Effect of Powdered Milk Containing *Lactobacillus paracasei* SD1 on Salivary Mutans Streptococci and Lactobacilli in Orthodontic Cleft Patients

Chontira Saetang

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Author Miss Chontira Saetang

Major Program Oral Health Sciences

Major Advisor :

……..……

(Asst. Prof. Wipapun Ritthagol)

Co-advisor :

……..……

(Prof. Dr. Rawee Teanpaisan)

Examining Committee :

……Chairperson

(Prof. Smorntree Viteporn)

……..……

(Asst. Prof. Wipapun Ritthagol)

……..……

(Prof. Dr. Rawee Teanpaisan)

……..……

(Prof. Dr. Winyou Mitarnun)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Oral Health Sciences

……

(Prof. Dr. Amornrat Phongdara)

Dean of Graduate School

ชื่อวิทยานิพนธ์	ผลของนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวันต่อเชื้อมิวแทนสเตรบโตคอคไคและแลคโตบาซิลไลในน้ำลายของผู้ป่วยปากแหว่งเพดานโหว่ที่ได้รับการจัดฟัน
ผู้เขียน	นางสาวชลธิรา แซ่ตั้ง
สาขาวิชา	วิทยาศาสตร์สุขภาพช่องปาก
ปีการศึกษา	2554

บทคัดย่อ

วัตถุประสงค์: 1.เพื่อประเมินผลของนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวันต่อเชื้อมิวแทนสเตรบโตคอคไคและแลคโตบาซิลไลในผู้ป่วยปากแหว่งเพดานโหว่ที่ได้รับการจัดฟันชนิดติดแน่น 2. ประเมินการคงอยู่ของเชื้อแลคโตบาซิลลัสพาราเคซิอายเอสดีวันในช่องปากภายหลังรับประทานนมผงโพรไบโอติก **วัสดุและวิธีการ**: จากกลุ่มตัวอย่างทั้งหมด 32 คน ได้รับการคัดออก 2 คนจึงเหลือผู้ป่วยที่เข้าร่วมการศึกษาทั้งหมด 30 คนซึ่งประกอบด้วยผู้ป่วยปากแหว่งเพดานโหว่ที่ได้รับการรักษาทางทันตกรรมจัดฟันที่โรงพยาบาลทันตกรรม คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ กลุ่มตัวอย่างจะถูกคัดเลือกแบบสุ่มเป็น 2 กลุ่มคือกลุ่มศึกษาและกลุ่มควบคุม โดยกลุ่มศึกษาประกอบด้วยผู้ป่วยจำนวน 15 คน (เพศหญิง 8 คน, ชาย 7 คน) อายุเฉลี่ย19.01± 4.24 ปี กลุ่มควบคุมประกอบด้วยผู้ป่วยจำนวน 15 คน (เพศหญิง 11 คน, ชาย 4 คน) อายุเฉลี่ย19.42± 3.11 ปี กลุ่มศึกษาจะได้รับนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวัน และกลุ่มควบคุมได้รับนมผงไม่ผสมเชื้อโพรไบโอติก โดยรับประทานนมผงปริมาณ 10 กรัม/น้ำ 50 ml/วัน ในช่วงหลังรับประทานอาหารเช้า เป็นระยะเวลา 30 วัน ทำการเก็บตัวอย่างน้ำลายเพื่อประเมินเชื้อมิวแทนสเตรบโตคอคไคและแลคโตบาซิลไล ค่าดัชนีแผ่นคราบจุลินทรีย์ ค่าความเป็นกรดด่างของน้ำลายจำนวน 6 ครั้งคือก่อนรับประทานนม (I) หลังรับประทานนมครบ 30 วัน(T0) สัปดาห์ละ 1 ครั้งหลังรับประทานนมครบ 30 วัน เป็นเวลา 1 เดือน (T1, T2, T3, T4) ตรวจวัดค่าดัชนีฟันผุ ถอนอุดก่อนรับประทานนม (I) และหลังรับประทานนมครบ 1 เดือน (T4) ตรวจลักษณะทางพันธุกรรมของเชื้อเพื่อดูความคงอยู่ของเชื้อแลคโตบาซิลลัสพาราเคซิอายเอสดีวันภายในช่องปากทุกสัปดาห์หลังรับประทานนม (T0-T4) โดยใช้วิธี arbitrarily primed polymerase chain reaction (AP-PCR) **ผลการศึกษา**:พบว่ากลุ่มศึกษามีปริมาณเชื้อมิวแทนสเตรบโตคอคไคลดลงอย่างมีนัยสำคัญทางสถิติ ($p < 0.001$) และปริมาณเชื้อแลคโตบาซิลไลเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($p < 0.001$) ในทุกสัปดาห์หลังรับประทานนม (T0-T4) เมื่อเปรียบเทียบกับก่อน

รับประทานนม สำหรับกลุ่มควบคุมปริมาณเชื้อมิวแทนสเตรบโตคอคไคและแลคโตบาซิลไล ก่อนและหลังการรับประทานนมมีค่าไม่แตกต่างกันทางสถิติ เมื่อเปรียบเทียบระหว่างกลุ่มศึกษาและกลุ่มควบคุมพบว่ากลุ่มศึกษามีปริมาณเชื้อมิวแทนสเตรบโตคอคไคลดลงอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) และปริมาณเชื้อแลคโตบาซิลไลเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ในทุกสัปดาห์หลังรับประทานนม (T0-T4) ไม่พบความแตกต่างทางสถิติของค่าความเป็นกรดด่างของน้ำลาย ค่าดัชนีแผ่นคราบจุลินทรีย์ก่อนและหลังการรับประทานนมในกลุ่มศึกษาและกลุ่มควบคุมไม่มีการเปลี่ยนแปลงของค่าดัชนีฟันผุ ถอนอุดในทั้งสองกลุ่ม การตรวจสอบทางพันธุกรรมของเชื้อพบแถบ DNA ของเชื้อแลคโตบาซิลลัสพาราเคซิอายเอสดีวันในทุกสัปดาห์หลังรับประทานนม (T0-T4)ในกลุ่มศึกษา **สรุป:** การรับประทานนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวันมีผลในการลดปริมาณเชื้อมิวแทนสเตรบโตคอคไคและเพิ่มปริมาณเชื้อแลคโตบาซิลไล อย่างมีนัยสำคัญทางสถิติในผู้ป่วยปากแหว่งเพดานโหว่ที่ได้รับการจัดฟันชนิดติดแน่น และพบการคงอยู่ของเชื้อแลคโตบาซิลลัสพาราเคซิอายเอสดีวันในช่องปากภายหลังรับประทานนมเป็นเวลา 4 สัปดาห์

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Major Program	Oral Health Sciences
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ABSTRACT

Objective: The aims of this study were to (i) evaluate the effect of short-term consumption of probiotic powdered milk containing *Lactobacillus paracasei* SD1 on the level of salivary mutans streptococci and lactobacilli, and (ii) investigate the oral persistence of *Lactobacillus paracasei* SD1 after short-term consumption of probiotic powdered milk in treated cleft patients with fixed orthodontic appliances**. Material and method:** From 32 subjects at the beginning of the study, two of them were excluded. Thirty cleft lip and cleft palate patients remaining in the study who had been undergoing treatment with fixed orthodontic appliances at the Dental Hospital, Faculty of Dentistry, Prince of Songkla University were divided into two groups; randomized and control groups. The randomized sampling group, consisting of 15 subjects in the probiotic group (8 females, 7 males) with mean age 19.01± 4.24 years, received 10g/50ml/day of probiotic powdered milk containing *Lactobacillus paracasei* SD1, while the 15 subjects in the control group (11 females, 4 males) with mean age 19.42± 3.11 years received 10g/50ml/day of control milk. Both groups were advised to drink the provided milk at breakfast time for 30 days. Salivary mutans streptococci, lactobacilli counts, plaque index (PI) and salivary pH were performed for pre-milk consumption as initial (I), immediately after post-milk consumption as T0 at one week intervals for 4 weeks, where post-milk consumption was recorded as T1, T2, T3, T4. The number of decayed, missing, and filled teeth (DMFT) was performed at initial and T4. The oral persistence of *Lactobacillus paracasei* SD1 was then investigated by a genotypic study using an arbitrarily primed polymerase chain reaction (AP-PCR) at T0-T4. **Results:** A statistically significant ($p < 0.001$) decrease of salivary mutans streptococci and an increase of lactobacilli ($p < 0.001$) were found at T0-T4 in the probiotic group, while no statistical

significance was found in the control group. Between the groups, a statistically significant ($p < 0.05$) decrease of salivary mutans streptococci and an increase of lactobacilli ($p < 0.05$) were found at T0-T4. There were no statistically significant changes among all cases of the control and probiotic groups in the salivary pH and plaque index of post-milk consumption periods and there were no changes of DMFT in both groups throughout the study. The genotype patterns of *Lactobacillus paracasei* SD1 were found at T0-T4 in the probiotic group. **Conclusion:** The short-term consumption of probiotic powdered milk containing *Lactobacillus paracasei* SD1 could decrease the salivary levels of mutans streptococci and increase lactobacilli in cleft patients who undergoing treatment with fixed orthodontic appliances. Moreover, *Lactobacillus paracasei* SD1 genotype patterns were found in all cases of the oral cavity over the post-milk consumption period.

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Chontira Saetang

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LISTS OF ABBREVIATIONS AND SYMBOLS

ml	=	milliliter
µl	=	microliter
et al.	=	and others
g	=	gram
h	=	hour
CFU	=	colony forming unit
PBS	=	phosphate buffer saline
min	=	minute
rpm	=	revolutions per minute
M	=	molar
°C	=	Celsius
ng	=	nanogram
Fig	=	figure
DMFT	=	the number of decayed, missing, and filled teeth
PI	=	plaque index
AP-PCR	=	an arbitrarily primed polymerase chain reaction
Initial (I)	=	pre-milk consumption
T0	=	immediately post-milk consumption
T1	=	1 week post-milk consumption
T2	=	2 weeks post-milk consumption
T3	=	3 weeks post-milk consumption
T4	=	4 weeks post-milk consumption
%	=	percent
/	=	per

CHAPTER 1

INTRODUCTION

Background and rationale

Cleft lip and palate (CLP) is a common congenital craniofacial abnormality that requires a multidisciplinary team approach for comprehensive treatment. Patients with cleft lip and palate generally display poor oral hygiene and higher caries prevalence.[1] This may be due to the difficulty in achieving adequate plaque control associated with dental anomalies and defects from the lips and palate.

Numerous dental and skeletal abnormalities such as hypodontia, supernumerary teeth, abnormality in tooth size and shape, discrepancy in skeletal base relationships and malalignment of teeth are encountered in patients with cleft lip and palate. To develop the arches and to align the teeth, several phases of orthodontic treatment are in almost all cases unavoidable. Fixed and removable orthodontic appliances facilitate the colonization of cariogenic mutans streptococci (MS) and the late colonizer lactobacilli (LB), hence providing a negative impact on caries susceptible patients with cleft lip and palate when they undertake orthodontic treatment.[2]

Patients who undergo orthodontic therapy have oral ecological changes such as a low pH environment, increased retentive sites for microorganism, and increased retention of food particles, which may lead to increased proportions and absolute numbers of saliva *S. mutans* and lactobacilli.[3-5] Mutans streptococci have been associated in particular with early demineralization while lactobacilli are implicated more with lesion progression and cavitation.[6] High saliva counts of lactobacilli seemed to reflect conditions that enhanced the risk of colonization of mutans streptococci that may cause enamel demineralization and dental caries, and recent systematic reviews have examined methods to prevent this side-effect in orthodontic treatment. Apart from fluoride exposure, very limited evidence was found and it was concluded that more high-quality clinical research would be needed to give evidence-based advice on the optimal caries-preventive strategy during orthodontic treatment.

