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Inhibition of *Staphylococcus aureus* by the Cotton Fabrics Treated with the Crude Finish Produced from *Streptomyces* sp. strain AC4

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Abstract

The isolated *Streptomyces* sp. AC4, was able to grow within one week, produced the purple-like pigment in Starch Casein Broth (SCB) and inhibited growth of *Staphylococcus aureus*. The crude samples of AC4 presenting the purple suspension in SCB were separated in two fractions, i.e. supernatants and cell pellets. These fractions were pasteurized for testing the thermal stability of the vegetative cells and spores, and the ability to inhibit *S. aureus*. The investigation showed that cells and spores of *Streptomyces* sp. AC4 in both fractions were inactivated by pasteurization at 73°C. Furthermore, both pasteurized fractions were able to inhibit the growth of *S. aureus*. Then, crude samples were applied to coat cotton fabrics by the simply heat dyeing at 70°C. Using American Association of Textile Chemists and Colorists (AATCC) 147-2004 test for testing antibacterial textile of coating cotton fabrics on *S. aureus*, the investigation indicated that the coating cotton fabrics had inhibition zone of 4.5 mm, indicating that the treated fabrics was able to inhibit *S. aureus*. In addition, AATCC 100-2004 test showed that the treated fabrics present 100% of inhibition of *S. aureus* for 12 and 24 h. Thus, the pasteurized crude samples of *Streptomyces* sp. AC4 will be applied to the natural finish that are environment friendly.

Introduction

The natural compounds of the organism products are investigated for application in various aspects. Especially natural products that could promote skin care and skin health, which is generated by the bioactive compounds that control the growth of the skin flora that live on normal human skin (1×10^5 CFU/cm^2) by the predominant bacterial flora found in the healthy human such as *Staphylococcus epidermidis* and *S. aureus* which are the opportunistic pathogenesis (Teruaki et al., 2017). The human skin flora grows and causes the fiber of textiles to spoil and this may cause unpleasant odor, dermal infection, allergic responses and opportunistic pathogenesis of the skin lesion (Teufel et al., 2010; Singh et al., 2005). These problems were related with consumers of the fabric product. Therefore, the bioactive compounds from the natural products were studied and

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developed for cosmeceuticals and cosmetic ingredients. Recently, Prompamorn and Ratcharin in 2018 studied the *Terminalia chebula* extracts for the gel formulation that showed the crude ethanol extract of *Terminalia chebula* that could protect against dermatitis inducing bacteria including *Staphylococcus aureus* and *Staphylococcus epidermidis* (Prompamorn & Ratcharin, 2018). Parkpoom et al in 2018 reported the leaf extract of *Piper betle* L. as promising for cosmetics with results showing that the test of antimicrobial capacity of the leaf extract of *Piper betle* L., from ethanol inhibited *S. aurues* with the best result having an inhibition zone at the concentration of 12.50 mg/ml. (Parkpoom et al., 2018). Meanwhile, the bioactive compounds for textile products using antimicrobial textiles were also interested in the usage. Currently, the use of fiber and textile are various aspects. In addition, the special fiber or textile containing properties with the ability to inhibit the microflora by itself. This has the capacity to decrease the risk or spread of the opportunistic infection from the flora skin. Moreover, the natural textile is made from natural fibers such as cotton, silk or wool. They have the chemical compounds that are the sources of nutrients such as cellulose, protein or lipid wax for the microbes. Thus, the microorganisms can be the decomposer to reduce the life time of products that the quality of fiber structure destroys. If the textile or natural fiber are coated with the antimicrobial finish, this also extends the life of these products (Singh et al., 2005; Gutarowska et al., 2013). The previous studies focused on the finishing process aimed to improve the quality of the fabric especially finishings that involved treatments with chemical compounds for the potential quality of the fabric including permanent press treatments, water proofing, softening, anti-static protection, soil resistance, stain release and microbial protection that were applied in the fabric treatments process (Moore & Ausley, 2004). On the other hand, the high potential compounds for finishing or dyeing are the synthetic substance which contaminate the natural waters which has become one of the biggest problems in the environment because the major source of dyes and finishes fail to adhere on the fibers during the dyeing step (Guaratini & Zanoni, 2000; Kunz et al., 2002). Therefore, the reasons that the current research about natural resources in the production processes is about natural dyes or finish is to increase the efficiency of the fiber and to reduce environment residual caused from the synthetic compounds. The natural dye and coatings are mostly naturally biodegradable, so it is environmentally friendly (Chequer et al., 2013; Joshi et al., 2009)

