The biotechnological production of lacewing silk: From gene to protein-based material

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Abstract
Structural proteins are in the spotlight of research and industry due to their versatile properties and the respective potential applications. Besides in-depth studied structural proteins such as spider silk proteins, the proteins within the egg stalk of green lacewings represent another interesting approach for material development. In order to convert such materials into products, biotechnological processes are required to generate a sufficient supply of raw materials. This work describes an innovative and efficient way to recombinantly produce and purify the genetically modified lacewing silk protein N[AS]8C (53 kDa) as well its exemplary conversion into silk films. By means of high cell density fermentations applying Escherichia coli (E coli) BL21(DE3) or E coli HMS174(DE3), the successful biosynthesis of the recombinant lacewing silk protein N[AS]8C was demonstrated. Interestingly, the formation of an intracellular, highly insoluble silk protein fraction was observed. A tailored purification strategy was developed, allowing the isolation and purification of the insoluble intracellular silk aggregates. Furthermore, the processing of the purified lacewing silk proteins into transparent films was also documented. Based on structural studies (ATR-FTIR), a dominant antiparallel β-sheet-orientation was observed in purified silk protein powder as well as in the processed silk materials, which can be attributed to the cross-β-structure characteristic to lacewing egg stalk silk proteins.

KEYWORDS
biomaterial, fermentation, lacewing silk, purification, silk

1 INTRODUCTION

The egg stalk of the green lacewing can be seen as an example for the fascinating mechanical properties of materials found in nature. It possesses a remarkable bending stiffness important to safely carry the egg, which is attached on the top of the fiber. For this egg stalk, a bending modulus three times higher than that of common silkworm fibers could be calculated.1 Silk is a term used to describe a specific type of structural proteins that are spun into fibers by a variety of
arthropods. In common silk proteins, α-helices and β-sheet structures predominate. Structural proteins that possess a high proportion of β-sheet structures were shown to have increased tensile strength (depending on the orientation of the β-sheet crystals in the material), but reduced extensibility. The crystalline character of the β-sheet structures is based on the hydrogen bonds forming between two β-strands.

Unlike most known silk proteins, the lacewing egg stalk is composed of silk proteins, which are structurally arranged in a cross-β-conformation. In the lacewing *Chrysopa carnea*, which is spread worldwide—with the exception of Australia—five proteins were identified in the spinning dope responsible for the formation of the egg stalk. In contrast, the lacewing *Mallada signata* (occurring in Australia and New Zealand) uses only two dominant proteins for formation of its egg stalk. These proteins, identified via cDNA databases, are termed MalXB1 and MalXB2. Both proteins, MalXB1 with a molecular weight of 109 kDa and MalXB2 with a molecular weight of 67 kDa, possess highly repetitive core domains, which occupy more than 70% of the respective protein and are composed of repeating sequence motifs (16 amino acids each). At the N-terminal and C-terminal ends, there are nonrepetitive regions located. Parker and Rudall first described the underlying cross-β structure of the egg stalk silk proteins in 1957. Furthermore, this structure was shown to be responsible for the high bending stiffness of the egg stalk. Tensile tests conducted with native lacewing egg stalks at different relative humidity followed by Raman spectroscopy of the elongated fiber revealed the special structure. Figure 1 schematically shows the cross-β-structure, which is built of antiparallel β-sheets perpendicular to the fiber axis.

In order to develop new innovative biomaterials made from lacewing silk, biotechnological processes are sought that enable a sufficient and cost-efficient supply of silk-based material. In prior studies, Bauer and Scheibel were able to generate an artificial protein, which is derived from the natural silk protein MalXB2. For this purpose, a 48-amino-acid consensus sequence was taken from the cross-β-structure core domain (module [AS]) and multimerized using a seamless cloning technique. Afterward, the native non-repetitive terminal domains (N-terminus and C-terminus) were ligated to the multimerized core domain. The artificial protein N[AS]8C generated in this way contains eight repeats of the module [AS] as well as the natural termini and has a molecular weight of 53 kDa.