Probiotics are bacterial cultures or living microorganisms which, upon ingestion in certain numbers exert health benefits beyond inherent general nutrition and support a good and healthy intestinal bacterial flora.[7] Hence, they are viable bacteria that beneficially affect the host by improving the intestinal microbial balance. These bacteria have to belong to the natural flora in order to be able to resist acid and bile, to survive during intestinal transit, to adhere to the intestinal mucosa, and to produce antimicrobial substances in order to retain the characteristics that contribute to their beneficial health effects.[8]

Traditionally, probiotics have been associated with gut health. However, during the last decade several investigators have also suggested the use of probiotics for oral health purposes. Recently, Nase *et al*. showed that long-term consumption of milk containing *Lactobacillus rhamnosus* GG (LGG) caused a significant reduction in caries risk in day-care children.[9] Cildir *et al*. found that short-term consumption of fruit yogurt containing bifidobacteria caused a statistically significant reduction of salivary mutans streptococci during consumption of yoghurt[10] and was a the key reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria such as strains of lactobacilli or bifidobacteria, and it can occupy a space in a biofilm that otherwise would be colonized by a pathogen. Caglar *et al*. found daily chewing on gums containing two strains of *Lactobacillus reuteri* ATCC 55730 and ATCCPTA 5289 reduced the levels of salivary mutans streptococci significantly.[11] Caglar *et al*. demonstrated that a short-term daily ingestion of lozenges or straws containing *L. reuteri* ATCC 55730 for 3 weeks reduced the level of salivary mutans streptococci in young adults.[12] Although there are many advantages yet also has several limitations, Yli-Knuuttila H. *et al*. have shown that *L. rhamnosus* GG and two different *L. reuteri* strains have been reported to colonize the oral cavity of 48–100% of volunteers consuming products containing *L. rhamnosus* GG and *L. reuteri*.[13-14] However, after a 14-day trial period, the occurrence of LGG in the oral cavity decreased gradually, indicating that no permanent colonization had occurred and that the oral persistence of LGG was only temporary. It may be that they did not cause potential strains for specific oral ecology. For these reasons, should be finding potentially probiotic microbial strains for specific oral ecology. *Lactobacillus paracasei* SD1 was a normal flora that was isolated from caries-free children; the advantage of this strain includes 1) growth inhibition of pathogenic strains; *S. mutans* and *S. sobrinus*, 2) less acidic produce than the other strains, and 3) ability to adhere to oral epithelial cells.

Thus, this strain may be useful for a potentially probiotic microbial strain for oral cavities.

Previous studies have suggested that probiotic supplements in dairy products may affect the oral microbial ecology. However, the possible effect of *Lactobacillus paracasei* SD1 on the oral cariogenic microbial flora and the effect in orthodontic cleft patients have not previously been reported. The aim of the present study was to investigate the effect of short-term consumption of powdered milk containing *Lactobacillus paracasei* SD1 on the levels of salivary mutans streptococci and lactobacilli in fixed orthodontic cleft patients and investigate the persistence of *Lactobacillus paracasei* SD1 in the oral cavity after short-term consumption of powdered milk containing *Lactobacillus paracasei* SD1.

Review of the literatures

Every day human beings ingest a large number of living microorganisms, predominantly bacteria. Although these organisms are naturally present in food and water, they can also be deliberately added during the processing of foods such as sausages, cheese, yogurt and fermented milk products. For several decades now, bacteria called probiotics have been added to some foods because of their beneficial effects for human health.[15] The bacteria in yogurt and fermented milk products constitute the most important source of probiotics for humans. The vast majority of probiotic bacteria belong to the general *Lactobacillus, Bifidobacterium, Propionibacterium* and *Streptococcus* groups. Several clinical studies have already demonstrated the effectiveness of certain probiotics in the treatment of systemic and infectious diseases such as acute diarrhea and Crohn disease.[15] Other studies have suggested potential applications in the treatment of cardiovascular disease, urogenital infections, oropharyngeal infections and cancers.[8,15,16] Probiotics may also prove useful in addressing problems arising from the excessive use of antibiotics, specifically the appearance of bacterial resistance. To date, however, the potential beneficial effects of probiotics for oral pathology have had only limited study.

Probiotics

Probiotics are bacterial cultures or living microorganisms which, upon ingestion in certain numbers, exert health benefits beyond inherent general nutrition and support a good and healthy intestinal bacterial flora , that beneficially influence the health of the host when used in adequate numbers.[7] This definition has been approved by the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO).[17] The establishment of standards and guidelines constituted an essential step in the acceptance of probiotics as legitimate health related products. To be called a probiotic, a bacterial strain must be fully characterized. The genus and species of the microorganism must be identified according to internationally accepted methods, and its nomenclature of Bacterial Names.[18] In addition, both in vitro and in vivo studies must be conducted to demonstrate the mechanism of action of the probiotic, to allow prediction of its scope of applicability and its potential side effects. The FAO and the WHO have recommended that probiotic bacterial strains are characterized by their spectrum of resistance to antibiotics, their metabolic and hemolytic activities, and their capacity to produce toxins, their infectious power in immunosuppressed animal models and their side effects in humans.[18]

The idea of probiotics dates back to the first decade of 1900 when the Ukrainian bacteriologist and Nobel Laureate Ilya Metchnikof (1908) studying the flora of the human intestine developed a theory that senility is caused by poisoning of the body by the products of some of these bacteria. To prevent the multiplication of these organisms he proposed a diet containing milk fermented by lactobacilli which produce large amounts of lactic acid and for a time this diet became widely popular.[19]

Mechanisms of probiotic action

Probiotic organisms are thought to act through a variety of mechanisms including:[20-21]

1. Competition with potential pathogens for nutrients or enterocyte adhesion sites
2. Degradation of toxins
3. Production of antimicrobial substances
4. Local and systemic immunomodulation

Some of the hypothetical mechanism of probiotics action in the oral cavity:[22]

- Direct interaction in dental plaque
- Involvement in binding of oral micro-organisms to proteins
- Action on plaque formation and on its complex ecosystem by competing and intervening with bacterial attachments
- Involvement in metabolism of substrate and production of chemicals that inhibit oral bacteria

Indirect probiotic actions are also featured such as[23]

1. Modulating systemic immune function
2. Effect on local immunity
3. Effect on non-immunologic defense mechanisms
4. Regulation of mucosal permeability
5. Probiotics as an antioxidants and produce antioxidants
6. Prevent plaque formation by neutralizing the free electrons

Ideal properties of a probiotic intended for use in disorders of the mouth:[16,18,19, 23]

1. Binding to dental surfaces
2. Production of antimicrobial substances against oral pathogens such as organic acids, hydrogen peroxide and bacteriocins
3. Alteration of environmental conditions of the mouth; modified the surrounding environment by modulating the pH and/or the oxidation–reduction potential, which may compromise the ability of pathogens to become established
4. Reduction of the inflammatory response; stimulating nonspecific immunity and modulating the humoral and cellular immune response

One mechanism of action of probiotics is suggested to be their modulation of host immune response. Immune inductive sites in the oral cavity are within the diffuse lymphoid aggregates of the Waldeyer's ring. Lingual and pharyngeal tonsils and adenoids contain most of the lymphatic tissue. The role of these anatomic structures as inductive sites of mucosal immunity has been shown by intranasally delivered vaccines.[24] Dendritic cells scattered in mucosal surfaces are pivotal in the front-line bacterial recognition (antigen presentation) and in activating T-cell responses. Depending on the signals from dendritic cells either immune tolerance or active immune response toward a specific antigen may occur.[25] A marked production of interleukin-10

by dendritic cells in gut mucosa has been registered after administration of a probiotic mixture[26] However, more studies on activation of the oral immune inductive sites after probiotic administration are needed before further conclusions can be drawn. Such investigations might even cast light on probiotic effects in general and substantiate their specific applications in the future.

Probiotic strains in the oral cavity

Microorganisms generally considered as probiotics may not have oral cavity as their inherent habitat and, subsequently, their possibility to confer benefit on oral health is then questionable. Paster *et al.*[27] in an attempt to determine bacterial diversity in the human subgingival plaque by using culture-independent molecular methods have estimated that the total species diversity in the oral cavity ranges between 500 and 600 species. This number was further extended by Kazor *et al.*[28] who detected 200 additional unknown species on the dorsum of the tongue, making the number of species in the mouth to reach 700. Lactobacilli make approximately 1% of the cultivable oral microflora.[29]

The most common lactobacilli species recovered from saliva in a study by Teanpaisan and Dahlen[30] were *L. fermentum, L. rhamnosus, L. salivarius, L. casei, L. acidophilus* and *L. plantarum.* Three of them, *L. rhamnosus*, *L. casei*, *L. acidophilus* are probiotic strains commonly used in dairy products. A similar diversity in the oral lactobacilli flora was observed by Colloca *et al.* [31] who found *L. fermentum, L. plantarum, L. salivarius* and *L. rhamnosus* to be the predominant species in healthy human mouth.

Koll-Klais *et al.*[32] observed a higher prevalence of homofermentative lactobacilli in healthy mouths compared to samples from patients with chronic periodontitis. These findings indicate that lactobacilli as members of resident oral microflora could play an important role in the microecological balance in the oral cavity. These studies further demonstrated that lactobacilli strains with probiotic properties may indeed be found in the oral cavity. Yet there is no evidence whether these lactobacilli strains were detected due to the frequent consumption of dairy products leading to temporary colonization only, or if the oral environment is their permanent habitat. There are no long-term follow-up studies published to answer this question.

