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Sericins exhibit ROS-scavenging, anti-tyrosinase, anti-elastase, and *in vitro* immunomodulatory activities

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ABSTRACT

Some biological properties of *Bombyx mori* sericins from twenty strains were investigated, fourteen fed with artificial diet, two with fresh mulberry leaves and four with both diets. Sericin exhibited ROS-scavenging, anti-tyrosinase and anti-elastase properties, the strain significantly influenced these properties, while diet only influenced the anti-tyrosinase activity. Sericins were clustered into 5 groups and one sericin from each group was further studied: sericins showed anti-proliferative activity on *in vitro* stimulated peripheral blood mononuclear cells; some strains decreased *in vitro* secretion of IFNγ, while no effects were observed on TNFα and IL10 release.

Therefore, a mixture of sericins extracted from the most promising strains may be useful for dermatological and cosmetic use.

1. Introduction

Sericins (collectively referred to as "sericin") are a family of water soluble proteins produced by the *Bombyx mori* silkworm. They bind fibroin fibers in order to create silk thread, which protects the worm during metamorphosis. In the textile industry *B. mori* silk has been used for more than a century. Silk proteins are also used for biomedical and cosmetic purposes; notwithstanding, some authors have reported that raw silk may elicit an immunogenic response, due to the presence of sericin [1]. However, recent studies have shown that sericin does not induce an immune response [2]. Therfore, new sericin applications have been developed, in particular in the pharmaceutical, biomedical, cosmetic and food industries [3]. The higher molecular weight sericin is used in the biomedical field [4], for enzyme immobilization [5], in material coating and photo-protective treatment [4,6], in wound dressings [7–10], as an anticoagulant agent [11], in corneal abrasion treatment [12], to promote cell proliferation [13], and as a component of serum-free cell culture media [14–17]. The lower molecular weight sericin (≤20 kDa) is generally used for the production of cosmetics for skin, nails and hair, for its well-known anti-oxidant and anti-tyrosinase properties [18,19].

Nowadays, a large number of *B. mori* strains have been identified. The CRA – Unit of Research of Apiculture and Sericulture, Padua, Italy (CRA-API) – is one of the most important silkworm germplasm banks in Europe, comprising about 200 *B. mori* strains [20,21]. The strains exhibit different properties such as cocoon shape, color, volume, total weight and amount of silk produced. These different characteristics reflect different chemical compositions that could influence the biological activities of silk sericin: in particular, the color of cocoon reflects the presence of carotenoids and flavonoids, compounds commonly known for their biological properties, such as anti-oxidant and anti-tyrosinase activities [22]; the total flavonoid contents depend on different strains [23]. Moreover, silkworms may be fed either an artificial diet or fresh mulberry leaves which influences some biological features, for example urea concentration in the haemolymph [24]. Discrepancies in feeding management could also explain variable reports on sericin properties.

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In this study different biological properties of sericin were investigated based on the *B. mori* strain and diet. The aim was to provide support for the employment of sericin in the dermatologic and cosmetic fields. For this purpose, the ROS-scavenging, anti-tyrosinase and anti-elastase activities of sericin from 20 different *B. mori* strains, fed with an artificial diet or fresh leaves, were evaluated. Furthermore, the correlation between activity was analyzed and finally, considering the differences in biological properties, the strains were splitted into five groups. One sericin per group was investigated *in vitro* for its anti-proliferative activity on human peripheral blood mononuclear cells (PBMCs) and for its effect on the *in vitro* TNFα, IFNγ and IL10. Correlations between the different cytokine release patterns were evaluated.

2. Materials and methods

2.1. Extraction of sericin

Twenty different strains of *B. mori*, fed with an artificial diet ($n = 14$) or fresh mulberry leaves (FL) ($n = 2$) or both ($n = 4$) as previously described, were supplied by CRA-API [20,21]. The artificial diet was composed by 25 g dried mulberry leaf powder, 36 g defatted soybean meal, 15 g wheat meal, 4 g corn starch, 5 g soybean fiber, 4 g citric acid, 2 g ascorbic acid, 3 g salt mixture, 4.2 g agar, 399 mg vitamin mixture, 200 mg sorbic acid, 691 mg propionic acid, 10 mg chloramphenicol and 500 mg β-sitosterol; the quantities referred to 100 g of dry weight. The powder was hydrated prior to administration (1 g dry powder: 2.6 g water) [20]. The following strains were used [25]: Nistari modificato, Nistari, Verde ovale, AP, Orgosolo, Daizo, Oro 208, Oro gigante, ADPR, Arancio, Rosa, 201A, R3G, Han-Han, Romagna, Sejaku green BG, Treotto rosa, SA48LB, PK12, G133 (Fig. 1). Six of them (ADPR, Nistari, Oro 208, PK12, Verde ovale and Treotto rosa) were fed with FL. All cocoons were degummed in an autoclave (Systec V-65, Wettenberg, Germany) at 120 °C for 1 h (40 mL water/g per cocoon), in order to obtain sericin solution concentrations dependent upon the original protein content. Solutions were dried to a powder using a Büchi Mini Spray Dryer (Flawil, Switzerland), with below process parameters: pump, 6 ml/min; inlet temperature, 120 °C; outlet temperature, 80 °C; air pressure, 3 bar; fluid flow, 500–600 ml/h.

