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The Effect of *Lactobacillus acidophilus* and Chito−Oligosaccharide on Antibacterial Activity and Organic Acid Production

Miksusanti[1]*, Harian Saputra[1], Sofia Sandi[2], Hermansyah[1]

[1]*Chemistry Department, Universitas Sriwijaya, Inderalaya 30662 Indonesia*
[2]*Animal Husbandry Department, Universitas Sriwijaya, Inderalaya 30662 Indonesia*
[*]*Corresponding Author: miksusalbi2000@yahoo.com*

Abstract

The effect of *Lactobacillus acidophilus* combined with and without Chito-Oligosaccharide (COS) on the growth of *Escherichia coli* and *Staphylococcus aureus* had been studied. The antibacterial activity of *L. acidophilus* before and after combination with COS 0.2% was tested on bacteria of *E. coli* and *S. aureus* with well-diffusion method. Incubation time was carried out in 44, 46, 48, 50, and 52 hours. Organic acids produced by *L. acidophilus* was measured by HPLC. The result showed that COS 0.2% can inhibit the growth of *E. coli* and *S. aureus* 37.2 mm^2 and 52 mm^2 respectively. Combination of *L. acidophilus* and COS 0.2% gave inhibition zone larger than *L. acidophilus* without COS 0.2%. Incubation time within 48 hours of *L. acidophilus* combined with COS 0.2% produced the largest inhibition zone against *E. coli* and *S. aureus* 367.92 mm^2 and 343.99 mm^2 respectively. Optical density measurement resulted in higher value for combination one but concentration of organic acid produced was lower compare to *L. acidophilus* without COS 0.2%.

Keywords: COS, L. acidophilus, Organic acid, antibacterial.

Abstrak (Indonesian)

Telah diteliti pengaruh kombinasi *Lactobacillus acidophilus* dan Chito-OligoSakarida (COS) terhadap pertumbuhan *Escherichia coli* dan *Staphylococcus aureus*. COS 0,2% dalam asam asetat 1% dan larutan asam asetat 1% diuji aktivitas antibakterinya terhadap bakteri *E. coli* dan *S. aureus.* Bakteri *L. acidophilus* dengan dan tanpa COS 0.2% diinkubasi dengan variasi waktu 44, 46, 48, 50, dan 52 jam serta diuji aktivitas anti-bakterinya terhadap bakteri *E. coli* dan *S. aureus* dengan metode difusi sumur. Asam organik yang diekskresikan oleh bakteri *L. acidophilus* sesudah dan sebelum kombinasi dengan COS 0,2% diukur menggunakan HPLC. Hasil penelitian menunjukkan COS 0,2% dapat menghambat pertumbuhan bakteri *E. coli* dan *S. aureus* berturut-turut sebesar 37,2 mm^2 dan 52,2 mm^2. Kombinasi *L. acidophilus* dan COS 0,2% memberikan luas zona hambat yang lebih besar daripada *L. acidophilus* tanpa kombinasi COS 0,2% terhadap kedua bakteri uji. Kombinasi pada waktu inkubasi 48 jam menghasilkan luas zona hambat yang terbesar terhadap bakteri *E. coli* dan bakteri *S. aureus* yaitu berturut-turut sebesar 367,92 mm^2 dan 343,99 mm^2. Kerapatan optik dari *L. acidophilus* yang dikombinasikan dengan COS 0,2% lebih besar daripada *L. acidophilus* kontrol. Konsentrasi asam organik yang dihasilkan oleh kombinasi *L. acidophilus* dan COS 0,2% lebih rendah dibandingkan bakteri *L. acidophilus* tanpa COS 0,2%.

Kata kunci: COS, L. acidophilus, Asam organik, antibacterial

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INTRODUCTION

Prebiotics and *probiotics* combination in improving health of body is called a symbiotic relationship. Symbiosis can improve life of bacteria and provide specific subtract for fermentation. The existence of symbiotic can very much help as antimicrobial, anti-carcinogenic, and anti-osteoporosis. Consuming *Prebiotic*, *probiotic* and symbiotic effect on micro flora, i.e. returning the balance of microbe that can be a great potential input for health B].

Lactate acid bacteria has potential to be probiotic such as *Lactobacillus plant arum* and *Lactobacillus acidophilus.* Several groups of resistant polysaccharide, fiber, oligosaccharide, alcoholic sugar and Chitosan are known as prebiotics [2]. Chito-Oligosaccharide (COS) that can be obtained from chitin derive from shrimp waste very potential and abundant in Indonesia, is oligosaccharide that has potential as prebiotics source [3].

