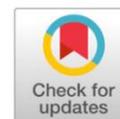




## Original Research



### *Antituberculosis agents of Lactobacillus plantarum and Pediococcus acidilactici Lactic Acid Bacteria in Breast milk isolates against Mycobacterium tuberculosis*



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**Abstract:** Tuberculosis (TB) is one of the infectious diseases that have become a major problem in Indonesia. This disease is caused by the *Mycobacterium tuberculosis* bacteria. The bacteria are commonly treated with antibiotics. However, the use of irrelevant antibiotics is the most influential factor of antibiotic resistance. Therefore, natural ingredients are required as novel antibiotic agents, some of which are sourced from lactic acid bacteria. This study aims at investigating the antituberculosis activities of *Pediococcus acidilactici* and *Lactobacillus plantarum* isolated from breast milk against *M. tuberculosis*. *Pediococcus acidilactici* and *Lactobacillus plantarum* breast milk isolates were rejuvenated with MRS broth media, and the supernatant was then neutralized to pH 7.0 using a pH meter by adding 1N NaOH solution. Antituberculosis activity test was performed with Lowenstein Jensen media to investigate the growth of *M. tuberculosis*. The results of this study showed that *Pediococcus acidilactici* had antituberculosis activity at 48 hours at concentrations of 80% and 90%, while the secondary metabolites of *L. plantarum* had antituberculosis activity at 24 hours and 48 hours at concentrations of 60%, 70%, 80%, 90%. Therefore, this study concludes that *P. acidilactici* and *L. plantarum* bacteria have the potential to be developed as antituberculosis agents.

**Keywords:** Antituberculosis, Lactic acid bacteria, *Pediococcus acidilactici*, *Lactobacillus plantarum*.

## INTRODUCTION

Indonesia is one of the most populous countries in the world, ranking fourth with a population of 273,523,615 people<sup>1</sup>. This large number requires the government to prosper its people because numerous problems are likely to arise, including political, social, economic, and health issues.

One of the prevalent health issues in Indonesia is tuberculosis (TB), an infection caused by *Mycobacterium tuberculosis* bacteria<sup>2</sup>, which can trigger symptoms in the respiratory tract. A person suffering from tuberculosis will experience symptoms of continuous cough, fever, and chest pain<sup>3</sup>. This disease can be transmitted through close contact with patients. Droplets resulted from coughing and sneezing are spread by the wind and then stick to other people. Rajni and Laxman<sup>4</sup> reported that this disease is one of the death-causing illnesses that are most difficult to control properly and effectively.

There have been many studies researching tuberculosis and *Mycobacterium tuberculosis*. Palomino and Anandi<sup>5</sup> reviewed the molecular basis

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and mechanism of drug resistance in *Mycobacterium tuberculosis*. Igarashi<sup>6</sup> also investigated the development of antituberculosis drugs derived from natural products. Furthermore, several related studies were conducted to obtain more effective drugs or methods for treating this disease, one of which is the potential of a secondary metabolite as an antimicrobial agent that can inhibit the growth of microbes<sup>7</sup>.

Secondary metabolites are antimicrobial peptides produced by bacteria that are effective to treat tuberculosis. These compounds serve as chemical defenses against microorganisms that are resistant to heat. In the pharmaceutical field, these have been widely developed in the food industry and have great potential as antimicrobials that can kill pathogens. Sharma et al<sup>8</sup> reported that secondary metabolites of *Bacillus subtilis* GAS101 can inhibit the growth of *Staphylococcus epidermidis* and *Escherichia coli* in broad ranges of temperature [30-121°C] and pH [2-12]. Aguilar-Perez et al<sup>9</sup> researched the ability of secondary metabolites that have antibacterial activity against *M. tuberculosis*.