The natural dyes or finish are derived from various types of the organism such as plant, fungi, bacteria or actinobacteria. Actinobacteria produce pigments and some species produce bioactive compounds. In addition, some pigments are also a biological substance. The natural resource in finishing and dyeing processes as bioactive compounds are studied in order to improve the quality of the fabric. Various organisms were studied; animal products such as chitosan and sericin, herbal plant products i.e. neem, curcumin, tannin, aloe vera, tea tree and aleppo oak (Gutarowska et al., 2013; Singh et al., 2005; Joshi et al., 2009), and microorganisms such as Monascus pigments and melanin pigments from fungi or actinobacteria. In a previous study, Ali et al studied the melanin pigments that were produced from *Streptomyces virginiae* strain and considered the activity of the bioactive compounds to apply in printing and dyeing of the wool fabric. The result showed that the culture broth of this strain could affect against *S. aureus, Pseudomonas aeruginosa* and fungi (Ali et al., 2011).

In addition to the compounds for finishing, the steps to operate the coating at the optimum temperatures have been a concern because the fiber of textile material such as, cotton, rayon, nylon, wool and some other fibers, dye well at temperatures of 100ºC or below. While polyester and some other synthetic fibers dye more conveniently at temperatures above 100ºC (Perkins, 1991). Thus, the thermo stability of the bioactive compounds for the antibacterial finishes were attempted at the optimum conditions to immobilize the fibers and remain the ability of bioactive compounds too.

The aim of this research is to study using the purple crude samples from *Streptomyces* sp. strain, AC4, cultivated in SCB media. The crude samples (the supernatants and the cell pellets) were tested to inhibit the *S. aureus*. Then, the study of the pasteurization of the crude samples by heat treatment. Furthermore, this results were applied to determine the pasteurization of the crude samples before the coating step. The cottons were coated with the crude finish then the treated fabrics was determined by AATCC Test Method 147-2004 and AATCC Test Method 100-2004 for assessing the antimicrobial properties of textiles. This coating cotton could help to promote the skin's hygiene. In addition, the process of the coating is environment-friendly and going apply to the manufacture of textile coating.

Materials and methods

1. Preparation of the starter of *Streptomyces* sp. AC4 and cultivation

The soil actinobacteria, *Streptomyces* sp. AC4, from the collection of the isolated strains of the Plant Genetic Conservation Project under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG) were cultivated by Starch Casein Agar (SCA: soluble starch 10 g, casein 0.3 g, KNO_3 2 g, NaCl 2 g, $MgSO_4.7H_2O$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO_4.7H_2O$ 0.01 g, K_2HPO_4 2 g, agar 15 g for distilled water 1000 ml) at 30°C for 4 to 7 days. The purple color colony from creating purple-like pigments were observed and subsequently selected. This pure colony for 3 to 5 colony were inoculated to the Starch Casein Broth (SCB) approximately 150 ml and incubated in incubator shaker at 30°C for 4 days (starter). The starter was then transferred to the new SCB (750 ml in the 1000 ml flask) approximately 5 to 10 percentages of final volume and incubated in incubator shaker at 30°C for 7 days. The cultured samples were collected with the incubation times at 0, 24, 48, 72, 96, 120, 144 and 168 h to plot the growth curve performed by the cell dry weights and to calculate the basic characteristics of the growth kinetics as the doubling time (t_d) and specific growth rate (μ) by the formula of the growth kinetics according to the mathematical equation described by Fattah et al. The exponential curve fitting function generated the equation (Fattah et al., 2018).

Exponential phase equation: $y = Ae^{Bx}$, where A and B are numbers

Doubling time equation (t_d): $y = Ae^{Bx} \rightarrow x = \ln (y/A)/B$

When $y = 1$, $x1 = \ln (1/A)/B$

When $y = 2$ (i.e. when y is doubled), $x2 = \ln (2/A)/B$

$t_d = x2 - x1 = [(\ln2 - \ln A) - (\ln1 - \ln A)]/B$ by $\ln1 = 0$

$t_d = \ln2/B = 0.693/B$

Growth rate (μ):

$\mu = \ln2/t_d$

The exponential curve fitting function was computed by the Excel 2016 program with the graph of the growth curve.