In biotechnological production of silk-type proteins in general, the highly repetitive structure of the gene sequences, the differential use of the codons of insects and bacteria, the homologous recombination of the bacteria as well as the generally poor protein solubility are only some of the challenges found in literature. Various attempts with varying success were made to produce synthetic silk proteins (mainly spider silk proteins) via *Escherichia coli* (*E. coli*), *Bacillus subtilis*, *Pichia pastoris*, in insect cells, in plants (tobacco, potato, cress) and in transgenic animals such as mice or goats. Despite the large number of expression systems used, the experiments that were published most...
often were conducted with *E. coli*. For example, Xia et al were able to produce artificial silk protein variants possessing different molecular weights in *E. coli* BL21(DE3). The artificial silk proteins ADF-3 and ADF-4 (origin: *Araneus diadematus*) were first produced in *E. coli* BLR (DE3) and later in *E. coli* HMS174 (DE3). The process strategy used to cultivate the microorganisms has a significant impact on the efficiency of recombinant protein production. The initial growth-phase of the microorganisms yields high cell densities, which make it possible to carry out the synthesis of the target product in the subsequent production phase with sufficient biomass, thereby increasing the volume-related product yield. High cell density cultivations (HCDC), particularly of *E. coli*, are frequently described in the literature.

With regard to the production of recombinant silk proteins, a wide variety of purification strategies has been pursued, depending on the individual properties of the artificial protein variants or the selected expression systems. In particular, the purification of silk proteins from the soluble fraction has been extensively studied and various methods have been demonstrated. However, an intracellular protein synthesis in *E. coli*, coupled with a strong overexpression, usually results in an accumulation of insoluble protein aggregates (ie, inclusion bodies). These partially highly pure, intracellularly deposited protein aggregates can, for instance, be purified further using nonionic detergents.

Based on prior experiences concerning the processing of silkworm proteins, similar procedures have been developed for recombinant silk proteins, especially spider silk proteins. Studies on the behavior of film formation and silk coatings were also published. A chronological overview and future perspectives for the very versatile use of silks in biomedical applications are presented by the group of Holland.

In this work, a process for the recombinant production of modified lacewing silk protein N[AS]C is reported, which includes HCDC and purification as well as further processing into materials and their respective characterization. In particular, the unique behavior regarding the solubility of the modified lacewing silk protein is discussed.

## 2 MATERIALS AND METHODS

### 2.1 Expression system

The expression system for lacewing silk protein N[AS]C is based on a pET29a vector, which was provided by AMSilk GmbH. The N[AS]C construct used is described by Bauer and Scheibel. The bacterial strains used in this study were *E. coli* BL21(DE3) and *E. coli* HMS174(DE3), both obtained from Merck KGaA, Darmstadt, DE.

### 2.2 Silk protein expression in flask cultivation

*E. coli* was grown at 37°C before induction. Expression experiments were conducted in a 250 mL flask containing 20 mL of LB medium in a shaking incubator at 175 rpm. Gene expression and protein production was induced with IPTG (Sigma). Samples were taken prior and after induction for SDS-PAGE analysis (silver staining and western blot).

