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Antibacterial activity of *Bombyx mori* and *Samia cynthia ricini* sericin proteins against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

Aims: This study was designed to investigate the antibacterial properties of the sericin protein *Bombyx mori* and *Samia cynthia ricini* against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* strains.
Methodology and results: Sericin protein was extracted from the cocoons of *B. mori* races A, B, C, D, F1-1, F1-2 and *S. ricini* from Indonesia using a degumming process. The extracted sericin protein was evaluated as anti-microbials using Kirby Breuer disc diffusion, spectrometer measurement in decreasing OD value and imaging scanning electron microscope (SEM). The tests revealed that sericin protein from *B. mori* was more capable of inhibiting bacteria *S. aureus* than *E. coli*. Sericin protein from *S. ricini* was also capable of strongly inhibiting both *S. aureus* and *E. coli* bacteria. The sericin proteins of *B. mori* and *S. ricini* were found to reduce the number of *S. aureus* and *E. coli* bacteria as the OD value decreased at a concentration of 9%. The SEM imaging suggests that sericin *B. mori* and *S. ricini* proteins caused changes in cell morphology in *S. aureus* and *E. coli* bacteria, resulting in bacterial cell function disruption and death.
Conclusion, significance and impact of study: *Bombyx mori* and *S. ricini* sericin proteins were found to act against *S. aureus* and *E. coli* bacteria. Therefore, sericin protein has a greater antibacterial activity against Gram-positive strain *S. aureus*.

Keywords: Antibacterial, *Bombyx mori*, *Samia cynthia ricini*, sericin

INTRODUCTION

Sericin is a water-soluble globular protein that can interact with other polymers (Borgohain, 2015). In the textile silk industry, the sericin protein will be wasted. Every year, approximately 50,000 tons of sericin waste are discarded globally from 1,000,000 tons of fresh cocoons (Aramwit *et al.*, 2012). In Indonesia, the average dry cocoon production per year is 250 tons, which equates to 31.25 tons of yarn. According to Bharathi *et al.* (2020), the percentage of sericin varies depending on the type of silkworm.

Silkworm cocoons, which contain both mulberry and non-mulberry silkworms, can be used to produce sericin protein. The cocoon process occurs in silkworms at the end of the larval stage when the silkworm stops eating and transforms into a pupa. Mulberry silkworms can only eat mulberry leaves. *B. mori* silkworms are mulberry silkworms. Non-mulberry silkworms are polyphagus silkworms that feed on a variety of host plants, including papaya, cassava and jatropha. Non-mulberry silkworms are *S. ricini*, which have been widely cultivated and domesticated in the East Indian Ocean and the Assam region of India.

Several studies have found that the protein sericin has anti-microbial properties. An anti-microbial is a substance or component that can either inhibit the growth of bacteria or molds (bactericidal or fungistatic) or kill bacteria or molds (bactericidal or fungicidal) (Tariq *et al.*, 2019). Bacteria may display many resistance genes that have been transmitted and recruited from other epidemic strains (Mohsin, 2020). However, protein has antibacterial properties that prevent bacteria from developing a tolerance to it. Xue *et al.* (2016) discovered that the antibacterial activity of sericin *B. mori* bivoltin against Gram-negative bacteria *E. coli* was suspected to be due to sericin destroying bacterial cell membranes. In addition, Ahamad and Vootla (2018) in his research found that tasar sericin in the Indian region known as *Antherae mylitta* has the potential as an antibacterial against pathogenic bacteria strains of *E. coli* and *S. aureus*. Therefore, this study aimed to study further the antibacterial properties of the sericin proteins *B. mori* and *S. ricini* against Gram-negative *E. coli* and Gram-positive strains of *S. aureus*.

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MATERIALS AND METHODS

Sericin preparation

The protein sericin was collected from the cocoons of *B. mori* and S. *ricini* in Indonesia. *B. mori* is a member of the multivoltine and crossbred races known as A, B, C, D, F1-1 and F1-2. The silkworm cocoons of *B. mori* and *S. ricini* were first washed with water to remove dirt from the cocoon surface. Following that, the floss (cocoon fibers) and pupae residue from the cocoon samples were removed. Cocoon shells that have been cleaned need to be dried at 80 °C for 24 h.

Sericin extraction

Extraction of sericin cocoons from *B. mori* and *S. ricini* was carried out using Endrawati's (2012) degumming method, which involved cutting the clean cocoons to a size of about 1 cm and placing them in a Schott bottle with a ratio of 1 g cocoons to 50 mL NaOH solution. *Bombyx mori* was dissolved in 33.3 percent 0.1 N NaOH, while *S. ricini* was dissolved in 33.3 percent 0.25 N NaOH. The Schott tube was then placed in an autoclave Tomy High Pressure Steam Sterilizer ES-315 for 40 min at 115 °C for *B. mori* and 34 min at 113 °C for *S. ricini*. After that, the degumming solution was filtered through a 450 mesh nylon filter. After that, the sericin liquid was neutralized by adding 2 N HCl solution until it reached pH 7. To obtain solid sericin, the sericin solution was purified with 96% technical ethanol solution and centrifuged for 10 min at 4 °C 10000 rpm using a Hettich Centrifuge Universal 320 R at 4 °C 10000 rpm. To remove the remaining ethanol solution, the protein solid sericin was evaporated with a Heidolph Rotary Evaporator.