2. Preparation of the crude samples

The crude samples were prepared by the cultured media that were divided into two parts including the supernatant and the cell pellets. The cell pellets were then separated from the supernatant by centrifugation at 5,000 rpm for 10 minutes. Next, the supernatant was removed to a new tube and cell pellets were stored in -80°C refrigerator and grinded by the mortar.

The parts of the cracked-cell pellets and the supernatants were used for the experiment of inhibiting the test organism and stabilizing of active compounds after the heat treatments. The cracked-cell pellets were resuspended by the distilled water for 1:10 w/v with the radio of the maximum concentration for the crude samples to permeate the 0.45 µm filter paper. The aseptic processes were sterilized to the aseptic conditions by 0.45 µm filter papers and pasteurized by the heat treatments (the details are shown in the next experiment). Furthermore, the parts of the undiluted supernatants were sterilized by the same methods with the cracked-cell pellets and then the samples were tested for disc diffusion.

Finally, the supernatants and the cracked-cell pellets were mixed as the crude finishing. The ratio of grams of the cracked-cell pellets to volume of the supernatants 1:10 (w/v) for the experiment of coating cotton fabrics. The mixture of the crude finishing were pasteurized by the optimal temperature that was selected by the result of the stabilizing of active compounds after the heat treatments. The optimal temperature for pasteurizing the crude finishing were determined by the inactivate growth of the vegetative cells and spores of the tested actinobacteria and stabilizing of the antibacterial activity.

3. Heat treatment of the crude samples for the pasteurization

The crude samples were treated by the autoclave at the pasteurization temperature of 62°C for 30 minutes and 73°C for 15 minutes. After the pasteurization, the ability of the crude samples to inhibit bacteria were tested by the disc diffusion method. The growth of the vegetative and spore of the actinobacteria, *Streptomyces* sp. AC4 were examined by the dropped plate method on SCA of 10 µl crude samples then cultivated at 30°C for 7 days.

4. Experiment of the crude samples inhibit to the growth of the test microorganisms

The ability of the crude samples to inhibit bacteria were tested by the disc diffusion method. The selected microorganisms which were used in the assays were *Staphylococcus aureus* and *Klebsiella pneumoniae,* according to the standards methods of American Association of Textile Chemists and Colorists (AATCC) for assessing the antimicrobial properties of textiles. The bacteria, *S. aureus* ATCC 25923 and *K. pneumoniae* ATCC 8216, were observed as the tested microorganism.

The pure colony of the test microorganisms were cultured in Muller Hinton Broth (MHB) (HiMedia) at 37°C for 16 to 18 h. The cell suspension was then adjusted to the standard concentration of the McFarland turbidity standards No. 0.5 (1.5 x 10^8 CFU/ml). The bacteria were spread by swabbing on Muller Hinton Agar (MHA) then onto the circular filter papers (6 mm diameter) and were placed on the surface of the MHA. The 20 µl of crude samples was dropped on each filter paper and incubated at 37°C for 18 to 24 h. A drop of distilled water, MHB and SCB was used for the negative control. Moreover, the chloramphenicol disc (30 mg/disc) was applied for the qualitative and positive control of the disc diffusion method. The diameter of an inhibited area (inhibition zone) was measured by the unit in millimeters.

5. Coating cotton Fabrics by crude samples of *Streptomyces* sp. AC4

The circular cottons (45 mm diameter) and the square cotton (25x40 mm) were used. The cotton fabrics were washed by the solution of laundry soap, sodium carbonate (Na_2CO_3) and water (5g:8g:500ml) that were mixed by boiling. This volume was used for 50 g of the cotton. The cotton fabrics were boiled with the solution for 1 h. The fabrics were rinsed by water for 5 times and the cotton was dried before applying the coating. The crude samples as the crude finishing were prepared by mixed suspension of the cracked-cell pellet and the supernatant at ratio 1:10 w/v, as mentioned previously. The coating process used heat at 70°C for 1 h by the ratio of grams of dry cotton material to volume of crude finishing 1:10 (w/v). The coating products were rinsed by water 3-5 times and dried after the coating. The coated fabrics were defined as the treated fabrics for the estimation of the antibacterial activity of the treated cotton (AATCC Test Method 147-2004 and AATCC Test Method 100-2004) while the non-coated fabrics were the untreated fabrics for the control.