### 2.3 Production of silk protein by HCDC

The recombinant *E. coli* was cultivated in a 10 L bioreactor (Proreact 5P 10 L; Heinrich Frings GmbH & Co. KG), containing modified minimal medium. Composition of batch medium: 25 g L⁻¹ glucose, 13.3 g L⁻¹ KH₂PO₄, 4 g L⁻¹ K₂HPO₄, 1.7 g L⁻¹ citric acid, 1.2 g L⁻¹ MgSO₄ · 7 H₂O; trace elements: 8.4 ml L⁻¹ EDTA, 2.5 ml L⁻¹ CoSO₄ · 7 H₂O, 15 ml L⁻¹ MnCl₂ · 4 H₂O, 1.5 ml L⁻¹ CuSO₄ · 5 H₂O, 3 ml L⁻¹ H₃BO₃, 2.5 ml L⁻¹ Na₂MoO₄ · 2 H₂O, 13 ml L⁻¹ ZnSO₄ · 7 H₂O, 100 ml L⁻¹ FeSO₄ · 7 H₂O. Composition of feed solution: 500 g L⁻¹ glucose, 10 g L⁻¹ MgSO₄ · 7 H₂O; trace elements: 1.68 ml L⁻¹ EDTA, 0.55 ml L⁻¹ CoSO₄ · 7 H₂O, 3 ml L⁻¹ MnCl₂ · 4 H₂O, 0.3 ml L⁻¹ CuSO₄ · 5 H₂O, 0.6 ml L⁻¹ H₃BO₃, 0.5 ml L⁻¹ Na₂MoO₄ · 2 H₂O, 2.6 ml L⁻¹ ZnSO₄ · 7 H₂O, 20 ml L⁻¹ FeSO₄ · 7 H₂O. Starting parameter of the cultivation: agitation speed: 1000 rpm, pH set point: 6.9, cultivation temperature: 37°C, headspace pressure: 750 mbar, aeration: 10 L min⁻¹. The end of the batch phase was defined by the sudden increase in the partial oxygen content (pO₂) in the
medium, which was used as the starting condition for the subsequent feeding phase. The feed solution was added until the end of the fermentation at a continuous metering rate. The pH was controlled at 6.9 by adding 25% (v/v) ammonia water. One hour prior to induction with IPTG the temperature was decreased to 30°C. The antifoam used was polypropylene glycol P2’000.

2.4 | SDS-PAGE and western blot analysis

Protein samples were boiled at 95°C for 10 minutes and loaded onto 10% SDS-polyacrylamide gels. The protein standards used for silver gels and western blot analysis were Roti-Mark BICOLOR (Carl Roth) and Precision Plus Protein Western C (BioRad), respectively. The silver staining was carried out according to the instructions of Heukeshoven and Dernick.46 For western blot analysis, proteins were transferred from gels to nitrocellulose paper using a Trans-Blot Turbo Transfer System (BioRad). The membrane was blocked with 5% (w/v) nonfat milk in 1× PBS supplemented with 0.3% (v/v) Tween 20 and then incubated with T7-Tag Antibody HRP Conjugate (Merck), diluted 1:5000 for 45 minutes. Immunoreactive bands were detected using the Luminata Forte Western HRP substrate (Merck) and the ChemiDoc MP Imaging System (BioRad).

2.5 | Purification of recombinant silk protein

N[AS]₈C was purified from HCDC of E coli HMS174(DE3) according to Schmidt et al.30 Briefly, the cells were harvested by centrifugation (7000×g, 15 minutes, 20°C) and the pellet was suspended in deionized water (33 g dry mass per liter). The dry mass was determined with a Moisture Analyzer MA 51 (Sartorius AG). Subsequently, cell disruption took place with cooling by means of three passes at 1000 bar through a high-pressure homogenizer (Panda PLUS 2000, GEA Niro Soavi). After centrifugation (3500×g, 15 minutes, 5°C), the cell lysate pellet was resuspended in 2% sodium dodecyl sulfate (SDS) (equal volume) and stirred for 90 minutes at room temperature. After an additional separation of the fractions (3500×g, 15 minutes, 5°C), the pellet was resuspended in an equal volume of deionized water and washed twice (centrifugation during washing steps: 7000×g, 20 minutes, 5°C). The purified protein pellet was lyophilized, weighed, and then stored at 4°C.

2.6 | Film casting with N[AS]₈C

For the production of silk films from lacewing silk protein, a modified procedure was carried out according to Bauer et al.9 For this purpose, the recombinant silk protein N[AS]₈C was resuspended with a concentration of 5% (w/v) in 98% formic acid. Subsequently, the protein solution was treated by means of an ultrasound probe (probe: MS72, ultrasound condition: 30 W, 2 × 30 seconds, pulse: 3 seconds on, 0.5 seconds off; Sonorex Digital 10 P, BANDELIN electronics GmbH & Co. KG). After sonication, glycerol (99.5%) was added in varying amounts (usually 2.5% (v/v)). After mixing the batch, the solution was degassed and transferred to a Teflon-coated fabric tape, spread with a doctor blade (0.7 mm), and then incubated at room temperature for 24 hours. After evaporation of the formic acid, the silk film could be removed from the Teflon matrix.