Lowry analysis

Standard solutions were prepared for the Lowry analysis by combining Bovine Serum Albumin (BSA) and demineralized water (DW). Dilutions with concentrations of 0, 10, 20, 50, 100, 200, 500, 1000 and 2000 g/mL were performed. A total of 100 samples of sericin *B. mori* and *S. ricini* were prepared for testing. In each tube, 100 mL of 2 N NaOH solution and 1 mL of reagent A were mixed with the standard solution and sericin sample. Reagent A was created by combining 2% Na_2CO_3, 1% $CuSo_4H_2O$ and 2% sodium potassium tartate in a 100:1:1 ratio. Furthermore, up to 100 Folin-Ciocalteau 10% was added to each tube to cause a color change and the tubes were left for 30 min before being measured (Lowry *et al.*, 1951). At a wavelength of 655 nm, absorbance measurements were taken using a Gene Quant 1300 spectrophotometer.

SDS PAGE analysis

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis was used to determine the molecular weight (SDS PAGE). Separating gel 12% was inserted vertically into the gel plate. After that, a 4% stacking gel solution and comb were applied to the dried gel plate. The mini gel was immersed in electrophoresis buffer while attached to the gel plate. Sericin sample was mixed with a 1:1 Laemmli buffer. The sericin was then denatured in a water bath at 100 °C for 5 min. The sample sericin *B. mori* and *S. ricini* were placed in a stacking gel column. Running electrophoresis is used with a voltage of 60 V and a current of 20 mA to ensure that the blue gel reaches the bottom of the comb. After the running gel results were obtained, the gel was washed with silver dye. SMOBIO PM 2700 ExcelBand 3-color Broad Range Protein Marker was used. The molecular weight analysis results are displayed as protein bands and are calculated using the molecular weight marker's standard curve (Laemmli, 1970).

Amino acid analysis

Amino acid analysis using High Performance Liquid Chromatography (HPLC) and the IPB Integrated Laboratory's HPLC protocol. HPLC begins with sample preparation and the injection of orthophthalaldehyde (OPA) reagent to Shimadzu HPLC with Thermo S Ods-hypersil column. OPA reagents will react with primary amino acids in an alkaline environment containing mercaptoethanol to form fluorescing compounds, allowing detection with a fluorescence detector. The retention time is the amount of time it takes for the sample sericin *B. mori* and *S. ricini* to leave the column. The sample retention time is used to identify the sample characteristics.

Antibacterial activity

The antibacterial activity was determined using the Kirby Bauer disc diffusion method (Bauer *et al.*, 1959). Muller Hinton Agar (MHA) solution was made by dissolving 3.8 g of MHA agar in 1 liter of demineralized water (DW). The MHA solution was then autoclaved at 121 °C for 15 min. The MHA was then poured into a sterile petri dish with a depth of 4 mm. Agar MH is solidified at room temperature and stored at 4 to 8 °C. Each MHA plate to be tested is labeled. *Staphylococcus aureus* and *E. coli* inoculum was prepared ahead of time and suspended in 2 mL of sterile NaCl solution. The microbial suspension was then vortexed and adjusted to 0.5 McFarland turbidity. Bacterial inoculum from *S. aureus* and *E. coli* was swabbed on top of the MHA media and evenly distributed across the entire surface of the MHA. Sericin protein *B. mori* and *S. ricini* was dissolved in DW and placed on a paper disk. The paper disk was placed on the surface of the MHA. After that, the samples were placed in an incubator at 37 °C for 24 h. The zone of inhibition was measured and recorded using a caliper after incubation.

Sericin protein optical density (OD) decrease

The term sericin protein optical density (OD) decrease refers to the method by Munfaati *et al.* (2015). Sericin

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proteins from *B. mori* and *S. ricini* were diluted in 1 mL of demineralized water (DW) at three different protein percentages: 3%, 6% and 9%. Forty-two test tubes were prepared, each with 8.8 mL of Nutrient Broth media, 200 µL of bacterial culture with population 10^7 and 1 mL of sericin protein. The first tubes contained *B. mori* protein in different percentages and *E. coli* bacteria, then *B. mori* protein and *S. aureus* bacteria, the other tubes with *S. ricini* protein and *E. coli* bacteria, and *S. ricini* and *S. aureus* bacteria. Furthermore, the optical density (OD) was measured before and after a 24 h incubation at 37 °C using a spectrophotometer Gene Quant 1300 with a wavelength of 630 nm.

