A Marine Photosynthetic Microbial Cell Factory as A Platform for Spider Silk Production

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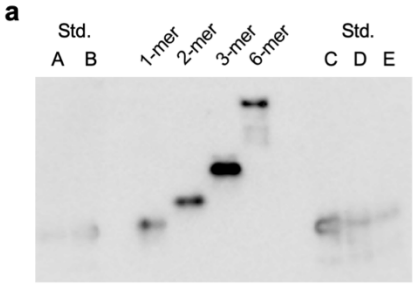
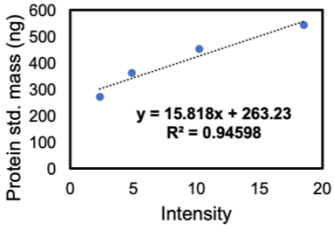
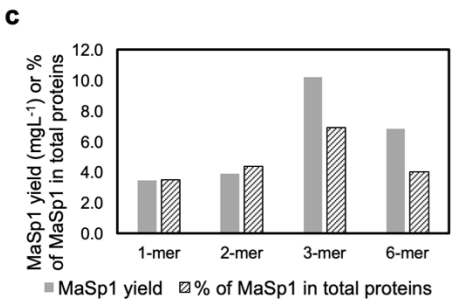
Supplementary Figures



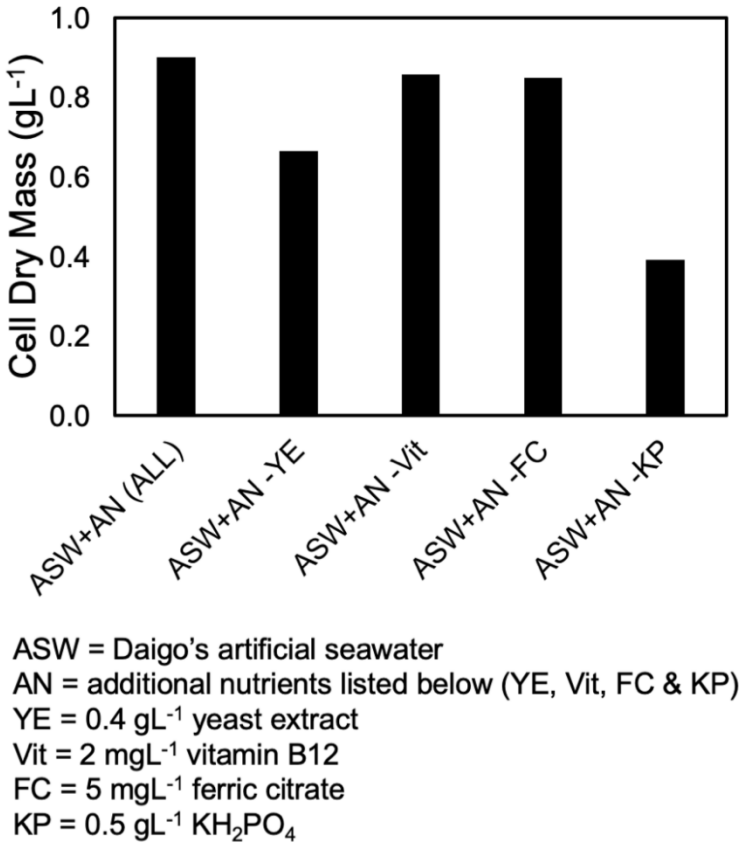
b

Std.	Std. vol. (µL)	Intensity after BG. subtraction	Std. amount (ng)
A	3	2.4	271
Average B & E	4	4.9	361
D	5	10.3	451
C	6	18.6	541

