

Bacterial degradation of sericin for degumming of silk fibers—A green approach

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ABSTRACT

Biodegumming is the process of degradation of sericin and its subsequent removal from the surface of silk fibers using microorganisms. Two proteins, viz., fibroin and sericin, make up the silk fiber. Fibroin is the fiber whereas sericin is the glue-like protein coat present on the surface of the fiber that needs to be removed. During the conventional degumming process, chemicals such as soap and soda are used for the removal of sericin from the fiber. This treatment requires a large quantity of water for the removal of the chemicals used. Also, the waste water released into the environment can lead to water pollution. In the following study, a microbiological degumming process was used for the silk fiber, using various bacterial cultures. Nine isolates were checked for their degumming activity. The biologically treated silk was found to be shinier, smoother, softer, and lustrous as compared to the chemically treated silk. Two promising isolates were identified. The treated silk was also tested for parameters like tensile strength and elongation. Silk was also tested for color fastness after dyeing with turmeric. As compared to the chemical process, the microbiological process was found to be more efficient, cost-effective, rapid, and most importantly, environment friendly.

1. INTRODUCTION

As per statistics by the International Sericultural Commission, global silk production was more than 1,00,000 metric tons in 2019. Asian countries produce about 98% of the total world's production. The largest manufacturer and supplier of silk and silk products is China making it the leader in raw silk production, it is closely followed by India [1]. The commonly employed silkworm, *Bombyx mori*, makes raw silk of very fine quality. It contributes to the highest fiber production for the silk industry. The worm feeds on the foliage of the mulberry plant, *Morus* spp. Hence, it is known as mulberry silkworm and the silk produced by it is known as mulberry silk [1].

Mainly two types of proteins, namely fibroin and sericin are present in the cocoons produced by *B. mori*. Silk fiber comprises 70% fibroin and 30% sericin. 20%–30% of the silkworm cocoon

consists of sericins, also called as “glue” proteins because of their gummy nature. They hold the fibroin fibers together and are glycoproteins that dissolve only in water at high temperatures. They are responsible for the formation of the fibroin–sericin composites that are resistant to many environmental conditions. Sericin is a protein with a very high content of the amino acid serine (40%) followed by considerable amounts of glycine (16%). It is made up of different polypeptides with molecular weights ranging from 24 to 400 kDa. These differences are due to differences in gene expression and protein synthesis [1]. Polypeptide fractions with molecular weights of 150, 250, and 400 kDa have been isolated from the sericin present in cocoons. Unlike fibroin, which consists majorly of antiparallel β -sheets, sericin protein consists of 35% β -sheet, 63% random coil, and no α -helices. It remains in a partially unfolded state because of the random coils and is amorphous in nature.

A series of steps is involved in the manufacture of silk products from silk fiber in the cocoons. These include: harvesting, degumming, dyeing, spinning, weaving, printing, and finishing. Degumming, i.e., the removal of sericin, is an important stage during which silk fibers acquire the characteristic lustrous aspect, soft handle,

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and elegance. These qualities are highly valued by consumers making silk an expensive fabric. Moreover, the presence of sericin hampers the penetration and absorption of liquor used for dyeing and processing of silk [5]. Furthermore, it is the primary cause of adverse reactions observed due to hypersensitivity and bioincompatibility to silk fibers. Fibroin solution of high purity is required to prepare silk-based biomaterials. For this, the removal of sericin from fibroin is of utmost importance since residual sericin can interfere with solubilization of fibers and also lead to inflammatory reactions in consumers [1][6].

The industrial process of silk extraction depends on the difference in the physicochemical properties of the fibroin and sericin. While the former is water-insoluble due to its highly ordered, crystalline and fibrous nature, the latter is amorphous in nature and can be easily dissolved by boiling in aqueous solutions containing alkaline soap and synthetic detergents. However, there could be a partial degradation of fibroin due to the high temperature, equal to approximately 95°C, and a high pH ranging from 8.0 to 9.0. In addition to this, strong chemicals used in the processing liquor create a significantly harsh environment for the fiber. This causes loss in fiber weight. Moreover, fiber degradation leads to a reduction of desirable aesthetic and physical properties. The silk fiber may appear dull with surface fibrillation and poor handle. Such fibers will also have poor tensile strength. The uneven texture of the fibers also affects dye absorption leading to uneven dyeing and poor value. A very important factor in this kind of chemical processing is the large consumption of water and energy. Also, the release of a large amount of harsh chemicals in the degumming liquor contributes to significant environmental pollution. Hence, the development of a novel, efficient, and cost-effective degumming method is the need of the hour [1].

