

IN VITRO ANTIBACTERIAL ACTIVITIES OF COMMERCIAL PROBIOTIC DRINKS AGAINST *Salmonella typhii*

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ABSTRAK

Demam tifoid yang disebabkan oleh *Salmonella typhii* menjadi masalah serius di Indonesia. Selain itu, pengetahuan dan penelitian tentang aktivitas probiotik antibakteri terhadap *Salmonella typhii* belum diketahui. Tujuan dari penelitian ini adalah untuk mengevaluasi dan membandingkan aktivitas antibakteri minuman probiotik, merek Y dan V, yang memiliki strain probiotik yang berbeda terhadap *Salmonella typhii*, agen penyebab demam tifoid, secara in vitro. Jumlah yang sama dari minuman Y atau V (1 mL) dan *S. typhii* dicampur. Satu mL aliquot dipindahkan ke cawan petri yang ditambahkan 19 mL lelehan agar nutrisi, divorteks, dan diinkubasi pada suhu 37°C selama 24 jam. Prosedur ini diulang 6 kali. Hasilnya menunjukkan, bakteri koloni tumbuh pada agar plate nutrisi dalam semua replikasi minuman Y dan V. Kesimpulan dari penelitian ini adalah aktivitas antibakteri terhadap *Salmonella typhii* secara in vitro tidak ditemukan dan tidak ada perbedaan antara minuman Y dan V. Hasil yang diperoleh dalam penelitian ini memberikan beberapa dukungan untuk penggunaan isolat probiotik atau isolat bakteriosin (agen antibakteri probiotik) sebagai substansi uji berikutnya probiotik dalam penelitian in vitro dan disarankan juga untuk melakukan percobaan vivo. (FMI 2012;48:109-114)

Kata kunci: probiotik, *Salmonella typhii*, aktivitas antibakterial, in vitro

ABSTRACT

Typhoid fever that caused by *Salmonella typhii* become serious problem in Indonesia. Besides, knowledge and study about antibacteria activity of probiotic against *Salmonella typhii* has not been known. The objective of the present study was to evaluate and compare antibacterial activity of probiotic drinks, they are Y and V, that have different probiotic strain against *Salmonella typhii*, a causative agent of typhoid fever. Equal amount of Y drink or V drink (1 mL) and *S. typhii* were mixed. One mL of aliquot was transferred to a petri dish added with 19 mL of melted nutrient agar, vortexed, and incubated at 37°C for 24 hours. The procedure was repeated 6 times. The result shows that bacteria colonies grew in nutrition agar plate in all replication of Y and V drinks. The conclusion of this experiment is that in vitro antibacteria activity against *Salmonella typhii* does not exist and there was no difference between Y and V drinks. The result obtained in this study supported the use of probiotic isolate or bacteriosin isolate (antibacterial agent of probiotic) as next substance for probiotic test in in vitro studies and we also suggest to undertake in vivo experiment. (FMI 2012;48:109-114)

Keywords: probiotic, Y drink, V drink, *Salmonella typhii*, antibacterial activity, in vitro

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INTRODUCTION

Salmonella typhii is a gram-negative bacteria causing typhoid fever with a high prevalence in Indonesia. This bacterial disease spread through the fecal-oral route, by contaminating food and beverages, especially water unhealthy. This is important, given the problems of sanitation in Indonesia, food and drink is still relatively poor. Many Indonesian citizens with the middle and lower economic classes still use river water contaminated with garbage and feces for bathing, washing, toilet, even to be consumed daily. Not only that, inclement weather and frequent natural disasters, such as floods and landslides, makes narrower access to

clean water sources. *S. typhii* infection may worsen because of its pathogenesis involving the circulatory system through the lymphatic vessels of the gut can invade any organ of the entire body (Brooks et al 2005).

According to the WHO in 2000, there are 17 million cases of typhoid and 60 thousand of them died with the highest prevalence of infection the same age as the national data, the age group 5-19 years (World Health Organization 2005). While national data is data Health Research (Riskesdas), 2007 (Agency for Health Research and Development Department of Health of the Republic of Indonesia in 2008), of the 970 thousand people who become members of the household sample,

the prevalence of typhoid fever in Indonesia is 1.6%. 12 of them had prevalence provinces above the national average, among others: NAD, West Java, NTB and NTT. Still from the same source, the prevalence of typhoid found in the school age group (5-14 years) and relatively higher in rural areas than urban and tends to be higher in the group with low education and low economic level.

