EFFECTS OF *LACTOBACILLUS SPP.* AGAINST VAGINAL CANDIDIASIS IN PREGNANT WOMEN

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EFFECTS OF *LACTOBACILLUS SPP.* AGAINST VAGINAL CANDIDIASIS IN PREGNANT WOMEN

by

ANG XIN YEE

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
BCAAs	Branched-chain amino acids
BSH	Bile Salt Hydrolase
BV	Bacterial vaginosis
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
CST	Community state types
CVL	Cervico vaginal lavage
DASS	Depression anxiety stress scales
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EMP	Embden-Meyerhof pathway
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FRT	Female reproductive tract
G6PD	Glucose-6-phosphate dehydrogenase
GI	Gastrointestinal
HACCP	Hazard Analysis Critical Control Point
HIV	Human immunodeficiency virus
HVS	High vagina swab
IFN-γ	Interferon-γ
IL-10	Interleukin-10
IL-1α	Interleukin-1 alpha

IL-1β	Interleukin-1 beta
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
ISO	International Organization for Standardization
JAKIM	Department of Islamic Development Malaysia
LAB	Lactic acid bacteria
LBW	Low birth weight
LVS	Low vagina swab
NF-κB	Nuclear factor--kappa B
O & G	Obstetrics and gynaecology
PKP	Phosphoketolase pathway
qPCR	Quantitative polymerase Chain Reaction
RBC	Red blood cells
RNase	Ribonuclease
RTV	Reccurent trichomoniasis
RVVC	Reccurent Vulvovaginal candidiasis
RVVI	Reccurent Vulvovaginal candidiasis
SGLT2	Sodium-glucose cotransporter-2
Th	T helper
THIDA	Taiwan Halal Integrity Development Association
TLR	Toll-like receptor
TNF-α	Tumour Necrosis Factor alpha
TQF	Taiwan Quality Food
TV	Trichomoniasis

UV	Ultraviolet
VEC	Virginia Employment Commission
VMB	Vagina microbiota
VSQ	Vulvovaginal symptom questionnaire
VVC	Vulvovaginal candidiasis
VVI	Vulvovaginal infection
WHO	World Health Organisation

KESAN SPECIES LAKTOBASILUS TERHADAP KANDIDIASIS FARAJ PADA WANITA HAMIL

ABSTRAK

Kajian ini bertujuan untuk menyiasat kesan probiotik lactobacilli dalam mencegah berulangnya kandidiasis faraj (VVC) dalam 78 wanita hamil dengan VVC (probiotik, n = 39; plasebo, n = 39) dan potensi faedah ke atas kualiti hidup. Probiotik lactobacilli (SynForU-HerCare; dua kapsul/hari 9.5 log CFU/kapsul) atau plasebo telah diberikan selama 8 minggu dalam kajian rawak, dua buta, dan terkawal plasebo. Subjek dinilai untuk keadaan kesihatan faraj dan usus pada minggu ke-0, 4, dan 8 melalui soal selidik dan menganalisis mikrobiota faraj dan perubahan keradangan. Soal selidik gejala vulvovaginal meliputi gejala vulvovaginal dan kualiti hidup. Kumpulan probiotik telah ditunjukkan mengurangkan tanda-tanda kerengsaan (P=0.023) dan pelepasan (P=0.011) bermula minggu-4 dan diteruskan selepas minggu-8 (P<0.05) berbanding plasebo. Kumpulan probiotik telah ditunjukan mengurangkan kebimbangan mengenai simptom selepas minggu-4 (P=0.010) dan diteruskan selepas minggu-8 (P=0.001), disertai dengan pengurangan kemerosotan aktiviti harian (P=0.012) dan berterusan selepas minggu-8 (P=0.026). Kumpulan probiotik telah ditunjukkan mengurangkan pengulangan kedua-dua tekanan emosi dan sosial berbanding plasebo pada kedua-dua minggu ke-4 dan minggu-8 (P<0.05). Kumpulan probiotik menunjukkan masa pembuangan air yang lebih tinggi setiap minggu pada minggu-4 (P=0.010) dan minggu-8 (P=0.001) berbanding kumpulan plasebo. Di kawasan faraj yang lebih rendah, kumpulan probiotik mengurangkan jumlah *Candida glabrata* selepas 8 minggu (P=0.009), berbanding kumpulan plasebo tidak menunjukkan sebarang perubahan dari semasa ke semasa. Di kawasan faraj dan servikovaginal yang lebih tinggi, kumpulan

