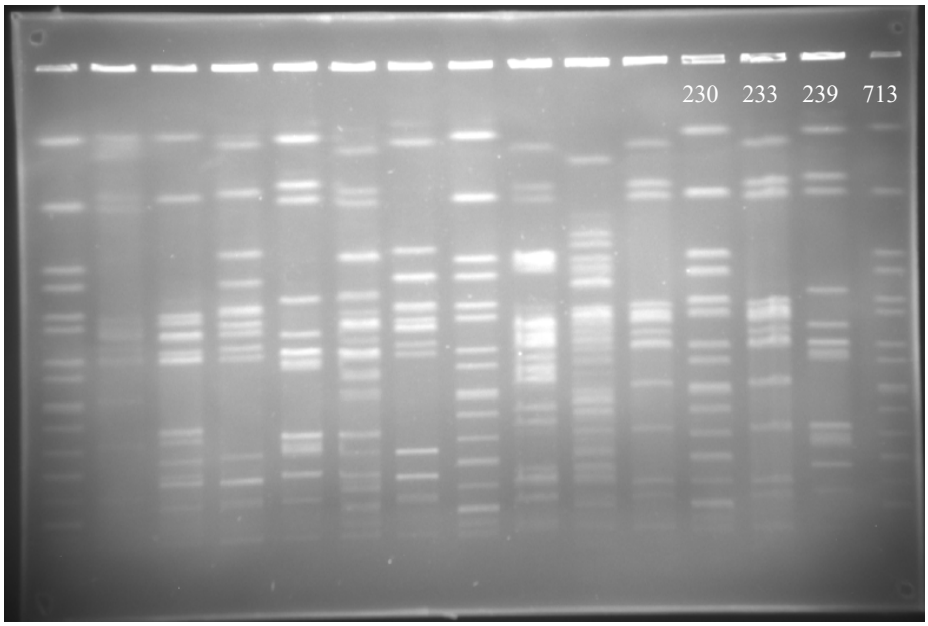


**Figure A.1** PFGE gel image of MET A2-230, 233, 239 (MET S1-713 represents the reference)

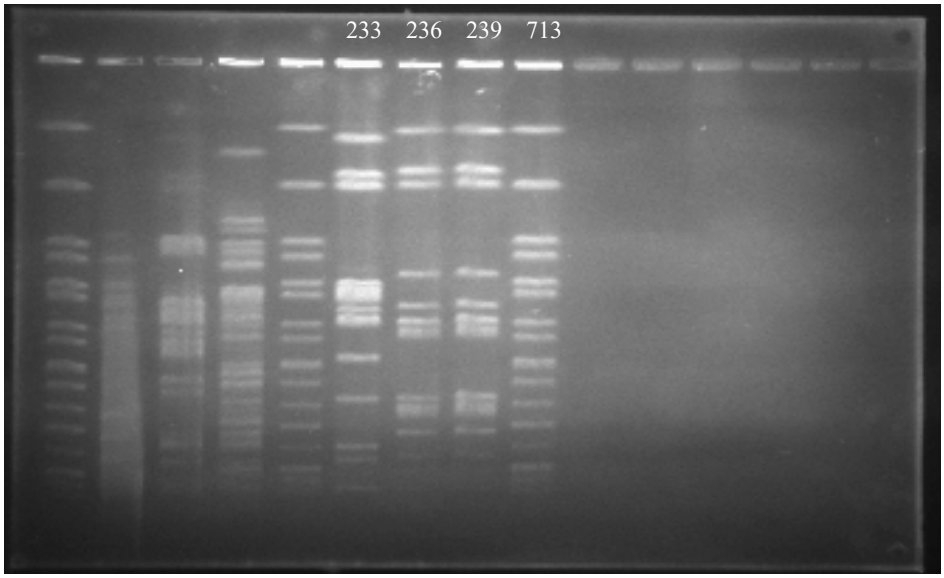


**Figure A.2** PFGE gel image of MET A2-233,236,239 (MET S1-713 represents the reference)

## B. PREPARATION OF MEDIA

**Table B.1** Brain Heart Infusion (BHI) Broth

| | |
|---|---|
| BHI Medium | 22.2 g |
| $dH_2O$ | 600 mL |

**Table B.2** Brain Heart Infusion (BHI) Agar

| | |
|---|---|
| BHI Medium | 18.5 g |
| Agar Bacteriological | 7.5 g |
| $dH_2O$ | 500 mL |