The most common probiotic strains

The most common probiotic strains belong to the general *Lactobacillus* and *Bifidobacterium*.[19]

1. *Lactobacillus* species from which probiotic strains have been isolated include *L. acidophilus, L. johnsonii, L. casei, L. rhamnosus, L. gasser,* and *L. reuteri*
2. *Bifidobacterium* strains include *B. bifidum, B. longum* and *B. infantis*

Application of probiotics to oral health

Probiotics and dental caries

Dental caries is a multifactorial disease of bacterial origin that is characterized by acid demineralization of the tooth enamel.[33] It appears following changes in the homeostasis of the oral ecosystem leading to proliferation of the bacterial biofilm, composed notably of streptococci from the mutans group. To have a beneficial effect in limiting or preventing dental caries, a probiotic must be able to adhere to dental surfaces and integrate into the bacterial communities making up the dental biofilm. It must also compete with and antagonize the cariogenic bacteria and thus prevent their proliferation. Finally, metabolism of food-grade sugars by the probiotic should result in low acid production. The advantage of incorporating probiotics into dairy products lies in their capacity to neutralize acidic conditions. For example, it has already been reported that cheese prevents demineralization of the enamel and promotes its remineralization.[34-35] Comelli and colleagues[36] reported that of 23 bacterial strains used in the dairy industry, *Streptococcus thermophilus* and *Lactobacillus lactis* ssp. lactis were the only ones with the capacity to integrate into a biofilm present on a hydroxyapatite surface and to interfere with development of the cariogenic species *Streptococcus sobrinus*. More recently, it was demonstrated that isolates of *W. cibaria* had the capacity to inhibit, both in vitro and in vivo, biofilm formation by *S. mutans* and to prevent proliferation of this bacterial strain.[37] In other studies, one strain of *L. rhamnosus* and the species *L. casei* inhibited in vitro growth of 2 important cariogenic streptococci, *S. mutans* and *S. sobrinus*.[9,38] More recently, Petti and colleagues[39] reported that yogourt containing *S. thermophilus* and *L. bulgaricus* had selective bactericidal effects on streptococci mutans. Several clinical studies have demonstrated that

regular consumption of yogourt, milk or cheese containing probiotics led to a decrease in the number of cariogenic streptococci in the saliva and a reduction in dental plaque.[9,11,40] More specifically, Nikawa and colleagues[40] reported that consumption of yogourt containing *Lactobacillus reuteri* over a period of 2 weeks reduced the concentration of *S. mutans* in the saliva by up to 80%. Comparable results were obtained by incorporating probiotics into chewing gum or lozenges.[11,12]

Nase and colleagues[9] published the results of a long-term (7-month) study of 594 children 1 to 6 years of age that evaluated the effects on dental caries of consuming milk supplemented with a strain of *L. rhamnosus*. The authors concluded that children consuming milk containing this probiotic, particularly those 3–4 years of age, had significantly fewer dental caries and lower salivary counts of *S. mutans* than controls. These promising results suggest a potentially beneficial application of probiotics for the prevention of dental caries.

Probiotics and periodontal disease

Periodontal disease is classified into 2 types: gingivitis and periodontitis. Gingivitis is characterized by inflammation limited to the unattached gingiva, whereas periodontitis is a progressive, destructive disease that affects all supporting tissues of the teeth, including the alveolar bone. The main pathogenic agents associated with periodontitis are *P. gingivalis, Treponema denticola, Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans*. These bacteria have a variety of virulent characteristics allowing them to colonize the subgingival sites, escape the host's defence system and cause tissue damage. The persistence of the host's immune response also constitutes a determining factor in progression of the disease.[41]

In one recent study, the prevalence of lactobacilli, particularly *Lactobacillus gasseri* and *L. fermentum*, in the oral cavity was greater among healthy participants than among patients with chronic periodontitis.[32] Various studies have reported the capacity of lactobacilli to inhibit the growth of periodontopathogens, including *P. gingivalis*, *Prevotella intermedia* and *A. actinomycetemcomitans*.[32] Together, these observations suggest that lactobacilli residing in the oral cavity could play a role in the oral ecological balance.

Krasse and colleagues[42] assessed the beneficial effect of *L. reuteri* against gingivitis. After 14 days of ingesting the probiotic incorporated into chewing gum, the oral cavity of patients with a moderate to severe form of gingivitis had been colonized by *L. reuteri* and the plaque index had been reduced. Although the exact mechanisms of action of *L. reuteri* remain to be elucidated, previous studies have suggested at least 3 plausible possibilities: 1. *L. reuteri* is known for its secretion of 2 bacteriocins, reuterin and reutericyclin, that inhibit the growth of a wide variety of pathogens[43-44], 2. *L. reuteri* has a strong capacity to adhere to host tissues, thereby competing with pathogenic bacteri ; 3.The recognized anti-inflammatory effects of *L. reuteri* on the intestinal mucosa, leading to inhibition of secretion of a direct or indirect beneficial effect of this bacterium on people with periodontal disease.[45-47] However, additional studies with larger patient cohorts are needed to confirm the long-term potential of *L. reuteri* in preventing and/or treating gingivitis.

Riccia and colleagues[48] recently studied the anti-inflammatory effects of *Lactobacillus brevis* in a group of patients with chronic periodontitis. The treatment, which involved sucking on lozenges containing *L. brevis* over a period of 4 days, led to improvements in the targeted clinical parameters (plaque index, gingival index, bleeding on probing) for all patients. In that study, a significant reduction in salivary levels of prostaglandin E2 (PGE2) and matrix metalloproteinases (MMPs) was also observed. The authors suggested that the beneficial anti-inflammatory effects of *L. brevis* could be attributed to its capacity to prevent the production of nitric oxide and, consequently, the release of PGE2 and the activation of MMPs induced by the nitric oxide. However, *L. brevis* may also be antagonistic, leading to a reduction in the quantity of plaque and therefore an improvement in the gingival index.

During the fermentation process in milk, *Lactobacillus helveticus* produces short peptides that act on osteoblasts and increase their activity in bone formation.[49] These bioactive peptides could thereby contribute to reducing the bone resorption associated with periodontitis.

Recently Shimazaki and colleagues[50] used epidemiological data to assess the relationship between periodontal health and the consumption of dairy products such as cheese, milk and yogourt. The authors found that individuals, particularly nonsmokers, who regularly consumed yogourt or beverages containing lactic acid exhibited lower probing depths and less loss of clinical attachment than individuals who consumed few of these dairy products. A similar effect was not observed with milk or cheese. By controlling the growth of the pathogens

responsible for periodontitis, the lactic acid bacteria present in yogourt would be in part responsible for the beneficial effects observed. Longitudinal studies are required, however, to clarify the observed relationship between regular consumption of products containing probiotics and periodontal health.

Sunstar (Etoy, Switzerland) recently began marketing the first probiotic specifically formulated to fight periodontal disease. Gum Perio Balance contains a patented combination of 2 strains of *L. reuteri* specially selected for their synergetic properties in fighting cariogenic bacteria and periodontopathogens. Each dose of lozenge contains at least 2×10^8 living cells of *L. reuteri* Prodentis. Users are advised to use a lozenge every day, either after a meal or in the evening after brushing their teeth, to allow the probiotics to spread throughout the oral cavity and attach to the various dental surfaces. Additional studies are required to evaluate the long-term effects of using these products.

Probiotics and halitosis

Halitosis has many causes (including consumption of particular foods, metabolic disorders, respiratory tract infections), but in most cases it is associated with an imbalance of the commensal microflora of the oral cavity.[51] More specifically, halitosis results from the action of anaerobic bacteria that degrade salivary and food proteins to generate amino acids, which are in turn transformed into volatile sulphur compounds, including hydrogen sulphide and methanethiol. Kang and colleagues reported the capacity of various strains of *W. cibaria* to inhibit the production of volatile sulphur compounds by *F. nucleatum*. They concluded that this beneficial effect resulted from the production of hydrogen peroxide by *W. cibaria*, which inhibited the proliferation of *F. nucleatum*. These authors also found that gargling with a solution containing *W. cibaria* was associated with a net reduction in the production of hydrogen sulphide and methanethiol and consequently a reduction in bad breath.[37] One recent study[52] showed that certain bacterial species, including *Atopobium parvulum*, *Eubacterium sulci* and *Solobacterium moorei,* predominate on the dorsal surface of the tongue among people with halitosis. Conversely, another species, *Streptococcus salivarius*, was detected most frequently among people without halitosis and is therefore considered a commensal probiotic of the oral cavity. *S. salivarius* is known to produce bacteriocins, which could contribute to reducing the number of bacteria that produce

volatile sulphur compounds.[53] The use of gum or lozenges containing *S. salivarius* K12 (BLIS Technologies Ltd., Dunedin, New Zealand) reduced levels of volatile sulphur compounds among patients diagnosed with halitosis.[54-55]

Administration of probiotics

Appropriate forms of administration of probiotic strains have been discussed in several articles. Dairy products supplemented with probiotics are a natural means of oral administration and easily adopted in dietary regime. However, for the purposes of prevention or treatment of oral diseases, specifically targeted applications, formulas, devices, or carriers with slow release of probiotics might be needed.

Montalto *et al.* [56] administered probiotic mix both in capsules and in liquid form without observing statistically significant difference, however, in the *S. mutans* counts between the two test groups. A specially designed straw with a reservoir containing probiotics has also been presented by Caglar *et al.*[12] who compared the effect of two non-dairy delivery methods, a Life top straw (BioGaia AB, Stockholm, Sweden) and a lozenge on the effectiveness of *L. reuteri* to reduce the number of *S. mutans*. Both means of administration showed significant reduction in salivary *S. mutans* levels in half of the patients when compared with subjects who received placebo.

A recent invention for caries prophylaxis is a chewing gum containing *L. reuteri* Prodentis. Consumed twice daily this was marketed to regulate *S. mutans* counts in the oral cavity.[19] The average content of *L. reuteri* was 10^8 CFU $ml.^{-1}$ However, we conclude that the most suitable means of delivery and dosages of probiotics for various oral health purposes have not been defined.

Residence time of probiotics in oral cavity

Residence time of probiotics in oral cavity after treatment withdrawal was studied by Çaglar *et al*.[14] A reduced of *S. mutans* level was shown after a two-week use of a *L. reuteri*-enriched yogurt; effects were observed during use and for a few days after discontinuation. A loss of *L. reuteri* colonization was observed by Wolf *et al.*[57] two months after having discontinued probiotic use. *L. rhamnosus* GG administration and oral cavity colonization

was studied by Yli-Knuuttila *et al.*[13]; the authors concluded that permanent colonization in oral cavity was unlikely (although possible in some cases) and suggested the probiotic to be used on a regular basis. Binding strength of 17 *Lactobacillus* strains and 7 bifidobacteria strains to saliva and oral mucous membrane was variable in different strains, according to a study by Haukioja e*t al.*[58]such a strength variation caused an increased residence time of probiotic in oral cavity. Latency time of probiotic *S. salivarius K12*, 4 tablets/day for 3 days, was assessed in several oral cavity areas in a 35-day follow-up, by Horz *et al.*[59]; probiotic could be found on oral mucous membrane, tongue and in stimulated saliva for more than 3 weeks, with a gradually reduced *S. salivarius K12* level being detected beginning 8 days after treatment withdrawal.

Safety aspects of probiotic

The issue of safety is of special concern during the past few years due to the increased probiotic supplementation of different food products. From the safety point of view, the putative probiotic microorganisms should not be pathogenic, should not have any growth-stimulating effects on bacteria causing diarrhea, and should not have an ability to transfer antibiotic resistance genes. The probiotics should rather be able to maintain genetic stability in oral microflora.[60]

The increased probiotic consumption inevitably leads to increased concentrations of these species in the host organism. *Lactobacillus* bacteremia is a rare entity, and data on its clinical significance are mainly found through case reports. For the last 30 years there have been approximately 180 reported cases.[61] Clinical characteristics of *Lactobacillus* bacteremia are highly variable, ranging from asymptomatic to septic shock-like symptoms. Any viable microorganism is capable of causing bacteremia, however, especially in patients with severe underlying diseases or in immunocompromised state. Nevertheless, the present literature supports the conclusion that the incidence of *Lactobacillus* bacteremia is unsubstantial and that all the cases where it has been registered are individuals with other systemic diseases such as diabetes, cardiovascular diseases, gastrointestinal disorders, malignancies, or organ transplant patients.[62-63] However, it is evident that careful monitoring is needed in this regard in the future.

