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Consortium Probiotic Fermented Milk using *Bifidobacterium sp.* and *Lactobacillus acidophilus* Protects against *Salmonella typhimurium* and Repairs the Intestine

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ABSTRACT

Background: This research investigates the implication effect of consortium microbiota probiotic *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium sp.* in yogurt on the zone of inhibition against *Salmonella typhimurium* in yogurt, also the activities of the enzyme.

Methods: The experiment used a randomized complete design (CRD) with four treatments and two replicates. T1: Yogurt A with *Streptococcus thermophilus*, *Lactobacillus acidophilus, Bifidobacterium spp* and *Lactobacillus bulgaricus.;* T2: Yoghurt A + S from cow milk + skim milk 5%.; T3: Pure cow milk; and T4: Amoxicillin 100 ppm. Analysis of variance was used to look at the data numerically and then Duncan's multiple range test was done. This study was carried out at Laboratory of Biochemistry Mathematics and Natural Sciences. The research started from February 2nd until 20th, 2023.

Result: Results indicated that the probiotic microbiota produced higher enzyme activities and showed the inhibition zone better than milk, even though there is no significant effect compared to antibiotic control.

Key words: *Bifidobacterium sp.*, Inhibition zone, Lactic acid, *Lactobacillus sp.*, *Salmonella typhimurium, Streptococcus sp.*

INTRODUCTION

The nutritional value of yogurt is determined by the milk as the sources and processing factors. These variables include temperature, heat duration and storage conditions. In addition, the nutritional and physiological properties of the product are contingent upon the specific probiotic strain utilised during the fermentation process (Mazza, 1998; Adriani, 2018). Milk is a valuable dietary source of essential vitamins, including B-6, B-12, riboflavin, niacin and folic acid. Folate is a B vitamin that can be produced by several species of lactic acid bacteria. Several bacterial species are utilized for milk fermentation and the potential of yogurt production to biosynthesize *S. thermophilus* and *Bifidobacteria*, which are known to generate folate, was evaluated. When *Bifidobacterium sp.* and *S. thermophilus* are used together, folate can increase six-fold (Crittenden *et al.*, 2003). Yoghurt, besides vitamins, provides 3-4 times the calcium intake through the feed. Essential minerals such as potassium, magnesium, phosphorus and zinc are found in yogurt (Kerry *et al.*, 2001). Calcium is involved in the processes of bone development and mineralization. According to the Recommended Dietary Allowance (RDA), adults' recommended daily calcium intake is approximately 900 mg, while teenagers and the elderly need 1200 mg/day (Gueguen and Pointillart, 2000).

In terms of disease prevention and health advantages, probiotics are acquiring popularity. Growth, adhesion and invasion of *Salmonella* cells can be inhibited by *Bifidobacterium sp.* according to *in vitro* studies. Likewise, it alters the immune response by reducing the secretion of interleukin (IL)-8 and the production of tumour necrosis factor (TNF) in the small intestine.

This study aims to determine how gut health and function are affected by the content of yogurt and Lactic Acid Producing Bacteria (LAB). *Salmonella* induced small intestinal damage may reduce villi-associated enzyme activity. This research is necessary because cases of fever due to *Salmonella typhi* or *typhimurium* in Indonesia range between 350-810 per 100,000 population. This disease affects 1.6% of Indonesia, the fifth most common infectious illness affecting all ages in Indonesia, which is 6.0% and ranks 15th for causes of death for all ages in Indonesia.

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MATERIALS AND METHODS

Materials

The milk is from Koperasi Peternak Sapi Perah Bandung Utara (KPSBU) Lembang, West Java, Indonesia. *Streptococcus thermophilus*, *Lactobacillus acidophilus, Bifidobacterium sp.* and *Lactobacillus bulgaricus* are all pure cultivated bacteria. Milk and Amoxicillin is the control treatment. Milk is a base for making yogurt. Amoxicillin is an antibiotic frequently used to kill pathogenic bacteria and *Salmonella sp.* in this research.

Statistical analysis

A completely randomized design (CRD) was used in the trial, with four treatments, each treatments has two replications. Analysis of Variance was used to look at the numbers of the data and then Duncan's multiple range test was done. This study was carried out at Laboratory of Biochemistry Mathematics and Natural Sciences. The research started from February 2 to 20, 2023. The following are four treatments:

T1: Yogurt A with *Streptococcus thermophilus*, *Lactobacillus acidophilus, Bifidobacterium sp.* and *Lactobacillus bulgaricus.*

T2: Yoghurt A + S from cow milk + skim milk 5%.

T3: Pure cow milk.

T4: Amoxicillin 100 ppm.