In Indonesia, which is the top choice of antibiotic therapy is Chloramphenicol typhoid fever. This is because antibiotics are relatively inexpensive, fairly sensitive and can be absorbed easily. Chloramphenicol can reduce fever average of 7 days. There is even mention of a decrease fever occurs after day 5 of treatment (Widodo 2009). On the other hand, the body has a physiological mechanism against invasion of any kind, including bacteria. Interestingly, in some parts of the body, the physiological mechanisms that can take place either by the presence of non-pathogenic bacteria that inhabit the site as normal flora. For example, normal intestinal flora is occupied by 10^5 - 10^{10} bacteria/gram with a variety of species. The normal flora of the intestinal mucosa dominates as a competitive inhibitor against pathogenic microorganisms (Brooks et al 2005).

Physiological mechanism was later adapted to make probiotic drink. The probiotic drink made from fermented milk containing live bacteria. Bacteria are expected to escape the extreme atmosphere of the gastrointestinal tract, the gastric acidity and bile, and may eventually reach the intestine and other normal flora joined to implement a competitive inhibitor function (US Probiotic 2007). In several recent studies, it was mentioned that probiotics have an antibiotic substance called lactosidin and bacteriosin (Godiosa et al 1993). Known, these substances increase the protective potential of probiotics against pathogens.

Probiotic drink products containing different bacterial strains as seed promotion in the community. Among them, Y containing *Lactobacillus casei* strain Shirota and V contains three species of bacteria at a time that is *Acidophilus digestiva*®, *Bifido defensia*®, *Casei imunita*®. Both of these products are in the last period is known by the people. However, the foundation of society eleksi not be based on their effectiveness against pathogens, but because other promotional points on offer eg taste, price and more attractive packaging.

Applications probiotic drink in public is rarely used as a treatment of gastrointestinal infections. During this time, people familiar drinks constipation probiotics as preventive measures a crowded promoted by these products. Not to many people who knew about the

antibacterial activity against pathogenic bacteria probiotic drink gastrointestinal tract, including gastrointestinal *S. typhii* as bacteria causing typhoid fever. Based on these descriptions, comparative studies on the antimicrobial activity of the probiotic drink products against *Salmonella typhii* should be done in vitro so it can be used as a new reference companion therapy for *S. typhii* infection. The purpose of this study was to determine the effect of probiotic drinks on the growth of *Salmonella typhii* in vitro.

MATERIALS AND METHODS

This type of research used in this study is true-experimental laboratory to compare the antibacterial activity of probiotic drinks on the growth of *Salmonella typhii* bacteria. The design of this study used a post-test only control group design. Evaluation is done by comparing the bacteria colonies formed in each treatment after 24 hours of administration of treatment on *Salmonella typhii* grown on nutrient agar plates.

The study population is the bacterium *Salmonella typhii*, while the sample is bacteria *Salmonella typhii* isolates stored at the Laboratory of Microbiology, Faculty of Medicine, University of Airlangga. Sampling technique in the study done by total random sampling. Replication is needed in this study using the formula Feerdeer (Murdiyanto 2005) is 15. Treatment (p) to be performed on each sample by mixing isolates of bacteria is bacteria in nutrient broth with 2 samples of probiotic drinks (Y and V), antibiotics Chloramphenicol and sterile distilled water each with the same ratio. By using the formula, found that replication of the required minimum is 6 times.

Suspension *Salmonella typhii* *Salmonella typhii* used was 0.5 McFarland. Available stock first cultured to obtain healthy growth (thrives and the logarithmic growth phase or not mutated or phase lag or dead). Suspension initial test was made equivalent to 0.5 Mc Farland turbidity (cloudiness mixture of barium sulfate and HCl) or proportional to the number of bacteria 1×10^8 CFU/mL (CFU: Colony Forming Unit) or 250-300 colonies on solid media. Taken a few colonies of bacteria then thinned or diluted with isotonic solution (PBS or PZ) so that the concentration in accordance with the concentration of 0.5 McFarland. The bacteria suspension was then diluted to concentrations 105. Concentrations have been adjusting to the research conducted by the probiotic drink Godiosa et al (1993).

The procedure used in this study to adjust premises n research on probiotic drink made by Godiosa et al (1993). 1 mL of *Salmonella typhii* suspension was

mixed with 1 mL of Y beverages as test material. Then, 1 mL of this mixture was poured in a petri dish and mixed with 19 mL of nutrient agar is still liquid. Before freezing, the mixture was shaken so that plate bacteria, nutrient agar, and the test material is mixed. Once frozen, the mixture is then incubated. The same procedure is performed on the other test materials that probiotic drink V and Chloramphenicol solution and sterile distilled water as a control.

The analysis was conducted by observing and comparing the presence or absence of bacteria colonies growing in culture medium of each treatment after 24 hours of incubation. Staining of bacteria was conducted to determine the type of bacteria colonies growing on culture medium. Staining was performed Gram staining.