2.7 | Attenuated total reflection-FTIR

The analysis of the amide I band, with respect to its individual structural components, provides information on the respective secondary structure components.47 The assignment of the vibrational bands in the amide I region was conducted according to Hu.48 For this work, lyophilized protein samples and silk films were analyzed between 500 and 4000 cm⁻¹ and the data were evaluated using the software OriginPro 9.1G (OriginLab Corporation). For the measurements, a Nicolet Nexus 670 FT-IR from Thermo and the software OMNIC 6.1a were used. Measurement parameters: Number of scans: 30; Resolution: 4 cm⁻¹; Reinforcement: 8; Speed: 0.6329; Aperture: 69; Detector: DTGS KBr; Accessories: Smart Performer.
2.8 | **Transmission electron microscopy**

Protein production and the formation of intracellularly insoluble aggregates were visualized by transmission electron microscopy (TEM) images. For this purpose, 500 μL of the fermentation broth was centrifuged at 5000×g for 5 minutes. Subsequently, the supernatant was discarded and the cell pellet washed with 10 mM Tris-HCl pH 7.2 and again centrifuged for 5 minutes at 5000×g. The pellet was fixed with 2.5% glutaraldehyde in fixative buffer (100 mM sodium cacodylate, 2 mM MgCl₂, pH 7.0) for 1 hour at room temperature. Afterward, the pellet samples were rinsed three times with fixative buffer. This was followed by post-fixation consisting of a 1-hour incubation period of the cells in fixative buffer at 22°C. After two washes with ultrapure water, the pellet samples were dehydrated using an acetone series (20%, 40%, 60%, 80%, 100%). For this purpose, the pellet samples were incubated for 10 minutes at each acetone concentration with gentle shaking (50 rpm, 22°C). The cell pellets present in 100% acetone were then embedded in agar low viscosity. The polymerization of the agar low viscosity used for embedding the bacteria was carried out at 60°C for 16 hours. Subsequently, ultrathin sections (60-80 nm) were made and transferred to carbon-coated copper grids. The TEM images were taken with the device CM200 (Philips) with 120 kV.

2.9 | **Scanning electron microscope**

The scanning electron microscope (SEM) images were taken with the device JSM6330F (JEOL Ltd.) with an acceleration voltage of 5 kV. Powder and film samples were either dusted or glued on a conductive substrate. Cross-sections of films were frozen in liquid nitrogen, broken, and adhered. Subsequently, the material samples were sputtered with a platinum layer (about 4 nm) to avoid electrical charging.

3 | **RESULTS AND DISCUSSION**

3.1 | **Expression kinetics of N[AS]₈C**

In order to analyze the synthesis of N[AS]₈C in *E. coli*, expression studies were carried out at different temperatures in order to be able to better assess the solubility behavior of the target protein and to determine an optimal temperature for the synthesis of recombinant N[AS]₈C. The growth behavior of *E. coli* BL21 (DE3) and *E. coli* HMS174 (DE3) was monitored spectroscopically (OD₆₀₀) (Figure 2A) and the respective protein synthesis was examined using Western blot analysis (Figure 2B). Following induction with IPTG and a simultaneous decrease in temperature, both *E. coli* strains showed a temperature-dependent decline in growth. Only *E. coli* BL21 (DE3) exhibited a further increase in growth at an induction temperature of 23°C and 30°C.

Other studies have shown that increased temperature during the induction phase can lead to a metabolic burden as well as different stress reactions. This affects growth, protein synthesis, and influences the formation of inclusion bodies.25,49,50 This cellular response is plausible, considering that after induction approximately 50% of the overall available energy is utilized for protein biosynthesis.51 This effect is depicted in more detail in the expression kinetics of Figure 2B. The induction temperature of 23°C showed a slower but constant increase of soluble silk protein over a longer period of time. This resulted in the detection of the most intense signals 20 hours after induction (*E. coli* HMS174(DE3)). Increasing the temperature showed a respective increase in the silk protein synthesis rate. Thus, the detection of the strongest bands for *E. coli* BL21(DE3) was observed after 3.5 hours and for *E. coli* HMS174 (DE3) after 2 hours at 30°C. Interestingly, for the 20 hours induction sample, a reduction of detected protein was observed in both strains. This effect is more evident in the induction samples cultured at 37°C. The signal reduction observed in *E. coli* BL21 (DE3) at 30°C and 20 hours after induction, resulted in complete signal loss at 37°C (20 hour induction time). The observed signal reduction up to complete signal loss at elevated induction temperature and increased induction time indicates intracellular deposition/aggregation of the synthesized silk proteins. A proteolytic degradation of the artificial N[AS]₈C proteins is unlikely due to the lack of respective degradation bands. This expression pattern, which first increases, then stagnates, and then decreases after longer induction time, was also observed in studies with different substrate-feed rates (fed-batch fermentations) and different inductor concentrations (data not shown).
3.2 HCDC with *E. coli* for the synthesis of N[AS]₈C and the optimization of protein solubilization