**Table B.3** Soft (0.6%) Luria-Bertani (LB) Agar

| | |
|---|---|
| LB Medium | 5 g |
| Agar Bacteriological | 1.5 g |
| $dH_2O$ | 250 mL |

**Table B.4** Luria-Bertani (LB) Agar

| | |
|---|---|
| LB Medium | 10 g |
| Agar Bacteriological | 7.5 g |
| $dH_2O$ | 500 mL |

**Table B.5** Luria-Bertani (LB) Broth

| | |
|---|---|
| LB Medium | 1 g |
| $dH_2O$ | 50 mL |

**Table B.6** Double Strength Tryptic Soy Broth (2xTSB)

| | |
|---|---|
| TSB Medium | |
| $dH_2O$ | 300 mL |

**Table B.7** Rappaport Vassiliadis Soya Peptone (RVS) Broth

| | |
|---|---|
| RVS Medium | 8.5 g |
| $dH_2O$ | 200 mL |

**Table B.8** Xylose Lysine Deoxycholate (XLD) Agar

| | |
|---|---|
| XLD Medium | 27.6 g |
| $dH_2O$ | 500 mL |

**Table B.9** Buffered Peptone Water (BPW)

| | |
|---|---|
| BPW Medium | |
| $dH_2O$ | 1 L |

**Table B.10** 0.85% NaCl Solution

| | |
|---|---|
| NaCl | 2.55 g |
| $dH_2O$ | 300 mL |

**Table B.11** Saline Magnesium (SM) Buffer

| | |
|---|---|
| $dH_2O$ | 800 mL |
| $MgSO_4.7H_2O$ | 2 g |
| NaCl | 5.8 g |
| Gelatin | 2% |
| 1M Tris-HCl pH:7.5 | 50 mL |
| Volume is adjusted to 1 L by adding sterile $dH_2O$ | |

**Table B.12** 0.5M EDTA-Stock Solution, pH: 8

| | |
|---|---|
| EDTA | 93.05 g |
| $dH_2O$ | 450 mL |
| pH is adjusted to 8 by 10M NaOH, mixture is diluted to 500 mL by $dH_2O$ | |

**Table B.13** 10x Tris-Borate EDTA Stock (TBE) Solution

| | |
|---|---|
| Tris | 54 g |
| Boric Acid | 27.5 g |
| EDTA | 4.65 g |
| $dH_2O$ | 500 mL |

**Table B.14** 1M Tris Stock Solution, pH:8

| | |
|---|---|
| Tris-HCl | 78.8 g |
| $dH_2O$ | 450 mL |
| pH is adjusted to 8 by 10M NaOH, mixture is diluted to 500 mL by $dH_2O$ | |

**Table B.15** 1M Tris Stock Solution, pH:8

| | |
|---|---|
| Tris-HCl | 78.8 g |
| $dH_2O$ | 450 mL |
| pH is adjusted to 8 by 10M NaOH, mixture is diluted to 500 mL by $dH_2O$ | |

**Table B.16** Cell Suspension Buffer (CSB)

| | |
|---|---|
| 1M Tris-HCl, pH: 8 | 20 mL |
| 0.5M EDTA, pH:8 | 40 mL |
| $dH_2O$ | 140 mL |

**Table B.17** Cell Lysis Buffer (CLB)

| | |
|---|---|
| 1M Tris-HCl, pH: 8 | 25 mL |
| 0.5M EDTA, pH:8 | 50 mL |
| N-Lauryl Sarcosine Sodium Salt | 5 g |
| $dH_2O$ | 400 mL |
| Mixture is first heated to 60 C while mixing and 25 mL sterile $dH_2O$ is added. | |

**Table B.18** Tris-EDTA (TE) Buffer, pH:8

| | |
|---|---|
| 1M Tris-HCl, pH: 8 | 5 mL |
| 0.5M EDTA, pH:8 | 1 mL |
| $dH_2O$ | 450 mL |
| pH is adjusted to 8 by 10M NaOH solution. Then diluted to 500 mL by adding $dH_2O$ | |

