

COMPARATIVE GENOMIC AND EVOLUTIONARY
RELATIONSHIP ANALYSIS IN IDENTIFICATION OF
Actinomyces naeslundii PROBIOTIC PROPERTIES-
RELATED PROTEINS

BY

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ABSTRACT

The genus *Actinomyces* encompasses a diversity of species that inhabit in human oral. Accordingly, their roles has come to debate as some claimed to cause actinomycosis, while the others claimed to give benefits, by inhibit the growth of *Streptococcus mutans*, thus prevent the formation of oral caries. The *Actinomyces* genus that are claimed to be beneficial in inhibiting the oral caries is *Actinomyces naeslundii* NCTC 10301. The objective of the study is to determine the probiotic-related proteins from *Actinomyces naeslundii* using bioinformatic tools. Here, genome comparison of *A. naeslundii* with the 26 known-probiotics was analyzed using the Mauve software. From the comparison, probiotic of *Bifidobacterium sp.* were found to have most similarity with *A. naeslundii* compared to the other probiotics. Thus, sequences of 16s rRNA afford a means to establish phylogenetic tree with the *A. naeslundii*, 26 known probiotics and 33 oral pathogens. This yield of tree, using the ClustalW and Mega-X software could provide view of species evolutionary relationship between *A. naeslundii*, probiotics and oral pathogens. The tree shows *A. naeslundii* was clustered together with its own genus as well as with *Bifidobacterium sp.* EMBOSS Needle was used to interpret the percent similarities between 16 potential proteins of *A. naeslundii* with probiotics proteins of probiotic porperties. InterPro and Biocyc.org software were used to interpret the details function of similar proteins found in the genome comparison between *A. naeslundii* and *Bifidobacterium sp.* From the analysis, 9 proteins were shown to have probiotics properties. From all the analysis, it shown that protein chaperonin GroEL in *A. naeslundii* to have the highest percent of similarities and predicted to function as the probiotic properties, in response to wide variety of stimuli including heat and stress. This finding conclude that the role of *A. naeslundii* was to give more benefits to human oral rather than harm.

خلاصة البحث

يتكون جنس الشعية الفطرية (*Actinomyces*) من مجموعة متنوعة التي تعيش في الفم البشري. فقد أصبحت أدوارها محل نقاش حيث ادعى البعض أنها تسبب داء الشعيات ، بينما ادعى البعض الآخر أنها تفيد ، عن طريق تثبيط نمو المكورات العقدية الطافرة ، إذا منع تكوين تسوس الفم. وجنس الشعية الفطرية التي تُزعم أنها مفيد في تثبيط تسوس الفم هو الشعية الفطرية النايسلودنية (*Actinomyces naeslundii* NCTC 10301). الهدف في هذه الدراسة هو تحديد البروتينات المرتبطة بالبروبيوتيك من الشعية الفطرية النايسلودنية (*Actinomyces naeslundii*) باستخدام أدوات المعلومات الحيوية. ومن هنا، مقارنة جينوم الشعية الفطرية النايسلودنية مع 26 البروبيوتيك المعروفة نُحَلَّل باستخدام برنامج موف (Mauve). ومن تحليل المقارنة، وُجِدَتْ بروبيوتيك بيفيدوباكتيريم (*Bifidobacterium* sp.) لديها معظم التشابه مع الشعية الفطرية النايسلودنية وهي أكثر من البروبيوتيك الأخرى . وكذلك تسلسل من الرنا الريباسي 16س (16s) يوفر وسيلة في إنشاء شجرة النشوء والتطور مع الشعية الفطرية النايسلودنية و26 بروبيوتيك معروف و33 من مسببات الأمراض الفموية . ويمكن أن توفر نتيجة من الشجرة، باستخدام برنامجين كلوستال (ClustalW) وعملق-X (Mega-X)، عرضًا للعلاقة التطورية للأنواع بين الشعية الفطرية النايسلودنية والبروبيوتيك ومسببات الأمراض الفمية. وتظهر الشجرة أن الشعية الفطرية النايسلودنية كانت متجمعة مع جنسها وكذلك مع بيفيدوباكتيريم (*Bifidobacterium* sp). اسْتُخْدِمَتْ إبرة نقش بارز (EMBOSS) لتفسير النسبة المئوية للتشابه بين 16 بروتيناً محتملاً من الشعية الفطرية النايسلودنية مع بروتينات البروبيوتيك ذات الخصائص البروبيوتكية . اسْتُخْدِمَ برنامج إنتربرو (InterPro) ومجموعة قاعدة بيانات (Biocyc.org) لتفسير وظيفة تفاصيل البروتينات المماثلة الموجودة في مقارنة الجينوم بين النايسلودنية و بيفيدوباكتيريم. ومن تنفيذ التحليل، تبين أن 9 بروتينات لها الخصائص البروبيوتكية. ومن جميع التحليلات السابقة، هذه تدل على أن البروتين تشافرونين غرول (*chaperonin GroEL*) في النايسلودنية تحتوي على أكثر نسبة من أوجه التشابه وتُنَبَّأ أن يؤدي مثل خصائص بروبيوتكية، في الاستجابة للمحفزات المتنوعة تتضمن أيضا من الحرارة والضغط.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Biobehavioural Health Science

