ISOLATION OF BACTERIOPHAGES SPECIFIC FOR *Lactobacillus casei* ATCC 393 STRAIN 03 AND *Streptococcus mutans* ATCC 35668 FROM HUMAN SALIVA

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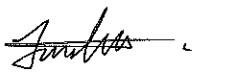
LIM JIA WEN	
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ABSTRACT

Streptococcus mutans and *Lactobacillus casei* are common microflora of the human oral cavity. Bacteriophages are important to ensure controlled microbial population in the oral cavity. Hence, isolation of the bacteriophages specific for *S. mutans* ATCC 35668 and *L. casei* ATCC 393 strain 03 from human saliva samples were done using the Double Agar Overlay Plaque Assay. Ten individuals aged 18-25 years old from INTI International University willingly provided the saliva samples for the isolation of bacteriophages of the mentioned bacteria. The saliva from all the individuals were collected at week 2, week 6 and week 9 of August 2014 session to study the effect of different stress level, diet, sleeping hours and oral hygiene practices to the presence of the bacteriophages. However, no bacteriophage specific for *S. mutans* ATCC 35668 and *L. casei* ATCC 393 strain 03 were isolated from the study.

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LIST OF ABBREVIATIONS

BHI	Brain heart infusion
Ca^{2+}	calcium(II) ion
MRS	de Man, Rogosa, Sharpe
°C	degree Celsius
EPS	exopolysaccharides
GSTB	glucose-sucrose-tellurite-bacitracin
Gtfs	glucosyltransferase
g	gram
IL-1	interleukin 1
IL-6	interleukin 6
L. casei	*Lactobacillus casei*
Mg^{2+}	magnesium(II) ion
µL	microliter
µm	micrometer
mL	milliliter
mM	millimolar
MSB	mitis salivarius with bacitracin
MSKB	mitis salivarius-kanamycin-bacitracin
MRS-V	MRS-vancomycin
NAG	N-acetylglucosamine
NAM	N-acetylmuramic acid
nm	nanometer
pfu	plaque forming unit

P1, P2, P3	plates of replicate 1, plates of replicate 2, plates of replicate 3
rpm	revolutions per minute
CSP-1	salivary protein-1
S. mutans	*Streptococcus mutans*
t	time
×*g*	times gravity
TYS20B	trypticase soy-sucrose-bacitracin
TYCSB	tryptone-yeast-cysteine-sucrose-bacitracin
TNF	tumor necrosis factor
UV	ultraviolet
v/v	volume/volume
w/v	weight/volume

CHAPTER 1

INTRODUCTION

The human oral cavity contains complex microflora population. There are more than 700 different bacterial species that have been found in the oral cavity (Dewhirst et al., 2010). Most of these bacterial species are commensal and the microbial balance is required as overrepresentation of pathogenic species might lead to oral diseases such as periodontal disease and dental caries especially the mutans streptococci and *Lactobacillus* (Belda-Ferre et al., 2012; Marsch, 2010; Metwalli, Khan, Krom, & Jabra-Rizk, 2013). The biological activities of these microflora are affected by the oxygen tension, nutrient availability, pH and carbohydrate sources in the oral cavity (Ahn, Ahn, Browngardt, & Burne, 2009). Apart from that, the microbial activity can be influenced by stress level, sleeping hours, oral hygiene practices and health state of the individual (Doyle & Bartold, 2012).

Streptococcus mutans plays a key role in initiating caries and is also the key factor that causes enamel decay while *Lactobacillus casei* may lead to further development of dental caries (Karpiński & Sckaradkiewicz, 2013). *S. mutans* is a coccibacillus Gram positive mesophile that is able to grow under aerobic and anaerobic conditions. They are also acidophilic whereby they are tolerant to low pH environment (Simon, 2007). *S. mutans* has the ability to metabolise a wide range of carbohydrates through glycolysis in acidic environment coupled with the formation of biofilms on teeth (Ahn et al., 2009; Karpiński, & Sckaradkiewicz, 2013; Metwalli et al., 2013). *L. casei* is a rod-shaped Gram positive bacterium and is able to maintain the microecological balance in the oral cavity and gastrointestinal tract by providing an acidic condition through the fermentation of glucose into lactic acid (Karpiński & Sckaradkiewicz, 2013). This helps in creating an acidic environment and thus preventing the growth of harmful bacteria.