Bacteriocins are produced by lactic acid bacteria (LAB). These bacteria are gram-positive, catalase-negative, and rod-shaped, and can perform carbohydrate fermentation<sup>10</sup>. LAB can be found or isolated from a variety of sources, including breast milk. Breast milk is known to contain various bacteria beneficial for babies, particularly those that aid in immune system development. *Staphylococcus*, *Lactobacillus*, *Pseudomonas*, *Enterococcus*, and other bacteria are frequently found in breast milk<sup>11,12</sup>. *Pediococcus pentosaceus* OZF is isolated from breast milk<sup>13</sup>.

Studies on the potential of *L. plantarum* and *P. acidilactici* bacteria as antituberculosis agents isolated from breast milk in inhibiting the growth of *M. tuberculosis* have not been carried out. Therefore, a study that investigates the potential of those bacteria as antimicrobials and their further development into antituberculosis agents that can inhibit the growth of *M. tuberculosis* bacteria is required.

## MATERIAL AND METHOD

### Rejuvenation of *Lactobacillus plantarum* and *Pediococcus acidilactici* bacteria

*L. plantarum* and *P. Acidilactici* bacteria isolates were obtained from breast milk<sup>14</sup>. The isolates were then rejuvenated in the medium of MRS agar with 1% CaCO<sub>3</sub> and incubated at 35±2°C for 2-3 days under anaerobic conditions. The clear zone formed around the bacterial colonies was suspected to be LAB.

### Isolation of secondary metabolites from *P. acidilactici* and *L. plantarum*

*P. acidilactici* and *L. plantarum* were cultured in MRS broth. After being cultured on the medium, the bacteria were incubated for 24 hours and then centrifuged to obtain the content of secondary metabolites in LAB. The supernatant produced was saturated with ammonium sulfate to 80% under cold conditions, and the precipitate was filtered and dried. It was then dissolved with potassium phosphate buffer at pH 7 and secondary metabolites with neutral pH were obtained.

### The anti-mycobacterium activity of *P. acidilactici* and *L. plantarum*

Antituberculosis activity test of secondary metabolites of *P. acidilactici* and *L. plantarum* isolates from breast milk against *M. tuberculosis* was performed using Lowenstein Jensen (LJ) media. Secondary metabolites of *P. Acidilactici* and *L. Plantarum* with concentrations of 90%, 80%, 70%, and 60% were incubated and then contacted with *M. tuberculosis* for 1 hour, 24 hours, and 48 hours.

## RESULTS AND DISCUSSION

Based on the results of the study, isolated microbes of *Lactobacillus plantarum* and *Pediococcus acidilactici* were obtained. *Lactobacillus plantarum* are gram-positive, rod-shaped, convex, and entire, while *Pediococcus acidilactici* are gram-positive, catalase-negative, round-shaped, and non-motile. From the two isolates, secondary metabolites which had been cultured on MRS media were isolated. Further, the secondary metabolites were tested for antimicrobial activity against *M. tuberculosis* as pathogenic bacteria that cause tuberculosis (TB).

The antimicrobial activity test of secondary metabolites of *L. plantarum* and *P. acidilactici* showed different results based on the variations in the contact periods, including 1 hour, 24 hours and 48 hours, as presented in Table 1. These results indicate that the secondary metabolites of each isolate have different abilities in inhibiting the growth of *M. tuberculosis*.

Table 1. The results of antimicrobial activity test of secondary metabolites of *L. plantarum* and *P. acidilactici* with various contact periods with *M. tuberculosis*

Concentration (%)	Period (hour)	Anti-tuberculosis Activity	
		<i>L. plantarum</i>	<i>P. acidilactici</i>
90	1	positive	positive
80		positive	positive
70		positive	positive
60		positive	positive
90	24	negative	positive
80		negative	positive
70		negative	positive
60		negative	positive
90	48	negative	negative
80		negative	negative
70		negative	positive
60		negative	positive

[Table 1](#) demonstrates that secondary metabolite of *L. plantarum* with contact period of 1 hour and concentrations of 60%, 70%, 80%, and 90% show positive results against *M. tuberculosis*. The results exemplify that secondary metabolites that are administered to *M. tuberculosis* are not effective to inhibit the growth of *M. tuberculosis*. With a one-hour initial contact period, the antimicrobial activity of secondary metabolites may not work optimally, allowing *M. tuberculosis* to continue actively growing and reproducing. [Figure 1](#) shows that *M. tuberculosis* is developing rapidly and a large number of yellow bacterial colonies grow on the media.