Location of the study was the Laboratory of Microbiology, Faculty of Medicine, University of Airlangga. Time study comparisons of antimicrobial activity against the growth of probiotic drinks *Salmonella typhii* bacteria held in October-December 2011. Data in the form of primary data, ie data taken from the experimental results of antibacterial testing

through modification pour plate method. We used a mixture of bacteria and antibacterial agents (Y, V and Chloramphenicol) and sterile distilled water. Then mixed with a liquid nutrient agar and allowed to freeze. After incubation for 24 hours, we noted the presence or absence of bacteria colonies growing on culture medium. *Salmonella typhii* colony growth data presented in each treatment in the table, and then compared with the control group. Analysis of samples of the antibacterial activity of probiotic beverages based on a comparison to the growth of colonies of bacteria that are subjected Chloramphenicol 0.1 mg/mL as a negative control and a colony of bacteria that are subjected to sterile distilled water as a positive control in this study.

RESULTS

The test results of the antibacterial activity of probiotic drinks *Salmonella typhii* growth in vitro by using a dilution method that, according to the research methods Godiosa et al (1993) are as follows:

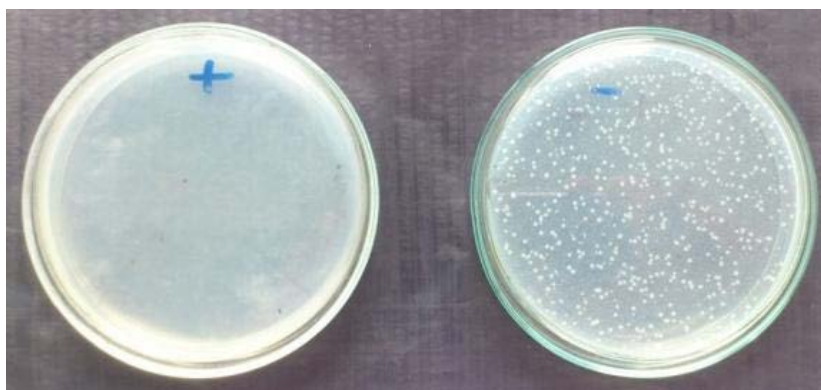
Table 1. Results of testing the antimicrobial activity of probiotic drinks and control materials

Replication	Materials			
	Y	V	Distilled water (control)	Chloramphenicol 0,1 mg/mL (control +)
1	✓	✓	✓	x
2	✓	✓	✓	x
3	✓	✓	✓	x
4	✓	✓	✓	x
5	✓	✓	✓	x
6	✓	✓	✓	x

Remarks:

V : No growth of bacteria colonies on culture medium

X : No growth of colonies of bacteria in the culture medium



Control +: no growth of bacteria colonies

Control -: growth of bacteria colonies is present

Figure 1. Growth of bacteria colonies in the control group as an indicator

Table 2. Results of Gram staining germ colonies in each treatment

Replications	Materials			
	Y	V	Aquadest (control +)	Chloramphenicol 0.1 mg/mL (control -)
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	contaminant	contaminant	-	-
6	contaminant	contaminant	-	-

In the first experiment showed that the test material is a probiotic drink Y and V showed no antibacterial activity against *Salmonella typhii*. This can be seen from the results of laboratory testing that there is a growing colony of bacteria on both the test material. These results were obtained after comparing with plate control group.

Control (-) on the research aims to prove that the *Salmonella typhii* bacteria grow in nutrient agar which had been treated sterile distilled water after incubation for 24 h at 37°C. Sterile distilled water is used because it is analogous to the test in the form of probiotic drinks/liquids. While the purpose of the control (+) is to examine the growth of *S. typhii* can be inhibited by antibiotics Chloramphenicol 0.1 mg/mL. In the control (-) obtained growth of bacterial colonies, whereas in the control (+) was not obtained growth of bacterial colonies.

To ensure the antibacterial activity of the test material did not show up, do Gram staining of bacteria colonies in each treatment and replication. Gram staining was also performed to determine whether or not the competitive nutrition to be one mechanism of action of probiotics. Of all the Gram stain of the colonies of bacteria to treatment with sterile Aquadest on replication to-1 up to the 6th, which means the red-stained bacteria was Gram-negative bacteria. Similarly, the Gram stain of the colonies of germs on treatment with Y and V drinks on replication to-1 through the 4th. While on treatment with Chloramphenicol 0.1 mg/mL, the Gram stain can not be done because there are no bacteria colonies formed. Gram-negative germs are assumed to *S. typhii* which is also a Gram-negative bacteria. Meanwhile, if the bacteria are stained blue bacterium Gram-positive bacteria. While all of the germs contained lactic acid bacteria in beverages Y and V are Gram-positive bacteria.