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DECLARATION

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Probiotics emerge a fascinating field in oral disease prevention and therapy. This concept introduces a new horizon on the relationship between diet and oral health. Besides, the use of probiotics to maintain healthy oral cavity is getting more attention (Gupta, 2011). Probiotics are defined by the Food and Agriculture of United Nations (FAO) (2001) as “living microorganisms that when administrated in adequate amounts, they can confer health benefit effects on the host” (Bhushan & Chachra, 2010).

The most common probiotics used in our daily life are *Lactobacillus* and *Bifidobacterium*. They are also known as Lactic Acid Bacteria (LAB). An example of LAB that is usually used and included in milk is *Lactobacillus rhamnosus* GG which has been shown to decrease dental caries in children (Näse *et al.*, 2001). LAB produces antimicrobial components such as lactic acid and hydrogen peroxide, which can inhibit the growth of oral pathogens such as *Streptococcus mutans* (Fang *et al.*, 2018).

In addition, LAB possesses anti-mutagenic effects and anti-carcinogenic properties. The bacteria have also been shown to improve lactose metabolism, reducing serum cholesterol, and stimulating an immune response (Nagendra, 2007). In this study, a list of well-known probiotics genomes was used to identify the *Actinomyces naeslundii* properties that may contribute to probiotic properties through genomic comparison.

The major focusing bacteria in this research is *A. naeslundii*, as it is one of the predominant bacteria in the normal oral cavity. As early as 2 months old, about one-third of infants are already colonized with *Actinomyces* (Könönen & Wade, 2015). As

they colonize, the bacteria have been shown to balance the oral microbiome by inhibiting the overgrowth of *S. mutans*, which may control the formation of oral carries (Arzmi *et al.*, 2016).

In previous study, the interactions between *Candida albicans* and *A. naeslundii* decreased *S. mutans* growth (Arzmi *et al.*, 2015). This inhibition could be due to the presence of specific proteins on the surface of *C. albicans* which interact with *S. mutans*, subsequently promotes the aggregation of *A. naeslundii* (Koo *et al.*, 2010 and Falsetta *et al.*, 2014). It was mentioned that the presence of *A. naeslundii* together with *C. albicans* increases the activity of mitochondrial dehydrogenase and succinate dehydrogenase, thus decreasing the metabolic activity of *S. mutans* in polymicrobial biofilms, consequently inhibit caries formation (Arzmi *et al.*, 2016).

Even though *A. naeslundii* is shown to have a good effect on promoting oral health. However, the bacterium is closely related to *Actinomyces israelii* and *Actinomyces gerencseria*, which have been shown to cause actinomycosis and periodontal disease (Baca *et al.*, 2014). Thus, further studies are needed to identify the specific protein(s) beneficial in maintaining a healthy oral cavity.

1.2 STATEMENT OF THE PROBLEM

Actinomyces species are the residential microbiota of the human oral cavity. However, the exact role of this bacterium is still poorly understood. The previous study stated its benefits in cellular signalling and communication during low pH cycles, which can promote a shift in the virulence of non-mutans *Streptococci* and *Actinomyces* species, thus increase the acid production of biofilms (Takahashi & Nyvad, 2008).