Lactate acid bacterial like *Lactobacillus acidophilus* can produce several organic acids such as lactate acid, acetate acid, propionate acid, malate acid, butyrate acid, oxalate acid and other organic acids. Organic acids produced by lactate acid bacteria can give activity to fight pathogen bacteria by weakening negative Gram bacteria and break outer layer of negative Gram bacteria. By breaking it, subtract of other antibacterial such as diacetyl, bacteriocin and hydrogen peroxide will penetrate into cytoplasm membrane [4]. In this study, synergetic effect analysis will be done by adding Chito-Oligosaccharide (COS) as prebiotic on lactate acid bacterial *Lactobacillus acidophilus* of local isolate as probiotic toward antibacterial characteristic and production of organic acid.

EXPERIMENTAL SECTION

Media for BAL was prepared from MRS-Agar media (6.2 g) and MRS-Broth media (5.22 g). Media preparation included homogenization and Sterilization for 15 minutes at 121°C, 15 lbs., whereas media for Pathogen was prepared from *Nutrient agar* media (2.3 g) and *Nutrient-Broth* media (0.8 g) in the similar procedure.

Preparation and Regeneration of Bacterial Culture

Bacterial Culture (*E. coli* and *S. aureus*) was prepared by taking bacteria from seaweed media and inoculated in 10 mL sterilized NB. The culture was incubated at 37°C for 24 hours and then planted on agar media. The bacterial colony was further incubated at 37°C for 48 hours, taken and then inoculated in 10 mL sterilized NB followed by incubation at 37°C temperature for 24 hours. Culture which was incubated in NB media will be used as testing bacteria.

Culture of *L. acidophilus* bacteria was inoculated in 10 mL sterilized MRS-Broth and then incubated at 37ºC for 48 hours. The next step was planted the bacteria on agar media followed by incubation at 37ºC for 48 hours.

Test of Antibacterial COS 0,2% in acetate acid 1%

Chito-Oligosaccharide 0,2 gram as prebiotik was dissolved into 100 mL acetate acid 0.175 M. Test of antibacterial on pathogen bacteria of *E. coli* dan *S. aureus* was conducted using COS 0.2% in acetic acid. The test also carried out by using only acetic acid 1% for comparison purpose. Antibacterial was tested using well-method and inhibition capacity was measured by using micrometers from four sides of inhibition zone

Incubation time Variation of the bacterial growth of *Lactobacillus acidophilus*

Culture of *Lactobacillus acidophilus* bacteria was inoculated in 10 mL MRS-Broth media and then incubated in various time: 44, 46, 48, 50, and 52 hours. After time set achieved, L. *acidophilus* bacteria was sentrifuged with speed 5000 rpm within 15 minute to separate its filtrate and its pelet. The filtrate of *L. acidophilus* bacteria was then filtered by filter membrane 0.2 µm and antibacterial activity on *E. coli* dan *S. aureus* bacteria was tested.

Combination of *L. acidophilus* and Chito-Oligosaccharide

0.9 ml of COS 0,2% was added into 9,1 ml solution of MRS-Broth media followed by 1 mL culture of *L. acidophilus* bacteria. The suspension was put into incubator and held for range of time 44, 46, 48, 50 and 52 hours. *L. acidophilus bacteria* was then sentrifuged at speed 5000 rpm within 15 minute to saparate its filtrate and its pelet. The filtrate was filtered by filter membrane 0.2 µm to acquired filtered filtrate which was used for testing the antibacterial activity on *E. coli* dan *S. aureus* bacterial.

Testing Antibacterial of *L. acidophilus* before and after combined with COS 0.2%

E. coli and *S. aureus* test were innoculated 10 µL into NA (*Nutrient Agar*). Three holes were created on Nutrient Agar by using sterilized pipet tips. The holes were set for inoculation of 3 different samples:100 µL filtered filtrate from filtering *L. acidophilus,* combination of *L. acidophilus and* COS 0,2% with different variety of time: 44, 46, 48, 50 dan

52 hours, and pure broth as control. The next step was incubation process for 24 hours at 37°C. Diameter of clean Zone formed around the well was measured as blocking zone toward pathogen bacteria [5].

Optical Density was measured to analyze the different growth of *L. acidophilus* combined with and without COS 0.2% for 48 hours' incubation time. The Optical Density was measured using spectrophotometer at 580 nm wavelength. Organic acids exerted by bacteria also was analyzed using HPLC instrument in acetonitrile solvent.