Bacteriophages are viruses that infect the bacteria and are believed to have the potential to alter human bacterial communities (Fischetti, 2006). Bacteriophages produce

lysin that would degrade the peptidoglycan in the cell wall of bacterial cells and this releases the progeny phages to infect more bacteria (Fischetti, 2006). However, there are limited number of studies on the isolation of bacteriophages from the oral cavity with only few successful isolation of bacteriophages from dental plaque or human saliva (Hitch, Pratten, & Taylor, 2004). Sixteen lytic bacteriophages specific for *S. mutans* strains and *Streptococcus sorbinus* had been successfully isolated (Armau, Bousque, Boue, & Tiraby, 1988, as cited in Ploeg, 2009). Three bacteriophages of *S. mutans* were also isolated and partially characterised by Delisle and Rostkowski (1993). The bacteriophages specific for *L. casei* were also successfully isolated by Meyers, Walter and Green (1958). However, the bacteriophages specific for *L. casei* ATCC 393 strain 03 and *S. mutans* ATCC 35668 has not been isolated.

Bacteriophages are very specific to their host bacteria and are believed to be present wherever their host bacteria are present (Pride et al., 2011). Therefore, the presence of a bacteriophage can be used to determine the presence of their host bacterium. Besides, the bacteriophages can also be used to treat the infectious diseases caused by the bacteria where this treatment is known as phage therapy. The specificity of bacteriophages allow phage therapy to be an alternative for treatment of dental caries and is found to be a powerful approach in treating oral pathogenic bacteria such as oral streptococci (Bhardwaj, 2014). In addition, phage therapy may help to overcome the problems of allergy and pathogenic bacteria resistance to the antibiotics as bacteriophages may mutate faster than the bacteria (Bhardwaj, 2014).

Therefore, the objectives of this project were to isolate the bacteriophages specific for *S. mutans* and *L. casei* from the human saliva of subjects aged 18-25 years old using the culture-dependent method and to preliminarily study the effect of stress level, diet, sleeping hours and oral hygiene practices on the presence of the specific bacteriophages in the oral cavity.

CHAPTER 2

LITERATURE REVIEW

2.1 HUMAN ORAL CAVITY

2.1.1 Human Saliva

Recently, researchers start to regard saliva as valuable fluid that is able to provide information about diseases (Kawas, Rahim, & Ferguson, 2012; Cheng, Rees, & Wright, 2014). There are two specific groups of saliva namely gland specific saliva and whole saliva (Kaufman & Lamster, 2002). The first type; gland specific saliva; is secreted from sublingual and submandibular salivary glands through the oral cavity via the Wharton's duct (Kaufman & Lamster, 2002). The accumulation of gland specific saliva is directly from the submandibular gland, parotid gland and sublingual gland as well as several small salivary glands (Kaufman & Lamster, 2002). Whole saliva on the other hand, is a combination of secretions and oral fluid from three pairs of main salivary glands together with many minor salivary glands (Kaufman & Lamster, 2002; Kawas et al., 2012). It also consists of non-adherent bacteria, keratin debris, desquamated oral epithelial cells, blood cells, mucous excretions from nasal cavity and pharynx, food residues and probably signs of medications or even chemical products (Castagnola et al., 2011).

In addition, saliva also contain various important chemical components such as water, organic compounds like non-proteins and lipids, inorganic compounds like ions, hormones, and proteins or polypeptides that help to achieve many biological functions of saliva such as digestion, antibacterial and antiviral activities, lubrication, maintaining normal taste sensation, and facilitating remineralisation of tooth enamel (Castagnola et al., 2011; Cheng et al., 2014). According to Schipper, Silletti and Vingerhoeds (as cited in Holtman, 2012), secretions of saliva ranges from 0.3 to 7.0 mL of saliva per minute with pH range from 6.2 to 7.4 provide optimal growth conditions for many microbes (Holtman, 2012). Saliva also

helps in microbial attachment by providing nutrients and salivary pellicle receptors. Thus, saliva is said to be important in maintaining the oral and tooth health (Haukioja, 2009).