Several studies have been carried out in immunocompromised patients. In a controlled study exposing 35 HIV-positive patients to *L. reuteri,* no clinically significant side

effects were noted[57]. Salminen *et al.* [64] found no increase in *Lactobacillus* bacteria in blood culture samples when screening the Finnish population for the period 1990–2000. Specifically, their study showed no increase related to the increasing probiotic use of LGG-containing commercial dairy products during that period. Further, Salminen[65] has recently reported no adverse effects caused by LGG ingestion or LGG treatment in general, on HIV positive patients. CD4+ cell counts or viral load levels were analyzed and all these patients received highly active antiretroviral therapy. Consequently, LGG-containing products are not likely to exert any major health risks among HIV-positive patients.

An indirect proof of safety might be the results of studies investigating lactobacilli species as live vectors in delivery of antigens at mucosal sites. Animal experiments have shown *that L. lactis, L. casei, L. plantarum, L. helveticus* and recombinant *L. plantarum* are capable of inducing both systemic and mucosal immune response against *S. pneumoniae* antigens and tetanus toxin, respectively, delivered by an intranasal route.[66-67]

The absence of acquired antibiotic resistances is another safety criterion to be tested in potential probiotic candidates. Some probiotics are closely related to opportunistic bacteria and this may also cause transferral of antimicrobial resistance genes in between microorganisms.[68] Several results from antibiotic susceptibility tests claim that the tet (W) and tet (S) genes in some probiotic lactobacilli and bifidobacteria strains are responsible for gentamycin, sulfamethoxazole, polymyxin B, and tetracycline resistance.[69] These investigations emphasize the need for a minimal safety evaluation during the selection of strains for probiotic use. However, further studies are also needed in this area because the increasing number of species that develop resistance to commonly used antimicrobial drugs is of great global concern. Hence, before any recommendations can be given for probiotic therapy in preventing and/or treating microbial infections instead of using antibiotic or antifungal drugs, transferral of resistance genes needs to be carefully investigated.

Objectives of research

1. To evaluate the effect of short-term consumption of powdered milk containing *Lactobacillus paracasei* SD1 on the level of salivary mutans streptococci, and lactobacilli in fixed orthodontic cleft patients
2. To investigate the persistence of *Lactobacillus paracasei* SD1 in the oral cavity after short-term consumption of powdered milk containing *Lactobacillus paracasei* SD1

Research Questions

1. Does the short-term consumption of powdered milk containing *Lactobacillus paracasei* SD1 can decrease salivary mutans streptococci and increase lactobacilli in fixed orthodontic cleft patient?
2. Does the *L. paracasei* SD1 can colonize in the oral cavity after short-term consumption of powdered milk containing *Lactobacillus paracasei* SD1?

Hypotheses

1. Short-term consumption of powdered milk containing *Lactobacillus paracasei* SD1 can decrease salivary mutans streptococci and increase lactobacilli in fixed orthodontic cleft patient.
2. *L. paracasei* SD1 can colonize in oral cavity after short- term consumption of powdered milk containing *Lactobacillus paracasei* SD1

Significance of the study

To use powdered milk containing *Lactobacillus paracasei* SD1 for reduction of mutans streptococci in the orthodontic cleft patients.

CHAPTER 2

RESEARCH METHODOLOGY

Subjects

The study was approved by the Ethics Committee of the Faculty of Dentistry, Prince of Songkla University. Subjects and/or their parents were informed consent to participate in the study. Thirty two cleft lip and palate patients from the Orthodontic clinic, Dental hospital, Faculty of Dentistry, Prince of Songkla University were recruited in this study based on the following inclusion criteria:

1. All subjects were undergoing treatment with fixed orthodontic appliances for at least 3 months with attachments on at least 20 permanent teeth
2. No systemic disease and without orofacial clefting as part of a craniofacial syndrome
3. No use of systemic antibiotics, antimicrobial drugs or any probiotic products within the past 2 weeks
4. No history of milk allergy and/or lactose intolerance
5. No active untreated carious lesions

Study design

The prospective investigation was a double blinded randomized placebo controlled study design. From 32 subjects at the beginning of the study, two of them were excluded. The 30 subjects remaining in the study were categorized into two groups, the probiotic and control group, by randomized sampling. There were 15 subjects in the probiotic group (8 females, 7 males, with mean age 19.01± 4.24 years) and 15 subjects in the control group (11 females, 4 males, with mean age 19.42± 3.11 years). The study group received probiotic milk and the control group received normal milk. All subjects were advised to drink the received milk at breakfast time for 30 days. No tooth brushing was allowed for at least 1 hour after milk consumption. During the experimental period, all subjects were instructed not to receive any other form of probiotic products and to maintain their normal life style.

Probiotic and control milks

The powdered milk used in this study was manufactured by the Faculty of Agro-Industry, Prince of Songkla University and divided into two forms of 10 g per pack powdered milk. The probiotic milk contained *Lactobacillus paracasei* SD1 at a concentration of 1.8×10^{9} colony forming units (CFU)/g, while the control milk was without viable bacteria (Fig.1). The powdered milk was kept in a refrigerator. A pack of powdered milk mixed in 50 ml. water was the daily intake at breakfast time by double blind randomized sampling for 30 days.

Fig.1: 10 g per pack of powdered milk

Data records and collections

Demographic data was taken for each of the subjects including their age, sex, medical history, etc. Oral clinical examinations (The number of decayed, missing, and filled teeth, plaque index, and salivary pH), salivary collection and culture were recorded at the following times

Initial (I) = pre-milk consumption

T0 = immediately post-milk consumption

T1 = 1 week post-milk consumption

T2 = 2 weeks post-milk consumption

T3 = 3 weeks post-milk consumption

T4 = 4 weeks post-milk consumption

Each parameter was recorded as follows:

1. Demographic data: at I
2. Microbial evaluation: at I, T0, T1, T2, T3, T4
3. Salivary pH value: at I, T0, T1, T2, T3, T4
4. Plaque index (PI): at I, T0, T1, T2, T3, T4
5. The number of decayed, missing, and filled teeth (DMFT): at I and T4
6. The arbitrarily primed polymerase chain reaction (AP-PCR): at T0, T1, T2, T3, T4

The summary of all procedures are demonstrated in Diagram 1.

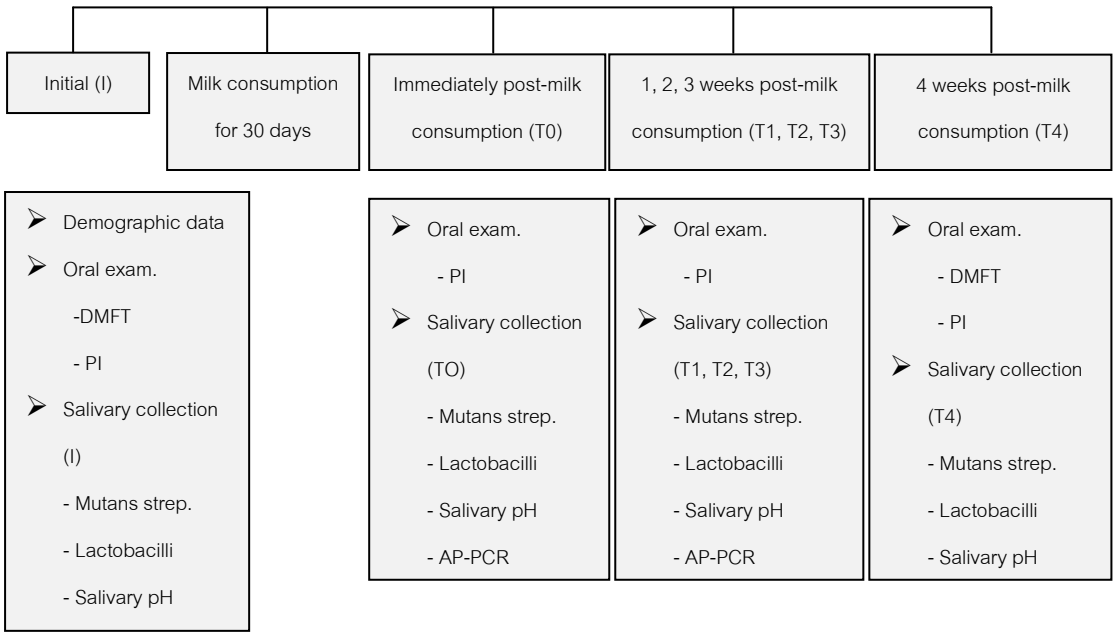
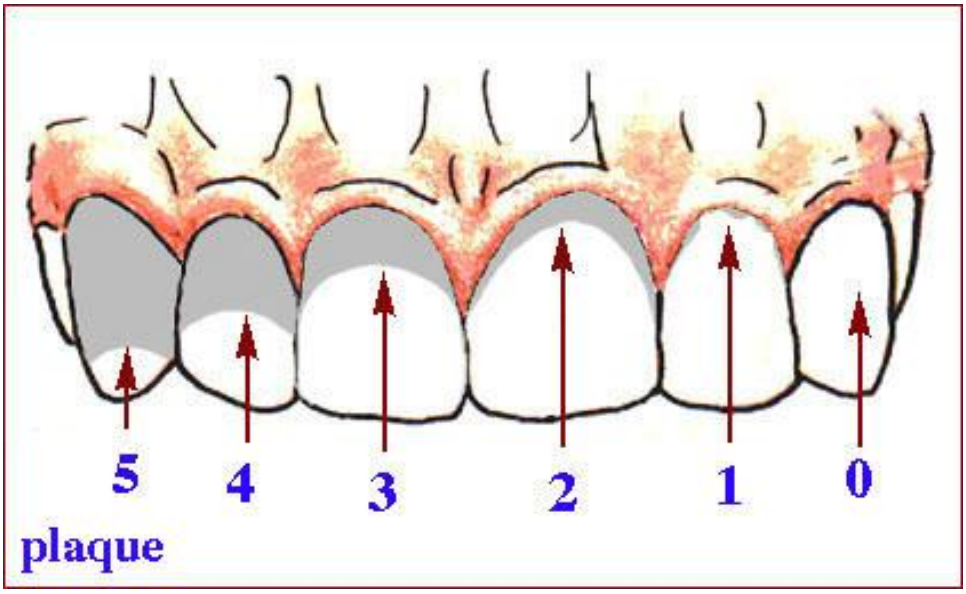


Diagram1: Clinical procedure

Plaque Index (PI)

Plaque Index (PI): Using Quigley and Hein[70], plaque index, 2 aspects of each tooth was examined: buccal and lingual. Plaque was disclosed with 0.2% erythrosine solution. Scores of 0 to 5 were given. Plaque score were expressed as the score number: A score of 0 or 1 is considered low, a score of 2 or more is considered high (Fig.2).