RESULT AND DISCUSSION

Test result of antibacterial activity of COS 0.2% and acetate acid 1%

Test result of antibacterial activity of COS 0.2% (dissolved in acetate acid 1%) and acetate acid 1% indicated a difference of inhibition zone resulted (Figure 1). Figure 1 shows inhibition zone resulted by COS 0.2% on *E. coli* was 290.36 mm^2 and on *S. aureus* was 323.86 mm^2. A lower result shows for the inhibition zone resulted from acetate acid solution 1% on *E. coli* bacteria was 253.16 mm^2 and on *S. aureus* bacteria was 271.66 mm^2.

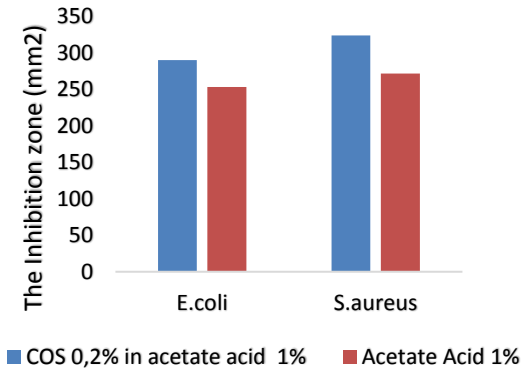

Figure 1. The inhibition zone of COS 0.2% and acetate acid 1%

From the data of inhibition zone of COS 0.2% (in acetate acid 1%) and acetate acid solution 1% we were able to calculated inhibition zone resulted from COS 0.2% on the bacteria of *E. coli* and *S. aureus* are 37.2 mm^2 dan 52.2 mm^2 respectively. Several mechanisms of microbe inhibition by COS have been proposed by several researchers but none of them are able to fully understood their interaction.

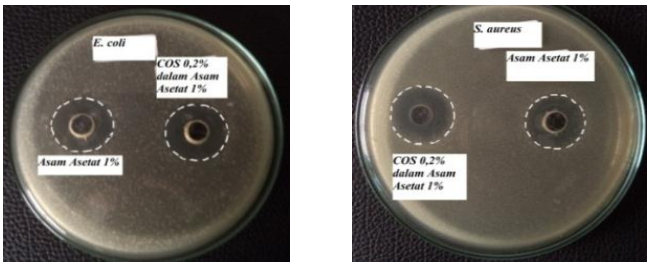

Figure 2. Test result of antibacterial activity of acetate acid solution 1% and COS 0.2 % solution in acetate acid 1% on the bacteria of *E. coli* and *S. aureus*

The most accepted mechanism commonly used is involve interaction between positively part of COS and negatively part of surface bacteria which induced permeability change of cell upper layer. The permeability change can cause the loss of protein, amino acid and glucose which composed protein. In this case, COS will inhibit micro organic metabolism and cause the death of cell [6]. Test result of antibacterial activity of COS 0.2% and acetate acid 1% against *E. coli* and *S. aureus* displayed on Figure 2.

Antibacterial activity of *L. acidophillus* bacteria in COS 0.2% against *E. coli* and *S. aureus*

The Inhibition Zone for *E. coli*

Antibacterial activity of *L. acidophillus* in combined with and without COS 0.2% against *E. coli* showed difference inhibition zone width. Combination of *L. acidophilus* with COS 0.2% give inhibition zone larger than without COS 0.2%. This result also found in all various time of incubation as shown in Figure 3. Figure 3 indicates combination of *L. acidophilus* with COS 0.2% is slightly more effective on inhibiting the growth of *E. coli*. Incubation time of *L. acidophilus* bacteria also shows influence on the growth of inhibition zone against *E. coli* bacteria. The inhibition zones resulted for *L. acidophillus* bacteria with COS combination within the range time 44, 46, 48, 50 and 52 respectively are 279.65; 318.04; 367.92; 332.46 and 292,65 mm^2 while for without combination are 258.44; 306.91; 332.46; 287.75 dan 266.86 mm^2. The biggest inhibition zone resulted from *L. acidophillus* on *E. coli* bacteria are in the same incubation time i.e. 48 hours. As well as smallest inhibition zone in 44-hour incubation.