plasebo mengurangkan kelimpahan *Candida albicans* hanya dalam tempoh 4 minggu ($P<0.05$) tetapi tiada perubahan dalam kelimpahan *C. glabrata* dari semasa ke semasa, manakala kumpulan probiotik secara berterusan mengurangkan kelimpahan *C. albicans* dan *C. glabrata* lebih 8 minggu ($P<0.05$). Kumpulan plasebo mengurangkan kelimpahan *Lactobacillus crispatus* dalam tempoh 4 minggu ($P=0.023$) dan menurunkan kelimpahan *L. jesenii* dalam tempoh 8 minggu ($P=0.001$) masing-masing di kawasan faraj dan servikovaginal yang lebih rendah. Sementara itu, kumpulan probiotik meningkatkan kelimpahan *L. crispatus* di kawasan faraj bawah selepas 8 minggu ($P=0.012$) dan *L. jesenii* selama 4 minggu di kawasan servikovaginal ($P<0.001$). Keradangan berlaku di kawasan faraj rendah dan tinggi sebagai peningkatan kepekatan sitokin pro-radang TNF-α dalam kumpulan plasebo ($P<0.05$). Sebaliknya, kumpulan probiotik memendekkan tempoh keradangan seperti yang diperhatikan daripada pengurangan keperluan untuk sitokin anti-radang IL-4 dan IL-10 dari semasa ke semasa ($P <0.05$). Diambil secara keseluruhan, probiotik lactobacilli bermanfaat kepada wanita hamil dalam mengurangkan gejala vulvovaginal dan berulangnya VVC, disertai dengan tekanan emosi dan sosial yang bertambah baik yang dikaitkan dengan VVC, modulasi mikrobiota faraj, dan persekitaran mikro dengan itu boleh menjadi strategi yang berpotensi untuk mengekalkan kesihatan faraj semasa mengandung.

EFFECTS OF *LACTOBACILLUS SPP*. AGAINST VAGINAL CANDIDIASIS IN PREGNANT WOMEN

ABSTRACT

This study aimed to investigate the effects of lactobacilli probiotics in preventing the recurrences of vulvovaginal candidiasis (VVC) in 78 pregnant women with VVC (probiotic, n = 39; placebo, n = 39) and the potential benefits on quality of life. The lactobacilli probiotic (SynForU-HerCare; two capsules/day of 9.5 log CFU/capsule) or placebo was administered for 8-weeks in a randomized, double-blind, and placebo-controlled study. Subjects were assessed for vaginal and gut health conditions at week-0, 4, and 8 via questionnaires and analyzed vaginal microbiota and inflammatory changes. The vulvovaginal symptom questionnaire covered vulvovaginal symptoms and quality of life. Probiotic group was exhibited the reduction of irritation (P=0.023) and discharge (P=0.011) starting week-4 and continued after week-8 (P<0.05) as compared to the placebo. Probiotic group was exhibited the reduction of concern about symptoms after week-4 (P=0.010) and continued after week-8 (P=0.001), accompanied by reduced impairment of daily activities (P=0.012) and continued after week-8 (P=0.026). Probiotic group was exhibited the reduction of recurrences of both emotional and social stress compared to the placebo at both week-4 and week-8 (P<0.05). Probiotic group showed higher defecation times per week at week-4 (P=0.010) and week-8 (P=0.001) compared to the placebo group. In lower vaginal region, probiotic group decreased the abundance of *Candida glabrata* after 8-weeks (P=0.009), compared placebo group did not show any changes over time. In higher vaginal and cervicovaginal regions, placebo group decreased the abundance of *Candida albicans* only within 4-weeks (P<0.05) but no changes in the abundance of *C.*

glabrata over time, while probiotic group continuously decreased the abundance of *C. albicans* and *C. glabrata* over 8-weeks ($P<0.05$). Placebo group reduced abundance of *Lactobacillus crispatus* over 4-weeks ($P=0.023$) and decreased abundance of *L. jensenii* over 8 weeks ($P=0.001$) in the lower vaginal and cervicovaginal region, respectively. Meanwhile, probiotic group increased abundance of *L. crispatus* in the lower vaginal region after 8-weeks ($P=0.012$) and *L. jensenii* over 4-weeks in the cervicovaginal region ($P<0.001$). Inflammation occurred in low and high vaginal areas as increased concentration of pro-inflammatory cytokine TNF-α in placebo group ($P<0.05$). In contrast, probiotic group shortened the period of inflammation as observed from the reduced need for anti-inflammatory cytokine IL-4 and IL-10 over time ($P<0.05$). Taken altogether, lactobacilli probiotics are beneficial towards pregnant women in reducing vulvovaginal symptoms and recurrences of VVC, accompanied by improved emotional and social distress attributed to VVC, modulation of the vaginal microbiota, and microenvironment thus could be a potential strategy for the maintenance of vaginal health during pregnancy.

CHAPTER 1

INTRODUCTION

1.1 Background

Vulvovaginal candidiasis (VVC) is the most prevalent of third of all cases of human candida infection that result of an inflammation of the vulva or vagina, or an exceedingly common lower female reproductive tract (FRT) mucosal infection in women of reproductive-age women and around 75% of all women have been estimated at least once in their lifetime to have candida vulvovaginitis (Jeanmonod & Jeanmonod, 2020), (Willems et al., 2020). VVC which induce mostly by the most common pathogen that caused by an opportunistic fungus known as *C. albicans* while the remaining cases were caused by *C. glabrata* which cause an invasion of the mucous membrane of the vagina, and contributes to an inflammatory response (Jeanmonod & Jeanmonod, 2020), (Willems et al., 2020). Vaginal scratching, irritating, swelling, discomfort, and redness are typical disease signs, while vaginal discharge also part of VVC symptoms consists of the sloughed epithelium, immune cells, yeast, and vaginal fluid (Jeanmonod & Jeanmonod, 2020), (Willems et al., 2020). Risk factors for VVC involve the consumption of antibiotics, sexual intercourse, oral contraception with excess estrogen, breastfeeding and unregulated diabetes mellitus. Recurrent Vulvovaginal candidiasis (RVVC) risk factors have not been established at present, though genome-wide interaction studies have started uncovering certain genetic susceptibility determinants (Willems et al., 2020). VVC is also the most prevalent of fungal infection among pregnant mothers which may cause systemic infections in neonate and has been associated with low birth weight and premature delivery (Rasti et al., 2014), (Ang et al., 2022). The increasing prevalence of vaginal *Candida* colonization during pregnancy is

assumed to be the result of higher levels of circulating estrogens and vaginal deposition of glycogen and other substrates (Sobel, 2007), (Ang et al., 2022).