6. Estimation of the antibacterial activity of the treated cotton by qualitative methods, AATCC Test Method 147-2004

The treated and the untreated fabrics were tested by modified AATCC Test Method 147-2004 (Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method) for assessment of the qualitative analysis (AATCC 147:2004; Pinho, et al., 2015). The square cottons (25x40 mm) were used and prepared the inoculum of the pure *S. aureus* into 10 ml of Brain Heart Infusion Broth (BHI) (HiMedia) and incubated at 37°C for 24 h. Then, the 1.0±0.1 ml from the inoculum in the enrichment medium, BHI (approximately 1.0x10^7 cells/ml), was transferred into the new BHI 9.0 ml (AATCC 147:2004; Pinho, et al., 2011; Pinho, et al., 2015). The diluted inoculum was streaked on BHI agar (approximately 15±0.5 ml in the petri dish) by making five streaks approximately 60 mm in length, spaced 10 mm on the surface of the central area of the media without refilling the loop and performed the same condition on Baird-Parker Agar (BP) (HiMedia) for the parallel control of the characteristic of *S. aureus*. The pasteurized step of the test swatches (treated cottons) was used by the autoclave at 73°C for 10 min. The test swatches were pressed by contact across the five inoculum streaks on the agar surface with the same condition as the untreated cottons for the control of the experiment. All of the plates were incubated at 37°C for 24 h. The incubated plates were matured by the unit in millimeters of the inhibition zone. The average width of zone was calculated using the equation, W = (T-D)/2, where: W = width of clear zone of inhibition in mm; T = total diameter of the test specimen and clear zone in mm; D = diameter of the test specimen in mm.

7. Antibacterial assessment of the treated cotton by quantitative methods, AATCC Test Method 100-2004

The circular swatches (45 mm) were used. The treated and the untreated fabrics, as mentioned previously, were tested by modifying AATCC Test Method 100-2004 (Antibacterial Finishes on Textile Materials: Assessment of) for assessment of the quantitative evaluation with *S. aureus*, the test bacteria (AATCC 100:2004; Pinho, et al., 2011; Pinho, et al., 2015). The pasteurized step of the test swatches was used by the autoclave at 73°C for 10 min. The number of swatches was considered by the property of the fiber type and fabric construction by the amount of fabric which could absorb the 1.0±0.1 ml of inoculum without free liquid. The number of swatches used per jar are reported. The number of cotton swatches in the experiment used by two cotton swatches (45 mm diameter) that amount of fabric will absorb the 1.0 ml of inoculum.

Inoculation of fabrics when using *S. aureus*, 24 h culture in BHI and the dilution of the organism to McFarland No.0.5 made in peptone water. The size of inoculum per two sample applied 1 ml showed counts of final concentration as 1-2x10^5 cells/ml. The two swatches were placed in sterile petri dishes and drop 1 ml of the inoculum carefully onto the swatches and used 1 ml peptone water to drop for the negative control. The inoculated swatches stacked in the 250 ml wide-mount

glass jar with screw cap. Each of incubation time (contact time) placed the swatches separately in the jar as 0, 6, 12 and 24 h. After incubated at the contact time, the sample added 100 ml of the normal saline (neutralizing solution) to each of the jars. The 10-fold serial dilution made by the normal saline and plated on the BHI agar and BP (parallel control of the characteristic of *S. aureus*) by dropping plate methods for 10 µl per drop (in triplicate). Finally, all of the plates were incubated 37°C for 24 h.

The bacterial counts were reported by percentage of reduction (% reduction; R) and the number of bacterial per sample. Consequently, the report was "absent (0) colony" counted at 100 dilution (in the jar mixed between swatches and neutralizing solution as the undiluted) as "less than 100". The percent reduction of bacteria by the formulas: R = 100(C-A)/C, where: R = % reduction, A = the number of bacteria recovered from the inoculated treated test specimen swatches in the jar incubated over the desired contact period, C = the number of bacteria recovered from the inoculated untreated control specimen swatches in the jar immediately after inoculation (at "0" contact time).

8. Data analysis

The different inhibition zones were measured in triplicate and compared by performing One-way ANOVA ranked with Duncan's multiple range tests. Statistical Package for the Social Sciences (SPSS) 24 Version was used for analysis of recorded data. The statistical results with $p<0.05$ were determined to be statistically significant. The formula of the growth kinetics as the doubling time (t_d) and specific growth rate (µ) and the graphs were performed and computed by the basic math calculations in Excel 2016.