2. MATERIALS AND METHODS

2.1. Silk

Mulberry silk yarns were obtained from the Textile committee of India, Prabhadevi, Mumbai.

2.2. Enrichment and Isolation

The sericin was extracted from raw silk fibers in three different ways. First, raw silk was boiled with distilled water for 10–15 minutes in a material:liquor (M:L) ratio of 1:2 and the supernatant was poured into a sterile Petri plate. Second, raw silk was autoclaved in distilled water and supernatant was poured into a sterile Petri plate and third, the silk waste solution, i.e., water released after conventional treatment was poured into a sterile Petri plate. All three plates were incubated at room temperature for 48 hours. Nine bacterial colonies were isolated from the plates and Gram nature was determined. Pure cultures were maintained on nutrient agar slants.

2.3. Screening of Bacterial Cultures

2.3.1. Test for sericin degradation

Saline suspensions of all isolates were prepared. These isolates were inoculated in a flask containing silk fiber and distilled water

in a ratio of 1:20. Flasks were incubated on a shaker at Room temperature (RT) for 48 hours. Absorbance of each flask was checked after the incubation period and isolates were selected on the basis of turbidity.

2.3.2. Proteolytic activity

Isolates were spot-inoculated on casein agar plates and milk agar plates. The plates were incubated at room temperature for 48 hours and checked for a zone of clearance around colonies [2].

2.4. Production of Crude Enzyme

Enzyme production was done in 100 ml of production medium at pH 7. After aseptic inoculation of the bacterial isolates, the production medium was kept at RT for 24 hours. The culture broth was then centrifuged at 3,000 rpm for 25–30 minutes. The cell free supernatant was decanted carefully and collected. A few drops of formaldehyde were added to the supernatant for preservation. This preparation was used as the crude enzyme.

2.5. Protein Content

Concentration of protein content in the degumming liquor was measured by Folin Ciocalteu method. Bovine serum albumin was used as the standard [11].

2.6. Degumming of Silk Using Cell Free Supernatant

The raw silk yarn was first washed in cold water followed by immersion in the cell free supernatant (crude enzyme) taken in a clean beaker. One gram of silk fibers was incubated with 20 ml of the crude enzyme in M:L ratio of 1:20 for degumming. The pH was adjusted to 7.0. The beaker was placed in an incubator and the temperature was maintained at 50°C for a duration of 3 hours. After this, the silk yarn was washed thoroughly with hot water followed by cold water, air-dried and weighed [9].

2.7. Conventional Degumming of Silk (Control)

Raw silk fibers were soaked for 24 hours in a solution containing 5.0 g/l of Marseille soap. The pH was adjusted to 9.5 and M:L ratio of 1:40 was used. After 24 hours, the silk fibers were immersed in an alkaline solution containing 10.0 g/l Marseille soap and 1.0 g/l sodium carbonate. The conditions maintained were 95°C for 2 hours at M:L ratio of 1:40 and pH 9.5. The fibers underwent degumming by this treatment. They were first rinsed at 50°C with 1.0 ml/l ammonia solution and twice at 40°C with 1.0 ml/l ammonia. Finally, the fibers were rinsed with cold water [2].

2.8. Degumming Efficiency

The percentage of sericin removed from the raw silk fibers was calculated using the following formula [3]:

$$S = (B - A) \div B \times 100$$

S = the percentage of sericin removed

B = the weight of silk before the treatment

A = the weight of silk after the treatment

Degumming efficiency was then calculated using the following formula [3]:

$$\text{Degumming efficiency (\%)} = (\text{Weight loss by crude enzyme treatment} \div \text{Weight loss by conventional chemical treatment}) \times 100$$

2.9. Optimization of Degumming Process

2.9.1. Substrate (for enzyme production)

Two proteinaceous substrates were used, namely, peptone and sericin for cultivation of selected isolates. Bacterial isolates were inoculated in media containing either 1% peptone or 1% sericin as the protein source and incubated at RT for 24 hours. The broths were centrifuged and supernatants were collected. Degumming activity was measured using the same procedure as given in Section 2.8.