The protection against oxidative stress was also observed in the coaggregation between *A. naeslundii* and *Streptococcus gordonii* (Jakubovics *et al.*, 2008). *A.*

naeslundii has also been correlated with biofilm ecological balance and is frequently associated with a healthy microbiome (Lee and Kim, 2014; Lobo *et al.*, 2019).

A. naeslundii also can increase local pH by producing ammonia and alkali (Nascimento *et al.*, 2009; Liu *et al.*, 2012) and can metabolize lactic acid into weaker acids (Takahashi & Yamada, 1999). However, when biofilms are frequently exposed to acid challenges, this species can express a more pathogenic phenotype and further contribute to the development of dental caries (Takahashi & Nyvad, 2008; Brighenti *et al.*, 2014). It has also been reported to contribute to dental caries and periodontal disease (Duran-Pinedo & Frias-Lopez, 2015).

Dental biofilms produce acids from carbohydrates that result in caries. The microbiome on enamel surfaces contains mainly non-mutans *Streptococci* and *Actinomyces*, in which acidification is mild and infrequent. This is compatible with the equilibrium of the demineralization/remineralization balance of shifts the mineral balance toward net mineral gain. When sugar is supplied frequently, acidification becomes moderate and frequent. This may enhance the acidogenicity and endurance of the non-mutans bacteria. In addition, more aciduric strains, such as 'low-pH' non-mutans *Streptococci*, increase selectively (Takahashi & Nyvad, 2010).

Actinomyces' abilities to adhere on tooth surface, form mixed-species biofilms with other potentially infectious bacterial partners, and to specifically interact with host cell receptors may be important steps in the pathogenesis of certain oral diseases, including root surface caries and gingivitis. Dental caries results from prolonged plaque acidification, leading to establishing a cariogenic microflora and demineralization of the tooth. These microbial acid-induced adaptation and selection processes, over time, may shift the demineralization/ remineralization balance toward net mineral loss,

leading to dental caries (Takahashi & Nyvad, 2010). The role of this species in caries and periodontitis initiation remains unclear (Dame-Teixeira *et al.*, 2016).

Even though many studies have been conducted to identify the properties of this bacterium, however, no study has been conducted to identify the details properties of *A. naeslundii* in terms of genomic, in determining whether the species are more prominent to induce pathogenicity or to promote a healthy oral cavity.

A comparative genomic approach can be very useful to elucidate this property, particularly in identifying molecular differences between species. This will promote further identification of its adaptation, colonization, and survival in the host, which will explain the probiotic properties of this species in the host.

1.3 PURPOSE OF THE STUDY

This study aims to determine *A. naeslundii* protein(s) that may share any similarity to probiotics bacteria in terms of their functions related to probiotic properties. Besides, this study will also determine the survival, adaptation, and colonization of *A. naeslundii* in the host that can contribute to the development of new oral probiotics in the future.

1.4 RESEARCH OBJECTIVES

The study aimed to achieve the following objectives:

1. To examine protein(s) in *A. naeslundii* that may share similarity or homology with other known probiotics, through genome comparisons.
2. To identify the evolutionary relationship between *A. naeslundii*, oral pathogens, and known probiotics by constructing the phylogenetic tree.
3. To investigate protein(s) that promote the survivability and adaptation of *A. naeslundii* in the host, which contribute to probiotic properties in terms of

function and mechanisms. These functions and mechanisms of proteins(s) were predicted using the InterPro and Biocyc.org website.

1.5 RESEARCH QUESTIONS

1. Does *A. naeslundii* show any protein(s) similarity with the known probiotics commonly used in humans daily through genome comparison?
2. How *A. naeslundii*, oral pathogens, and known probiotics are evolutionary related?
3. What are the probiotic properties possessed by *A. naeslundii*?

1.6 RESEARCH HYPOTHESIS

A. naeslundii proteins have similarities in terms of functions and mechanisms with any compared known probiotics, which contribute to probiotics properties, thus promoting human oral health.