SEM (Scanning Electron Microscope)

S. aureus and *E. coli* 200 µL were cultured in Nutrient Broth 8.8 mL media before being treated with 1 mL of protein sericin *B. mori* and *S. ricini*. The samples were then incubated at 37 °C for 24 h. The sample was then centrifuged for 10 min at 4 °C and 10000 rpm to isolate bacterial cell solids. Bacterial cells were placed on stubs and then coated with thin gold before being analyzed by SEM with an EVO-MA10 electron microscope.

RESULTS AND DISCUSSION

Lowry analysis

The silkworm builds cocoons made of silk strands towards the conclusion of its larval stage. The major components of silkworm cocoons are fibroin and sericin. The Lowry method was used to determine the concentration of sericin in protein at OD 655 nm, which was obtained during the degumming process. Sericin protein from *B. mori* races A, B, C, D, F1-1 and F1-2 had a protein percentage value of 24-38% (Table 1). *B. mori* race F1-1 had the highest protein percentage of sericin *B. mori* (38.10%), whereas *B. mori* race A had the lowest (24.27%). The difference percentage of sericin protein is probably due to the differences in the races that show different morphology of the cocoons. This is in accordance with the statement of Murthy *et al.* (2014) that bivoltine silkworms are superior to cross and multivoltine in biochemical content; the difference between silkworms is caused by the genetic endowment of races that affect the quantity and biochemical quality of silkworm cocoons. *S. ricini* had sericin protein at 23.58% lower than *B.mori* (Table 1). Furthermore, unlike mulberry silkworms, the fibroin structure of non-mulberry silkworms has a hydrophobic poly-Ala sequence, which leads to a highly organized crystal structure (Kundu *et al.*, 2014). As a result, non-mulberry silkworms had lower sericin protein levels than mulberry silkworms.

SDS Page analysis

The sericin proteins from *B. mori* and *S. ricini* silkworm cocoons were extracted with NaOH to get a low molecular weight. According to Kumar and Mandal (2017), alkaline-degraded cocoons produced sericin proteins ranging in size from 15 to 75 kDa. According to the results of SDS-PAGE electrophoresis, the proteins of sericin *B. mori* and *S. ricini* had molecular weights ranging from 15 to 93 kDa. According to Zhao *et al.* (2013), sericin protein is divided into two types: big sericin with a molecular weight of 191-339 kDa and small sericin with a molecular weight of 61-132 kDa. The molecular weights of sericin proteins in *B. mori* race A, F1-2 and *S. ricini* varied from 15.68 to 45.70 kDa, according to the findings. The molecular weights of *B. mori* race B, C and D sericin proteins range from 15.68 to 51.47 kDa. *B. mori* race F1-1 sericin protein has a molecular weight ranging from 15.68 to 93.25 kDa (Figure 1).

Figure 1: Molecular weight of the proteins sericin *Bombyx mori* and *Samia ricini* by the SDS-Page electrophoresis method.

Table 1: Sericin protein percentage in silkworm cocoons *Bombyx mori* and *Samia ricini*.

Silkworm cocoon	Protein percentage in OD 655 nm
Bombyx mori A	24.270 ± 5.599
Bombyx mori B	34.743 ± 2.532
Bombyx mori C	29.682 ± 7.932
Bombyx mori D	30.704 ± 7.298
Bombyx mori F1-1	38.098 ± 9.863
Bombyx mori F1-2	24.982 ± 11.200
Samia cynthia ricini	23.576 ± 4.062

The numbers in the same column that are not followed by letters are not significantly different at the 5% test level (P>0.05).

Amino acid analysis

Sericin is composed of 18 amino acids, most of which have highly polar side groups such as hydroxyl, carboxyl

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Table 2: *Bombyx mori* and *Samia cynthia ricini* protein sericin amino acid analysis.

Amino acid	*B. mori* A	*B. mori* B	*B. mori* C	*B. mori* D	*B. mori* F1-1	*B. mori* F1-2	*S. ricini*
Asp	12.89	13.05	13.16	11.79	11.91	10.98	1.79
Glu	5.92	6.10	6.31	5.56	5.56	5.30	0.98
Ser	10.11	10.27	10.83	8.90	8.75	9.05	1.17
His	1.34	1.31	1.23	1.22	1.22	0.92	0.63
Gly	28.69	25.48	30.87	24.64	24.03	28.29	5.98
Thr	11.87	15.14	14.32	1043	8.30	7.86	1.73
Arg	1.11	1.15	1.13	1.07	1.13	0.72	0.72
Ala	24.84	24.98	27.52	22.08	19.65	25.71	13.03
Tyr	11.08	11.10	11.67	10.21	9.20	10.89	2.97
Met	0.34	0.40	0.36	0.43	0.30	0.64	0.04
Val	4.32	4.40	4.68	3.96	3.79	4.90	0.31
Phe	2.42	2.47	2.62	2.19	2.19	3.62	0.27
Isoleu	2.67	2.15	2.99	2.61	2.63	4.77	0.25
Leu	3.08	3.49	2.91	2.89	3.14	7.01	0.36
Lys	6.39	6.29	7.30	5.41	5.67	11.05	0.62

Table 3: *Bombyx mori* and *Samia cynthia ricini* sericin protein clear zone area against *Staphylococcus aureus* and *Escherichia coli*.