2.9.2. Temperature and pH (for enzyme activity)

Silk fiber was incubated with the crude enzyme in a M:L ratio of 1:20 at different temperatures ranging from 45°C to 65°C at pH 7 and at pH such as 6, 7, 8, 8.5, and 9 at 50°C [10][7]. The experiments were performed in triplicates.

2.10. Identification of Bacterial Cultures

Biochemical tests were performed to identify A1 and A6 cultures.

2.11. Evaluation

2.11.1. Subjective evaluation

All the degummed silk fiber samples were cut into equal sizes of 15 cm, mounted on a black sheet of paper and visually evaluated for their lustre, color, softness, and hand value by a panel of 200 judges comprising of students and teachers [10].

2.11.2. Objective evaluation

Raw untreated sample, conventionally treated control, and enzyme treated test samples were subjected to standard yarn tests such as loss in weight, tensile strength, and elongation using Electronic Fabric Strength Tester (YG026T). Standards used: GB/T3923.1 GB/T3923.2 [4].

2.12. Dyeing Test of Silk Yarn

Natural dye was prepared using turmeric which yielded shades of brilliant yellow. Pieces of turmeric were oven-dried at 60°C for 10 hours. The dried pieces were then crushed using a mortar and pestle and re-dried. They were finely powdered and used for dye extraction. 10 g of the powder was boiled in 250 ml of water for an hour. The solution was cooled and filtered [8]. The filtrate contained the water-soluble dye.

3. RESULTS AND DISCUSSION

3.1. Isolation and Screening of Bacteria

A total of nine bacterial isolates were selected from the sericin-containing plates and screened for sericin-degrading ability. The

cultures were inoculated in broth containing raw silk fibers. The turbidity of the broth was taken as a measure of growth and sericin degradation. Growth indicated that the bacterial culture was using sericin present on the fibers as a source of nutrition since the broth contained sericin as the sole source of carbon and nitrogen. Out of nine, two isolates showed good growth in the medium (Table 1) and were used for further study.

3.2. Proteolytic Activity

The bacterial isolates selected on the basis of above screening method were checked for their ability to degrade casein. Bacterial isolates were spot-inoculated on 1% casein agar and milk agar plates. These plates are turbid due to the presence of the insoluble milk protein casein. A zone of clearance around the bacterial growth indicates that the casein has been degraded or utilized. No zone of clearance was seen around the colonies of these isolates indicating that these bacteria were not capable of degrading casein. Hence, the proteolytic activity of these isolates was specific for sericin degradation [3].

3.3. Determination of Protein Content

There was an increase in protein content after the degumming process of silk (Table 2) which indicates the protein sericin was degraded and released in the liquid. Hence, the enzymes from cultures A1 and A6 were capable of degrading the sericin present on the fibers.

3.4. Degumming Efficiency

Degumming efficiency of both the cultures was found to be 87%. The efficiency is a comparison of the sericin removal by the enzymatic process with the conventional one. This indicates that required amount of sericin is efficiently removed from the raw silk fibres by treatment with the crude enzyme obtained from these two cultures.

Table 1: Turbidity of bacterial cultures grown in sericin medium.

Isolate number	Absorbance at 540 nm	Gram nature
A1	0.78	Gram positive
A2	0.19	Gram negative
A3	0.3	Gram negative
A4	0.47	Gram positive
A5	0.27	Gram positive
A6	0.53	Gram negative
B2	0.32	Gram negative
B3	0.15	Gram positive
B4	0.49	Gram negative

A—Isolates from autoclaved raw silk supernatant sample.

B—Isolates from boiled raw silk supernatant sample.

Table 2: Concentration of protein in treatment liquor.

Isolates	Protein concentration (mcg/ml) (before degumming)	Protein concentration (mcg/ml) (after degumming)
A1	170	240
A6	110	180

3.5. Optimization Studies

3.5.1. Substrate for enzyme production

For optimizing substrate for enzyme production, two substrates were used in enzyme production media, namely, peptone and sericin. Enzymes produced using sericin as a substrate gave better results in terms of texture and visual appearance as compared to enzymes produced using peptone as substrate.

3.5.2. Optimization of temperature and pH conditions for enzyme activity

Silk fiber was incubated with the crude enzymes at different temperatures and pH conditions. As seen in Figures 1–4, enzymes from both A1 and A6 have better activity at temperature 50°C at pH 7. The results clearly indicate that harsh pH and temperature are not required for sericin removal using bacterial enzymes, unlike the conventional chemical methods. In conventional treatment, boiling temperatures and highly alkaline pH are used which damage the silk fibroin. Thus, raw silk fibers can be degummed with these enzymes at 50°C and neutral pH.