Positive results were also shown by the secondary metabolite activity of *P. acidilactici* with one hour period and the same concentration variations of 60%, 70%, 80%, and 90% without any inhibitory activity against the growth of *M. tuberculosis* bacteria. Even at a concentration of 90%, the bacteria that cause tuberculosis could continue to grow and reproduce.



Figure 1. The results of the activity test of secondary metabolites (*L. Plantarum* and *P. acidilactici*)<sup>15</sup> from breast milk isolates against *M. tuberculosis* with contact periods of 1 hour, 24 hours, and 48 hours

After 24 hours of incubation, significant inhibitory activity was shown by the secondary metabolite of *L. plantarum* against *M. tuberculosis*. All concentration variations (60%, 70%, 80% and 90%) of *L. plantarum* secondary metabolites demonstrated the ability to inhibit *M. tuberculosis*, as indicated by nearly the absence of *M. tuberculosis* bacteria in the growth media.

Further, within the 24 hour incubation period, secondary metabolites of *L. plantarum* were effective in hindering the growth of *M. tuberculosis* even at a concentration of 60%. The study by Lin<sup>15</sup> also reported that secondary metabolites produced by *L. plantarum* showed effective antimicrobial activity against *Vibrio parahaemolyticus*. Moreover, Hasan et al<sup>16</sup> found the activity of secondary metabolites as pathogenic antimicrobials in the isolates from yogurt.

However, different results were shown by secondary metabolites of *P. acidilactici*. With various concentrations (60%, 70%, 80% and 90%) and the same incubation period of 24 hours, the secondary metabolites did not demonstrate any inhibitory activity against *M. tuberculosis*, as summarized in [Figure 1](#). The bacteria causing TB kept growing and reproducing well.

Furthermore, with an incubation period of 48 hours, the secondary metabolite of *L. plantarum* appeared to show better inhibitory (antimicrobial) activity, as proven by no more growth of *M. tuberculosis* on MRS media. In other words, the medium was free from *M. tuberculosis*. Secondary metabolites of *P. acidilactici* with an incubation period of 48 hours also demonstrated the ability to inhibit the growth of *M. tuberculosis*. However, the inhibitory activity only occurred at high concentrations (80% and 90%). Meanwhile, at the concentrations of 60% and 70%, *P. acidilactici* did not show any growth inhibition activity. Gaspar et al. (2018) reported that secondary metabolites can block the growth of urogenital pathogenic bacteria, such as *Gardnerella vaginalis*, *Streptococcus agalactiae* and *Pseudomonas aeruginosa*.

Therefore, these results signify that secondary metabolites are effective in inhibiting the growth of *M. tuberculosis* pathogenic bacteria. In particular, secondary metabolites of *L. plantarum* isolated from breast milk have a better inhibiting ability than *P. acidilactici*. Thus, further research is required to investigate their potential as antituberculosis agents and identify the benefits.

## CONCLUSION

Secondary metabolites of *Lactobacillus plantarum* and *Pediococcus acidilactici* can inhibit *M. tuberculosis* bacteria. The secondary metabolites of *L. plantarum* can inhibit the growth of *M. tuberculosis* during the 24 hour incubation period with concentrations of 60%-90%. Meanwhile, the secondary metabolites of *P. acidilactici* demonstrate inhibitory activity at 48 hours of incubation with concentrations of 80% and 90%, respectively. Thus, the secondary metabolites of *L. plantarum* have better antimicrobial ability than *P. acidilactici*. However, both secondary metabolites have the potential as pathogenic antimicrobials to kill *M. tuberculosis*.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally to this work.

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## DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

## DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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