Types of bacteria	Clear zone area (mm)						
	Bombyx mori A	*Bombyx mori* B	*Bombyx mori* C	*Bombyx mori* D	*Bombyx mori* F1-1	*Bombyx mori* F1-2	*Samia cynthia ricini*
Staphylococcus aureus	8.670 ± 2.043^{c}	8.287 ± 2.032^{c}	9.727 ± 1.522^{bc}	17.023 ± 1.560^{a}	10.307 ± 1.916^{bc}	6.850 ± 0.847^{c}	13.568 ± 1.521^{ab}
Escherichia coli	0.000^{d}	9.970 ± 2.763^{bc}	0.000^{d}	7.513 ± 0.633^{c}	6.797 ± 0.349^{c}	0.000^{d}	13.270 ± 1.808^{ab}

The numbers in the same column followed by different letters were significantly different at the 5% test level (P<0.05).

and amino groups. In this study, the primary polar amino acids aspartic acid, glycine, alanine, tyrosine and serine, were isolated from *B. mori* and *S. ricini* cocoon sericin protein (Table 2). Previous research discovered that aspartic acid, glycine, glutamic acid, threonine and serine were the most abundant amino acids in sericin protein recovered from *B. mori* bivoltine cocoons (Züge *et al.*, 2017). The sericin protein of *S. ricini* found in silkworm cocoons comprises three primary amino acids: serine, glycine and threonine (Bungthong and Siriamornpun, 2021). The discrepancy was caused by the fact that the sericin *B. mori* protein employed in this work was derived from two distinct races, namely multivoltine and crossing. The cocoon extraction technique for *S. ricini* and *B. mori* varies from prior research that employed NaOH solution rather than Na_2CO_3 and Distilled Water (DW) in general. According to Kumar and Mandal (2019), the amino acid content of sericin changes depending on the silk type and the extraction process.

Antibacterial activity of sericin proteins

The Kirby Bauer clear zone test technique was used to evaluate the antibacterial activity of the sericin protein. Treatment with sericin *B. mori* race A, B, C, D, F1-1, F1-2 and *S. ricini* proteins revealed a clear zone with substantially different values P<0.05 against Gram positive *S. aureus* and Gram negative *E. coli* (Table 3). David and Stout (1971) categorized the zone of inhibition into five categories: very weak for diameters less than 5 mm, weak for diameters larger than 5 mm, medium to 5-10 mm diameter, strong for diameters more significant than 10-20 mm and very strong or most sensitive for diameters greater than 20-30 mm. As a consequence, sericin *B. mori* protein has an intermediate to high inhibitory zone capacity against *S. aureus* bacteria, with sericin *B. mori* race D protein having the biggest area of inhibition at 17.023 mm (Table 3). Meanwhile, sericin *B. mori* protein showed an intermediate category of inhibition zone capacity against *E. coli* bacteria, although *B. mori* races A, C and F1-2 did not. The sericin protein from *S. ricini* was discovered to have a significant inhibitory zone capacity against *E.coli* and *S. aureu*s bacteria, with clear zones of 13.270 mm and 13.568 mm, respectively (Table 3).

Antibacterial activity of *B. mori* races A, B, C, D, F1-1 and F1-2 have different antibacterial potentials based on clear zone area. This can be happened due to *B. mori* have different sericin protein percentage for each race and bivoltine race are considered as resistant to bacterial (Gajalakshmi and Raja, 2015). Antibacterial activity of *B. mori* races A, B, C, D, F1-1, F1-2 and *S. ricini* sericin proteins against pathogenic bacteria. Sericin protein contains polar side groups which are formed by diverse amino acid compositions. *S. aureus* and *E. coli* bacterial cells have different cell membrane components, with *S. aureus* cell membranes comprised of peptidoglycan, teichoic acid and teicuronic acid and *E. coli* cell