2.2. Characterization of sericin powder

Granulometric analysis of sericin powder was performed with a laser light scattering granulometer (Beckman Coulter LS230, Miami, Florida) equipped with a small cell volume (120 ml volume, obscuration 5%); the refractive index was set at 1.359 for ethanol. Powder suspended in ethanol was sonicated for 4 min, transferred to the measurement cell and run in 5 replicates of 90 s each.

Sericin powder morphology was evaluated by scanning electron microscopy (JEOL JSM-6380LV, Tokyo, Japan).

Sericin molecular conformation was investigated with a Bruker Alpha-E spectrometer (Billerica, Massachusetts, USA) to obtain the Fourier Transform Infrared Spectroscopy (FTIR).

2.3. ROS-scavenging activity

To evaluate ROS-scavenging activity of silk sericin, the DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) method was used, according to Fan et al. [18]. In detail, silk sericin was tested at different concentrations (1.6, 0.8, 0.4, 0.2 mg/mL) by dissolving powder in water.

Three hundred microliters of aqueous sericin solution were mixed with 2700 microliters of methanolic solution containing DPPH (Sigma–Aldrich) 0.0028% w/v. Samples were incubated in the dark for 1 h at 25 °C, centrifuged for 8 min, and absorbance was measured at 515 nm wavelength with a UV–vis spectrophotometer Uvikin 930 (Kontron Instruments, Everett, Massachusetts, USA). Reaction mixture alone was used as a negative control, while ascorbic acid was used as a positive control at the same concentrations as sericin samples. ROS-scavenging activity was calculated with the following formula: % activity = $(A - B)/A \times 100$, where A is the absorbance of the negative control and B is the absorbance of the test solution. Analyses were performed in three replicates, and results are reported as the mean ± standard deviation of the activity percentage.

2.4. Anti-tyrosinase activity assay

The anti-tyrosinase assay was performed according to Aramwit et al. [22], with some modifications. Silk sericin powder was tested at different concentrations, dissolving microparticles in an aqueous reaction solution (final concentrations 6.4, 3.2, 1.6 mg/mL). Mushroom tyrosinase (3933 units/mg; Sigma Aldrich) was solubilized in phosphate buffer (pH 6.8) in order to obtain 100 units/mL. One hundred and twenty-five microliters of tyrosinase solution were pre-incubated for 10 min with sericin samples, then 250 microliters L-tyrosine 2 mM (Fluka) were added. The final mixture volume was 2500 μL and reaction kinetics were evaluated at 480 nm wavelength with a UV–vis spectrophotometer Uvikin 930 (Kontron Instruments). The assay was carried out in triplicate. Reaction mixture without samples was used as a negative control, while arbutin (Sigma–Aldrich) was used as a positive control at the same concentrations as the sericin samples. Results are reported as the

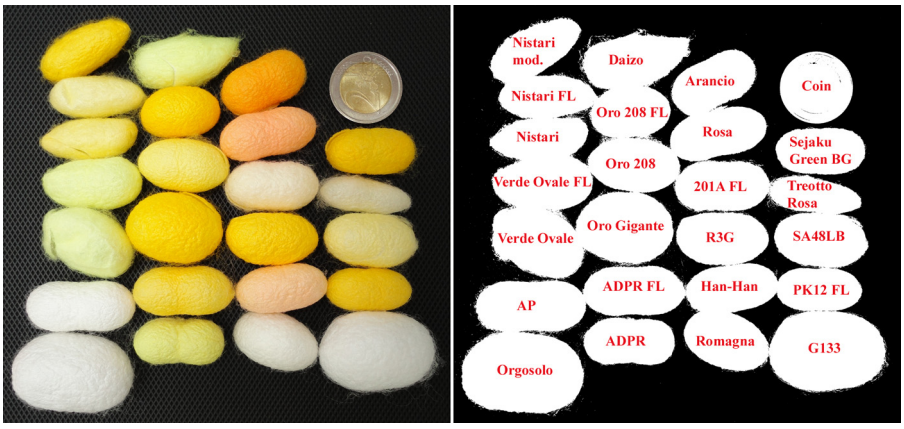

Fig. 1. Image of the twenty-four cocoon types employed in the study. Twenty strains are represented, fed with artificial and/or mulberry leaf diet (FL).