In order to provide sufficient amounts of silk protein for material development and to study the expression behavior under a defined and controlled process, high cell density fermentations with *E. coli* were performed (Figure 3A).

Based on the experience from the expression studies, the induction phase was carried out at 30°C with 0.5 mM IPTG. The constant glucose feed rate of 8.3 g L⁻¹ h⁻¹ enabled a linear growth behavior (μ = 0.06 h⁻¹). The temperature for the induction phase was lowered to 30°C 1 hour before the addition of IPTG. At the end of the fermentation, an OD₆₀₀ of 132 and a cell dry mass of 53 g L⁻¹ was achieved. The synthesis of the silk protein N[AS]₈C was verified by expression kinetics using Western-Blots. The standard analysis (solvent system: 8 M urea, 50 mM H₃PO₄) was compared with a stronger denaturing solvent system (10 M ZnCl₂, 5% β-mercaptoethanol) to dissolve the highly insoluble intracellular protein aggregates more efficiently (high molar guanidinium thiocyanate, guanidinium hydrochloride, lithium chloride/-bromide each showed insufficient solubilization). With respect to the western blot analysis with the urea/H₃PO₄ solvent system, a steady increase in signal intensity could only be detected up to 5 hours after induction (Figure 3B). The samples at 23 hours and 24 hours after induction showed again a decrease in band intensity. The solvent system (10 M ZnCl₂, 5% β-mercaptoethanol) showed a significant signal increase in the recombinant lacewing protein for the long-term induced samples (Figure 3C). Similar behavior could be observed within the expression kinetics of a high cell density fermentation with *E. coli* HMS174 (DE3) (Figure 3D and E) (data not shown for fermentation). The strongest detected signals were observed for the solvent system urea/H₃PO₄ after 4 hour induction and for the zinc chloride/β-mercaptoethanol system at the end of the fermentation (22-28 hours). This indicates the formation of insoluble aggregates during expression that cannot be dissolved under standard denaturing conditions. The formation of insoluble intracellular protein aggregates could also be detected by transmission electron microscopy (Figure 3F).

The possible N[AS]₈C synthesis in *E. coli* is illustrated by means of a simplified scheme in Figure 4.
FIGURE 3  Expression kinetics of N[AS]₈C during high cell density cultivations (HCDC) of *E coli* BL21(DE3) and *E coli* HMS174(DE3) using a more optimal solvent system. (A) HCDC with *E coli* BL21(DE3)-pET29a-N[AS]₈C. Induction with IPTG took place after 16.7 hours of cultivation. The achieved OD₆₀₀ at the end of the process was 132. (B, C) Expression kinetics with *E coli* BL21(DE3), (B) Western blot with solvent system 8 M urea, 50 mM H₃PO₄, (C) Western blot with solvent system 10 M ZnCl₂, 5% β-mercaptoethanol. (D, E) Expression kinetics with *E coli* HMS174(DE3), (D) Western blot with solvent system 8 M urea, 50 mM H₃PO₄, (E) Western blot with solvent system 10 M ZnCl₂, 5% β-mercaptoethanol. 0 hour = before induction. The data 1, 2, … , 24 hours refer to the time (hours) after induction with IPTG. Expression kinetics were monitored with whole cells. TEM images of induced *E coli* BL21(DE3) cells before and after induction with IPTG. (F) Before induction, (G) induction with 0.5 mM IPTG, harvest sample. Arrows mark intracellular agglomerates. Scale bar in (F) represents 300 nm. Scale bar in (G) represents 500 nm.