There are various methods used in collection of saliva. However, there are two preferred methods; collection of whole saliva with or without stimulation and collection by swabbing (Holtman, 2012). Collection of whole saliva with stimulation requires a person to chew a paraffin wax for 5 minutes and expectorates into a tube whereas collection of whole saliva without stimulation needs a person to expectorate saliva into a tube or allow saliva to drip off the lower lip without any mechanical stimulation (Holtman, 2012).

The collection of whole saliva is easy and is able to be stored and transported at low cost as no special equipment is required (Kawas et al., 2012). It is also easier to handle as compared to blood plasma because it will not clot. The collection techniques used for whole saliva is non-invasive. This in turn reduces individuals' anxiety and discomfort when repeated samples are needed over a period of time. However, centrifugation or filtration is needed for whole saliva to remove large debris, cellular contaminants, precipitated mucins and some proteins (Kawas et al., 2012).

2.1.2 Oral Ecology

There are more than 700 bacterial species found in human oral cavity. However, the number of species present in an individual oral cavity may be varied between 30 and 80 species (Haukioja, 2009). Several species can be found in most of the oral sites while others can only be found on specific sites (Haukioja, 2009). For example, *Streptococcus mitis* can be found in most of the oral sites whereas *Streptococcus salivarius* were specifically found on tongue dorsum (Aas, Paster, Stokes, Olsen, & Dewhirst, 2005). The oral microbiota of an adult is relatively stable at strain level whereby colonisation of new species is infrequent unless there are changes in the oral cavity like loss of teeth, dental caries, periodontitis, and changes in salivary flow or diet (Haukioja, 2009).

Besides, large populations of viruses may also be found in human oral cavity and they play important roles in maintaining microbial ecology (Ly et al., 2014). Most of these viruses are bacteriophages and were shown to have the ability to trigger host immune responses (Ly et al., 2014). Several studies showed that the oral viruses carry substantial gene functions that are able to affect pathogenic functions of their host bacteria (Ly et al., 2014). However, there are limited number of studies regarding the potential role of virus communities (Ly et al., 2014).

Studies also suggest that prolonged stress may affect oral ecology (Doyle & Bartold, 2012; Malathi & Sabale, 2013). When an individual is under stress, it would lead to the activation of autonomic nervous system to release adrenaline (Malathi & Sabale, 2013). This may increase the blood flow in order to perform vigorous muscle activity (Doyle & Bartold, 2012). Besides, it would also increase blood glucose concentration, cellular metabolism and rate of blood coagulation as well as affecting regulation of immune cell activities (Doyle & Bartold, 2012). The increment in the level of adrenaline due to stressful event may also cause deregulation of immune system and increased susceptibility to diseases (Doyle & Bartold, 2012). Stress hormone may modulate the immune response either indirectly through induction of cytokine such as interferon γ, tumor necrosis factor (TNF), interleukin 1 (IL-1) and interleukin 6 (IL-6); or directly through the binding of hormone to its receptor at the surface of cells (Malathi & Sabale, 2013).

Besides, some studies showed that stressful life events might increase the risk of poor health such as poor sleep patterns, less exercise, poor nutrition, and greater tendency for substance abuse (Doyle & Bartold, 2012). Stressful life events might also lead to poor oral hygiene practices, alteration in food intake, failure in seeking dental care and bruxism (Doyle & Bartold, 2012). Thus, it is possible that the oral ecology might be affected by one's stress level. Studies also show that increase in bacterial plaque accumulation and gingival inflammation in medical students who under academic examination was more common as compared to control group who did not undergo academic examination (Doyle & Bartold, 2012).