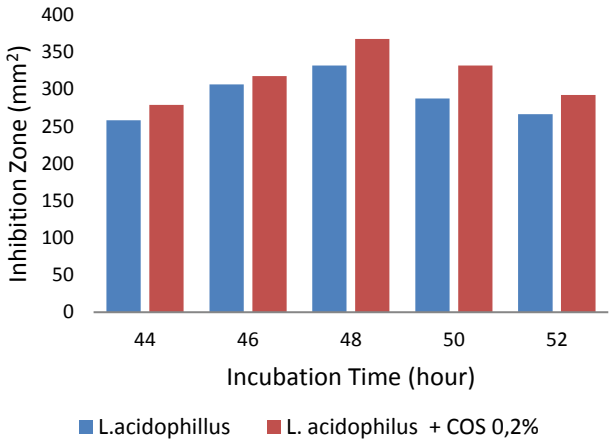

Figure 3. Inhibition zone of antibacterial character of *L. acidophillus* and combination on *L. acidophilus* + COS 0,2% on *E. coli* bacteria.

Comparing inhibition zone of *L. acidophilus* and combination of *L. acidophilus* with COS 0.2% shows *L. acidophillu* bacteria that has been combined with COS 0.2% yield bigger inhibition zone. It is also confirmed that combination of *L. acidophilus* and COS 0,2% with 480-hours incubation in this study gave a good combination method to inhibit the growth of *E. coli.* The result of antibacterial activities of *L. acidophilus* before and after combination with COS 0.2% on *E. coli* bacteria can be seen on Figure 4.

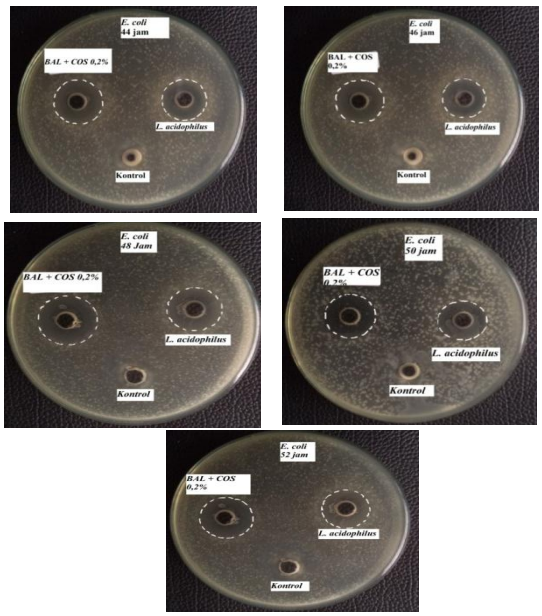

Figure 4. The result of antibacterial activities of *L. acidophilus* before and after combination with COS 0.2% on *E. coli* bacteria

Inhibition Zone for *S. aureus* Bacteria

Combination of *L. acidophilus* and COS 0.2% shows growth inhibition of *S. aureus* bacteria larger than without combination. Filtrate of *L. acidophilus* without COS 0.2% gave inhibition zone on *S. aurues* bacteria at various incubation times 44, 46, 48, 50, and 52 are 274.16; 295.96; 306.86; 266.26, dan 245.2 mm^2 respectively.

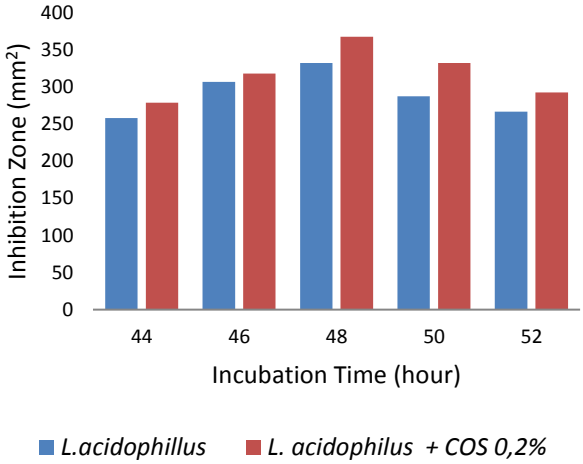

Figure 5. The inhibition zone of antibacterial character of *L. acidophillus* and combination of *L. acidophilus* + COS 0.2% on *S. aureus* bacteria.

Figure 5 above showed that after 48-hour incubation time, inhibition zone on *S. ureus* bacteria is 306.86 mm^2, while the smallest inhibition zone occurred when incubation time was 52 hours i.e. 245.2 mm^2. Figure 5 also showed that incubation time of *L. acidophilus* combined COS 0.2% affects inhibition zone against *S. aureus* bacteria. The inhibition zone of *L. acidophilus* combined with COS 0.2% at different incubation time i.e. 44, 46, 48, 50 and 52 hours respectively are 305.86; 321.11; 343.99; 287.75 and 279.65 mm^2. It can be seen that 48-hours incubation time showed good growth inhibition of *S. aureus* i.e. 343.99 mm^2, while 52-hours incubation gave lowest inhibition zone compare to other incubation time i.e. 279.65 mm^2. Combination of *L. acidophilus* and COS 0.2% in this study has proofed as good alternative for probiotic and prebiotic due to ability to inhibit pathogen bacteria growth either for gram-negative or gram-positive bacteria.