According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics were living microorganisms that provide health benefits to host individuals when supplied in appropriate amounts. The primary and higher types of gastrointestinal microbiota Bifidobacterium and *Lactobacillus* are the most often consumed probiotic bacteria in many functional foods and supplements (Bermudez-Brito et al., 2012).

For this project, lactobacilli probiotic STP4 was chosen and was manufactured under a HACCP and ISO 22000 certified manufacturing plant (Synbio Tech Inc., Taiwan). The manufacturing plant also was certified by the Taiwan Quality Food (TQF) Scheme which met the requirement of the Food Industry Research and Development Institute with the scope of processing Ambient stable products. Lactobacilli probiotic STP4 did not contain any porcine or bovine ingredients and obtained the HALAL certification from Taiwan Halal Integrity Development Association (THIDA), Taiwan, which was recognized by JAKIM, Malaysia. Lactobacilli probiotic STP4 capsules several strains of lactobacilli. The strains are *Lactobacillus plantarum* LP115, *Lactobacillus helveticus* LA25, *Lactobacillus rhamnosus* LRH10, *Lactobacillus paracasei* LPC12, *Lactobacillus fermentum* LF26, and *Lactobacillus delbrueckii* subsp. lactis LDL114. One capsule contains not less than 9.5 CFU of lactobacilli. The other ingredients are maltodextrin added with the ingredients and a capsule shell made of hydroxypropyl methylcellulose.

However, probiotics maintain a healthy vaginal microbiota, maintaining a healthy vaginal microbiome is critical for optimal pregnancy outcomes (Barthow et al., 2016). Due to infection recurrence and drug-resistant yeast, probiotics are preferred over azole-

based antifungal drugs like fluconazole and metronidazole used for VVC treatment (Ang et al., 2022). Overuse of antifungals might lead to the development of antifungal-resistant yeast, that's a major problem. As a result, it was critical to look for additional ways to prevent VVC, such as probiotics.

1.2 Aim and objectives of research

Problem statement:

1. Most of the studies focused only on 1 vaginal region (Cervico/ High/ Low)
2. Mental stress worsened among pregnant women if they were infected by Vulvovaginal Candidiasis
3. Lack of studies conducted on the use of probiotics to prevent the re-occurrence of VVC among pregnant women

To evaluate the effects and efficiency of oral administration of lactobacilli probiotics STP4 for 8 weeks against vaginal yeast infection in pregnant women.

Specific objectives:

1. To assess mental stress due to infection and/or gastrointestinal discomfort in pregnant women with yeast infection.
2. To evaluate microbiota profiles, concentrations of proteins of pregnant women with yeast infection.
3. To assess clinical symptoms in pregnant women with yeast infection.

CHAPTER 2

LITERATURE REVIEW

2.1 Vulvovaginal Infection (VVI)

Vulvovaginal infection is an infection of the vagina, and outer female genitals including conditions caused by bacteria, yeast, etc. VVI is the most common gynecological illness among women. The three common vulvovaginal infections are bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomoniasis (TV). Recurrences are defined as more than three episodes per year and affect nearly 8% of women globally (Willems et al., 2020). Moreover, recurrent vaginal infections (RVVI) were defined as repeated experiences of common vaginal infection, this was causing a lot of concern among researchers these days. BV, VVC, and TV were the three most frequent RVVIs. The recurrence rate of BV (RBV) is as high as 30–50% within three months, whereas recurrent VVC was defined as four repetitive episodes of VVC in 12 months (RVVC). Similarly, cases of recurrent tv (RTV) have been documented, with recurrence rates as high as 5–8% within two months of initial diagnosis (Kalia et al., 2019). Changes in the vulvovaginal regions caused by these infections frequently provide a niche for the pathogenesis of other infections, resulting in mixed infections and co-infections that, if left untreated, can lead to death. VVI will have an impact on not just female reproductive health, but may also result in various foster infections/diseases and pregnancy outcomes (Kalia et al., 2019).

2.1.1 Vulvovaginal Candidiasis (VVC)

Vulvovaginal candidiasis (VVC) is the most prevalent of third of all cases of human candida infection that result superficially penetrating the mucosal lining of the vagina, an inflammation of the vulva or vagina, or an exceedingly common lower female reproductive tract (FRT) mucosal infection in women of reproductive-age women.

