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 precipitationfollowed by a fractional ammonium sulfate precipitation. | 1.2 g/L | Tenacity:508 ± 108 MPaElongation: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 precipitationfollowed by a fractional ammonium sulfate precipitation. | 2 g/L or 63 mg/g cell dry weight. | Tensile strength:525-1031 MPaYM: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 MPaToughness:144 ± 44 MJ/m3YM: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/m3Tensile 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 GPaElasticity: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-1µmLengths: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 MPaBreaking energy:84.8 ± 14.4 MJ/m3YM: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* | MaSpIMaSp2 |  | ER-PR1b and KDEL | 60.358.5 |  | 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 % TSP6.7 % TSPNone | 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  |  | None18 % TSP8.2 % TSP | N.D. | Yang et al., 2005 |
| *Arabidopsis* leaves*Arabidopsis* seedsSoybean 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(SO1-100$×$ELP) |  | 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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