3.6. Identification of Bacteria (As Per Bergey's Manual)

Bacterial isolates A1 and A6 were identified as *Bacillus subtilis* and *Pseudomonas fluorescens*, respectively (Table 3). Both these bacteria are soil and water inhabitants, and are non-pathogenic saprophytes. They do not pose any danger to the workers or the environment.

3.7. Subjective Evaluation

3.7.1. Visual evaluation of degummed silk yarn

The samples were visually evaluated for lustre, color, softness, and hand value. All the three treated samples and a raw untreated silk sample (Figs. 5–8) were evaluated by more than 200 people. All of them found the crude enzyme treated samples to be very soft, lustrous, and shiny as compared to the chemically treated silk

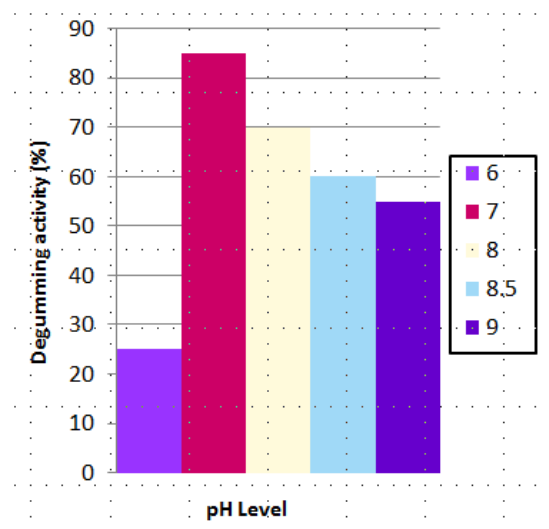


Figure 1: Optimum pH for activity of enzyme from A1.

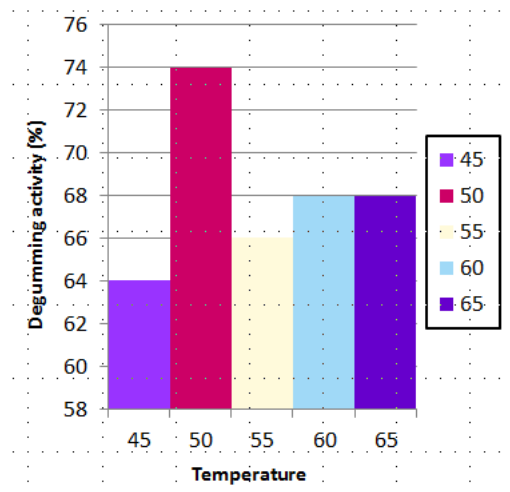


Figure 2: Optimum temperature for activity of enzyme from A1.

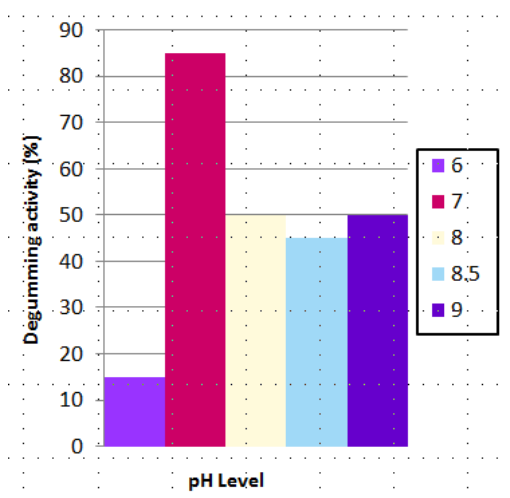


Figure 3: Optimum pH for activity of enzyme from A6.

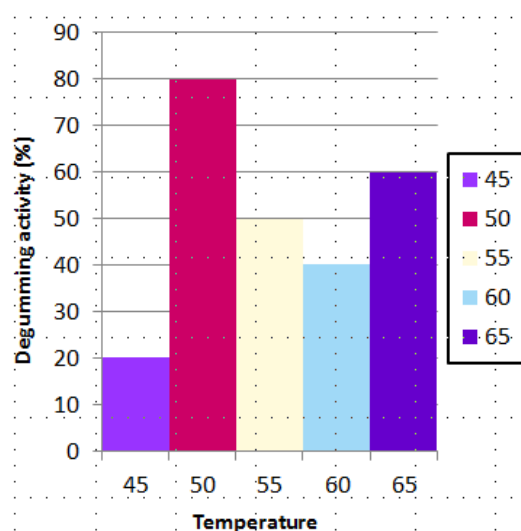


Figure 4: Optimum temperature for activity of enzyme from A6.

