CONSORTIUM OF MICROBIOTA IN PROBIOTIC YOGURT IN INHIBITING THE GROWTH OF SALMONELLA TYPHII WHICH CAUSES TYPHUS

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Abstract

Yogurt is usually made by using two types of lactic acid bacteria (LAB), Lactobacillus bulgaricus and Streptococcus thermophilus as starters, but the bacteria are not surviving in a very high acid condition. To develop probiotics in yogurt, the addition of lactic acid bacteria (LAB) with probiotic such as Lactobacillus acidophilus and Bifidobacterium are needed. Yogurt is expected to improve human health, specifically gut health. This study aims to study the effect of probiotic yogurt with a consortium of many microbiotas to inhibit the growth of Salmonella typhii which causes typhus. The literature research method used Google Scholar and PubMed from 2005 to 2022 which obtained 40 journals. Based on observations, it was shown that lactic acid in bacterial isolates was able to inhibit the growth of Salmonella typhii due to the formation of an inhibition zone. Therefore, yogurt has an important ability to inhibit the growth of Salmonella typhii, besides that Salmonella typhi is the most sensitive bacteria to probiotic fermented milk.

Key words: *Yoghurt probiotic, consortium microbiota, Salmonella typhi, inhibit zone*

INTRODUCTION

Yogurt is a processed product of fermented milk or reconstituted milk using *Lactobacillus delbrueckii subsp. bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* or other suitable lactic acid bacteria (LAB), with or without the addition of other food ingredients and permitted food additives (National Standardization Agency of Indonesia, 2009). However, the two LABs used in making yogurt cannot live in an environment with very high acidity. If these bacteria could die when they reach the small intestine, and the benefits of bacteria for the digestive tract health will be reduced (Helferich and Westhoff 1980; Adriani et al., 2015).

According to Adriani et al., 2017, yogurt has many benefits for the body, including regulating the digestive tract, increasing immunity, antidiarrhea, preventing colon cancer, helping people with lactose intolerance, and regulating fat levels in the blood.

Probiotic yogurt needs to be added, such as *L. acidophilus, L. casei*, and *Bifidobacterium* which can live and metabolize in the intestine. This was confirmed by Lengkey & Adriani (2009), who stated that *L. acidophilus* is a lactic acid bacteria (LAB) that is resistant to gastric acid and can maintain the number of live bacteria up to 10^7 colonies mL^{-1}. In addition, the benefits of probiotic *L. acidophilus* have been shown to inhibit the growth of pathogenic bacteria (Adriani & Lengkey, 2009).

The term probiotic is defined as "a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance". Probiotic bacteria may produce various compounds that inhibit the growth of pathogens, which include organic acids (lactic and acetic acids) and bacteriocins. The organic acids not only could lower the pH but also affect the growth of the pathogen, (Adriani et al., 2019). Commonly claimed benefits of probiotics include the decrease of potentially pathogenic gastrointestinal microorganisms, the reduction of gastrointestinal discomfort, the strengthening of the immune system, the improvement of bloating, the protection of DNA, the protection of proteins and lipids from oxidative damage, and the maintaining

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The manuscript was received: 03.10.2022
Accepted for publication: 03.11.2022

of individual intestinal microbiota in subjects receiving antibiotic treatment (Lesmana et al., 2021). However, scientific evidence has been insufficient to substantiate any anti-disease claims or health benefits from consuming probiotics.

Yogurt, a dairy product produced by milk fermentation, contains lactic acid bacteria (LAB) which ferment the lactose (producing lactic acid) and affects milk peptides and proteins (Algaron et al., 2004). As a dairy product, yogurt is rich in variable minerals such as calcium, magnesium, potassium, and zinc, and vitamins such as vitamin B. Yogurt is also a good source of various other nutrients and energy. Interestingly, higher levels of proteins, vitamins, and minerals have been reported in yogurt than in milk, supporting its role in improving the nutritional status and health of older adults and possibly healthy and active aging (El-Abbadi et al., 2014). The use of probiotic yogurt at a dose of 1.25-2% in experimental rats could inhibit the growth of pathogenic bacteria (Lesmana et al., 2021).