FIGURE 4  Model presentation of the N[AS]₈C synthesis in *E coli*. T = temperature, IPTG = isopropyl-β-D-thiogalactopyranoside, Vₚₛ = protein synthesis rate.
The N[AS]_8C synthesis initiated after induction with IPTG initially results in soluble silk protein (temperature and inductor concentration dependent protein synthesis rate [V_Ps]). As soon as the maximum intracellular solubility limit is reached (temperature-dependent), the silk protein aggregates and deposits intracellularly. The nucleation induced by aggregation results in a decrease in the intracellularly dissolved N[AS]_8C in the further induction process. Similar nucleation processes and aggregation kinetics could be shown for amyloid cross-β structures. 52

### 3.3 Purification of the insoluble lacewing silk protein N[AS]_8C

Within this work, a purification procedure for the isolation of intracellularly aggregated N[AS]_8C protein was established. For this purpose, a harvest sample from a HCDC was used. The distribution of the lacewing silk protein N[AS]_8C during the purification process can be traced using western blot (Figure 5A).

The majority of the silk protein can be detected within the respective pellet fractions. After homogenization and treatment with 2% SDS, a weak but specific N[AS]_8C signal could additionally be detected in the gel pockets of the supernatants (Figure 5A, arrow with *). The treatment of the pellet fraction with Triton X-100 instead of SDS showed an insufficient purification effect (data not shown). The purification efficiency is demonstrated with the silver-stained SDS-PAGE-gels (Figure 5B). The separation or solubilization of the host cell proteins from the insoluble silk protein fraction via cell disruption, SDS treatment, and washing steps shows an N[AS]_8C band with only minor protein-based impurities. This process enabled the production of up to 5 g silk protein per liter of fermentation broth.

### 3.4 Production and characterization of N[AS]_8C films

The film forming properties of the recombinant lacewing silk protein N[AS]_8C were investigated on the basis of the methods described in the literature for the production of silk-based films. 53 Purified N[AS]_8C was dissolved in 98% formic acid in a concentration of 5% (w/v). Additionally, glycerol was added as plasticizer. According to the initial sample weight, the silk film obtained after evaporation of formic acid consisted of 61% N[AS]_8C and 39% glycerol (calculated data). Figure 6A shows a transparent silk film clamped in a frame. As seen from the wrinkling in the film, the silk film obtained had elastic properties. The glycerol-plasticized films were not brittle and stable to gentle manipulation. A compact, closed-film surface could be shown by SEM images (Figure 6B). No porosity was observed within the film structure. The film cross-section also showed a solid structure and no porosity could be observed. Based on the SEM images, a layer thickness of approximately 9-10 μm was determined.

**FIGURE 5** Purification process of the lacewing silk protein N[AS]_8C. The purification process is illustrated by Western blot analysis (A) and the silver-stained polyacrylamide gel (B). (1) Supernatant before HPH, (2) pellet before HPH, (3) supernatant after 3 × HPH, (4) pellet after 3 × HPH, (5) supernatant after 2% SDS, (6) pellet after 2% SDS, (7) supernatant after 2 × washing step with deionized water, and (8) Pellet after 2 × washing step with deionized water. The loading per lane was always carried out with a comparable sample volume. Arrow marks the silk protein N[AS]_8C. Arrow with (*) marks the silk protein present in the gel pockets (N[AS]_8C-aggregates). HPH = high pressure homogenization.
However, the use of glycerol as plasticizer for film preparation counteracts the flexural rigidity of the silk protein-based material so that bending stiffness is no longer to be expected. The preparation of a flexurally stiff film, which is comparable to the natural model in its mechanical properties, is unlikely by using only one type of lacewing silk protein. This point is discussed in more detail at the end of this chapter using the egg stalk fiber as an example. Nevertheless, the stability of the silk films containing glycerol seems to be sufficient for tensile tests. However, further investigations have to be carried out to obtain detailed information about the mechanical properties.