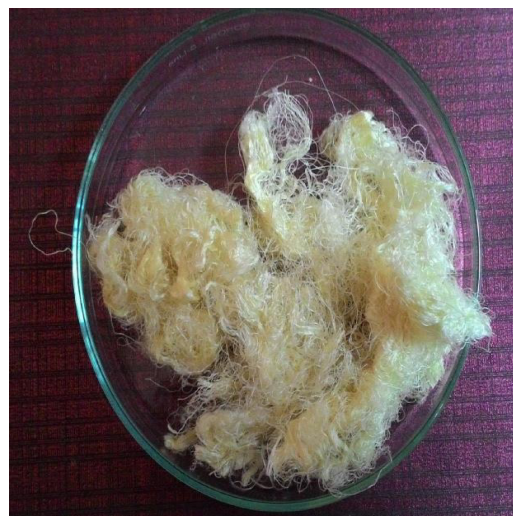
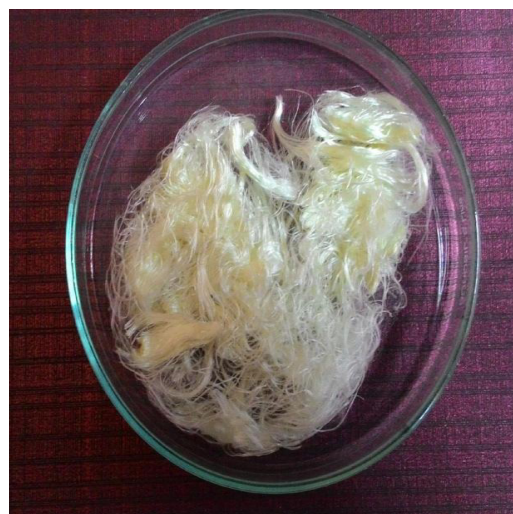
Table 3: Biochemical results.

Sr. no.	Biochemical tests	A1	A6
1	Gram staining	Gram positive	Gram negative
2	Glucose fermentation	–	–
3	Catalase	–	+
4	Mannitol fermentation	–	–
5	Nitrate reduction	–	–
6	Urease reduction	–	+
7	Methyl red test	–	–
8	Indole	–	–
9	Voges Proskauer	+	–
10	Oxidase	–	–
11	Sporulation	+	–
12	Pigment production	–	+
13	Gelatin hydrolysis	+	+
14	Starch hydrolysis	+	–
15	Arginine dihydrolase	–	+
16	Growth on King's B medium	–	+
17	Growth on cetrimide agar	–	+
18	Growth at 4°C	–	+
19	Growth at 41°C	–	–
20	UV fluorescence	–	+
Results		<i>B. subtilis</i>	<i>P. fluorescens</i>

+ = Positive, – = Negative.

**Figure 5:** Raw silk fibre (control).

fiber [10]. The overall rating given by the judges was maximum for the enzyme treated fiber samples followed by conventionally treated sample. The raw untreated silk sample was poor in all the criteria considered for visual evaluation. There was a vast and clear difference in the quality of the enzyme-treated sample as compared to controls. The silk yarn degummed using crude enzymes from bacterial isolates was the best in general appearance, smoothness, texture, and lustre.

**Figure 6:** Chemically treated silk fiber.**Figure 7:** Biologically treated silk fiber (A1).**Figure 8:** Biologically treated silk fiber (A6).

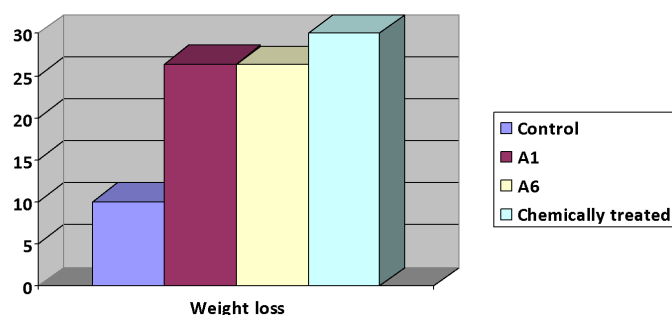


Figure 9: Percentage weight loss of fiber after treatment.