Salmonellosis is one of the most common foodborne infections that can impact the gastrointestinal tract by disrupting normal function and causing diarrhea. The use of antibiotic therapy for *salmonellosis* is being questioned due to the rise of multidrug-resistant strains of *Salmonella*, which are a prominent cause of pediatric morbidity and mortality in developing countries (Birosova & Mikulasova, 2009). Therefore, alternative treatments and safety precautions are necessary.

Pathogens in the digestive system can trigger a variety of host immunological reactions and pathological outcomes, including changes to epithelial function that facilitate penetration across the epithelial barrier (reviewed in Lu and Walker, 2001). Salmonella is a pathogen that attaches to the intestinal epithelial cells and causes membrane ruffling and microvilli degeneration (Lu and Walker, 2001). According to this, the function of the small intestine is impacted and reduces the enzyme activity in the brush border (Correa-Matos et al, 2001; Chitra et al., 2002). Reduced activity of brush border enzymes, such as sucrase-isomaltase and maltase, leads to undigested food in the intestine, resulting in maldigestion diarrhea (Jung et al., 2006), which contributes to the gastrointestinal symptoms of Salmonella infection. The root causes of the decreased enzyme activity are still not fully understood. Many studies have only investigated enzyme activity in the ileum as Salmonella is known to appear in the terminal cecum, ileum, and colon; (Frost et al., 1997) however, because the jejunum took a major role in nutrient digestion and absorption, it is important to determine whether infection affects jejunal brush border enzymes. Furthermore, studies have focused on the effect of infection on disaccharidase enzymes involved in carbohydrate digestion, such as sucrase-isomaltase and maltase. Intestinal alkaline phosphatase (ALP), a brush border enzyme unrelated to the breakdown of carbohydrates, is hypothesized to play a role in controlling the absorption of fat by catalyzing the hydrolysis of phosphomonoesters in an alkaline environment (Narisawa et al., 2003). Due to the potential impact on fat digestion of changes to this enzyme's activity, it is especially crucial to evaluate it in disease conditions.

The use of probiotics for health advantages and illness prevention is getting more popular. According to in vitro studies, certain probiotic strains may inhibit Salmonella growth, adhesion, and cell invasion (Forestier et al., 2001; Fernandez et al., 2003), as well as alter immune responses, such as by reducing overall interleukin (IL)-8 secretion (O'Hara et al., 2006) and reducing tumor necrosis factor (TNF)-a production in the small (Castillo et al., 2011). Few in-vivo studies have been performed, but report increased survival, reduced intestinal damage (Silva et al., 2004; de LeBlanc et al, 2010), and decreased translocation of Salmonella to the liver and spleen (Lin et al., 2007). In addition, the probiotic *Bifidobacterium infantis* reduces the inflammatory activity associated with Salmonella infection by the induction of T regulatory cells (O'Mahony et al., 2008; Konieczna et al., 2012).

No study has assessed the effect of probiotics on brush border enzyme activity following Salmonella infection. It was hypothesized that Salmonella would significantly reduce the digestive enzyme activity of the gastrointestinal tract, and that treatment with the probiotic *B. longum subsp. infantis* would attenuate the activation of the inflammatory immune response and reduce the gastrointestinal damage.

MATERIAL AND METHOD

The literature research used Google Scholar and PubMed from 2005 to 2022 which obtained 40 journals. The results of the selection analysis obtained six journals that match the inclusion criteria. Based on the observations, it was shown that lactic acid in the isolated bacteria was able to inhibit the growth of *Salmonella typhii* due to the formation of an inhibition zone. The presence of antibacterial activity against pathogenic bacteria is indicated by the formation of an inhibition zone in the form of a clear zone around the pit.

RESULTS AND DISCUSSIONS

Fermented milk has a different antibacterial activity in inhibiting pathogenic bacteria. In a study by Khikmah (2015), probiotic yogurt fermented milk has antibacterial activity against *S. typhii*, and *B. cereus*. Meanwhile, pure yogurt did not have antibacterial activity against all test pathogenic bacteria. The results are presented in Table 1.