Plaque Scoring System for Quigley and Hein	Score
No plaque	0
Flecks of stain at the gingival margin	1
Definite line of plaque at the gingival margin	2
Gingival third of surface	3
Two thirds of surface	4
Greater than two thirds of surface	5

Fig.2: Plaque scoring system by Quigley and Hein[70]

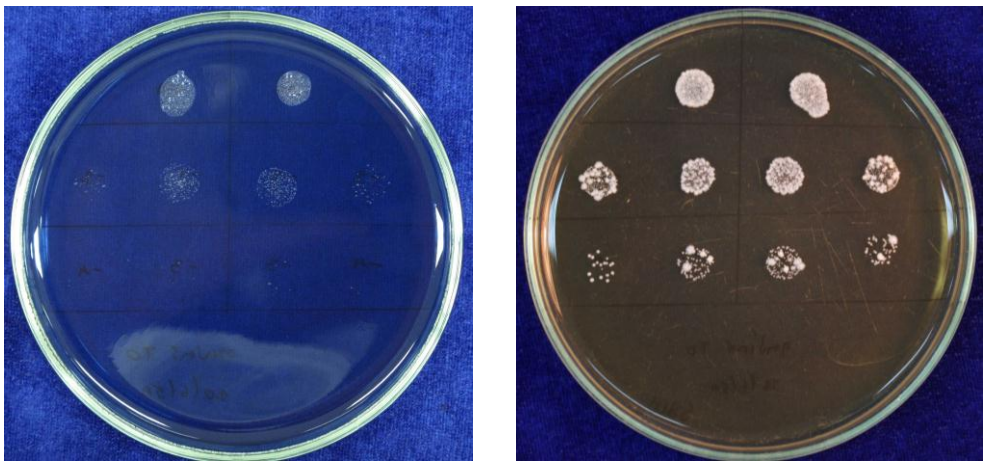
Salivary collection and bacterial culture

Unstimulated saliva of all subjects was collected for pH measurement. To determine salivary pH value, the reactive strip of the pH-indicator strip (Merck universal indicator strips) was submerged in non-stimulated saliva for 10 seconds; the color obtained was compared with the chart (Fig.3).

Bacterial evaluation was performed using an oral rinse method. Briefly, samples with sterile phosphate-buffered saline (PBS) an ten millimeters of sterile phosphate-buffered saline (PBS; 0.1 M; pH 7.0) was held in the mouth for 1 min prior to collection in a sterile container. Each rinse was centrifuged (4,000 rpm; 10 min), the supernatant was removed, and the pellet was resuspended in 1 ml of PBS. The mixture was diluted with sterile phosphate-buffered saline by serial dilution into 1:10, 1:100, 1:1000, 1:10,000 dilutions. A portion (10 µl) of each dilution was placed on selective agar plates. Selective media consisted of (1) Mitis salivarius bacitracin agar for determination of mutans streptococci was incubated in a candle jar (a carbondioxide-rich environment) at 37°C for 48 h, (2) MRS agar for determination of lactobacilli was incubated in an anaerobic chamber at 37°C for 48 h. The colonies were identified on the basis of their morphology and counted under a microscope with 10 times magnification and the latter were multiplied by the dilution factor to yield the CFU/ml. of the original oral rinse sample and converted to log10 value (Fig.4). All microbial evaluation was made in duplication at the same time by the same examiner.

The procedures were performed pre-milk consumption as I, immediately post-milk consumption as T0 and one week interval for four weeks post-milk consumption as T1, T2, T3, and T4.

Fig.3: The pH-indicator strip for determination of salivary pH value



A B

Fig.4: A; Mitis salivarius bacitracin agar for determination of mutans streptococci, B; MRS agar for determination of lactobacilli

Genotype investigation of *Lactobacillus paracasei* SD1

After incubation in MRS agar, the colonies were identified as lactobacilli based on their growth on MRS agar and colonial morphology. Then, 5 colonies / samples were collected from the primary culture plates. After pure culture, all isolates were brought to DNA extraction for identification for *Lactobacillus paracasei* SD1. DNA was extracted according to protocol of the manufacturer. The genotypic study for *Lactobacillus paracasei* SD1 were performed using an arbitrarily primed polymerase chain reaction[30] (AP-PCR) with the primers ERIC1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2R (5'-AAGTAAGTGACTGGGGTGAGCG-3'). The reaction mixture in a 50 µl reaction volume consisted of 100 ng template DNA, 5 ml 10× buffer, 1.0 unit Taq DNA polymerase, 0.2 mM of dNTP, 1.0 µM of each primer and 2.5 mM $MgCl_2$. Amplification proceeded using a PCR System 2400 (Applied Biosystems) programmed as follows: 35 cycles of denaturation at 95°C for 1 min, annealing at 35°C for 1 min and extension at 74°C for 2 min, with a final extension at 74°C for 5 min. PCR products were run on 7.5% polyacrylamide gel, stained with silver staining. The genotype patterns were compared between strains collected at each time interval (T0-T4) with the template of *Lactobacillus paracasei* SD1.

Data analysis

1. Microbial evaluation: The mutans streptococci and lactobacilli counts were compared pre (I) and post (T0, T1, T2, T3, T4) milk consumption
2. Salivary pH value: The salivary pH was compared pre (I) and post (T0, T1, T2, T3, T4) milk consumption
3. Plaque index were reported and compared pre (I) and post (T0, T1, T2, T3, T4) milk consumption
4. The DMFT were reported and compared pre (I) and post (T4) milk consumption
5. The oral persistence of *Lactobacillus paracasei* SD1 was reported the duration of the persistence of this strain in the oral cavity (T0-T4)

Statistical analysis

All microbial evaluation was made in duplication at the same time by the same examiner and calculated with averages to yield the CFU/ml.

Inferential test

The Shapiro-Wilk Test was conducted for proving normal distribution of data. All numeric data are presented with means and standard deviation. The log10 value of salivary mutans streptococci and lactobacilli counts within each group were statistically analyzed with a Paired t-test to compare pre and post-milk consumption but between the study and control groups were analyzed with an independent t-test. The salivary pH, DMFT and plaque index were statistically analyzed within the groups with the Wilcoxon Signed-Rank test and between the groups with Mann-Whitney U-test. A p-value < 0.05 was considered statistically significant.

CHAPTER 3

RESULTS

From 32 subjects at the beginning of the study, two of them were excluded due to one subject not drinking milk regularly and the other one could not return for a saliva collecting sample at a specified time period. The 30 subjects remaining in the study were categorized into two groups; 15 subjects in the probiotic group (8 females, 7 males, with mean age 19.0± 4.24 years) and 15 subjects (11 females, 4 males, with mean age 19.4± 3.11 years) in the control group (Table 1). All subjects completed the trial without any side effects reported. The records of salivary microbial parameters (mutans streptococci, lactobacilli) and dental health status (DMFT, salivary pH and plaque index) were demonstrated in Table 2 as initial data and there were no statistically significant differences between the groups related to the levels of salivary mutans streptococci and lactobacilli, DMFT, salivary pH and plaque index.

Table 1: Distribution of subjects in the probiotic and the control group

Group	Number of subjects			Mean ages
	Male (%)	Female (%)	Total (%)	(years ± SD)
Probiotic	7 (46.7)	8 (53.3)	15 (100)	19.01 ± 4.24
Control	4 (26.7)	11 (73.3)	15 (100)	19.42 ± 3.11
Total	11 (36.7)	19 (63.3)	30 (100)	19.22 ± 3.66

Table 2: Salivary microbial parameters and dental health status at initial data

Parameters	Control group	Probiotic group	*p*-value	Significant
	Mean scores	Mean scores		
Mutans streptococci[a]	5.51±0.58	5.90±0.72	0.109	NS
Lactobacilli[a]	6.40±0.53	6.34±0.51	0.776	NS
DMFT	5.8±2.62	6.33±3.33	0.753	NS
Salivary pH	6.58±0.46	6.51±0.43	0.859	NS
PI	2.96±0.38	3.04±0.47	0.885	NS

[a]The value in the table denotes the log10 CFU/ml.; PI, plaque index; NS, not significant

Microbial evaluation

1. Pre and post-milk consumption (within the group)

Mutans streptococci counts

After powdered milk consumption in the probiotic group, the salivary mutans streptococci counts at post milk consumption periods (T0-T4) decreased statistically significant ($p < 0.001$) when compared with the initial record. However, there were no statistically significant differences demonstrated in the control group (Table 3).

Table 3: Distribution of salivary mutans streptococci scores at pre and post-milk consumption (within the groups)

Groups	Mutans streptococci	*p*-value	Significant
	Mean scores		
Control group			
Initial	5.51±0.58		
T0	5.59±0.59	0.058	NS
T1	5.54±0.57	0.110	NS
T2	5.68±0.57	0.085	NS
T3	5.51±0.52	0.512	NS
T4	5.58±0.53	0.109	NS
Probiotic			
Initial	5.90±0.72		
T0	4.92±0.66	< 0.001	**
T1	4.99±0.63	< 0.001	**
T2	5.06±0.62	< 0.001	**
T3	4.99±0.54	< 0.001	**
T4	5.05±0.59	< 0.001	**

[a]The value in the table denotes the log10 CFU/ml.; ** $p < 0.001$; NS, not significant

Lactobacilli counts

In the probiotic group, the salivary lactobacilli counts at post milk consumption periods (T0-T4) increased statistically significant ($p < 0.001$) when compared with the initial record. However, there were no statistically significant differences demonstrated among the subjects in the control group (Table 4).

Table 4: Distribution of salivary lactobacilli scores at pre and post-milk consumption (within the groups)

Groups	Lactobacilli	*p*-value	Significant
	Mean scores		
Control group			
Initial	6.40±0.53		
T0	6.36±0.5	0.480	NS
T1	6.33±0.51	0.207	NS
T2	6.34±0.63	0.155	NS
T3	6.35±0.55	0.197	NS
T4	6.33±0.49	0.084	NS
Probiotic			
Initial	6.34±0.51		
T0	7.22±0.72	< 0.001	**
T1	7.20±0.66	< 0.001	**
T2	7.19±0.75	< 0.001	**
T3	7.12±0.62	< 0.001	**
T4	6.96±0.56	< 0.001	**

[a]The value in the table denotes the log10 CFU/ml., ** $p < 0.001$; NS, not significant

2. Pre and post-milk consumption (between the groups)

When comparing between the groups, there were no statistical significance at the initial record but the decreased salivary mutans streptococci count and the increased lactobacilli count at post milk consumption periods (T0-T4) were statistically significant ($p < 0.05$) (Table 5, 6).

Table 5: Distribution of salivary mutans streptococci score at pre and post-milk consumption (between the groups)

Time	Control group	Probiotic group	*p*-value	Significant
	Mean scores	Mean scores		
Initial	5.51±0.58	5.90±0.72	0.109	NS
T0	5.59±0.59	4.92±0.66	0.007	*
T1	5.54±0.57	4.99±0.63	0.020	*
T2	5.68±0.57	5.06±0.62	0.019	*
T3	5.51±0.52	4.99±0.54	0.019	*
T4	5.58±0.53	5.05±0.59	0.018	*

[a]The value in the table denotes the log10 CFU/ml., * $p < 0.05$; NS, not significant

Table 6: Distribution of salivary lactobacilli score at pre and post-milk consumption (between the groups)

Time	Control group	Probiotic group	*p*-value	Significant
	Mean scores	Mean scores		
Initial	6.40±0.53	6.34±0.51	0.776	NS
T0	6.36±0.5	7.22±0.72	0.001	*
T1	6.33±0.51	7.20±0.66	0.001	*
T2	6.34±0.63	7.19±0.75	0.007	*
T3	6.35±0.55	7.12±0.62	0.003	*
T4	6.33±0.49	6.96±0.56	0.003	*

[a]The value in the table denotes the log10 CFU/ml., * $p < 0.05$; NS, not significant

Salivary pH value

There were no statistically significant changes of salivary pH within the groups and between the group throughout the study (Table 7 and Fig 5).