Around 75% of all women have been estimated to have candida vulvovaginitis at least once in their lifetime (Jeanmonod & Jeanmonod, 2020), (Willems et al., 2020). VVC which induced mostly by the most common pathogen that caused by an opportunistic fungus known as *C. albicans*, while the remaining cases are caused by *C. glabrata* which cause an invasion of the mucous membrane of the vagina and contributes to an inflammatory response (Jeanmonod & Jeanmonod, 2020), (Willems et al., 2020). Fungal overgrowth in the vaginal then contributes to epithelial invasions and the production of virulence effectors, resulting in symptomatic infection. All the mechanisms result from exuberant mucosal inflammation, leading to the appearance of candidiasis (Ribeiro et al., 2020). Vaginal scratching, irritating, swelling, pain during sexual intercourse, soreness, discomfort, and redness are typical disease signs, while vaginal discharge also part of VVC symptoms consists of the sloughed epithelium, immune cells, yeast, and vaginal fluid (Okonkwo & Umeanaeto, 2020), (Jeanmonod & Jeanmonod, 2020), (Willems et al., 2020). CDC also reports that women who are more likely to get vaginal candidiasis are those who are pregnant. It is the most common form of fungal infection in pregnant mothers, which may cause systemic infections in neonates, particularly with low birth weight (LBW) and prematurity after delivery (Rasti et al., 2014). The increasing prevalence of vaginal *Candida* colonization during pregnancy is assumed to be the result of higher levels of circulating estrogens and vaginal deposition of glycogen and other substrates (Sobel, 2007), (Ang et al., 2022).

Furthermore, recurrent VVC (identified as >3 episodes per year) affects roughly 8% of women worldwide, whereas it affects fewer than 5% of healthy women (Sobel, 1997), (Willems et al., 2020). A recurring VVC is referred to as RVVC in 4 recurring episodes in 2 months (Kalia et al., 2019). Frequently, RVVC requires anti-fungal maintenance therapy for six months with azole drugs, which have shown effectiveness in attenuating

disease reemergence, proven by several studies (Sobel, 1997), (Willems et al., 2020). The stationary activity and lack of immune-mediated elimination of azoles from the most recurrent anti-fungal drug products are essential to treating the disease. Risk factors for VVC involve the consumption of antibiotics, sexual intercourse, oral contraception with excess estrogen, and unregulated diabetes mellitus. RVVC risk factors have not been established at present, though genome-wide interaction studies have started uncovering certain genetic susceptibility determinants (Willems et al., 2020).

2.1.2 Tendency of vaginal yeast infection

Yeast infections can occur on any female. A healthy vaginal contains *Lactobacillus* and several bacteria and a mix of yeast such as *Candida*. In around 30% of women, the vaginal microbiota contains *Candida,* the most common *C. albicans.* The prevalence of non-*albicans* (such as *C. glabrata*) species with the range of 10-30% among women with vaginal *Candida* varies (Tortelli, B. A et al., 2020).

Administration of *Lactobacillus* was maintaining a healthy vaginal microbiota and preventing yeast overgrowth in the vaginal. VVC occurs when there is an imbalance of vaginal microflora. The signs and symptoms of a yeast infection are caused by an overgrowth of *Candida* or penetration of the fungus into deeper vaginal cell layers (Sobel, 2007).

Overgrowth of vaginal yeast infection can be caused by clothing (particularly underwear) that is too tight or made of materials that trap moisture and air, causing yeast infections more likely. Infection can also be caused by poor hygiene practices. Douching and using scented sanitary products might disrupt the vaginal microbial balance, resulting in yeast infections more prevalent. Furthermore, taking antibiotics kills a variety of microorganisms and includes beneficial bacteria in the vaginal region,

resulting in the overgrowth of yeast (Hirsch, 2020). Furthermore, consumption of oral contraceptives or hormone treatment raises estrogen levels, which promotes yeast to overgrow, and estrogen levels rise consistently during pregnancy. (Wilson D.R, 2017), (Sian Ferguson, 2019). A yeast infection can also be caused by uncontrolled diabetes. Glycogen, a polysaccharide that the body uses to store glucose, people with diabetes also have higher glycogen levels. Increased acidity is correlated to extra glycogen in the vaginal region, which promotes yeast overgrowth (Carrara et al., 2009). Lastly, the impaired immune system will become more prone to vaginal yeast infection. Women with lower immunity, such as HIV infection, as their CD4 count drops below 350 cells/mL, become more prone to get a yeast infection, and their infection may be more severe (Koenig D, Hosein SR, 2016).

2.1.3 Vaginal yeast infection in pregnant women

In Malaysia, recurrent vaginal candidiasis was expected to affect 501,138 otherwise healthy women annually at a rate of 4800 per 100,000 females (Velayuthan, R. D et al., 2018).

Symptomatic recurrences among pregnant women are also higher during pregnancy's second and third trimesters. Increased levels of circulating estrogens and glycogen deposition that promote yeast adherence to vaginal mucosal epithelial cells during these phases of pregnancy are thought to be the consequences of increased levels of circulating estrogens and glycogen deposition that promote yeast adherence to vaginal mucosal epithelial cells during these phases of pregnancy (Aguin & Sobel, 2015), (Sobel, 2007).

Evidence has suggested that VVC during pregnancy are associated with an increased risk of premature rupture of membranes and poor pregnancy outcome. Although VVC induced chorioamnionitis is rare, several cases have been reported of

intraamniotic infection caused by *C. albicans*, and *C. glabrata* cause preterm rupture of membranes or preterm labor where such a progression could lead to fetal fatality (Meizoso et al., 2008).