1.7 SIGNIFICANCE OF THE STUDY

This study will determine the role of *A. naeslundii* as a pathogenic microorganism or as a probiotic that promotes a healthy oral cavity. It is expected that *A. naeslundii* is a safe and effective potential probiotic that may prevent the formation of dental caries that act against the *S. mutans* growth. This safe and effective potential probiotic of *A. naeslundii* could help develop mouthwash in the prevention of oral diseases.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Actinomyces naeslundii*

Actinomyces' taxonomy has undergone considerable changes and expansions during the past ten years, with plenty of new species defined. All of the bacteria from genus *Actinomyces* are Gram-positive, facultative anaerobes, with some are strict anaerobes. The *A. naeslundii* was shown to have an irregular bacillus shape, as in Figure 2.1 (Mishra *et al.*, 2007).

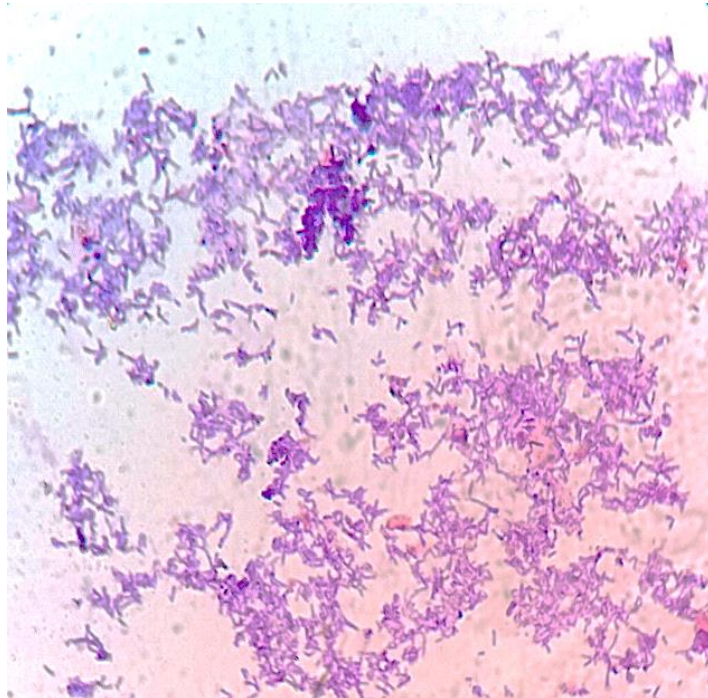


Figure 2.1 *Actinomyces naeslundii* at 100X magnification as observed under the light microscope

Members of this genus have a high GC content, indicating that most genes have been involved in genetic evolution (Chen *et al.*, 2019). The DNA GC content was

around 67.9%, with the length of genome assembly of 3.12 Mb and with protein-coding genes of 2517 (Henssge *et al.*, 2009).

This genus was first established in 1877 by the Harz and his colleagues as a member of the *Actinobacteria* phylum (Schaal, Yassin & Stackebrandt, 2006). *Actinomyces bovis* is the earliest isolated species of this genus. Today, about 47 species of *Actinomyces* have been identified with validly published names and nomenclature. The number of discovered *Actinomyces* species starts to increase from the 1980s, corresponding to the utilization of PCR. *Actinomyces* species are known to be ubiquitous, colonizing soil, animals, or humans. In humans, they are mainly present on the oral mucosa or urogenital and intestinal tracts (Acevedo, Baudrand, Letelier & Gaete, 2008).

A. naeslundii consists of three genospecies (1, 2, and WVA 963) which can only be identified based on these six partial gene sequences of housekeeping genes known as *atpA*, *rpoB*, *pgi*, *metG*, *gltA*, and *gyrA*. The partial sequences of *atpA* and *metG* differentiate three genospecies of *A. naeslundii* genospecies 1 and 2 formed two distinct clusters, well separated from both genospecies WVA 963 and *A. viscosus* (Henssge *et al.*, 2009).