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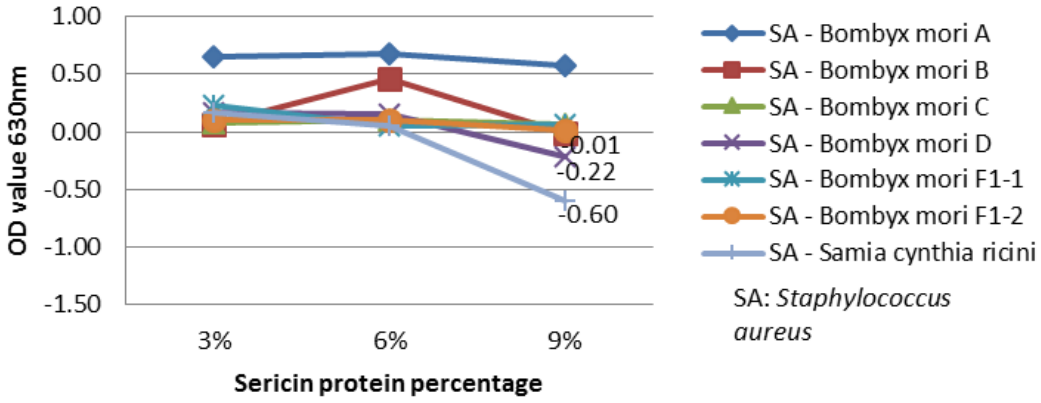

Figure 2: Decreased OD value against *S. aureus* bacteria with different percentages of sericin *B. mori* and *S. ricini* protein treatments.

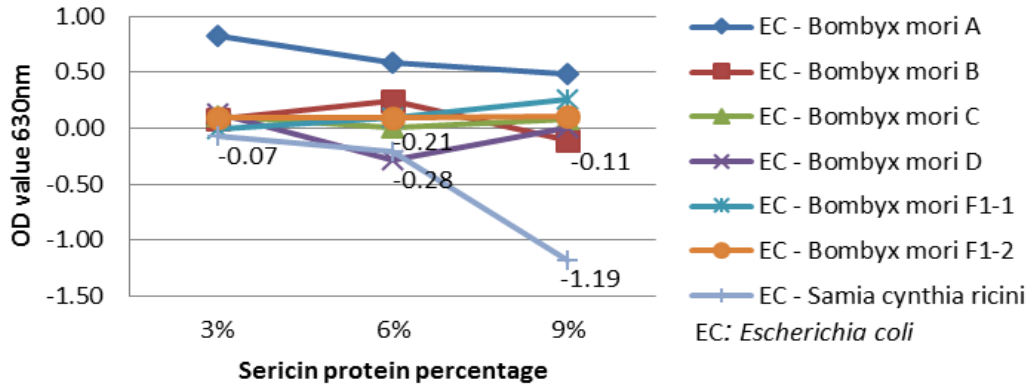

Figure 3: Decreased OD value against *E. coli* bacteria with different percentages of sericin *B. mori* and *S. ricini* protein treatments.

membrane components composed of phospholipids and little peptidoglycan. According to Husniah and Gunata (2020), polar protein molecules permeate the peptidoglycan layer, which is likewise polar in Gram-positive bacteria, more effectively than non-polar lipid layers. *Staphylococcus aureus* cell wall includes trichoic acid polysaccharide, which is polar, allowing sericin protein to enter the bacterial cell and damage the metabolic activity of the bacterial cell. This may be seen in the clear zone region (Table 3). Sericin protein's antibacterial activity is more susceptible to *S. aureus* than *E. coli*.

Decreasing optical density (OD) value of protein sericin in different concentrations

The value of optical density decreased in *E. coli* and *S. aureus* bacteria treated with sericin *B. mori* and *S. ricini* proteins at varying percentages of 3%, 6% and 9% (OD). At a concentration of 9%, the sericin proteins of *B. mori* race B, D and *S. ricini* decreased OD to the point -0.01, -0.22 and -0.60 to the *S. aureus* bacterial cells, respectively (Figure 2). In E. *coli* bacterial cells treated with sericin *B. mori* race B protein at a concentration of 9%, the OD decreased to the point -0.11, *B. mori* race D at a concentration of 6%, the OD decreased to the point -0.28 and *S. ricini* at concentrations of 3%, 6% and 9%, the OD decreased to the point -0.07, -0.21 and -1.19, respectively (Figure 3). Increasing the protein concentration of sericin against *S. aureus* and *E. coli* bacteria causes an increase in hydrogen and covalent bonds between functional groups, which then sets a spatial boundary for charged functional groups, inhibiting binding to the bacterial cell wall (Manesa *et al.*, 2020), as indicated by a negative decrease in the OD value.

SEM imaging of *Staphylococcus aureus* and *Escherichia coli* bacteria

The impact of sericin *B. mori* and *S. ricini* proteins on *E. coli* and *S. aureus* bacteria grown in nutrient broth medium for 24 h was studied morphologically using an electron microscope at 20000× magnification. *S. aureus* bacteria without sericin protein displayed a cocci-shaped morphology (Figure 4A). The treatment of *S. aureus* bacteria with sericin *B. mori* race D produced a rough cell