Table 1 Average Diameter of the Clear Zone of Fermented Milk against Pathogenic Bacteria (Khikmah, 2015)

Fermented Milk	Diameter of Clear Zone (mm)			
	S. typhii	*E. coli*	*B. cereus*	*S. aureus*
A	2,4	0	0	0
B	2,2	0	0	0
C	0	0	1,4	0
D	0	0	0	0

Note: Inhibitory zone diameter data is the average based on 5 (five) replications
B – D = probiotic yogurt, E = pure yogurt

According to Khikmah (2015), the differences in inhibition was due to differences in lactic acid bacteria that ferment milk, so the amount and activities of the antibacterial compounds produced were also different. The tested pathogenic bacteria also have different sensitivities to antibacterial compounds. The consortium of lactic acid bacteria used in the experimental fermented milk is presented in Table 2.

Table 2 A consortium of lactic acid bacteria used in the experimental fermented milk (Khikmah, 2015)

Fermented Milk	Lactic Acid Bacteria
A	*Bifidobacterium BB-12, L. acidophilus LA-5,*
B	*S. thermophilus, L. bulgaricus, L. acidophilus, Bifidobacterium*
C	*L. acidophilius, Bifidobacterium, L. casei*
D	*S. thermophilus, L. bulgaricus*

Based on Kaboosi's research (2011), *S. typhii* could be inhibited by yogurt in bacteriostatic and bactericidal inhibition categories, such as *Lactobacillus sp., Streptococcus sp.*, and *Bifidobacterium sp.* Yesillik et al. (2011) also stated that *S. typhimurium* was the most sensitive bacteria to fermented milk, both in pure yogurt products (homemade and commercial), commercial kefir, or probiotic yogurt.

One of the main characteristics of probiotics is their ability to antagonize pathogenic bacteria and, thus, improve host health. A summary by Markowiak and Śliżewska (2017) stated that the capacity to reach this goal is achieved by four different mechanisms, such as the production of antimicrobial substances (bacteriocins, SCFA, etc.), competition for the adhesion sites in the intestinal epithelium and nutrients, modulation of the immune system of the host, and blockage of the toxin production by pathogenic bacteria. Among them, *Lactobacillus rhamnosus* HN001 can produce bacteriocin-like substances (Aguilar-Uscanga et al., 2013), which is not demonstrated for *Bifidobacterium longum subsp. infantis* yet, and has just been proven to synthesize a peptide against rotavirus (Muñoz et al., 2011).

Regarding *Bifidobacterium longum subsp. infantis*, benefits against infectious agents have been described. Based on a study of mice with Salmonella infection that were pre-treated with probiotics, showed diminished enterocyte damage and a reduced expression of interleukins IL-8 and IL-10 (Symonds et al., 2012). This down-regulation of pro-inflammatory cytokine is consistent with results reported by O'Mahony et al. (2008), where mice consuming the probiotic showed a decrease in the release of IFN-γ, TNF-α, and IL-10 following CD3/CD28 stimulation by a challenge with *Salmonella typhimurium*. In addition, other authors have reported more CD4+CD25+ cells in the spleen, associated with pro-inflammatory cytokine inhibition (Maloy et al., 2003; Scully et al., 2013). In this case, the probiotic could reduce pathogen intestinal colonization and modulated the immune response with an increase in the intraepithelial lymphocytes at the ileal level. In the present trial, this suggests that the combined use of these two strains (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) does not generate a clear immunomodulatory activity. Another research by Lengkey & Adriani (2009) showed that probiotics with a well-balanced amount of consortium can inhibit the growth of pathogenic bacteria such as *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

CONCLUSIONS

In conclusion, probiotic yogurt with the correct consortium and balance will inhibit the growth of several pathogenic bacteria, such as *Salmonella typhii, S. typhimurium, Pseudomonas aeruginosa, staphylococcus aureus, E. coli,* and *Bacillus cereus.* The results presented here provide evidence that some *Bifidobacterium* strains exert antimicrobial activity against pathogenic bacteria like *Salmonella typhimurium*. This production seems to be varied among *Bifidobacterium* species. The combination of Yoghurt Mixed Culture with *Bifidobacterium* strains caused the pH of the culture to drop; which caused a high death ratio for the population of *Salmonella spp.* Such combinations between Bifidobacterium species and some lactic acid bacteria have a great advantage in increasing the protective effects of Bifidobacteria for the gastrointestinal tract against enteric pathogens also ensuring the safety and quality of many dairy products.

ACKNOWLEDGEMENTS

We would like to thank the Ministry of Research and Technology and Academic Leadership Grant (ALG) for the funding and facilities provided during the research.

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