Analysis of the same genes in *Actinomyces gerencseriae*, *A. israelii*, *A. meyeri*, *A. odontolyticus*, and *A. georgiae* indicated that these species exhibited <90% similarity with the *A. naeslundii* genospecies. Based on the data, the new name for *Actinomyces* is being proposed; *Actinomyces oris* sp. nov. (strain ATCC 27044T = CCUG 34288T) for *A. naeslundii* genospecies 2 and *Actinomyces johnsonii* sp. nov. (type strain ATCC 49338T = CCUG 34287T) for *A. naeslundii* genospecies WVA 963. *A. naeslundii* genospecies 1 remain as *A. naeslundii sensu stricto* (strain ATCC 12104T = NCTC 10301T = CCUG 2238T) (Henssge *et al.*, 2009).

A. naeslundii is the predominant microbial in human oral (Uta *et al.*, 2009). The *Actinomyces* constitute a significant proportion of the complex microbial flora that inhabits the human oral cavity. *A. naeslundii* and *A. viscosus* have become the subject of increasing study within the past two decades because of their suspected role in periodontal infections, affecting the cervical areas of the teeth and the supporting tissues (Baca *et al.*, 2004).

However, another previous study has also shown that *A. naeslundii* is poorly correlated with differential gene expression of gingival tissue, proving that this bacterium exhibits non-pathogenic characteristics, suggesting that the role of *A. naeslundii* in the oral cavity remains unclear (Papapanou *et al.*, 2009).

2.2 PROBIOTICS AND HEALTH BENEFITS

The word probiotic is derived from the Greek word, *probiotikos* which is defined as “improving life” (Azad *et al.*, 2018). Probiotics can play a vital role in combating drug-resistant bacteria and use as prophylaxis against certain diseases. Another benefit of probiotics is to provide a balance niche in the oral cavity and promote the growth of other beneficial microbes by modifying pH and other growth conditions (Gupta, 2011).

Fermented milk is an example of a probiotic-based product consumed worldwide for thousands of years as a treatment for gastroenteritis. A study has shown that the metabolites of probiotics can improve immune response, promoting a healthy gastrointestinal tract (Aragón *et al.*, 2014).

Among probiotics, *Lactobacillus* and *Bifidobacterium* are the most common bacteria used in the preparation of probiotic-based products which; these bacteria are

among the intestinal microbiome that was reported to ferment lactic acid (Fernández *et al.*, 2015).

From Table 2.1, Lactic acid bacteria (LAB); *Bifidobacterium*, and *Lactobacillus* are effective bacteria that can eradicate *Helicobacter pylori* activity, exhibit inhibitory activity against tumors, preventing allergy as well as producing several antimicrobial compounds such as bacteriocin (Annuk *et al.*, 2003; Segawa *et al.*, 2008). These probiotics also reported producing antimicrobial agents such as lactic acid and hydrogen peroxide that can inhibit oral pathogens like *S. mutans* (Fang *et al.*, 2018). Recently, *Lactobacillus kefiranofaciens* (ZW3) was reported to have exo-polysaccharide properties, with therapeutic properties in human health. It is also being used for co-culturing yogurt in the industrial sector (Vinderola *et al.*, 2006).

Table 2.1 List of probiotics bacteria that are claimed for health-promoting effects (Fijan, 2014).

Genus	Species	Health benefits
<i>Lactobacillus</i>	<i>L. rhamnosus</i>	<ul style="list-style-type: none"> • Shortening of rotavirus diarrhoea • Immune modulation • Relief of inflammatory bowel disease • Treatment and prevention of allergy • Reduce caries • Helpful in children chronic constipation
	<i>L. acidophilus</i>	<ul style="list-style-type: none"> • Reduced antibiotic-associated diarrhoea
	<i>L. plantarum</i>	<ul style="list-style-type: none"> • Relief of irritable bowel syndrome • Reduction of LDL-cholesterol
	<i>L. casei</i>	<ul style="list-style-type: none"> • Shortening of rotavirus diarrhoea • Reduced recurrence of superficial bladder cancer • Immune modulation • Anti-cariogenic activity
	<i>L. delbrueckii</i> <i>spp. bulgaricus</i>	<ul style="list-style-type: none"> • Slow the ageing process by suppressing proteolytic bacteria in the colon
	<i>L. brevis</i>	<ul style="list-style-type: none"> • Ability to combat gastric ulcers by inhibiting a major cause of ulcers, <i>Helicobacter pylori</i>. • Anti-inflammatory effects against periodontal disease