2.2 Emergence of probiotics

According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics were living microorganisms that provide health benefits to host individuals, maintaining or improving microflora balance in the intestine, maintaining a healthy vaginal microbiota, and preventing yeast overgrowth in vaginal when supplied in appropriate amounts (FAO/WHO 2001), (Mattila-Sandholm et al., 2002), (Bermudez-Brito et al., 2012). Properties of probiotics have been studied in a variety of genera of bacteria and fungi, most of which are probiotic strains belonging to *Bifidobacterium* and *Lactobacillus* primary and subdominant present in gastrointestinal microbiota. In contrast, other probiotic strains such as *Saccharomyces*, *Enterococcus*, *Escherichia*, and *Bacillus* are contained in the gut intestine, while *Streptococcus* strains used in probiotics provide health benefits to pharyngotonsillitis and maintain oral health. These probiotics were the most widely used probiotic bacteria and can be found in a variety of functional and nutritional supplements, including yoghurt (Bermudez-Brito etal.,2012),(Fijan,2014).*Lactobacillus*, *Lactococcus*, *Enterococcus*, *Oenococcus*, *Pedi ococcus*, *Streptococcus*, and *Leuconostoc* species which are from Lactic acid bacteria (LAB) groups commonly used in dairy products for manufacturing fermented products and have been used for a long time (Fijan, 2014). In earlier of the twentieth century, it was proposed that the consumption of fermented dairy products contributed to the longevity of Bulgarian by Ilja Metchnikoff. In 1906, Henry Tissier noticed that only a small amount of Y-shaped bacteria remained in children's stools with diarrhea, while a large percentage were found in healthy children. Lilly and Stillwell, in 1965, the word "probiotic" was used to describing substances released by one organism that promoted the development of another one (Zawistowska-Rojek & Tyski, 2018). After a

long history of beneficial probiotics in fermented dairy products and increased recognition of their beneficial health effects, the food industry has been increasingly focused on certain microorganisms. Tolerance to digestive conditions (gastric acid and bile) was also a criterion for probiotic selection (Bermudez-Brito et al., 2012).

In the oral cavity, ileum, and colon, the *Lactobacillus* strains are found in healthy humans, and they are the major microorganisms in the vagina (Borriello et al., 2003). As commensals of the human microbiome, *Lactobacillus* and Bifidobacterium strains are harmless and non-pathogenic. No evidence consuming probiotic lactobacilli or bifidobacterial strains raises the risk of infection beyond that of commensal bacteria. The number of infections generated by *Lactobacillus* or *Bifidobacterium* strains is extremely low, ranging from 0.05 to 0.4 percent for infective endocarditis and bacteremia, respectively (Zawistowska-Rojek & Tyski, 2018). Lactobacilli are Gram-positive, non-spore-forming rods or coccobacilli with a low G+C DNA content below 50 mol%. They are strictly fermentative, aero-tolerant or anaerobic, aciduric, or acidophilic. They have complex nutritional requirements, such as carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivatives, and vitamins (Hammes & Vogel, 1995).

The genus *Lactobacillus* belongs to the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, and family *Lactobacillaceae*, with the genus *Pediococcus* as its closest relative within the same family. *Lactobacillus* species are phylogenetically diverse, with over 100 species documented to date (1). *Lactobacillus* thrives mostly in carbohydrate-rich environments, including milk, meat, cereals, plants, and mucosal surfaces (oral, gastrointestinal [GI] tract, and reproductive tracts) of animals and humans, reflecting their particular nutritional value requirements. They are typically low G + C Gram-

positive, non-spore-forming rods or coccobacilli, catalase-negative, anaerobic or aerotolerant, and acid-tolerant. Lactobacilli are lactic acid bacteria (LAB) members, a broadly defined family of microorganisms that ferment various hexoses into primarily lactic acid. They are chemo-organotrophs, strictly fermentative, and are biochemically subdivided to either homofermentative (generate mainly lactic acid via the glycolytic Embden-Meyerhof pathway [EMP]), heterofermentative (produce lactic acid, acetic acid, formic acid, carbon dioxide, and ethanol, via the phosphoketolase pathway [PKP]), or facultative heterofermentative (ferment hexoses via the EMP and pentoses via the PKP).

One key trait of *Lactobacillus* species is their complex dietary requirements and prevalence of transporters in the genome. The genome analyses indicated that most of the species are extremely auxotrophic, with some at intermediate auxotrophy, including *L. plantarum* and *L. salivarius.* All species lack major biosynthetic enzymes, if not all vitamins and cofactors, which explains these components' minimal growth medium requirements. It has been reported that *L. reuteri* CRL1098 can synthesize cobalamin (vitamin B12), a unique feature among lactobacilli. Concomitantly, only the *L. reuteri* genome was found to encode putative enzymes for cobalamin synthesis, of which the closest orthologs were identified in *Listeria*, *Yersinia*, *Salmonella*, and *Streptococcus sanguinis* (Goh & Klaenhammer, 2009).

2.2.1 Classification and beneficial effects of probiotics

To define a viable bacterial strain as probiotic, probiotic strain should be a must to be safe for human consumption. Most of which are probiotic strains belonging to *Bifidobacterium* and *Lactobacillus* are isolated from healthy humans (Zawistowska-Rojek & Tyski, 2018). The probiotic strain must be good viability and provide a

beneficial effect on the gastrointestinal effect to the host (Zawistowska-Rojek & Tyski, 2018). There are some criteria for choosing a preferred probiotic strain that must be evaluated (Mattila-Sandholm et al., 2002). The strain must include acid tolerance and tolerance to human gastric juice; bile tolerance, which is an important factor for survival in the small intestine; adhesion to epithelial surfaces and persistence in the human GI-tract; immunostimulation but no pro-inflammatory effect; antagonistic activity against pathogens such as *Salmonella spp*., *Helicobacter pylori, Clostridium difficile*, and *Listeria* monocytogenes; and antimutagenic activity (Mattila-Sandholm et al., 2002).