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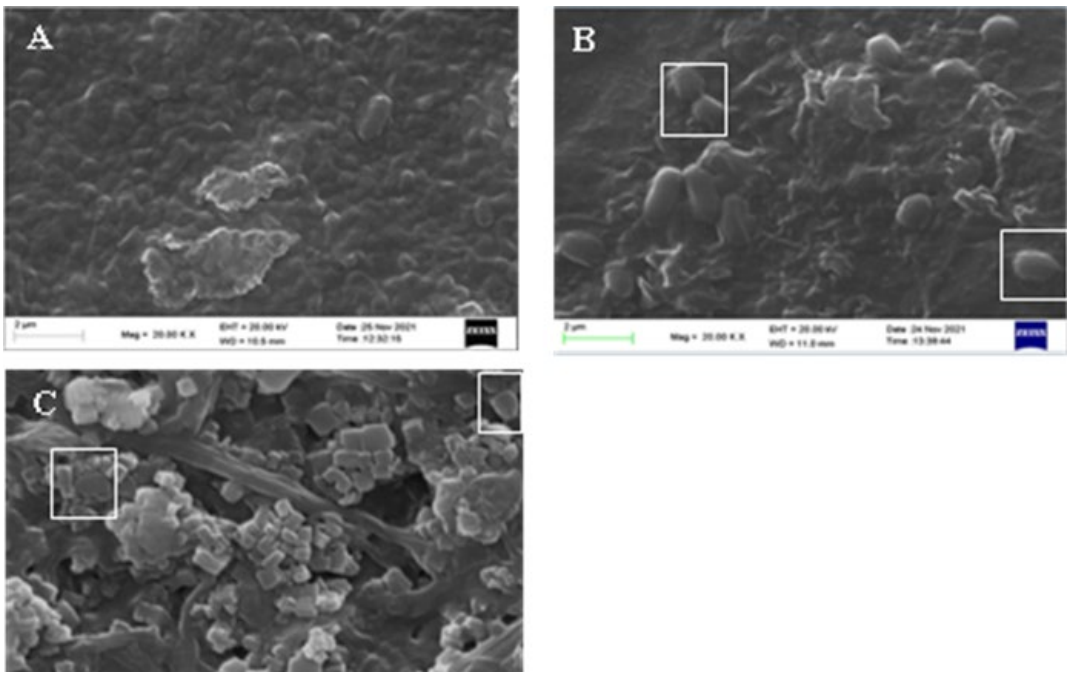

Figure 4: SEM magnification 20000×. (A) Control *S. aureus*; (B) *S. aureus* treated with *Bombyx mori* race D; (C) *S. aureus* with treatment *Samia cynthia ricini*.

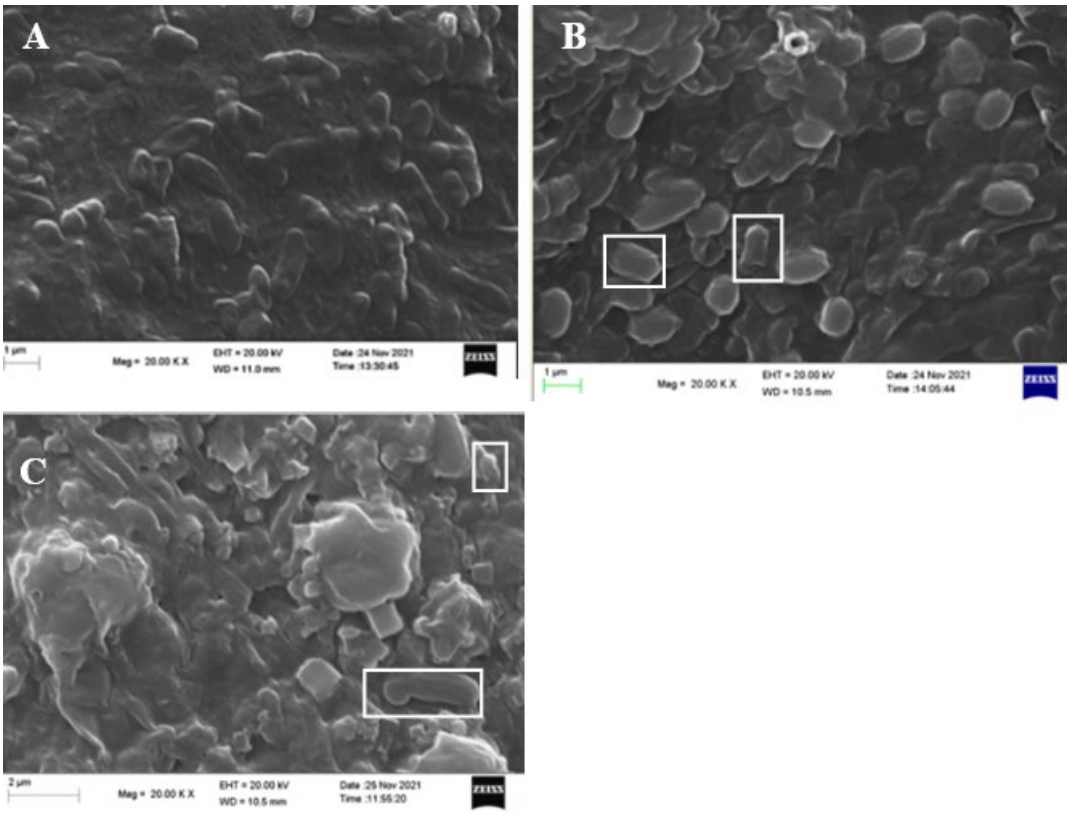

Figure 5: SEM magnification 20000×. (A) Control *E. coli*; (B) *E. coli* treated with *Bombyx mori* race D; (C) *E. coli* with treatment *Samia cynthia ricini*.