Gram staining on treatment with Y drinks and beverages V, replication of the 5th and 6th germs stained blue, which means Gram Positive bacteria. Although Gram has properties similar to probiotics, it is estimated that

germ is a germ contaminants because of its morphology is not characteristic morphology of probiotic bacteria. *Lactobacillus bacillus* shaped, thin and form a chain, while *bifidobacterium* shaped bacillus, thin and form a chain with the composition Y or V (Murray et al 2007). In addition, this contamination can actually be seen from the colonies of germs on the surface of the plate. Colonies of bacteria on the plate surface treatment Y drinks and beverages V replication 5th and 6th round shape. While colony isolates of probiotic bacteria in general diffuse irregular shape. Contamination is caused by environmental factors that can not be controlled lab.



Y: treatment with Y beverages
V: treatment with a drink V

Figure 2. Results of Testing with Diffusion Method The wells

Experiments were then performed by adjusting research methods Godiosa et al (1993). This method was chosen because it has several advantages to prove the antibacterial effect of probiotic drinks. Dilution method that was commonly used method to evaluate the testing techniques other antibiotics. With this method, the heterogeneity of the population and is more easily detected microbial contamination.

In this method, Y and V drinks mixed with bacteria suspension concentrate 105 of the same volume, ie each 1 mL. 1 mL of a mixed germ-test material was mixed

with 18 ml of molten nutrient agar plate after plate shaken in order to mix evenly. After freezing, the plate was incubated for 24 hours. Same way done in distilled water and Chloramphenicol solution of 0.1 mg/mL as a control group. After that, the second showed that probiotic drinks contained colonies of bacteria growing. These results were together with the results of the negative control treatment that is distilled. This suggests that the antibacterial activity against *S. typhii* from both probiotic drinks do not appear on this method.

To ensure that the colony is not germ-growing bacteria contained in probiotic drinks, do a simple test, the Gram stain. As a result, all the Gram staining, both in the test group Y and V probiotic drinks and distilled water control group, indicating that the bacteria colonies formed are Gram-negative bacteria are also one of the profiles of *Salmonella typhii*. This suggests that the antibacterial activity against *S. typhii* from beverages Y and V do not appear.

This study is expected to provide results that probiotic drinks test showed antibacterial activity against *S. typhii* in vitro. However, the results of laboratory tests showed no such expectations. This may be due to the probiotic tested is still in the form of a drink, instead of isolates. In some studies, bacteriosin, probiotics antibacterial peptides, produced by probiotic isolates were obtained from samples. In the study conducted by Godiosa et al (1993), *Lactobacillus casei* Shirota strain isolates of Y has 50% effectiveness inhibits the growth of bacteria that cause diarrhea within 5 minutes. Research Chuayana et al (2003), revealed a bacteriostatic effect against *Pseudomonas aeruginosa* and fungistatic effects on *Candida albicans* appear after probiotic microorganisms isolated.

Although the potential effects of probiotics against *S. typhii* obstacles not seen in this study, but this barrier function against pathogenic bacteria in the gastrointestinal tract is quite rational though consumed in beverage form. Simply put, is a probiotic drink fermented milk products containing probiotic bacteria. When in the gastrointestinal tract, the milk will be mechanically and enzymatically digested like other foods. However, probiotic microorganisms are not nutrients so it was not 'digested'. Probiotics have resistance to gastric acid and other upper gastrointestinal barrier. After reaching the intestine, probiotic starts working. When probiotics bind to the receptor, a new probiotic active in carrying out its antibacterial activity, namely nutrition competitors pathogenic bacteria, produce bacteriosin (analogous to microsin or lactosidin), producing VFA (Volatile Lactic Acid) which induces cytoplasmic acidification of the

cytoplasm so that there arose a decrease in intestinal pH which makes the environment intestinal hostile against pathogens (Ogawa et al 2001).

Optimal bacteriocin activity takes place in the intestinal lumen, supported by Lengkey and Soeharsono 2010 which revealed that many bacteriosin of lactic acid-producing bacteria are active in a certain range of temperature and pH. Additionally, Martini et al, 2002 in Lengkey and Soeharsono 2010 cites the opinion of Tagg et al, 1976 that bacteriosin activity directed against species that share the same ecological environment.

Thus, it can be concluded that in this study, although nutrients are met by the culture medium, but not in a state of active probiotics, which isolates but not in the form of a drink. In addition in the form of isolates, probiotics can only be active if there are factors other intestinal lumen and in this study these factors are not met so that the antibacterial activity of probiotic drinks, both Y and V, on the growth of *S. typhii* in vitro does not appear.

CONCLUSION

Not found the antibacterial activity of probiotic drinks on the growth of *Salmonella typhii* in vitro. No differences were found between the antibacterial activity of probiotic drinks Y and V against *Salmonella typhii* in vitro

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