The *Lactobacillus* and *Bifidobacterium* strains are safe and not pathogenic as commensals to the human microbiome, *Lactobacillus* in the oral cavity, ileum, and colon which present in healthy humans; moreover, they are major vaginal microorganisms. There is no indication that this increases the risk of infection beyond what is associated with commensal strains. Lactobacilli or bifidobacterial strains are probiotics that may be consumed. (Zawistowska-Rojek & Tyski, 2018).

Probiotics have three different modes of action that may be classified. (i) Probiotics may be able to control the host's defensive mechanisms, including the innate and acquired immune systems. This mechanism of action is expected to be beneficial in the prevention and treatment of infectious diseases, as well as the treatment of (chronic) inflammation of the digestive tract or sections thereof. Furthermore, this probiotic mechanism may be important in the elimination of neoplastic host cells. (ii) Other microorganisms, commensal and pathogenic bacteria may be affected directly by probiotics. In many cases, prevention and treatment are critical for infections and the restoration of microbial balance in the gut. (iii) Finally, probiotic effects could be influenced by mechanisms that affect microbial products such as toxins, host products such as bile salts, and food ingredients. In the gut, such mechanisms may occur in the

inactivation of toxins and the detoxification of host and dietary components. Infection defense, cancer prevention, and establishing or maintaining the physiological equilibrium between the gut microbiota and its host are all likely to be involved in all three modes of probiotic activity mentioned above (Oelschlaeger, 2010).

Certain lactobacilli and bifidobacteria may generate antimicrobial peptides known as bacteriocins that inhibit certain infections from increasing. The term 'resistance to colonization' relates to probiotics used to prevent or treat enteric infections. Small cationic compounds of ~30-60 amino acids constitute bacteriocins. Those chemicals work in cytoplasmic bacterial membranes to impair the proton-motive force by targeting energy-driven membrane vesicles. Bacteriocins were classified into 4 major types by their structures, molecular weights, post-translational modifications, and genetic features. For example, some of these compounds produced by *L. plantarum* or *L. acidophilus* have been discovered to limit the development of *Helicobacter*, *C. difficile*, rotaviruses, multidrug-resistant *Shigella spp.*, and *E. coli* in various gastrointestinal situations, as well as activity against several uropathogens (Plaza-Diaz et al., 2019).

2.2.2 General benefits of *Lactobacillus*

Probiotic provides health benefits by enhancing the immune system of lactose intolerance to deter and strengthen bowel diseases. Furthermore, probiotics have been found to have hypocholesterolemic effects via cholesterol absorption, cholesterol binding to the cellular surface, and cholesterol co-precipitation. Probiotics also play a role in blocking the formation of micelles for intestinal absorption, bile acid-binding by Bile Salt Hydrolase (BSH), and enhancing lipid profiles by blocking anti-hypercholesterolemic and anti-hypertensive effects, and relieving postmenopausal disorders. Apart from these well-known benefits, probiotics have been studied for their ability to prevent atopic eczema, promote wound and scar healing, and have skin-rejuvenating characteristics. Probiotics may have favorable dermal benefits through producing bacterial substances that promote specific immune responses and improve skin barrier functions, according to some research. Oral diseases are treated using probiotics to prevent and cure them. Dental caries and periodontal infection can be used to prevent cariogenic bacteria and periodontopathogens from growing.

Additionally, they have been shown to reduce nitric oxide production, which in turn reduces prostaglandin and matrix metalloproteinase levels in the saliva. Furthermore, probiotics can help with halitosis by limiting the formation of volatile sulfide-producing species, which create an unpleasant odor from the mouth. Furthermore, probiotics' ability to alleviate stress-related mental diseases including anxiety and depression via regulating the gut-brain axis has increased the importance of probiotics (Shi et al., 2016). Last but not least, probiotic acts as an anti-fungal agent which plays an important part in this project.

Multi-strains probiotic bacteria were chosen in lactobacilli probiotic STP4 because of multi-strain benefits for treating non-infectious disorders, preventing pathogens like

C. albicans, and enhancing human health. Administering azoles combined with multi-strain probiotics such as *L. acidophilus* and *L. rhamnosus* improves the treatment of vaginal candidiasis in patients. This study further confirms the benefits of combining multi-strain probiotics for the treatment and prevention of recurrence of vaginal candidiasis, particularly in cases of azole-resistant mycosis (Kwoji ID et al., 2021).

However, this probiotic maintain a healthy vaginal microbiota, maintaining a healthy vaginal microbiome is critical for optimal pregnancy outcomes (Barthow et al., 2016). Due to infection recurrence and drug-resistant yeast, probiotics are preferred over azole-based antifungal drugs like fluconazole and metronidazole used for VVC treatment (Ang et al., 2022). Overuse of antifungals might lead to the development of antifungal-resistant yeast, that's a major problem. As a result, it's critical to look for additional ways to treat VVC, such as probiotics.