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wall surface on the bacterial cells (Figure 4B). In *S. aureus* bacteria treated with *S. ricini* sericin protein, there was cell deformation with hollows on the bacterial cell surface (Figure 4C). In control bacteria, *E. coli* that had not been treated with sericin protein, a bacillus-shaped morphology was found (Figure 5A). *Escherichia coli* bacteria treated with sericin *B. mori* race D exhibited a rough surface on the bacterial cell membrane, leading in ruptured bacterial cells (Figure 5B). The presence of blebs on the bacterial cell surface produced morphological alterations in *E. coli* bacteria treated with the sericin protein *S. ricini* (Figure 5C).

Sericin *B. mori* race D protein clings to the cell surface of *S. aureus* and *E. coli* bacteria, as demonstrated by the presence of a rough surface on the bacterial cells. After conversion from smooth to the rough surface, cell surface roughness contributes to bacterial adherence (Khan *et al.*, 2014). In *E. coli* bacterial cells, there is a rupture that occurs during adhesion and the bacterium will seek to sustain its development by widening the contact area with multiple anchoring sites and when the cell wall reaches the strain threshold (Das *et al.*, 2017). Cell rupture might occur as Gram-negative cells are less resistant to stretching effects than Gram-positive cells (Pulingam *et al.*, 2019). *Samia cynthia ricini* sericin protein is densely packed, which may cause cell deformation owing to pressure differences generated by sericin protein on both sides of the bacterial cell wall (Szwast *et al.*, 2012). Hollows in the cell wall of *S. aureus* suggested cell deformation, whereas blebs indicated cell deformation in *E. coli*. Blebs in *E. coli* bacteria develop as a result of the bacteria releasing membrane vesicles (MV), which are created by controlled blebbing of the outer membrane of Gram-negative bacteria. Blebs appear in bacterial cells due to environmental stress or a shortage of nutrients, which is frequently produced by antibiotics and results in cell lysis (Toyofuku *et al.*, 2019).

CONCLUSION

The presence of a clear zone indicated that the sericin protein had antibacterial activity. Sericin of *B. mori* inhibited the growth of *S. aureus* bacterial colonies more significantly than *E. coli*, whereas Sericin from *S. ricini* inhibited both. In addition, sericin protein at a concentration of 9% was shown to be substantially more efficient in inhibiting pathogenic bacterial growth. Antibacterial sericin proteins from *B. mori* and *S. ricini* were discovered to have bactericidal activity against *S. aureus* and *E. coli* bacteria. In *B. mori*, the bacterial cell surface seems rough to the point of rupture, but in *S. ricini*, the cell appears distorted due to the presence of hollows and blebs on the cell surface.

REFERENCES

Ahamad, M. S. I. and Vootla, S. K. (2018). GC-MS analysis of anti-microbial compounds from *Antheraea mylitta* cocoons. *International Journal of Pharmaceutical Sciences and Research* **9(7), 1000-1007.**

Aramwit, P., Siritientong, T. and Srichana, T. (2012). Potential applications of silk sericin, a natural protein from textile industry by-products. *Waste Management and Research* **30(3), 217-224.**

Bauer, A. W., Perry, D. M. and Kirby, W. M. M. (1959). Single-disk antibiotic-sensitivity testing of *staphylococci*: An analysis of technique and results. *AMA Archives of Internal Medicine* **104(2), 208-216.**

Bharathi, M. C., Umakanth, R. S. and Ashoka, K. S. (2020). Extraction of sericin from cocoon shell and raw silk of bivoltine and multivoltine breeds of the Silkworm, *Bombyx mori* L. *Journal of Entomology and Zoology Studies* **8(4), 869- 871.**

Borgohain, A. (2015). Silk and its biosynthesis in silkworm *Bombyx mori*. *Journal of Academia and Industrial Research* **3(12), 626-628.**

Bungthong, C. and Siriamornpun, S. (2021). Changes in amino acid profiles and bioactive compounds of Thai silk cocoons as affected by water extraction. *Molecules* **26(7), 2033.**

Das, S., Sinha, S., Das, B., Jayabalan, R., Suar, M., Mishra, A., Tamhankar, A. J., Lundborg, C. S. and Tripathy, S. K. (2017). Disinfection of multidrug resistant *Escherichia coli* by solar-photocatalysis using Fe-doped ZnO nanoparticles. *Scientific Reports* **7, 104.**

David, W. W. and Stout, T. R. (1971). Disc plate method of microbiology antibiotic assay. *Applied Microbiology* **22(4), 659-665.**

Endrawati, Y. C. (2012). Sericin protein extraction from *Attacus atlas* cocoon and its characterization as a biomaterial by animal science. M.Sc. Thesis. IPB University, Indonesia.