Table 7: Distribution of salivary pH value at pre and post-milk consumption (between the groups)

Time	Control group	Probiotic group	*p*-value	Significant
	Mean scores	Mean scores		
Initial	6.58±0.46	6.51±0.43	0.859	NS
T0	6.62±0.45	6.56±0.42	0.650	NS
T1	6.61±0.44	6.59±0.42	0.925	NS
T2	6.66±0.43	6.62±0.46	0.948	NS
T3	6.63±0.42	6.64±0.42	0.752	NS
T4	6.62±0.40	6.60±0.47	0.857	NS

NS; not significant

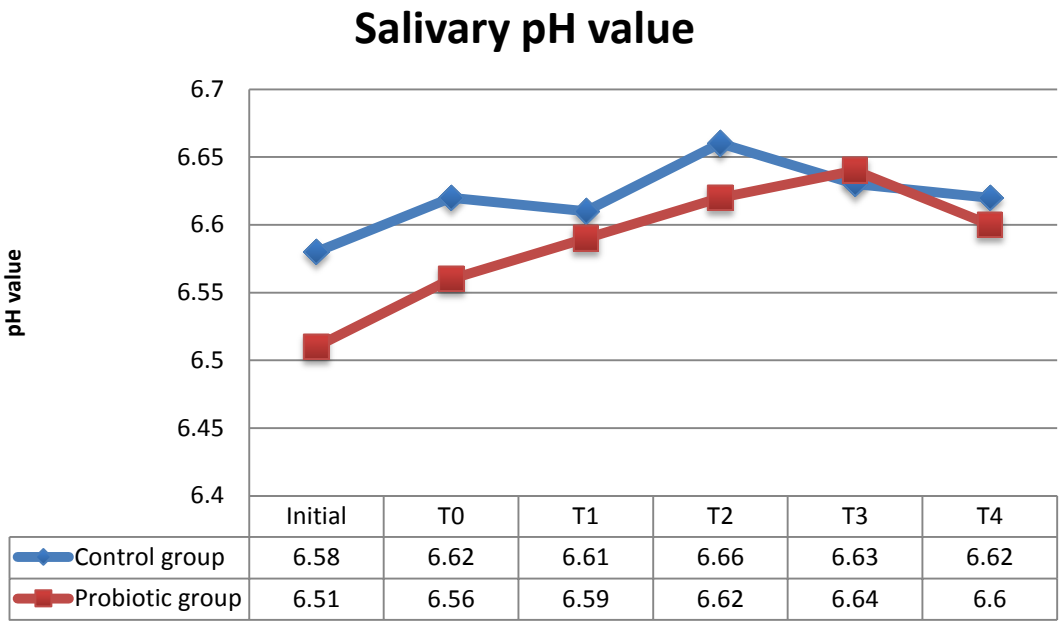


Fig 5: Salivary pH value at pre and post-milk consumption, no statistically significant changes of salivary pH value within the groups and between the groups throughout the study.

Plaque index value

There were no statistically significant changes of plaque index value within the groups and between the groups throughout the study (Table 8 and Fig. 6).

Table 8: Distribution of plaque index scores at pre and post-milk consumption (between the groups)

Time	Control group	Probiotic group	*p*-value	Significant
	Mean scores	Mean scores		
Initial	2.96±0.38	3.04±0.47	0.885	NS
T0	2.92±0.35	3.02±0.47	0.724	NS
T1	2.79±0.39	3.03±0.46	0.301	NS
T2	2.86±0.38	3.14±0.49	0.198	NS
T3	2.91±0.38	3.01±0.50	0.918	NS
T4	2.92±0.32	3.03±0.44	0.663	NS

NS; not significant

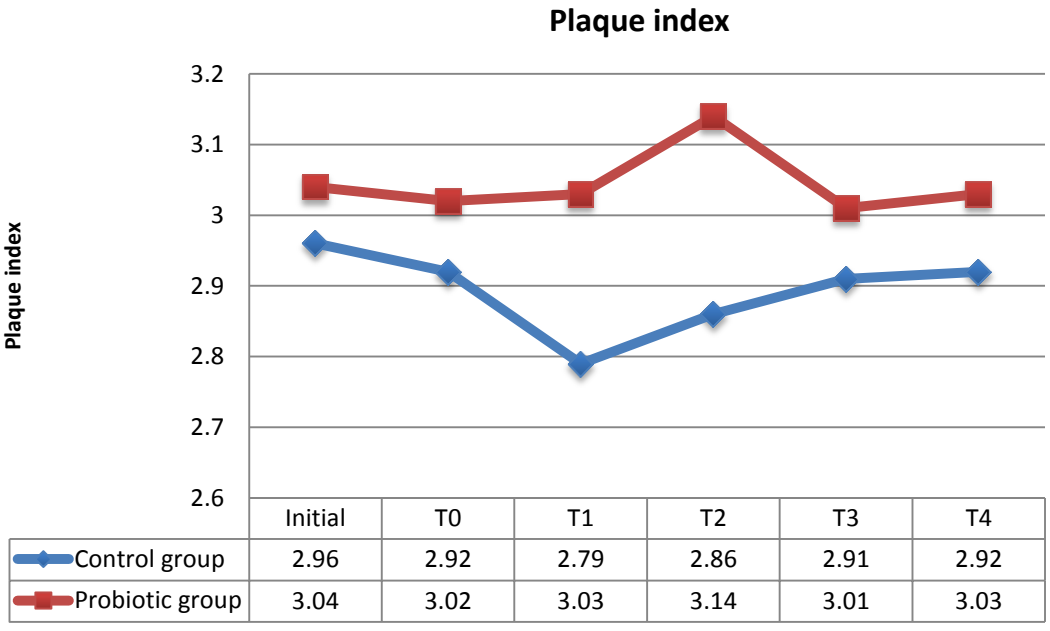


Fig 6: Plaque index value at pre and post-milk consumption, no statistically significant changes of plaque index value within the groups and between the groups throughout the study.

The number of decayed, missing, and filled teeth (DMFT)

There were no changes of DMFT in the probiotic groups and the control group throughout the study (Fig.7)

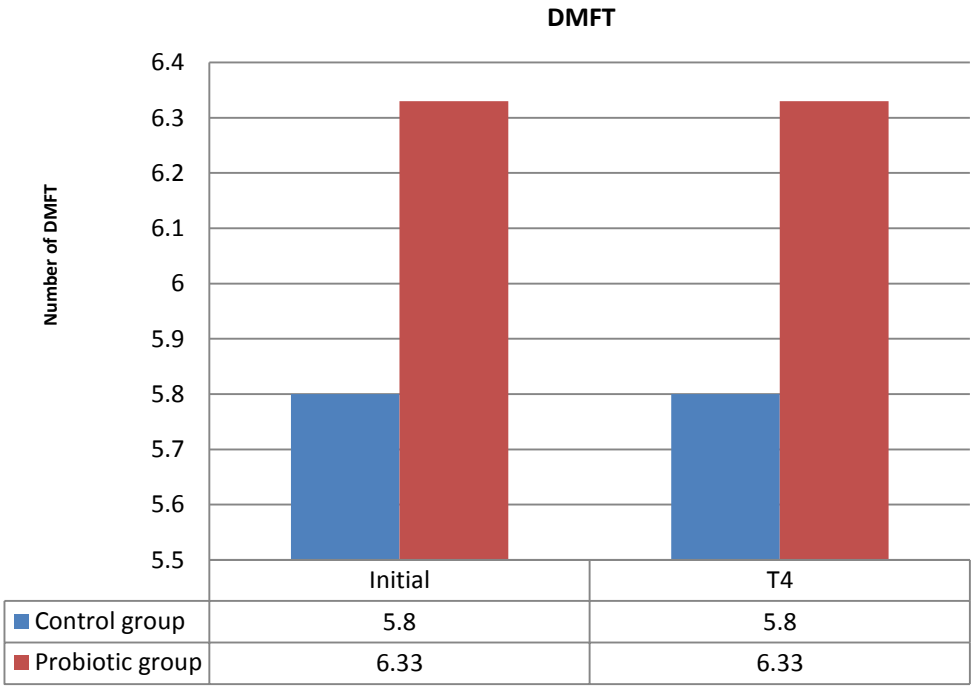


Fig7: DMFT at pre and post-milk consumption, no changes of DMFT in the probiotic groups and the control group throughout the study.

The oral persistence of *Lactobacillus paracasei* SD1

In the present study, an arbitrarily primed polymerase chain reaction (AP-PCR) was used to detect genomic DNA and evaluate the persistence of *Lactobacillus paracasei* SD1. The genotype patterns of *Lactobacillus paracasei* SD1 found at post milk consumption periods (T0-T4) in the probiotic group were the same as the genotype pattern template of *Lactobacillus paracasei* SD1 (Fig 8).

A. B. A. C.

Fig 8: The oral persistence of *Lactobacillus paracasei* SD1; A. genotype pattern template of *Lactobacillus paracasei* SD1, B. genotype pattern of lactobacilli at T0, C. genotype pattern of lactobacilli at T4 in the probiotic group.

CHAPTER 4

DISCUSSION

Probiotics have been found to be advantageous in combating various diseases such as gastro-intestinal infections, cancer prevention, irritable bowel syndrome, constipation, periodontal diseases, dental caries and many others.[71,72] As the mouth represents the first part of the gastrointestinal tract there is every reason to believe that at least some probiotic mechanisms may also play a role in this part of the system.[15] Although many studies with *L. rhamnosous* GG, *L. reuteri* have defined their potential in interacting with *Streptococcus mutans* by reducing the number of this caries pathogen, thus suggesting a role of probiotics in caries prophylaxis[9,38] but the potential beneficial effects of probiotics containing these microorganism have had only limited study. *Lactobacillus paracasei* SD1[73] is a normal oral microorganism which was isolated from the mouths of caries-free children and powdered milk containing *Lactobacillus paracasei* SD1 was chosen as the vehicle for the probiotic supplement in this study because of its convenience to storage and use, long shelf life, health benefits, and low cariogenic potential.

In this study, the effect of short-term consumption of powdered milk containing *Lactobacillus paracasei* SD1 in cleft lip and palate patients were undergoing treatment with fixed orthodontic appliances who display higher caries prevalence due to their specific oral environment[1,74]was investigated. The intervention had started at 3 months after the orthodontic fixed appliances were placed to avoid the confounding effect of an immediate decrease in bacterial counts that may take place at appliance insertion.[75] The subjects in this study had no recent antibiotic therapy within the past 2 weeks, no active untreated carious lesions and were not using any other commercially available probiotic products to prevent the effect that influences on mutans streptococci, lactobacilli counts, and which also control the pretreatment microbial counts.