2.2.3 Oral admission of probiotic against VVI

Lactobacillus protects the vaginal mucosa from pathogenic organisms by inducing antimicrobial agents such as hydrogen peroxide and bacteriocins, co-aggregation, competing for nutrients, adhering to epithelial surfaces, maintaining acidity and lower vaginal pH through lactic acid production, and dominating the vaginal microbiota with a low pH (pH 3.5-4.5), and immune modulation (Barthow et al., 2016), (Borges et al., 2014) Probiotics colonizing the vaginal tract can help to keep urogenital health and prevent or cure infections. The inclusion of natural antimicrobials produced by probiotics that limit pathogen growth would improve the survival and stimulate the development of native vaginal microbiota, hence enhancing natural defense against pathogenic microorganisms (Borges et al., 2014).

The healthy flora of the vagina can also be restored by administrating specific lactobacilli strains orally, possibly providing a practical way to lower the risk of vaginal

infections. Such probiotic products might help reduce pain and difficulties associated with infection in both developed and developing countries, as well as provide a vehicle to deliver additional nutritional advantages (Reid et al., 2001).

2.2.4 Mechanisms of *Lactobacillus* against *Candida*

The initial stage in the pathophysiology of *C. albicans* is the adhesion to the organism's host cell surface, allowing for the colonization of a fungus in a specific niche and infection development (Ribeiro et al., 2020).

Once the procedure has been inhibited, *C. albicans* is unable or readily removable, which results in the non-colonization of tissue (H. Martin et al., 2018), (Hans et al., 2019), (Simon et al., 2019). Some *Lactobacillus* strains suggest that *Candida* adhesion to mucosal surfaces can be inhibited by several mechanisms such as exclusion, competition for receptor sites, and the adherence of yeast cells (Parolin et al., 2015; Ribeiro et al., 2020).

Five different *Lactobacillus* strains have been reported to have an anti-adhesive activity against *C. albicans* (Verdenelli et al., 2014). The efficacy of all strains tested to inhibit the yeast from adhering. However, this profile was strain-dependent and suggested that various action mechanisms may have differed from microorganisms belonging to the same species. to against *C. albicans* (Ribeiro et al., 2020).

Parolin and the team were studying the adhesion mechanisms of *Lactobacillus* on *C. albicans* and examined the three distinct adherence mechanisms of HeLa cells such as exclusion, competition, and displacement for 13 *Lactobacillu*s strains which derived from the vaginal cell (Parolin et al., 2015). The results revealed that the adhesion of *C. albicans* was minimized by ten lactobacilli strains were tested across all pathways, with *L. crispatus* BC2, *L. gasseri* BC10, and *L. gasseri* BC1 being the strains having the most inhibitory activity (Ribeiro et al., 2020).

The vaginal epithelial cells that *C. albicans* attached undergo morphological alterations that trigger cellular endocytosis. Treatment with *Lactobacillus spp*. of these infected cells reduced *Candida* adherence, hyphal development, and proliferation of *candida* (Zangl. I et al., 2020).

Lactobacillus spp. ability's to limit *Candida* adherence on host tissues has been directly linked to the cell surface hydrophobicity of lactobacilli, since these features impact its attachment to epithelial tissue, creating a mechanical barrier against *Candida* adhesion (Kang et al., 2018; Itapary Dos Santos et al., 2019). In 2018, Aarti and the researcher team confirmed that *L. pentosus* strain LAP1, isolated from Hentak (a fermented fish of Manipur, India), showed a significant level of auto-aggregation and hydrophobicity percentage, as well as anti-fungal activity against *C. albicans*, *C. tropicalis*, and *C. krusei* (Aarti et al., 2018). For these characteristics, this strain might be a good candidate for biotherapeutics products against *Candida* infections. However, because this species was isolated from Hentak, a fermented fish, more research was needed to determine whether *L. pentosus* strain LAP1 can colonize the host and maintain its inhibitory abilities against *C. albicans*, as all of the findings in vitro may not be reproducible in vivo in people (Ribeiro et al., 2020).

2.2.5 Benefits of probiotic for pregnant women against VVI

When the administration of probiotics via orally or used vaginally, probiotic strain should be a must to be safe for human consumption and well-tolerated (Zawistowska-Rojek & Tyski, 2018). Due to infection recurrence and drug resistance, probiotics are frequently preferred to antibacterial drugs such as clindamycin and metronidazole, which are taken orally or intravaginally for the treatment of bacterial vaginosis (Jang et al., 2017), (Younus et al., 2017). Metronidazole is the preferred primary medicine for

BV treatment. After treatment, symptoms appear again after a year in more than 50% of cases. Failure to recover lactobacilli can also acts a significant role in recurrence in addition to drug resistance. Probiotic lactobacilli were used for restoration and maintaining a healthy vaginal microbiota among non-pregnant and pregnant women. In the presence of microbial imbalance, probiotics colonize the vagina, restore its microbiota, and eliminate or reduce the occurrence of urogenital infections (Husain et al., 2020). Vaginal microbiota (VMB) was defined as a vaginal community of commensal, symbiotic, and pathogenic microorganisms that colonize the vagina. Probiotics have been consumed as a food supplement to reduce proneness to common illnesses and improve metabolic health in humans. The risk of probiotic-induced bacteremia and fungemia is low; probiotics are unlikely to enter the fetus's systemic circulation, and hence are unlikely to cause harm (Zawistowska-Rojek & Tyski, 2018).