Results and discussion

1. Culturation and characterization of the crude samples of *Streptomyces* sp. AC4

The pure actinobacteria isolated from soil samples and identified belong to *Streptomyces* by 16SrDNA sequencing. The actinobacteria were known that *Streptomyces* species could produce plenty of the secondary metabolites as bioactive compounds such as antimicrobial compounds (Lam, 2006). The test strain, AC4, consistent with the research that was performed by screening of inhibited the gram-positive bacterial growth (the result not shown). *Streptomyces* sp. isolated strain, AC4, was used by property of inhibition of *S. aureus* growth (next result). Their ability produced the purple-like pigment on SCA. When cultured in SCB, AC4 growth presented the purple suspension and difficultly separated between cell and supernatant by centrifugation. Although, most of actinobacteria grow slowly in the habitat (Goodfellow, 1985) but the researchers screened some of them that could produce the bioactive compounds and grow significantly to apply to produce the benefit products. The basic characteristics of the growth kinetics of the strain AC4 showed the result that AC4 strain belonged to the significant growth of the actinobacteria group which produced the bioactive compounds by the doubling time (t_d) of the strains AC4 incubating in SCB as 72.19 h and specific growth rate (µ) as 0.0069 h^{-1} (Fig. 1). In addition, the research of Sejiny (1991) noted that the *Streptomyces* MY18, which produced antibiotic agents, showed the progressive to increase the biomass and had the highest antibiotic activity as recorded in the stationary phase of the growth during the first 4-7 days of incubation. The characteristic of significant growth might be applied to produce the benefit products.

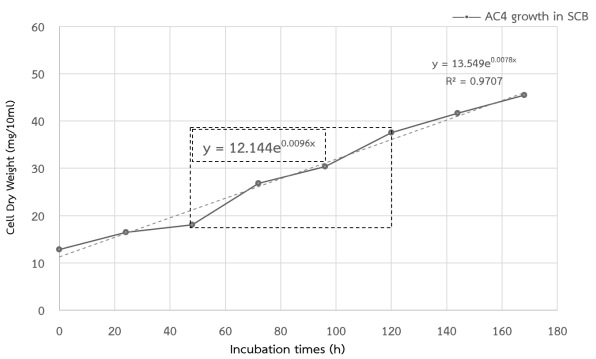


Fig. 1 The growth curve of the actinobacteria, *Streptomyces* sp. AC4

This *Streptomyces* strain, AC4, was performed by screening of inhibition the growth of the gram-positive bacteria, *S. aureus*, that relates to the skin flora and opportunistic pathogens. So, *S. aureus* was related with the fabric products that are consumed in the human. Then, the antibacterial activity to *S. aureus* of the crude samples from AC4 were interested to apply to the research of the antimicrobial textiles.

2. Antibacterial activity of the crude samples by disc diffusion method

The cultivated suspension of AC4 was then separated into two parts including the undiluted supernatants and the cracked-cell pellets (re-suspending with the distilled water 1:10 w/v that was sterilized with the 0.45 µm filter paper). The test bacteria of inhibition that concerned the skin flora and the standard organisms by the standard methods of American Association of Textile Chemists and Colorists (AATCC) for assessing the antimicrobial properties of textiles as *S. aureus* and *K. pneumonia*. The qualitative and positive control of the tested bacteria could be reliable with the standard range for the inhibition zone of the chloramphenicol disc (30 mg/disc) for *S. aureus* and *K. pneumonia* as 19.22±0.55 mm and 24±0 mm, respectively (CLSI, 2016). The ability of the crude samples to inhibit bacteria was tested by the disc diffusion method at the standard concentration of 1.5 x 10^8 CFU/ml on Mueller-Hinton agar (MHA). The diameter of the *S. aureus* inhibited zone of the supernatants and the cracked-cell pellets was measured as 17±0.5 mm and 18.00±0 mm, respectively. Both of the tested samples could not inhibit *K. pneumonia* (Table 1). Ali et al. (2011) studied the melanin pigments that were produced from *Streptomyces virginiae* strain which could have the proper activity of the bioactive compounds to apply in printing and dyeing of the wool fabric. The result showed that the culture broth of this strain could inhibit against *S. aureus*, *Pseudomonas aeruginosa* and fungi (Ali et al., 2011). There were bacteria on normal human skin (1×10^5 CFU/cm^2) by the predominant bacterial flora found in the healthy human such as *S. aureus* (Teruaki et al., 2017). The human skin flora grows and causes the fiber of textiles to spoil and this may cause unpleasant odor, dermal infection, allergic responses and opportunistic pathogenesis of the skin lesion (Teufel et al., 2010; Singh et al., 2005). The problems were related with consume of the fabric product. Therefore, the study of the crude samples from AC4 strains for coating the cotton were interesting. The crude samples of AC4 had narrow spectrum for gram-positive bacteria and did not effect to gram-negative bacteria the result shows the standard gram-negative bacteria of the standards methods as *K. pneumonia*. However, the other characteristic could pasteurize, significantly grow for 3-7 days and be environmental friendly from the natural finish by itself (Ali et al., 2011). The result of the characteristic of the crude samples of AC4 could apply to the study of the antibacterial coating.

