Supplementary Table 1. Recombinant production of spider silk proteins in different organisms. MS: moleculare mass, RP: recombinant protein, kDa: kilodalton, YM: Young’s modulus, MPa: megapascal, GPa: gigapascal, TSP: total soluble protein, mg: milligram, L: litre, ER: endoplasmic reticulum, TD: terminal domain, Ni-NTA: nickel nitrilotriacetic acid, HisPrep FF: histidine prepacked fast flow, HiPrep SP: histidine prepacked small protein, SSP: sporamin signal peptide, SP: sporamin propeptide, FPLS: fast protein liquid chromatography, His-tag: histidine tag, tCUP: tobacco cryptic constitutive promoter, USP-FIC: unknown seed protein promoter-flag intein c-myc, ITC: heat denaturing and inverse transition cycling, ELP: elastin-like polypeptide, LeB4: seed specific legumin B4 protein, PR1b: pathogenesis-related 1b protein, N.D.: no data.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Organism** | **Spider species** | **Protein** | **Method** | **Target compartment- Signal peptide** | **MS of RP (kDa)** | **Purification method** | **Yield** | **Mechanical properties** | **Reference** |
| **Bacteria** | | | | | | | | | |
| *E. coli* BL21(DE3) | *N. clavipes* | MaSpI |  |  | 284.9 | Acidic precipitation followed by a fractional ammonium sulfate precipitation. | 1.2 g/L | Tenacity:  508 ± 108 MPa  Elongation:15 ± 5%  YM:21 ± 4 GPa | Xia et al., 2010 |
| *E. coli*  NEB 10-beta (NEB10β) | *N. clavipes* | MaSpI | Split inteins-based multimerization |  | 556 | Acidic precipitation followed by a fractional ammonium sulfate precipitation. | 2 g/L or 63 mg/g cell dry weight. | Tensile strength:  525-1031 MPa  YM:7.8-13.7 GPa | Bowen et al., 2018 |
| *E. coli* BL21(DE3) | *L. hesperus* TD,  *C. moluccensis* core domain | MaSpIs |  |  | 42 | Ni-NTA sepharose column | 300-400 mg/L of induced culture medium | Strength:282 ± 66 MPa  Toughness:  144 ± 44 MJ/m3  YM:1.5 ± 0.3 GPa | Thamm and Scheibel, 2017 |
| *R. sulfidophilum* |  | MaSp1 |  |  | ~31 | HisPrep™ FF 16/ 10 20 mL column | 3-10 mg/L | N.D | Foong et al., 2020 |
| *E. coli* | *L. hesperus* | Mixture of different spidroins |  |  | 11.5-81.4 | Ni-NTA beads | 50-100 mg/L | Both libraries had higher elastic moduli rather than natural silk proteins | Jaleel et al., 2020 |
| *E. coli* | *A. ventricosus*  *A. trifasciata* | Flag-AcSp1 |  |  | 36.8 | Ni-NTA columns | 50 mg/L | Toughness:~33.1 MJ/m3  Tensile strength:  ~261.4 MPa | Tian et al., 2020 |
| **Yeast** | | | | | | | | | |
| *P. pastoris* GS115 (*his4*) | *N. clavipes* | MaSp1 |  |  | 65 |  | 663 mg/L | N.D. | Fahnestock and Bedzyk, 1997 |
| *P. pastoris* | *N. madagascariensis* | 2E12 |  |  | 113 | HiPrep SP column in the FPLS system. |  | N.D. | Bogush et al., 2011 |
| **Organism** | **Spider species** | **Protein** | **Method** | **Target compartment- Signal peptide** | **MS of RP (kDa)** | **Purification method** | **Yield** | **Mechanical properties** | **Reference** |
| *S. cerevisiae* | *N. clavipes* | MaSp1 (1F9) |  |  | 94 |  | 450 mg/L | Tnsile strength:  0.1-0.15 GPa  Elasticity:5-15 % | Bogush et al., 2009; Sidoruk et al., 2015 |
| **Insect** | | | | | | | | | |
| Cell line Sf9, derived from  *S. frugiperda* | *A. diadematus* | ADF-3  ADF-4 |  | Cytosol | 60 |  | 50 mg/L of insect cell culture | Diameters:200 nm-1µm  Lengths:up to 100 µm | Huemmerich et al., 2004 |