**Table B.19** 20 mg/mL Proteinase K (ProK) Stock Solution

| | |
|---|---|
| Pro K | 0.01 g |
| $dH_2O$ | 500 μL |

**Table B.20** 20% Sodium Dodecyl Sulfate (SDS) Solution

| | |
|---|---|
| SDS | 10 g |
| $dH_2O$ | 500 mL |

**Table B.21** 1% SeaKem Gold (SKG) Agarose: 1% SDS

| | |
|---|---|
| SKG | 0.15 g |
| TE Buffer, pH:8 | 14.1 mL |
| Mix is microwaved and cooled at 55°C for 10 min, pre-warmed 20% SDS solution is added as 750 μL to agarose. | |

**Table B.22** CLB/Pro K Solution

| | |
|---|---|
| CLB | 5 mL |
| 20 mg/mL Pro K | 25 μL |

**Table B.23** H Buffer Solution

| | |
|---|---|
| $ddH_2O$ | 180 μL |
| H Buffer | 20 μL |

**Table B.24** Xba1 Enzyme Solution

| | |
|---|---|
| $ddH_2O$ | 175 μL |
| H Buffer | 20 μL |
| Xba1 enzyme | 5 μL |

**Table B.25** SKG Agarose

| | |
|---|---|
| SKG | 1.5 g |
| 10x TBE | 7.5 mL |
| $ddH_2O$ | 142.5 mL |

**Table B.26** Running Buffer

| | |
|---|---|
| 10x TBE | 110 mL |
| $ddH_2O$ | 2090 mL |

**Table B.27** 10 mg/mL Thiourea Solution

| | |
|---|---|
| Thiourea | 0.5 g |
| Sterile $ddH_2O$ | 50 mL |

## C. CHEMICALS AND MATERIALS

**Table C.1** The list of chemicals and materials with their suppliers

| **Chemicals** | **Producers** |
|---|---|
| American Bacteriological Agar | Condalab (Madrid, Spain) |
| Luria Bertani (LB) Broth | Condalab (Madrid, Spain) |
| Buffered Peptone Water | Merck (Darmstadt, Germany) |
| Xylose-Lysin-Desoxycholat (XLD) Agar | Merck (Darmstadt, Germany) |
| Rappaport-Vassiliadis *Salmonella* Enrichment Broth | Merck (Darmstadt, Germany) |
| Brain Heart Infusion Broth | Merck (Darmstadt, Germany) |
| Sodium chloride | Merck (Darmstadt, Germany) |
| Magnesium sulfate hexahydrate ($MgSO_4*6H_2O$) | Merck (Darmstadt, Germany) |
| SeaKem Gold Agarose | Lonza (USA) |
| Boric Acid | Sigma-Aldrich (St. Lois, MO, USA) |
| Pro K | Roche |
| Xba1 | Roche |
| H buffer | Roche |
| DirectLoad PCR 100 bp Low Ladder | Sigma-Aldrich (St. Lois, MO, USA) |
| 0.45 μm and 0.22 μm poresize syringe filters | ISOLAB |