3. Antibacterial activity of the crude samples after treated by pasteurized temperature

After the pasteurization, the ability of the crude samples to inhibit *S. aureus* was tested by the disc diffusion method (Table 1 and Fig. 2B). The diameter of the *S. aureus* inhibited zone was measured for the cracked-cell pellets at 62°C and 73°C as 20.41±0.40 mm and 20.16±0.95 mm, respectively, which are not significantly different. For the undiluted supernatants, the diameter of inhibited zone was measured at 62°C and 73°C as 19.30±0.72 mm, 18.19±0.44 mm and the statistical results were not different. The statistical results showed that both the cracked-cell pellets and the

Table 1 Antibacterial activity of the crude extraction after treated by pasteurized temperature and the thermal stability of the vegetative cells and spores of *Streptomyces* sp. AC4 in the crude extract

Temperature (°C) for treatment of crude samples	Inhibition zone (diameter in mm)				The number of the vegetative cells and spores of *Streptomyces* sp. AC4 (CFU/ml)	
	S. aureus		*K. pneumoniae*			
	Supernatant	Cell Pellets	Supernatant	Cell Pellets	Supernatant	Cell Pellets
No treatment	17.00±0.50^b	18.00±0^b	0^b	0^b	>300	>300
62	19.30±0.72^a	20.41±0.40^a	0^b	0^b	2.95x10^4	>300
73	18.19±0.44^{ab}	20.16±0.95	0^b	0^b	0	0
Chloramphenicol* (30 mg)	19.22±0.55		24.00±0			

Remark: Values were means ± SD
a, b with different letter in the column show significant statistical difference ($p < 0.05$)
* = The chloramphenicol disc (30 mg/disc) are applied for the qualitative and positive control of the disc diffusion method.

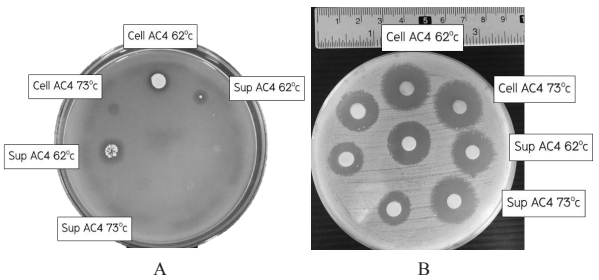


Fig. 2 The thermal stability of *Streptomyces* sp. AC4 growth and antibacterial activity after heat treatments (A) The growth of the vegetative and spore of the actinobacteria, *Streptomyces* sp. AC4 (B) the ability of the crude extract to inhibit *S. aureus* after heat treatments by the disc diffusion method on MHA (Sup: Supernatant, Cell: the cracked-cell pellets)

supernatants at 62°C had a higher inhibition zone that is significantly different when compared to the non-heat treatment of the crude samples (the cracked-cell pellets was measured as 17±0.5 mm and 18.00±0 mm, respectively) and were not different to the cracked-cell pellets and the supernatants treated at 73°C. The stability of the antibacterial activity for the high temperatures of finishing were important because the high temperatures were suitable for immobilization of the dye or finishes. The steps to operate the coating at the optimum temperatures have been a concern because the fiber of textile materials such as cotton and some other well dyed fibers at the high temperatures at 100ºC or below (Perkins, 1991). The result of the stability of the antibacterial activity supported the coating step and produced the antibacterial fabric from the conventional methods of the dyeing. The diameter of inhibited zone with the undiluted supernatants and the cracked-cell pellets after being treated at 62°C and 73°C, were not different from the statistical results but the choice of the temperature for the coating process were considered to be consistent with the pasteurization due to the crude samples from microorganisms used as finishers.