	<u><i>L. johnsonii</i></u>	• Improved oral vaccination
	<u><i>L. fermentum</i></u>	• Reduced colonization by <i>Helicobacter pylori</i>
	<u><i>L. reuteri</i></u>	• Shortening of rotavirus diarrhoea
	<u><i>L.salivarius</i></u>	• Growth inhibitory effect against <i>S. mutans</i>
<i>Bifidobacterium</i>	<u><i>B. infantis</i></u>	• Useful therapy for infant diarrhoea
	<u><i>B. animalis</i> spp. <i>lactis</i></u>	• Reduce the levels of selected caries-associated microorganisms in saliva
	<u><i>B. bifidum</i></u>	• Anti-cancerogenic activity
	<u><i>B. longum</i></u>	• Improvement of constipation
	<u><i>B. breve</i></u>	• Reduced symptoms of irritable bowel disease
	<u><i>B.lactis</i></u>	• Treatment of allergy • Shortening of rotavirus diarrhoea • Reduced incidence of traveller's diarrhoea • Improved oral vaccination
<i>Saccharomyces</i>	<u><i>S. cerevisiae</i></u>	• Fewer relapses of inflammatory bowel disease
<i>Lactococcus</i>	<u><i>L. spp. lactis</i></u>	• Antimicrobial activity
	<u><i>L. spp. cremoris</i></u>	
<i>Enterococcus</i>	<u><i>E. faecium</i></u>	• Promotes a balanced gut environment
	<u><i>E. durans</i></u>	• Reduce LDL (bad) cholesterol
<i>Streptococcus</i>	<u><i>S. thermophiles</i></u>	• Improve the function of the gastrointestinal tract and immune system
<i>Pediococcus</i>	<u><i>P. acidilactici</i></u>	• Balance the intestinal micro flora and reduce the risk of suboptimal health
	<u><i>P. pentosaceus</i></u>	• Promote a healthy inflammatory response in the intestines
<i>Leuconostoc</i>	<u><i>L. mesenteroides</i></u>	• Produce a proteinaceous substance with antibacterial activity
<i>Bacillus</i>	<u><i>B. coagulans</i></u>	• Colitis
	<u><i>B. subtilis</i></u>	• Crohn's disease
	<u><i>B. cereus</i></u>	• Diarrhoea • Irritable bowel syndrome
<i>Escherichia</i>	<u><i>E. coli</i></u>	• Fewer relapses of inflammatory bowel disease • Treating infectious intestinal disorders

Bifidobacteria comprises a major portion of the gut microbiota. These bacteria are mostly rod-shaped, gram-positive, strictly anaerobic, and non-spore-forming (Schrezenmeir and Vrese, 2008). Nearly 50 species of beneficial bacteria have been

discovered, which are all suggested to have different functions in the human system (Bottacini *et al.*, 2014).

Among the benefit of *Bifidobacteria* in humans is reducing the levels of selected caries-associated microorganisms in saliva, improving constipation, reducing symptoms of irritable bowel disease, and treating allergy to reduce the incidence of traveller's and rotavirus diarrhea (Fernández *et al.*, 2015). As for oral probiotics, from 23 dairy bacterial strains to prevent dental caries, only two types of strains; *Streptococcus thermophilus* and *Lactococcus lactis* can adhere to saliva-coated hydroxyapatite and further successfully incorporated into a biofilm similar to the dental plaque. Later, it modulated the growth of the oral bacteria and diminished the colonization of *Streptococcus oralis* (Comelli *et al.*, 2002).

2.2.1 Probiotic and Its Importance Properties

The most important properties to even be considered for probiotic use include resistance to gastric acidity, bile acid resistance, adherence to mucus and/or human epithelial cells and cell lines, antimicrobial activity against potentially pathogenic bacteria or fungi, ability to reduce pathogen adhesion to surfaces, bile salt hydrolase activity, enhancing viability of probiotics (Anwar *et al.*, 2014). All these properties required specific genes/proteins to function as probiotic properties, thus promoting human health.

In environment adaptation factor and stress resistance, the *IS30* elements contain in bacteria have been proved to be associated with these factors. This is due to certain genes involved in carbohydrate metabolism that benefit from gastrointestinal microflora colonization (El *et al.*, 2012).