In the present study, the results showed that daily consumption of powdered milk containing *Lactobacillus paracasei* SD1 for 30 days statistically significant decrease mutans streptococci counts in saliva during the post-milk consumption period compared with the control group that reinforced several previous findings with bifidobacteria and lactobacilli-derived probiotics.[10,11,12] The reason for the bacteria hindering effect is not fully known but may be

explained by several pathways; They prevent cellular adhesion and invasion of pathogenic bacteria[76]and mutans streptocooci[77] or probiotics may competitively inhibit streptococci by replacement because of the direct contact with the oral tissue and biofilm.[78] They interact and modulate the local and systemic inflammatory immune response.[22]

In accordance with previous studies, no effect on the level of salivary lactobacilli were note; Caglar *et al*.[12] found that lactobacillus levels were unchanged after receiving the lozenges or straws containing *L. reuteri* ATCC 55730 for 3 weeks and Cildir *et al.*[10] showed that no significant alterations of the salivary lactobacilli counts were observed after ingesting fruit yogurt containing *Bifidobacterium animalis* subsp. *lactis* DN-173010. In this study, the daily consumption of powdered milk containing *Lactobacillus paracasei* SD1 for 30 days showed a statistically significant increase in lactobacilli count in the probiotic group compared with the control group during the post-milk consumption period which was different from previous studies. The bacterial strain used in each study was isolated from different sites of the human body. *L. reuteri* ATCC 55730 and *Bifidobacterium animalis* subsp. *lactis* DN-173010 were isolated from human intestine while *Lactobacillus paracasei* SD1 was a normal flora that was isolated from oral cavities in caries-free children.[73] Because our bodies contain numerous different environments and each environment possesses certain advantages and disadvantages and different microorganisms have adapted to certain regions of the body for their particular needs, so *Lactobacillus paracasei* SD1 may has better ability to adhere to oral epithelial cells than the others.

In addition, when using PCR to detect genomic DNA and evaluate the oral persistence of *Lactobacillus paracasei* SD1, the genotype patterns of *Lactobacillus paracasei* SD1 were found in all cases of the post-milk consumption period in the probiotic group. This finding confirmed that *Lactobacillus paracasei* SD1 was permanently colonized longer in the oral cavity than others which differed from previous studies.[13,22]

There were no statistically significant changes among the probiotic groups in salivary pH and DMFT during the post-milk consumption period. This result was reinforced in the in vitro study[79] that this strain had less acidic produce than the other strains. This might imply that the powdered milk containing *Lactobacillus paracasei* SD1 had no apparent adverse effect on salivary pH. Moreover, there were no statistically significant changes in the plaque index at pre-milk consumption and during the post-milk consumption period. This finding supported the

result, a statistically significant decrease of mutans steptococci and increases of lactobacilli as a result of powdered milk containing *Lactobacillus paracasei* SD1 only and was not affected by the amount of plaque deposits on the teeth. Although, it was found that there were no significant changes in plaque index, salivary pH and DMFT at pre and post-milk consumption period. This finding showed that during the study period the subjects oral ecology were stable and was interested for further study whether it was effected by this intervention or not.

In this study, without any adverse effect of probiotic milk consumption, it could be concluded that *Lactobacillus paracasei* SD1 is a normal oral microorganism suitable and safe for use as a potentially probiotic in the oral cavity.

Regarding further study, it would be interesting to study the long-term effect and mechanism of probiotic bacteria on the oral microbial counts more clearly with an increased the sample size. The results of the present study showed that probiotic intervention could be beneficial to those with the highest mutans streptococci. In any case, it seems obvious that cleft lip and palate patients with fixed orthodontic appliances constitute a very suitable group for further study.

CHAPTER 5

CONCLUSIONS

The present study demonstrated that short-term consumption of powdered milk containing *Lactobacillus paracasei* SD1 could statistically significant decreased the salivary levels of mutans streptocooci and increased of lactobacilli count in treated with fixed orthodontic appliance cleft patients. Moreover, *Lactobacillus paracasei* SD1 DNA were found in the oral cavity during 4 weeks post - milk consumption period. No apparent adverse effect of probiotic milk consumption during the trail was registered. Further studies are needed to clarify the long term effect of *Lactobacillus paracasei* SD1 as an alternative probiotic for caries prevention in oral cavity.

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APPENDICES

ที่ ศธ 0521.1.03/ 520

คณะทันตแพทยศาสตร์
มหาวิทยาลัยสงขลานครินทร์
ตู้ไปรษณีย์เลขที่ 17
ที่ทำการไปรษณีย์โทรเลขคอหงส์
อ.หาดใหญ่ จ.สงขลา 90112

หนังสือฉบับนี้ให้ไว้เพื่อรับรองว่า

โครงการวิจัยเรื่อง "ผลของนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวันต่อเชื้อมิแทนสเตรปโตคอคไคและแลคโตบาซิลไลในน้ำลายของผู้ป่วยปากแหว่งเพดานโหว่ที่ได้รับการจัดฟัน"

หัวหน้าโครงการ ทันตแพทย์หญิงชลธิรา แซ่ตั้ง

สังกัดหน่วยงาน นักศึกษาหลังปริญญา ภาควิชาทันตกรรมป้องกัน คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์

ได้ผ่านการพิจารณาและได้รับความเห็นชอบจากคณะกรรมการจริยธรรมในการวิจัย (Ethics Committee) ซึ่งเป็นคณะกรรมการพิจารณาศึกษาการวิจัยในคนของคณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ แล้ว ในคราวประชุมครั้งที่ 6/2553 เมื่อวันที่ 23 ธันวาคม 2553

ให้ไว้ ณ วันที่ 11 พ.ค. 2554

(รองศาสตราจารย์ ดร.รวี เถียรไพศาล)
รองคณบดีฝ่ายวิจัย
ประธานกรรมการ

.....กรรมการ
(ผู้ช่วยศาสตราจารย์ ทพ.นพ.สุรพงษ์ วงศ์วัชรานนท์)

.....กรรมการ
(ผู้ช่วยศาสตราจารย์ ทพญ.สรียา ศรีสินทร)

.....กรรมการ
(ผู้ช่วยศาสตราจารย์ ดร.ทพญ.อังคณา เธียรมนตรี)

.....กรรมการ
(ผู้ช่วยศาสตราจารย์ นพ.พรชัย สถิรปัญญา)

.....กรรมการ
(อาจารย์วิจารณ์ หอประยูร)

.....กรรมการ
(อาจารย์วศิน สุวรรณรัตน์)

.....กรรมการ
(อาจารย์ ทพญ.สุพัชรินทร์ พิวัฒน์)

.....กรรมการ
(อาจารย์ ทพ.กมลพันธ์ เนื่องศรี)

ใบเชิญชวน

ขอเชิญเข้าร่วมโครงการวิจัยเรื่อง ผลของนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวันต่อเชื้อมิวแทนสเตรบโตคอคไคและแลคโตบาซิลไลในน้ำลายของผู้ป่วยปากแหว่งเพดานโหว่ที่ได้รับการจัดฟัน

เรียน ท่านผู้อ่านที่นับถือ

ข้าพเจ้า ทพญ.ชลธิรา แซ่ตั้ง นักศึกษาระดับปริญญาโท สาขาทันตกรรมจัดฟัน ภาควิชาทันตกรรมป้องกัน คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ขอแจ้งรายละเอียดเกี่ยวกับโครงการวิจัยและขอเชิญชวนท่านผู้สนใจเข้าร่วมโครงการวิจัยดังนี้

โครงการวิจัยนี้จัดทำเพื่อศึกษาและประเมินผลการเปลี่ยนแปลงของปริมาณเชื้อมิวแทนสเตรบโตคอคไคและแลคโตบาซิลไลในน้ำลายของผู้ป่วยปากแหว่งเพดานโหว่ที่ได้รับการจัดฟันจากการรับประทานนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวันและการคงอยู่ของเชื้อแลคโตบาซิลลัสพาราเคซิอายเอสดีวันภายในช่องปากหลังรับประทานนม

ในผู้ป่วยปากแหว่งเพดานโหว่ที่ได้รับการจัดฟันจะมีความเสี่ยงในการเกิดฟันผุและปัญหาในช่องปากที่มากกว่าผู้ป่วยที่ไม่ได้รับการจัดฟันอันเนื่องมาจากการติดเครื่องมือจัดฟันชนิดติดแน่นจะส่งเสริมการยึดเกาะของแบคทีเรียซึ่งจะส่งผลให้มีการเพิ่มจำนวนของแบคทีเรียที่ก่อโรคฟันผุ โพรไบโอติกคือแบคทีเรียที่มีประโยชน์และเมื่อได้รับในปริมาณที่เหมาะสมจะเป็นประโยชน์สำหรับสุขภาพ กลไกของโพรไบโอติกที่มีผลต่อการเสริมสร้างสุขภาพในช่องปาก คือ 1) ลดจำนวนจุลินทรีย์ที่ก่อโรคโดยการแย่งอาหารในการเจริญเติบโต 2) แข่งขันเพื่อพื้นที่สำหรับการเกาะติด 3) สร้างสารที่เป็นพิษต่อจุลินทรีย์ที่ก่อโรค 4) เสริมสร้างระบบภูมิคุ้มกันทั้งแบบเฉพาะที่และทั้งระบบ

ได้มีการศึกษามาแล้วในภาคโอษฐวิทยา คณะทันตแพทยศาสตร์มหาวิทยาลัยสงขลานครินทร์เกี่ยวกับเชื้อแลคโตบาซิลลัสพาราเคซิอายเอสดีวัน ในขั้นต้นพบว่าแลคโตบาซิลลัสที่แยกได้จากช่องปากของเด็กโดยเฉพาะสายพันธุ์พาราเคซิอายเอสดีวันที่แยกได้จากช่องปากของเด็กที่ไม่มีฟันผุมีข้อดีหลายประการคือ 1) เป็นสายพันธุ์หนึ่งที่มีความสามารถสูงในการยับยั้งการเจริญเติบโตของเชื้อซึ่งเป็นแบคทีเรียสาเหตุของฟันผุ 2)แลคโตบาซิลลัสพาราเคซิอายเอสดีวันสร้างกรดได้น้อยกว่าเมื่อเปรียบเทียบเชื้อสายพันธุ์อื่นๆ ที่มีความสามารถในการยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียที่เป็นสาเหตุของฟันผุได้ในระดับที่เท่ากัน 3)แลคโตบาซิลลัสพาราเคซิอายเอสดีวัน สามารถเกาะติดเยื่อบุผิวในช่องปากได้ดี จึงกล่าวได้ว่าเชื้อแลคโตบาซิลลัสพาราเคซิอายเอสดีวัน เป็นสายพันธุ์ที่เหมาะในการใช้เป็นโพรไบโอติกในช่องปาก หากการศึกษานี้แสดงผลว่านมผงผสมแลคโตบาซิลลัสพาราเคซิอายเอสดีวันสามารถลดปริมาณเชื้อที่ก่อให้เกิดโรคฟันผุได้และคงอยู่ภายในช่องปากได้เป็นระยะเวลานานรวมทั้งไม่ส่งผลเสียใดๆแก่ผู้ป่วย ในอนาคตอาจใช้วิธีการนี้เพื่อใช้ลดปริมาณเชื้อที่ก่อให้เกิดโรคฟันผุในผู้ป่วยที่มีลักษณะปากแหว่งเพดานโหว่และได้รับการจัดฟันได้

ถ้าท่านตัดสินใจเข้าร่วมในโครงการนี้จะมีขั้นตอนของการศึกษาที่เกี่ยวข้องกับท่านคือ ผู้เข้าร่วมวิจัยจะได้รับการตรวจและบันทึกข้อมูลซึ่งประกอบด้วยการซักประวัติ ตรวจนอกช่องปากและภายในช่องปาก ย้อมสีฟันเพื่อตรวจปริมาณคราบจุลินทรีย์ที่ผิวฟันในครั้งแรกที่มารับการรักษาและทำการเก็บตัวอย่างน้ำลายของผู้เข้าร่วมโครงการวิจัย หลังจากนั้นผู้เข้าร่วมโครงการวิจัยจะถูกแบ่งแบบสุ่มเป็นกลุ่มทดลอง และกลุ่มควบคุม ท่านอาจถูกจัด