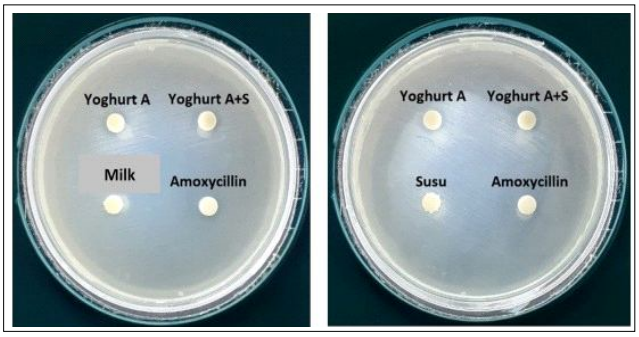

Fig 1: Antibacterial against *Salmonella typhimurium* ATCC 14028.

RESULTS AND DISCUSSION

Table 1 shows the results. The results showed that Yoghurt A with the consortium amount of *Streptococcus thermophilus*, *Lactobacillus acidophilus, Bifidobacterium sp.* and *Lactobacillus bulgaricus.* Yogurt A+S is fermented milk with the addition of 5% skim milk. The results showed that yoghurt, made only from fermented milk with a mixture of 5% skim milk, showed better results than consuming only unfermented milk. However, the use of 100 ppm amoxicillin showed the best results. Yogurt with a mixture of skim milk showed a higher amount of lactose than youghurt without the addition of skim milk.

Salmonella is a bacteria that causes gastroenteritis, which disrupts normal gut function and causes diarrhea. However, *Salmonella* will induce an unknown disease when it interacts with its host.

Brush border enzyme activity is reduced by *Salmonella typhimurium*. These alterations are most likely the result of a combination of minor intestinal morphological changes and the inflammatory response of the host immune system, which may alter enzyme gene expression and protein activity. LPS administration showed physiological changes, decreased body weight and the administration of probiotics containing *Bifidobacterium.*

Lactic acid bacteria (LAB) used in fermentation contribute to sustaining the nutritional value of various foods. The starter culture is a source of proteolytic enzymes during fermentation, ultimately contributing to product preservation as it produces inhibitory metabolites (O'Keeffe and Hill, 1999). In the digestive tract, lactic acid bacteria are important microbes (Holzapfel *et al.*, 2001). Lactic acid and acetic acid production were produced more by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* than *Lactobacillus acidophilus* and *Bifidobacterium sp.* (Adriani and Lengkey,

Table 1: Antibacterial against *Salmonella typhimurium* ATCC 14028.

Sample	Concentrate	Inhibitation diameter (d/mm)		Average diameter (mm)	Result
Yogurt A	-	8.40	8.60	8.50±0.14	Active*
Yogurt A + S	-	8.60	8.70	8.75±0.07	Active
Milk	-	6.00	6.00	6.00±0.00	Inactive
Amoxicilin	100 ppm	15.40	15.60	15.50±0.14	Active

Note: The diameter of disc used is 6 mm.

*Inhibitation zone are not clear.

Table 2: Enzyme activities.

Yogurt starters	Enzyime activities (Units/ml)	
	Protease	Lipase
Bifidobacterium spp: *Lactobacillus acidophilus* (1 : 1)	1.70	0.45
Lactobacillus bulgaricus: *Streptococcus thermophilus : Bifidobacterium spp* (1 : 1 : 1)	1.62	0.27
Lactobacillus bulgaricus: *Streptococcus thermophilus : Lactobacillus acidophilus* (1 : 1 : 1)	1.65	0.19
Lactobacillus bulgaricus: *Streptococcus thermophilus* (1 : 1)	1.45	0.18

2009). The bacterial starter culture *Lactobacillus acidophilus* and *Lactobacillus plantarum* may offer health benefits that improve glucose and protein level in the blood (Latipudin *et al*., 2018; Adriani *et al*., 2021), also blood lipid (Adriani *et al*., 2018). Antibacterial compounds produced by probiotics are able to control intestinal pathogens as competition for nutrients and adhesion sites (Fig 1). In addition, probiotics produce lactic acid, acetic acid and substances such as antibiotics resulting in increase the enzyme activity and antibody levels (Hose and Sozzi, 1991). Based on Table 2, yogurt with *Bifidobacterium sp.* starter and *Lactobacillus acidophilus* had higher lipase activity (0.45 units/ml) than *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (0.18 units/ml) (Adriani *et al*., 2009).

CONCLUSION

It is believed that consuming yogurt, including consortium lactic acid bacteria with probiotics, could inhibit the growth of *Salmonella typhimurium*, which causes typhus and enhances gut health. Several of these beliefs are supported by numerous studies on the potential health benefits of yogurt in preventing gut-related diseases. In addition to the health benefits of yogurt with live and active cultures such as *L. bulgaricus, S. thermophilus, L. acidophilus* and *B. bifidum*, the protection against *Salmonella typhimurium* is one of the most intriguing preventive effects because it produces protease and lipase enzyme. These results are exciting and should lead to more studies into how yoghurt does what it does and what parts of yogurt are most important for that to happen.

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Competing interest

The authors say there are no competing interests.

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