## D. IMAGES OF ISOLATED *SALMONELLA* BACTERIOPHAGES

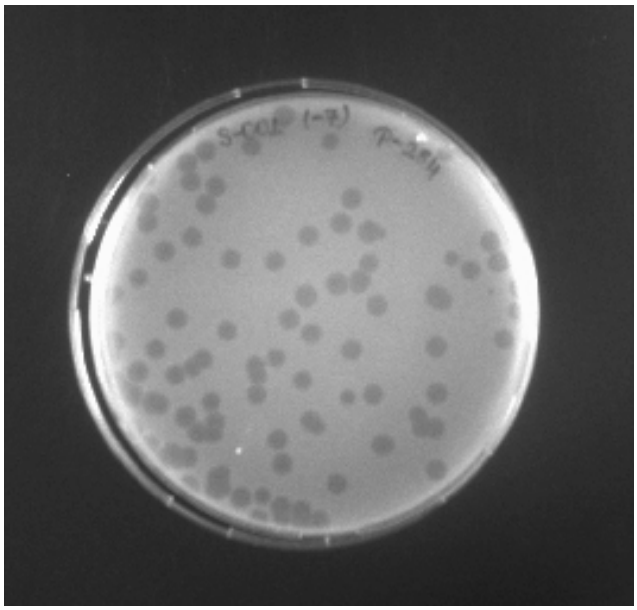

**Figure D.1** MET P1-254 double plaque assay results

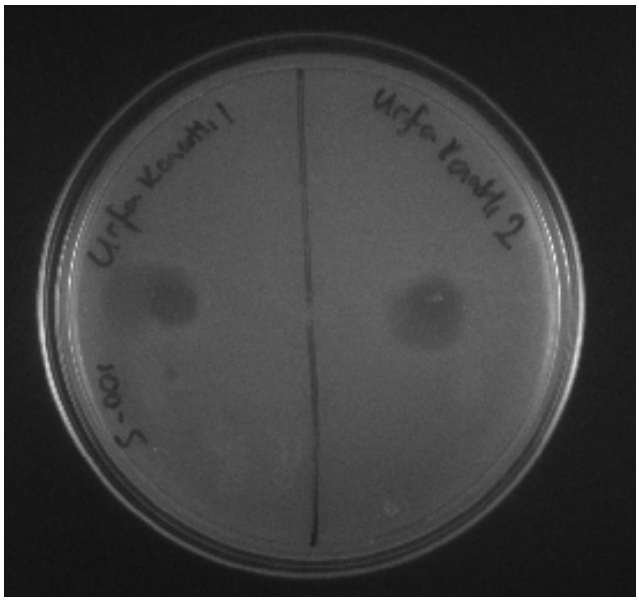


**Figure D.2** Spot test results of MET P1-217 and MET P1-220

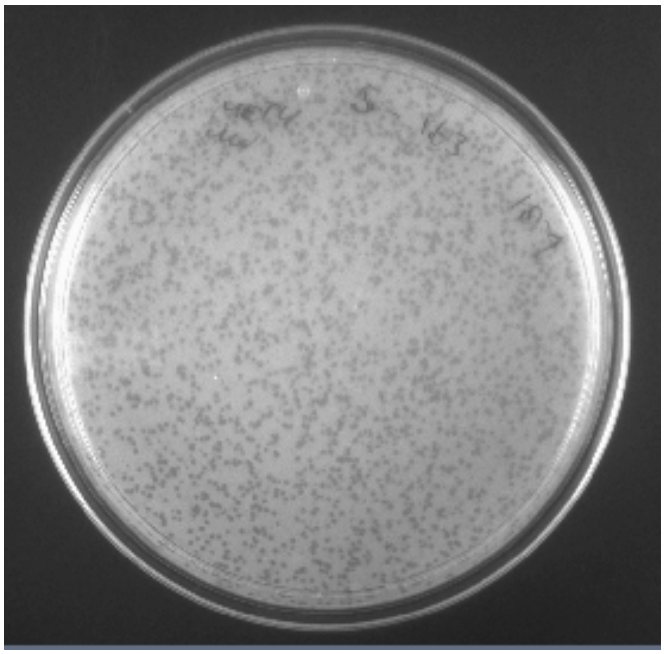

**Figure D.3** Double plaque assay results of MET P1-233

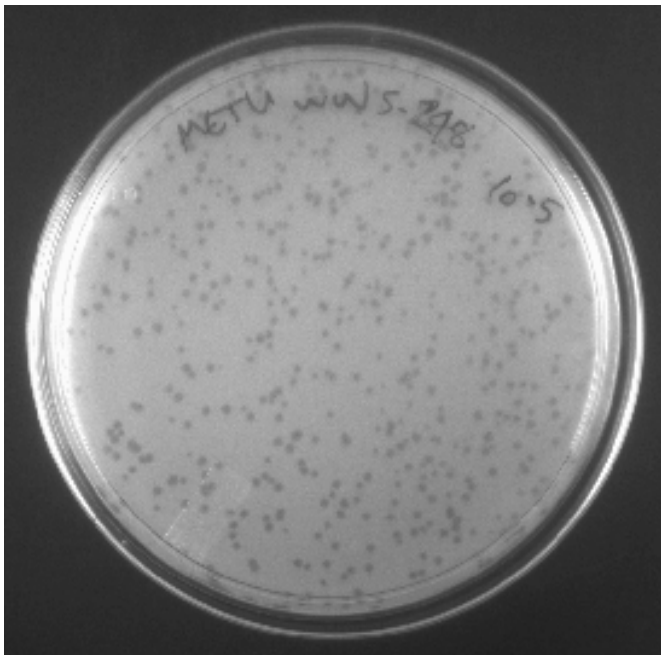