4. The effect of the thermal stability of the vegetative cells and spores of *Streptomyces* sp. AC4 in the crude samples

After the pasteurization, the growth of the vegetative and spore of *Streptomyces* sp. AC4 was examined by the dropped plate method on SCA of 10 µl crude extract then cultivated at 30°C for 7 days. The result showed that the cracked-cell pellet and the supernatant at 62°C could not inhibit the growth of the vegetative and spore with the colony count more than 300 colony and $2.95x10^4$ CFU/ml, respectively, but the higher the temperature could inhibit the growth of AC4 at 73°C (Fig. 2A).

The pasteurization at 73°C killed all the vegetative and spore of *Streptomyces* sp. AC4 and surprisingly the ability of the crude extract to inhibit bacteria could still exit hence this might be the reasons for selecting this temperature for pasteurization of the crude samples. In addition, the crude finish of AC4 could apply to coat the cotton with the traditional process by heat at 70-100°C because their anti-*Staphylococcus aureus* activity could tolerate the temperature for pasteurization at 73°C. Thus, the ability of *S. aureus* inhibition could be available for the coating process at 70°C. The optimal temperature for dyeing the cotton with cellulose component was at 70-100°C (Perkins, 1991).

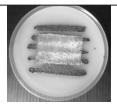
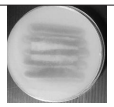
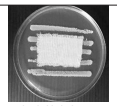
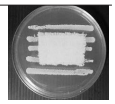
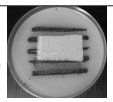
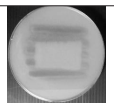
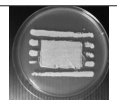
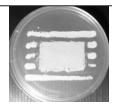
5. Antibacterial activity of the treated fabrics by qualitative methods

The supernatant and the cracked-cell pellet were mixed and pasteurized as the crude samples (crude finish) for the coating. The untreated (control) and treated fabrics were tested by AATCC Test Method 147-2004 for assessment of the qualitative analysis. The result shows that the treated fabrics had the average width of inhibited zone as 4.5 mm (Table 2). For the parallel control of the characteristic of *S. aureus* on BP, the five inoculum streaks on the agar surface were black line that showed the characteristic of the pure *S. aureus* on BP. However, the inhibited zone on BP could not be measured because BP is a hydrophobic media, nevertheless the process of coating and the crude finish was hydrophilic compound. The treated fabrics of AC4 showed clear ability to antimicrobial activity against *S. aureus* by Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method. This method was quick and easily to screen and determine the antibacterial activity of diffusible antimicrobial agents on the treated swatches. The size of the inhibition zone was different. This does not mean that the treated sample had more or less biocidal. The zone size of inhibition depends on the permeated property of the antibacterial finish to diffuse into the agar, therefore, it does not rely on the efficacy of the bioactive compounds (Varesano et al., 2011).

6. Antibacterial assessment of the treated cotton by quantitative methods

The fabrics were tested by AATCC Test Method 100-2004 for assessment of the quantitative evaluation with *S. aureus*. The calculation of percent reduction of the inoculated untreated fabrics at contact time for 24 h was a significant increase in the number of bacteria over the number of bacteria at contact time for 0 h. The result at 0 h on BHI and BP show $4x10^5$ CFU/sample and $1.3x10^5$

Table 2 Inhibition of *S. aureus* of the treated cottons by AATCC Test Method 147-2004

Samples	Inhibition zone (mm)			
	BP		BHI	
	Front side	Back side	Front side	Back side
Control (untreated fabrics)				
	No inhibition zone			
AC4 (treated fabrics 1:10 w/v)				
	4.5 mm			