Table 4: Objective evaluation of silk fibers using electronic fabric strength tester Model YG0260.

Sr. No.	Tests	Results (mean values)
1.	Weight loss	
	Enzyme treated	26.12%
	Chemical treated	31.34%
	Raw sample	11%
2.	Tensile strength of degummed silk yarn	
	Enzyme treated	1.397 kg
	Chemical treated	0.875 kg
	Raw sample	1.307 kg
	Dyed enzyme treated sample	1.735 kg
	Dyed chemically treated sample	0.448 kg
3.	Elongation of degummed silk yarn	
	Enzyme treated	3.702 cm
	Chemically treated	1.524 cm
	Raw sample	3.422 cm
	Dyed enzyme treated	4.317 cm
	Dyed chemically treated	3.455 cm

3.8. Objective Evaluation

The percentage weight loss of silk fiber after conventional chemical treatment and microbial crude enzyme treatment is represented in Figure 9. There was a 30% weight loss in chemically treated silk fiber and 26% weight loss in the crude enzyme treated fiber. Since raw silk contains approximately 30% sericin, this indicates that the sericin was removed by these treatments. The crude enzyme treated fiber is as good as chemically treated fiber with respect to sericin removal. The treated and untreated silk fibers were subjected to tensile strength and elongation tests. Crude enzyme treated silk fiber had higher tensile strength and elongation characteristics as compared to chemically treated samples (Table 4). The dyed enzyme treated samples also gave better results compared to dyed chemically treated ones. The high tenacity and good elongation indicate that the protein fibroin is not damaged by the enzymes. This could be due to the specificity of the enzymes to sericin thereby leaving fibroin unaffected.

3.9. Dyeing Property

After dyeing with turmeric extract, the chemically treated fiber appeared brownish yellow, whereas biologically treated ones looked

brilliant yellow and after washing chemically treated fiber lost its color turning light brown and biologically treated retained the color.

Color fastness was checked through washing tests (as per ISO 105 C06 standard). The dyed fiber was washed for five cycles and below are the ratings given after the washing cycles.

Crude enzyme treated sample: Colour-5 (rating)

Chemically treated:- Colour-3 (rating)

Ratings: 1 = Very poor; 2 = Poor; 3 = Average; 4 = Good; 5 = Excellent

Rating was based on the visual change in the color of fiber. Color of the crude enzyme treated dyed silk fiber did not change much after five cycles of washing. This indicated absorbance of the dye onto the fiber due to absence of sericin. Better retention of the dye could be due to better quality of silk fiber with less damage and fibrillation.

4. CONCLUSION

The textile industry is the largest industry in terms of scale of production and product value. However, it is also the foremost in toxic effluent generation and thereby as the major cause of water pollution. Textile manufacturers need to focus on reducing pollution-causing processes in textile production as it is the need of the hour and adopt a greener approach in their manufacturing process. Because of their non-toxic and environment-friendly nature, the use of biological systems in the textile industry is becoming increasingly popular. The research should be carried out to replace the use of harsh chemical methods with biological processes and simultaneously make it cost-effective. Effluent water reduction and recycling are important factors to be considered. Hence, this study explored the use of crude bacterial enzymes for degumming as a green and cost-effective alternative to conventional degumming methods.

In the present study, the quality of silk after bacterial-enzymatic treatment is better than chemical treatment. It is softer, lustrous, and shinier. The crude enzymes obtained from the isolates *B. subtilis* and *P. fluorescens* give better degumming properties than conventional processes. This is because of the specificity of sericin-degrading enzymes. The enzymes produced by the isolates did not degrade other proteins, including fibroin. The degumming efficiency was 87%. The strength and dyeing properties of the silk fibres was better after enzymatic degumming.

The use of these crude enzymes was found to be a good method because they selectively degrade the sericin while keeping the fibroin intact. Bacterial degumming is not just a green alternative to conventional methods, but is also more cost-effective since the degumming liquor can be used for many cycles. The results of this work prove the potential of bacterial degumming as a suitable and necessary replacement of traditional non-environment friendly silk degumming processes.

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6. CONFLICT OF INTEREST

Authors declare that they do not have any conflicts on interest.

7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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9. ETHICAL APPROVALS

Not Applicable.

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