2.3 Treatment against VVC

2.3.1 Mechanism of clotrimazole against vaginal yeast infection

Our subjects applied an anti-fungal drug named Candid V3 pills for two weeks to treat vaginal candidiasis while at the same time they took lactobacilli probiotics STP4 (oral probiotic) or placebo. Candid V3 is clotrimazole (anti-fungal), an FDA-approved drug (Khatter & Khan, 2021). Clotrimazole against certain Gram-positive bacteria such as *Candida sp*p. Clotrimazole inhibits VVC-related damage by disrupting the fungal cytoplasmic membrane's permeability barrier. Prolific hyphal dissemination was prevented by clotrimazole within the epithelium (Ross et al., 1995; Khatter & Khan, 2021). By preventing fungal activation of the epithelial "danger response," clotrimazole treatment may reduce local inflammation and neutrophil infiltration. In the absence of fungal cells, clotrimazole has been proven to suppress TNF-α induced IL-8 production and NF-B activity, indicating that this azole may have further anti-inflammatory mechanisms. (Kasper et al., 2015; Ross et al., 1995).

2.3.2 Mode of mechanism of oral probiotic against vaginal yeast infection

The route of delivery of oral probiotics was orally transit through the intestine and ascending into the vaginal without practical intervention via gut-vagina microbiota crosstalk (Amabebe & Anumba, 2020; Reid et al., 2001; Baldewijns et al., 2021).

Despite the close proximity of the anus to the vagina (Cribby et al., 2008). Among healthy conditions of vaginal homeostasis, the dominant microbial in VMB which was known as lactobacilli, 4 strains were commonly found in VMB such as *L. crispatus, L. gasseri, L. iners, and L. jensenii*. Lactobacilli produce lactic acid exclusively from sugars such as glycogen and glycogen hydrolyzed as carbon and nitrogen sources. *Lactobacillus spp*. dominance associated with intact vaginal amino acids includes glutamate and branched-chain amino acids (BCAAs) such as leucine, isoleucine, and valine. Lactic acid-producing bacteria (*Lactobacillus spp*.) also develop BCAAs, which were found in high concentrations in the vaginal region, which was another indicator of *Lactobacillus* dominance. The major bacteria in the VMB was *L. crispatus*, which was a potent lactic acid producer and followed by *L. jensenni,* which was a bacterial source of lactic acid production in the vagina (Baldewijns et al., 2021).

Lactobacilli suppress pro-inflammatory responses in cervicovaginal epithelial cells produced by Toll-like receptor (TLR) agonists (Mata Forsberg et al., 2019; Delgado-Diaz et al., 2020). Lactic acid may play a role in these immunomodulatory responses. We discovered that IL-10 enhances B lymphocyte proliferation by modifying the Th cell environment and suppresses inflammation and macrophage activity by limiting the formation of interferon (IFN-γ) and tumour necrosis factor (TNF-α). The role of IL-10 in pathogenesis during infection was considered to be hinge on microorganism clearance via promoting adaptive immune effector mechanisms such as clonal

proliferation and maturation of Th2 cells. Th2 responses, which were characterized by IL-4, are enabled by the suppressive influence of IL-10 towards macrophages and Th1 responses. B cells, antibody production, and alternatively activated macrophages are all involved by this effect on humoral effector mechanisms. Probiotics suppress adaptive immune responses and reduce tissue damage caused by inflammation. This resembles the role of IL-10 in limiting immunopathology caused by fulminating infection and inhibiting the synthesis of several pro-inflammatory molecules. In an IL-10-dependent way, several probiotic treatments have shown the ability to control particular viral illnesses. It existed to involve a decline in IFN-γ and TNF-α secretion (Delgado-Diaz et al., 2020).

2.4 Vaginal microflora

2.4.1 Vaginal microbiota

The vaginal microbiota (VMB) was defined as a vaginal community of commensal, symbiotic, and pathogenic microorganisms that colonize the vagina, and VMB as one of the first lines of defense against VVC (Łaniewski & Herbst-Kralovetz, 2018; Wagner et al., 2012). Among healthy reproductive-age women, the dominant microbial in VMB which known as lactobacilli, 4 strains were commonly found in VMB such as *L. crispatus, L. gasseri, L. iners, and L. jensenii* which tend to prevent the overgrowing of other bacterial species such as yeast by the synthesis of lactic acid and antimicrobial products. However, these bacterial populations, on the other hand, might differ significantly across individuals and over time (Łaniewski & Herbst-Kralovetz, 2018; Wagner et al., 2012). Despite its near proximity of the vagina to the anus, the microbial diversity of the vagina was far lower than in the gut. The reason for this decline diversity was unknown; however, it might be due to inadequate vaginal receptivity, altered nutrition supply from the stomach, or competition with native species. Some bacteria present in the gut, such as *E. coli* and *Streptococcus*, may also be found in the vaginal region, indicating that these organisms have the proper receptors, nutrients, and oxygen tension in place for these organisms to survive. Estrogens, vaginal pH, glycogen, and progesterone were needed to enhance the environmental conditions for lactobacilli at the start and during the reproductive phase of women (Cribby et al., 2009). Estrogens assist the adherence of the vaginal epithelium and induce intraepithelial glycogen, whereas progesterone facilitates the cytolysis of epithelial cells and releases glycogen. Lactobacilli and other bacteria can metabolize the glycogen into glucose and maltose, which can then be converted to lactic acid. This resulted in a vaginal pH of 3.8–4.4, considered a healthy pH value in VMB (Mendling, 2016). When estrogen levels