ให้เข้าในกลุ่มทดลองซึ่งจะได้รับนมผงที่มีเชื้อแลคโตบาซิลลัสพาราเคซิอายเอสดีวันหรือกลุ่มควบคุมซึ่งได้รับนมผงปกติที่ไม่มีเชื้อโพรไบโอติก โดยรับประทานนมผงปริมาณ 10 กรัมผสมกับน้ำดื่ม 50 มิลลิลิตรวันละ 1 ครั้ง ในตอนเช้าโดยหลังจากรับประทานนมงดการแปรงฟันอย่างน้อย 1ชั่วโมง เป็นระยะเวลา 30 วัน เมื่อครบ 30 วัน ทำการเก็บตัวอย่างน้ำลายของผู้เข้าร่วมโครงการวิจัย เพื่อตรวจนับเชื้อต่าง ๆ และวัดค่าความเป็นกรดด่างของน้ำลาย สัปดาห์ละ 1 ครั้ง เป็นเวลา 1 เดือน รวมเป็นจำนวน 5 ครั้ง ผู้ป่วยจะต้องมารับการรักษาทั้งสิ้นประมาณ 7 ครั้ง โดยใช้เวลาในการรักษาแต่ละครั้ง ดังนี้คือ

- ซักประวัติและตรวจครั้งแรก 1 ชั่วโมง
- รับนมผงและข้อปฏิบัติระหว่างการศึกษา 1 ชั่วโมง
- เก็บน้ำลายครั้งละ ½ ชั่วโมง จำนวน 5 ครั้ง

หลังจากนั้นผู้ป่วยจะได้รับการรักษาทางทันตกรรมจัดฟันตามขั้นตอนมาตรฐานต่อไป

ถ้าท่านตัดสินใจเข้าร่วมโครงการวิจัยนี้ จะมีขั้นตอนของการวิจัยที่จำเป็นต้องขอความร่วมมือของท่านตามที่กล่าวมาข้างต้น และผู้เข้าร่วมโครงการฯต้องมารับการรักษาและติดตามผล ณ คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์โดยไม่เสียค่าใช้จ่ายใดๆทั้งสิ้น เนื่องจากอยู่ในโครงการความร่วมมือการรักษาผู้ป่วยปากแหว่งเพดานโหว่

จากการศึกษาไม่พบผลข้างเคียงของการรับประทานผลิตภัณฑ์นมที่ผสมโพรไบโอติกแต่หากเกิดผลข้างเคียงก็เป็นผลข้างเคียงที่ป้องกันได้ หรือรักษาได้โดยง่าย มีความปลอดภัยต่อชีวิตและการดำรงชีวิตในกรณีผู้เข้าร่วมการศึกษาเกิดอาการผิดปกติจากการบริโภคนมที่ได้รับจากโครงการ เช่น ท้องเสีย มีไข้ ผู้เข้าร่วมการศึกษาสามารถติดต่อผู้ดำเนินการวิจัยได้ตลอด 24 ชั่วโมง ตามเบอร์โทรศัพท์ที่ได้ให้ไว้โดยหากผู้เข้าร่วมการศึกษาได้รับผลเสีย หรืออันตรายใดๆ ที่พิสูจน์ได้ว่าเป็นผลที่เกิดจากการดื่มนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวันทางคณะผู้ดำเนินการวิจัยจะรับผิดชอบค่ารักษาทั้งหมดตามมาตรฐานการรักษาของโรงพยาบาลสงขลานครินทร์

ไม่ว่าท่านจะเข้าร่วมในโครงการวิจัยนี้หรือไม่ ท่านจะยังคงได้รับการรักษาที่ดีเช่นเดียวกับผู้ป่วยคนอื่นๆ และถ้าท่าน ต้องการที่จะถอนตัวออกจากการศึกษานี้เมื่อใด ท่านก็สามารถกระทำได้อย่างอิสระ ถ้าท่านมีคำถามใดๆ ก่อนที่จะตัดสินใจก่อน เข้าร่วมโครงการนี้ โปรดซักถามคณะผู้วิจัยได้อย่างเต็มที่

ขอขอบคุณอย่างสูง

.....

ทพญ.ชลธิรา แซ่ตั้ง

(หัวหน้าโครงการ)

หมายเหตุ :- กรุณาอ่านข้อความให้เข้าใจก่อนเซ็นชื่อยินยอมเข้าร่วมโครงการ

แบบยินยอมเข้าร่วมการศึกษา

โครงการวิจัยเรื่อง ผลของนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวันต่อเชื้อมิวแทนสเตรบโตคอคไคและแลคโตบาซิลไลในน้ำลายของผู้ป่วยปากแหว่งเพดานโหว่ที่ได้รับการจัดฟัน

วันที่_____เดือน_____พ.ศ._____

ข้าพเจ้า_____อายุ_____ปี อาศัยอยู่บ้านเลขที่_____หมู่_____ถนน_____ตำบล_____อำเภอ_____จังหวัด_____ ได้รับการอธิบายถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตรายที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่จะเกิด ขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

หากข้าพเจ้ามีข้อสงสัยประการใด หรือเกิดผลข้างเคียงจากการวิจัยจะสามารถติดต่อกับ ทพญ.ชลธิรา แซ่ตั้ง ได้ที่ ภาควิชาทันตกรรมป้องกัน คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ หมายเลขโทรศัพท์ 084-8553682 หรือ เมื่อมีปัญหาใดๆ เกิดขึ้นเนื่องจากการทำวิจัยในเรื่องนี้ ข้าพเจ้าสามารถร้องเรียนได้ที่คณบดี คณะทันตแพทยศาสตร์ มหาวิทยาลัย สงขลานครินทร์ อ.หาดใหญ่ จ.สงขลา 90112 หมายเลขโทรศัพท์ 074-287510

จากการศึกษาไม่พบผลข้างเคียงของการรับประทานผลิตภัณฑ์นมที่ผสมโพรไบโอติกในกรณีผู้เข้าร่วมการศึกษาเกิดอาการผิดปกติจากการบริโภคนมที่ได้รับจากโครงการ เช่น ท้องเสีย มีไข้ ผู้เข้าร่วมการศึกษาสามารถติดต่อผู้ดำเนินการวิจัยได้ตลอด 24 ชั่วโมง ตามเบอร์โทรศัพท์ที่ได้ให้ไว้โดยหากผู้เข้าร่วมการศึกษาได้รับผลเสียหรืออันตรายใดๆ ที่พิสูจน์ได้ว่าเป็นผลที่เกิดจากการดื่มนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวัน ทางคณะผู้ดำเนินการวิจัยจะรับผิดชอบค่ารักษาทั้งหมดตามมาตรฐานการรักษาของโรงพยาบาลสงขลานครินทร์

หากผู้วิจัยมีข้อมูลเพิ่มเติมทั้งทางด้านประโยชน์และโทษที่เกี่ยวข้องกับการวิจัยนี้ ผู้วิจัยจะแจ้งให้ข้าพเจ้าทราบอย่างรวดเร็วโดยไม่มีปิดบัง

ข้าพเจ้ามีสิทธิ์ที่จะของดการเข้าร่วมโครงการวิจัย โดยจะแจ้งให้ทราบล่วงหน้าโดยการงดการเข้าร่วมการวิจัยนี้จะไม่มีผลต่อการได้รับบริการหรือการรักษาที่ข้าพเจ้าจะได้รับแต่อย่างใด

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะที่เกี่ยวกับตัวข้าพเจ้าเป็นความลับ จะไม่เปิดเผยข้อมูลหรือผลการวิจัยของข้าพเจ้าเป็นรายบุคคลต่อสาธารณชน จะเปิดเผยได้ในรูปที่เป็นสรุปผลการวิจัย หรือการเปิดเผยข้อมูลต่อผู้มีหน้าที่ที่เกี่ยวข้องกับการสนับสนุนและกำกับดูแลการวิจัย

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ จึงได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ โดย ผู้วิจัยได้ให้สำเนาแบบยินยอมที่ลงนามแล้วกับข้าพเจ้าเพื่อเก็บไว้เป็นหลักฐานจำนวน 1 ชุด

ลงชื่อ.....ผู้ยินยอม

( )

ลงชื่อ.....ผู้รับผิดชอบโครงการวิจัย

(ทพญ.ชลธิรา แซ่ตั้ง)

ลงชื่อ.....บิดา/ผู้ใช้อำนาจปกครอง

( )

ลงชื่อ.....มารดา/ผู้ใช้อำนาจปกครอง

( )

ลงชื่อ.....พยาน

( )

ลงชื่อ.....พยาน

( )

ข้อมูลผู้ป่วย

ข้อมูลทั่วไป

ชื่อ-สกุล.....

HN.....ON.....

เพศ ☐ ชาย ☐ หญิง วันเกิด.....อายุ.....

ที่อยู่.....

เบอร์โทรศัพท์.....

ชนิดของ Cleft.....

โรคประจำตัว……

ท่านได้รับประทานยาปฏิชีวนะหรือยาฆ่าเชื้ออื่นๆภายใน 2 สัปดาห์นี้หรือไม่

☐ไม่..... ☐ใช่(ระบุชื่อยา).....

ประวัติการแพ้นม ☐ไม่..... ☐ใช่(ระบุชนิดของนม).....

ข้อมูลการจัดฟัน

1.ลักษณะของเครื่องมือจัดฟันชนิดติดแน่น

☐ Fixed full mouth (จำนวนซี่.....)

☐ Partial fix (จำนวนซี่…….....)

☐ อื่นๆ.....

2. วันที่เริ่มติดเครื่องมือ.....

3. ชื่อทันตแพทย์.....

การตรวจภายในช่องปาก

1. DMFT.....

D..... (ระบุจำนวน)

M.....

F.....

3. Salivary pH.....

4. *S. mutans* (I)……

5. *Lactobacillus* (I)……

6. Candida (I)……

2. Plaque index (PI) ……

3. T0 Plaque index (PI) ……

Salivary pH……

ตารางบันทึกช่วงเวลาในการรับประทานนม

วันที่	เวลา	หมายเหตุ
1……..		
2……..		
3……..		
4……..		
5……..		
6……..		
7……..		
8……..		
9……..		
10……..		
11……..		
12……..		
13……..		
14……..		
15……..		
16……..		
17……..		
18……..		
19……..		
20……..		
21……..		
22……..		
23……..		
24……..		
25……..		
26……..		
27……..		
28……..		
29……..		
30……..		

VITAE

Name Miss Chontira Saetang

Student ID 5310820017

Educational Attainment

Degree	Name of Institution	Year of Graduation
Doctor of Dental Surgery	Srinakharinwirot University	2005

Work-Position and Adress

Dental Department, Nongkae hospital, Saraburi, Thailand

List of Publication and Proceeding

Saetang C, Teanpaisan R, Ritthagol W. Effect of powdered milk containing *Lactobacillus paracasei* SD1 on salivary mutans streptococci in the orthodontic cleft patients. Proceedings of the 23rd National Graduate Research Conference; 2011 December 23-24; Nakhon Ratchasima, Thailand. Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan; 2011