The spectroscopic analysis of the processed silk film using attenuated total reflection (ATR)-FTIR (Figure 6C) showed a comparable structure pattern to the processed silk protein powder (Figure 6D). As demonstrated in the purified silk protein powder, a significant \( \beta \)-sheet signal between 1697 and 1703 cm\(^{-1} \) could be observed. The significant absorption of the purified silk material as well as the processed silk film within the \( \beta \)-sheet regions (1703-1697 cm\(^{-1} \) and 1637-1605 cm\(^{-1} \))\(^{48,54} \) verify the crystalline structures occurring in N[AS]\(_6\)C. The wavenumber range around 1610, 1619, and 1629 cm\(^{-1} \) is associated with strong intermolecular hydrogen bonds between \( \beta \) strands.\(^{55} \) While the wavenumber range of 1637-1630 cm\(^{-1} \) is assigned to rather parallel \( \beta \)-sheets,\(^ {56} \) the range of 1630-1605 cm\(^{-1} \) could be assigned to antiparallel \( \beta \)-sheets by analysis of amyloid fibrils (cross-\( \beta \)-structure).\(^ {55,56} \) In contrast to parallel \( \beta \)-sheet orientations, a characteristic absorption in the higher wavenumber range (1703-1697 cm\(^{-1} \)) can additionally be detected in antiparallel \( \beta \)-sheet structures.\(^ {55-58} \) X-ray diffraction data showed the films to be partially crystalline with d-spacing values for antiparallel \( \beta \)-sheets (Figure S1 in Supporting Information).

The material could potentially be used in various applications in the medical sector as well as in the textile industry, where lateral stiffness is required. Lintz and Scheibel have summarized an overview of three selected protein fibers with outstanding properties (spider dragline, lacewing egg stalk, and mussel byssus) and their potential for material development.\(^ {59} \) As shown by Bauer and Scheibel, no significant adhesion of fibroblasts and myoblasts to films could be observed with N[AS]\(_6\)C.\(^ {9} \) N[AS]\(_6\)C could also be a candidate for the coating of implants, as already described for spider silk proteins.\(^ {44} \) However, further studies are required to show the extent to which the lacewing silk protein is biocompatible or triggers immunological reactions.

Furthermore, in form of a rigid fiber, the silk could be used, for example, as a reinforcing fiber in composite materials. However, and in order to obtain comparable fiber qualities as found in natural egg stalks, the second silk protein variant MalXB1 should also be produced recombinantly and used for future experiments. Both proteins (MalXB1 and MalXB2)
differ in their core domain by the amino acids present in the $\beta$-turns. While MalXB2 mainly contains lysine and thus presents positively charged side chains, the core domain of MalXB1 shows negatively charged side chains (aspartic acid).\textsuperscript{1} MalXB1 and MalXB2 also contain asparagine and serine in the $\beta$-turns. The interaction of both proteins could take place for example between asparagine and serine via hydrogen bonds, between lysine and glutamic acid (or aspartic acid) via ionic bonds and through cysteines via disulfide bridges.\textsuperscript{60} The interaction of both silk proteins in the natural egg stalk can thus be understood as a kind of composite material.

4 | CONCLUSION

In order to implement the production of novel biomaterials from green lacewing silk, processes are sought that enable a sufficient and cost-effective supply of lacewing silk protein. In this work, it could be shown that recombinant lacewing silk proteins can be produced by high cell density fermentation of \textit{E. coli}. The recombinant proteins can be purified efficiently and in remarkably high yields. Expression studies have shown that a highly insoluble intracellular silk protein fraction can only be visualized by SDS-PAGE under suitable fermentation conditions and/or a suitable denaturing solvent system. The lacewing silk proteins can be processed into films, in which they possess a structurally similar conformation to their native proteins (related to the protein secondary structure). The achieved N[AS]$_6$C yields allow a future large-scale production of recombinant lacewing silk protein. Especially for egg stalk silk proteins of lacewings, the structurally mediated bending stiffness provides an interesting starting point for the development of biocompatible scaffold materials with outstanding mechanical properties in future.

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CONFLICT OF INTEREST

Authors have no conflict of interest relevant to this article.

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