| *B. mori* silkworm | *N. clavipes* | MaSp1 |  |  | 67 |  | 35.2% of composite proteins | Breaking stress:  371.5 ± 27.5 MPa  Breaking energy:  84.8 ± 14.4 MJ/m3  YM:8.9 ± 1.3 GPa | Xu et al., 2018 |
| *B. mori* silkworm | *N. clavipes* | MaSp1 |  |  | 120-300 |  | N.D. |  | Zhang et al., 2019 |
| **Mamalian cells** | | | | | | | | | |
| Bovine mammary cells and baby hamster kidney (BHK) cells | *N. clavipes*  and *A. diadematus* | MaSpI, MaSpI (2)  MaSpII, ADF-3  ADF-3- His,  ADF-33, ADF-333 |  |  | 59,106,59, 60, 63, 110,  140 | His-tag was used for purification | 25 to 50 mg/L | Toughness (gpd):  0.645-0.895  Modulus (gpd):  42.8-110.6  Tenacity (gpd):1.8-2.26 | Lazaris et al., 2002 |
| **Transgenic animals** | | | | | | | | | |
| Goat |  | MaSp1 and MaSp2 |  |  | 65 |  | N.D. |  | Karatzas et al., 2007; Copeland et al., 2015 |
| Goat |  | MaSp1 |  |  | ~70 |  | N.D. | 21-73 MPa | Decker, 2018 |
| Mouse |  | MaSp1 and MaSp2 |  |  | 40 |  | 11.7 mg/L |  | Xu et al., 2007 |
| Sheep embryo |  |  |  |  |  |  | Offspring was not produced |  | Li et al., 2020 |
| **Plant** | | | | | | | | | |
| Tobacco | *N. clavipes* | MaSpI  MaSp2 |  | ER-PR1b and KDEL | 60.3  58.5 |  | 0.025% TSP in the 35S plants, <0.005% TSP in the tCUP plants. | N.D. | Menassa et al., 2004 |
| *Arabidopsis* leaves | *N. clavipes* | MaSpI  (DPIB) |  | Apoplast, ER, vacuole /  Combinations of SSP, SP, and KDEL | 64 |  | 8.5 % TSP  6.7 % TSP  None | N.D. | Yang et al., 2005 |
| **Organism** | **Spider species** | **Protein** | **Method** | **Target compartment- Signal peptide** | **MS of RP (kDa)** | **Purification method** | **Yield** | **Mechanical properties** | **Reference** |
| *Arabidopsis* seeds | *N. clavipes* | MaSpI  (DPIB) |  | Apoplast, ER, vacuole/  Combinations of SSP, SP, and KDEL | 64 |  | None  18 % TSP  8.2 % TSP | N.D. | Yang et al., 2005 |
| *Arabidopsis* leaves  *Arabidopsis* seeds  Soybean somatic embryos | *N. clavipes* | MaSpI (DPIB)  MaSpI (DPIB)  MaSpI (DPIB) |  | Without protein-targeting approach | 64, 127    64, 127    64, 127 |  | 0.34% and 0.03%  1.2% and 0.78%  1% and None  (%TSP) | N.D. | Barr et al., 2004 |
| Tobacco and potato leaves | *N. clavipes* | SO1  (SO1-100ELP) |  | ER | 94.2 | Heating, acidification and salt fractiation | 80 mg /kg of tobacco leaves | N.D. | Scheller et al., 2004 |
| Tobacco leaves | *N. clavipes* | FLAG | Intein-based multimerization | ER /  LeB4and KDEL | > 250 | Based on Ni-NTA agarose | 1.8 mg/50 g leaf material. | Length:500 µm  Diameters:1-2 m | Hauptmann et al., 2013 |
| Tobacco leaves | *N. clavipes* | MaSp1 | Transglutaminat-ion | ER | > 250 | ITC | Up to 400 mg from 6 kg of tobacco leaves | YM E:3.29±0.03 GPa | Weichert et al., 2014; Heppner et al., 2016 |
| Tobacco seed | *N. clavipes* | FLAG | Intein-based multimerization | ER /  LeB4 and KDEL | > 460 | Semi-quantitative analysis | 20-190 mg/kg for USP-FIC lines |  | Weichert et al., 2016 |
| Alfalfa  (*M. sativa*) | *N. clavipes* | MaSp2 |  |  | 80 |  | N.D. |  | Hugie, 2019 |
| Rice  (*Oryza sativa*) | *A. ventricosus* | AvMaSp |  |  | 22 |  | N.D. |  | Park et al., 2019 |

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