The result of antibacterial of *L. acidophilus* before or after combined with COS 0,2% on *S aureus* bacteria with the biggest inhibition zone can be seen on figure 6.

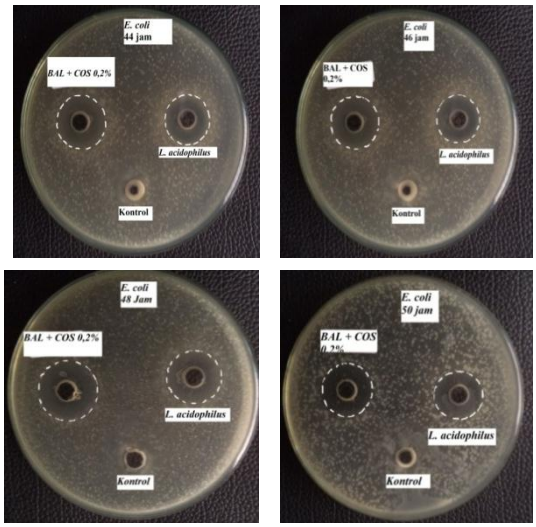

Figure 6. Antibacterial activity of *L. acidophilus* before or after combine with COS 0.2% against *S aureus* bacteria.

Optical Density of *L. acidophilus* before and after combination with COS 0.2 %

The difference in growth of *L. acidophilus* before and after combination with COS 0.2% was measured by conducting absorbance measurement using spectrophotometer at wavelength 580 nm. The result showed that the absorbance of *L. acidophilus* combined with COS 0.2% was bigger than the absorbance of *L. acidophilus* without combination.

Culture of *L. acidophilus* combined with COS 0.2% shows absorbance 0.956 and for *L. acidophilus* without combination with COS 0.2% 0.863. Higher absorbance indicates the growth of *L. acidophilus* and COS 0.2% was larger than *L. acidophilus* without combination. Addition of COS 0.2% on MRS-Broth media could stimulated the growth and activity of *L. acidophilus* probiotic. Chito-Oligosaccharide (COS) is a prebiotic that can stimulate the growth of BAL and enlarge inhibition zone on the growth of pathogen bacteria [7].

Organic Acid Produced by *L. acidophilus*

Measurement of organic acids produced by bacteria activities was carried out in Balai Pasca Panen Bogor. The aim of this measurement was to evaluate the influence of COS 0.2% addition on organic acids produced by *L. acidophilus* after 48-hours incubation. The result of the HPLC measurement can be seen on Table 1.

Table 1 showed that more organic acids were produced by *L. acidophilus* without COS 0.2% compare to *L. acidophilus* combined with COS 0.2%. Addition of COS 0.2% obviously affected the production of organic acids. The inhibition zone produced by combination of *L. acidophilus* and COS 0.2% against pathogen bacteria, *E. coli* and *S. aureus* was bigger than without COS 0.2%.

Table 1. Organic acids produced by *L. Acidophilus* before and after combined with COS 0.2%

Organic Acids (mg/L)	*L. acidophilus*	*L. acidophilus* + COS 0,2%
Acetate acid	3.925	2.943
Propionate acid	101.585	78.973
Lactic acid	25.046	22.482
Oxalic acid	4.554	3.589
Butyrate acid	114.25	101.719
Citric acid	21.789	14.534
Malic acid	18.383	16.142

CONCLUSION

COS 0.2% gave inhibition zone 37.2 mm^2 on *E. coli* bacteria and 52,2 mm^2 on *S. aureus* bacteria. Combination of *L. acidophilus* with COS 0.2% gave bigger inhibition zone than *L. acidophilus* without COS within 48-hours incubation against bacteria *E. coli* and *S. aureus*. i.e. 367.918 mm^2 and 343.99 mm^2. Optical Density for combination *L. acidophilus* and COS 0.2% was 0.956 and for *L. acidophillus* without COS 0.2% was 0.863. The result of organic acids from *L. acidophilus* before and after combined with COS 02% showed as follow: acetate acid, propionate acids, lactic acid, oxalic acid, butyrate acid, citric acid and malic acid.

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