Another element contributing to probiotic adaptation factors is PTS and ABC transporter containing lacS, galP, rafP, and metQ, which provide various transport

pathways for the probiotic to adapt in the host (Chen *et al.*, 2019). Furthermore, fructosyltransferase (sacB) elements that are available in carbohydrate transport may possess biochemical function that contributes to probiotic stress resistance (Porrasmórguez *et al.*, 2015).

The previous study has also shown that the probiotic genes promote the complete pathways for synthesizing the essential amino acid (valine, lysine, and methionine) and vitamins such as thiamine and folate. Genes for exopolysaccharide (EPS) and antioxidant production (hydro-peroxidases) were also noted, which help the probiotic strains to adapt themselves in the human gut (Ghattargi *et al.*, 2018).

2.2.2 Probiotic and Its Specific Strains

A specific strain of probiotics promises a different kind of effect on human health. For example, Minoru Shirota from the University of Kyoto found a strain of lactic acid bacteria that strongly restricted harmful bacteria living in the intestine known as *Lactobacillus casei* strain *Shirota*.

Through his finding, a commercial lactic bacteria beverage was developed, known as Yakult. Based on the Shirota's hypothesis, intake of a beverage containing *L. casei* strain *Shirota* daily can improve the internal environment, especially in the intestine, by preventing food poisoning and infectious diseases. Yakult and similar fermented milk products contribute to a healthy intestinal tract, good health, and longer life span of Japanese people (Chorley, 2014).

The most common probiotics strain used in daily human life are come from fermented cheese, milk (*L. reuteri* DSM20016), French yogurt (*Lactococcus lactis* subsp. *lactis* UR1), cocoa in producing chocolate (*L. plantarum* CH3, CH41), and preservatives foods and pickles; for example kimchi, a traditional Korean food

(*Lactococcus lactis* subsp. *lactis* A164) (Chong, 2014). Table 2.2 below shows detail of the specific strain of each probiotic.

Table 2.2 Probiotics and its specific strains.

Genus	Species	Strain
<i>Lactobacillus</i>	<i>L. rhamnosus</i>	GG, ATCC53103
	<i>L. acidophilus</i>	BG2FO4 (19,23), La5, ATCC4356
	<i>L. plantarum</i>	CH3, CH41, SAU96, 299V
	<i>L. casei</i>	CNRZ387, GG (12), BL23, Shirota
	<i>L. delbrueckii</i> spp. <i>bulgaricus</i>	ATCC11842, DN-100107
	<i>L. brevis</i>	SAU105, FFC199
	<i>L. johnsonii</i>	La1
	<i>L. fermentum</i>	CH58, KLD
	<i>L. reuteri</i>	DSM20016, SD2112
	<i>L. salivarius</i>	UCC118
<i>Bifidobacterium</i>	<i>B. infantis</i>	LW420
	<i>B. animalis</i> spp. <i>lactis</i>	Bb12, HN019
	<i>B. bifidum</i>	BG4, NCIMB 41171
	<i>B. longum</i>	BB536, NCC2705
	<i>B. breve</i>	NCIMB8807
<i>Saccharomyces</i>	<i>S. cerevisiae</i>	<i>boulardi</i>
<i>Lactococcus</i>	<i>L. lactis</i> spp. <i>lactis</i>	A164, UR1
	<i>L. lactis</i> spp. <i>cremoris</i>	MG1363
<i>Enterococcus</i>	<i>E. faecium</i>	SF68
	<i>E. durans</i>	LAB18s
<i>Streptococcus</i>	<i>S. thermophiles</i>	LMG18311, CNRZ1066
<i>Pediococcus</i>	<i>P. acidilactici</i>	K15
	<i>P. pentosaceus</i>	ATCC25745
<i>Leuconostoc</i>	<i>L. mesenteroides</i>	NRRL B-512, UL5
<i>Bacillus</i>	<i>B. coagulans</i>	GBI-30, 6086
	<i>B. subtilis</i>	KS03
	<i>B. cereus</i>	toyoi
<i>Escherichia</i>	<i>E. coli</i>	<i>Nissle 1917</i>