**Figure D.4** Double plaque assay results of MET P1-239

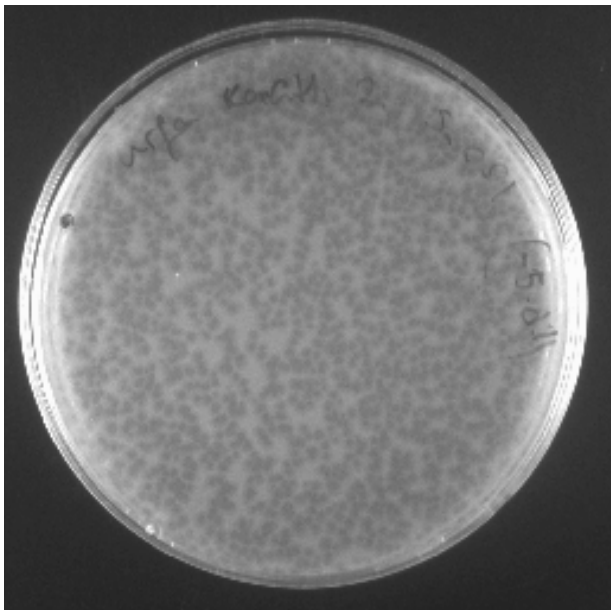

**Figure D.5** Double plaque assay results of MET P1-220

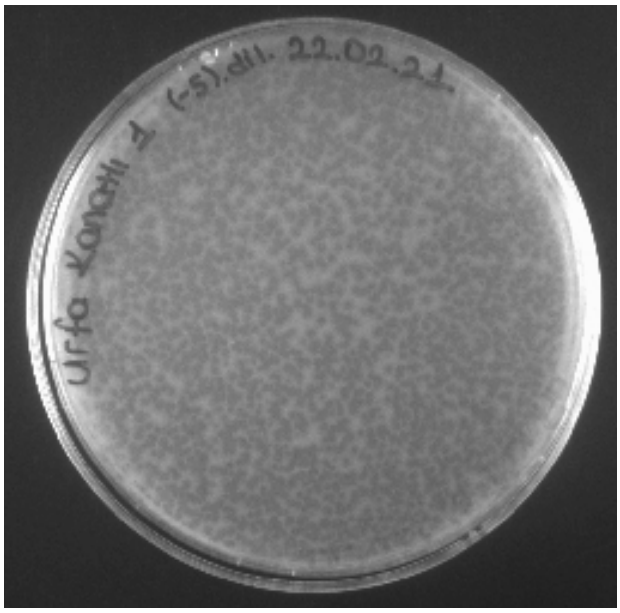


**Figure D.6** Double plaque assay results of MET P1-217

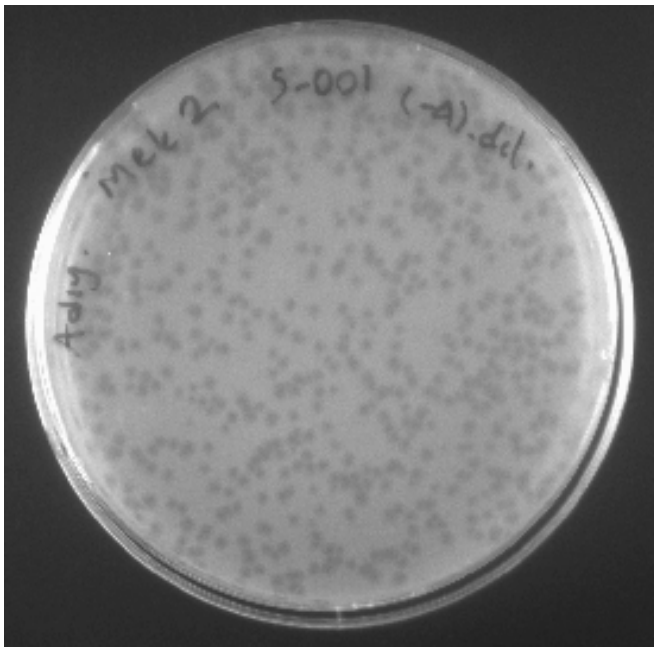


**Figure D.7** Double plaque assay results of MET P1-221

## E. HOST RANGE ANALYSIS RESULTS

**Table E.1** Spreadsheet of Host range determination