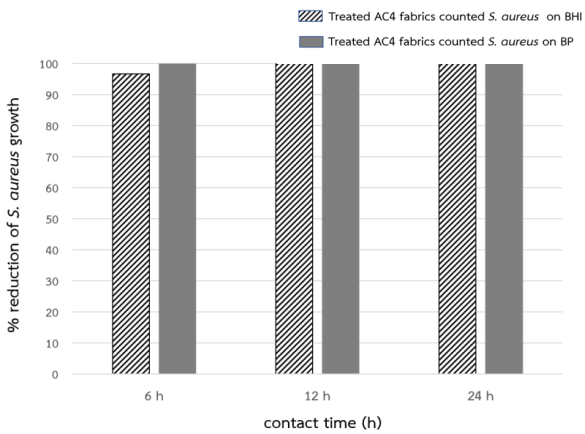


Fig. 3 The percentage of reduction (% reduction) of *S. aureus* growth by the treated fabrics

CFU/sample, respectively. The results of percentage of reduction (% reduction; R) showed that the treated fabrics could reduce *S. aureus* growth 96 to 100% for contact time at 6, 12 and 24 h (Fig. 3) on BHI. The reduction activity was determined that prevented microbial growth and killed bacteria bio-cidal activity. Additionally, the parallel control of the characteristic of *S. aureus* could count on BP for contact time at 12 and 24 h, that shows the treated fabrics could reduce *S. aureus* growth as 100% as same as the result of percent reduction on BHI agar (Fig. 3). For the parallel control of the characteristic of *S. aureus* on BP, the colony on the agar surface were black color that shows the characteristic of the pure *S. aureus* on BP for the easy counts the colony forming of *S. aureus*. The research showed the information that the cotton contained of largely cellulose, natural waxes and proteins. The mainly hydroxyl groups on the cellulose created a high potential of the water absorption that could be useful for immobilization the water-soluble compounds (Chequer et al., 2013). In this study, the hydroxyl groups that presented on the cellulose could immobilize the antibacterial agents from the *Streptomyces* sp. AC4 crude extract by the capability of *S. aureus* inhibition on the cotton fabrics which could remain and reduce *S. aureus* growth to 100%.

This study shows that the crude finish from *Streptomyces* sp. AC4 culture could correspond the cotton fabrics by the traditional batch process as the simply heat dyeing and the capability of *S. aureus* inhibition on the cotton fabrics could be exit.

The finishing was concerned with chemical agents purposed to improve the quality of the fabric such as microbial protection of fabric applied in the finishing process. Nevertheless, the contamination of natural waters from chemical synthesis from finishing and dyeing process is a problem for human health and environment because the major source of dye and finish loss immobilized during the textile fiber coating step and releases harmful effluents into the environment. So, the economical use of this natural resource in production processes has gained special attention (Chequer et al., 2013). Furthermore, the crude finish from *Streptomyces* sp. AC4 is a natural product during the finishing step and will release friendly effluents. Nonetheless, during the produced metabolite process, the microorganisms showed a significant growth of the organisms as evidenced by *Streptomyces* sp. AC4 which could culture for 4-7 days (t_d in SCB as 72.19 h and μ as 0.0069 h^{-1}) and create the bioactive compounds.

Conclusion

Streptomyces sp. AC4 crude samples could inactive its cells and spore by the temperature for pasteurization at 73°C with the ability to inhibit remaining that showed the diameter of the *S. aureus* inhibited zone of supernatant and the cracked-cell pellet was measured as 18.19±0.44 mm and 20.16±0.95 mm, respectively. The immobilization of the antibacterial agents with the cotton fabrics were performed by the traditional batch process as the simply heat dyeing at 70°C. The agents were treated by two steps of high temperature but the capability of *S. aureus* inhibition on the cotton fabrics could be remain that showed clear zone of inhibition against *S. aureus* as 4.5 mm on BHI by using AATCC

147-2004 test on *S. aureus*, the investigation indicated that the coating cotton fabrics had inhibition zone of 4.5 mm, indicating that the treated fabrics was able to inhibit *S. aureus*. In addition, the results of % reduction showed that the treated fabrics could reduce *S. aureus* growth as 100% for contact time at 12 and 24 h when the inoculated treated fabrics were incubated and calculated in both media (BHI and BP). Although the *Streptomyces* sp. AC4 crude extract was the narrow spectrum for gram-positive bacteria, the other characteristic could pasteurize, significantly grow for 3-7 days [the doubling time (t_d) of the strains AC4 incubating in SCB as 72.19 h and specific growth rate (μ) as 0.0069 h^{-1}] and be environmental friendly from the natural finish by itself which were useful to improve and optimize the proper condition of finishing that could apply to produce the antibacterial fabrics in the future.

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