Gajalakshmi, P. and Raja, A. (2015). Bacterial induced immunity studies in selected silkworm (*Bombyx mori* L.) germplasm strains. *Biomedical and Pharmacology Journal* **3(1), 269-271.**

Husniah, I. and Gunata, A. F. (2020). Pineapple peel extract as antibacterial. *Jurnal Penelitian Perawat Profesional* **2(1), 85-90.**

Khan, M. N., Mirani, Z. A., Wang, J., Lin, H. and Khan, S. I. (2014). Effect of traditional food preservatives on biofilm formation by food pathogens. *Malaysian Journal of Microbiology* **10(2), 126-132.**

Kumar, J. P. and Mandal, B. B. (2017). Antioxidant potential of mulberry and non-mulberry silk sericin and its implications in biomedicine. *Free Radical Biology and Medicine* **108, 803-818.**

Kumar, J. P. and Mandal, B. B. (2019). The inhibitory effect of silk sericin against ultraviolet-induced melanogenesis and its potential use in cosmeceutics as an anti-hyperpigmentation compound. *Photochemical and Photobiological Sciences* **18(10), 2497-2508.**

Kundu, B., Kurland, N. E., Yadavalli, V. K. and Kundu, S. C. (2014). Isolation and processing of silk proteins for biomedical applications. *International Journal of Biological Macromolecules* **70, 70-77.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227, 680-685.**

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemis*try **193(1), 265-275.**

Manesa, K. C., Kebede, T. G., Dube, S. and Nindi, M. M. (2020). Profiling of silk sericin from cocoons of three Southern African wild silk moths with a focus on their anti-microbial and antioxidant properties. *Materials* **13(24), 5706.**

Mohsin, M. I., Al-Shamarti, M. J., Abbas, S. A., Mohsin, R. I. and Al-Sahaf, S. (2020). Approach to enhance antibiotics efficiency towards uropathogenic bacteria. *Malaysian Journal of Microbiology* **16(3), 193-202.**

Munfaati, P. N., Ratnasari, E. and Trimulyono, G. (2015). Aktivitas senyawa antibakteri ekstrak herba meniran (*Phyllanthus niruri*) terhadap pertumbuhan bakteri *Shigella dysenteriae* secara *in vitro*. *Lentera Bio* **4(1), 64-71.**

Murthy, V. N. Y., Ramkumar, B., Jayaram, G. N. and Lokesh, G. (2014). Critical biochemical analysis in different body tissues in three commercial silkworm (*Bombyx mori* L.) races. *Asian Journal of Natural and Applied Sciences* **3(2), 20-30.**

Pulingam, T., Thong, K. L., Ali, M. E., Appaturi, J. N., Dinshaw, I. J., Ong, Z. Y. and Leo, B. F. (2019). Graphene oxide exhibits differential mechanistic action towards Gram-positive and Gram-negative bacteria. *Colloids and Surfaces B: Biointerfaces* **181, 6-15.**

Szwast, M., Suchecka, T. and Piątkiewicz, W. (2012). Mathematical model for biological cell deformation in a cylindrical pore. *Chemical and Process Engineering* **33(3), 385-396.**

Tariq, S., Wani, S., Rasool, W., Shafi, K., Bhat, M. A., Prabhakar, A., Shalla, A. H. and Rather, M. A. (2019). A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. *Microbial Pathogenesis* **134, 103580.**

Toyofuku, M., Nomura, N. and Eberl, L. (2019). Types and origins of bacterial membrane vesicles. *Nature Reviews Microbiology* **17(1), 13-24.**

Xue, R., Liu, Y., Zhang, Q., Liang, C., Qin, H., Liu, P., Wang, K., Zhang, X., Chen, L. and Wei, Y. (2016). Shape changes and interaction mechanism of *Escherichia coli* cells treated with sericin and use of a sericin-based hydrogel for wound healing. *Applied and Environmental Microbiology* **82(15), 4663-4672.**

Zhao, R. B., Han, H. F., Ding, S., Li, Z. H. and Kong, X. D. (2013). Effect of silk sericin on morphology and structure of calcium carbonate crystal. *Frontiers of Materials Science* **7(2), 177-183.**

Züge, L. C. B., Silva, V. R., Hamerski, F., Ribani, M., Gimenes, M. L. and Scheer, A. P. (2017). Emulsifying properties of sericin obtained from hot water degumming process. *Journal of Food Process Engineering* **40(1), e12267.**

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