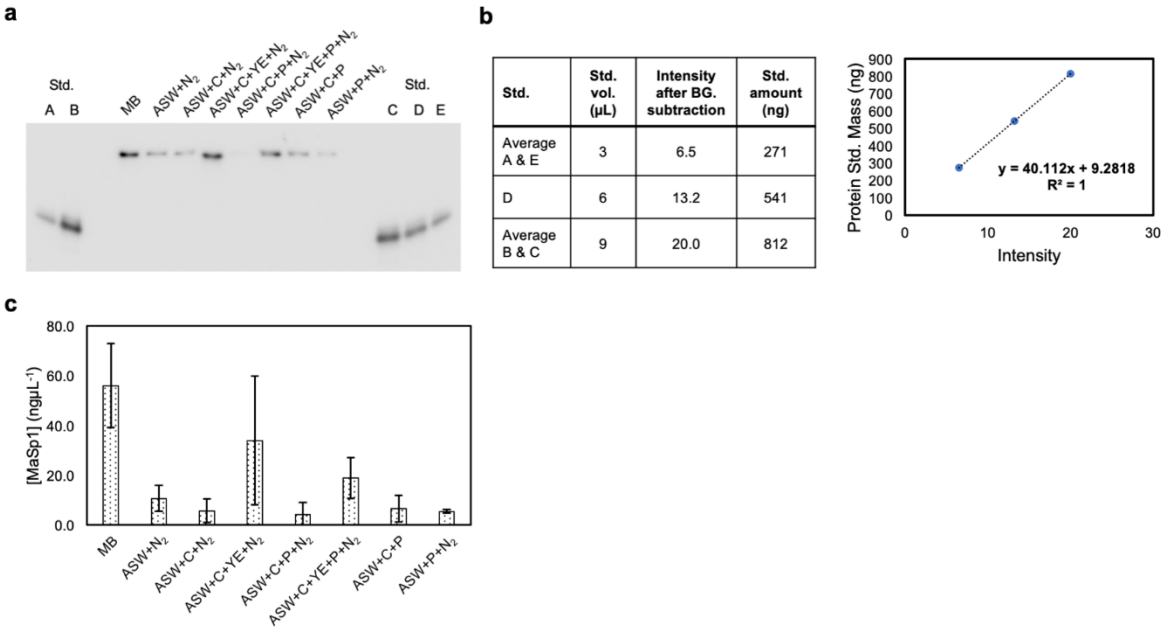




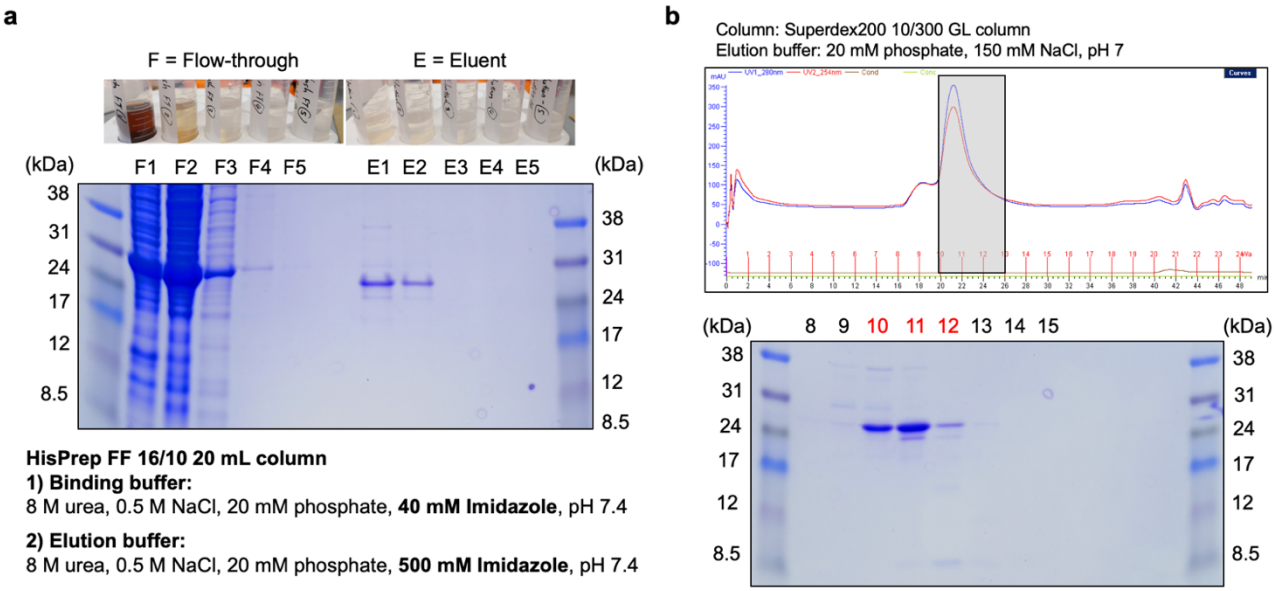
Supplementary Fig. 1: Semiquantitative measurement of MaSp1-(1-mer, 2-mer, 3-mer and 6-mer) expression in recombinant *R. sulfidophilum* under photoheterotrophic conditions (as in Fig. 1d). Recombinant *R. sulfidophilum* cells harboring pBBR1-P_{trc}-MaSp1-(1-mer, 2-mer, 3-mer and 6-mer) were cultivated in 50 mL centrifuge tubes containing 50 mL of marine broth with 100 mg L^{-1} kanamycin at 30°C with continuous far-red LED light (730 nm, 20 to 30 W m^{-2}) for 4 days. **a,** Western blot using a monoclonal anti-His•Tag antibody, which targeted histidine-tagged MaSp1-(1-mer, 2-mer, 3-mer or 6-mer) proteins. **b**, Purified MaSp1-(1-mer) with a known concentration was used as a standard protein to generate a calibration curve. **c**, MaSp1 protein yield and percentage of MaSp1 in total proteins were quantified using Fiji/ImageJ version 1.52p and calculated based on a calibration curve. (Abbreviation: std. = standard; vol.= volume; BG. = background).



Supplementary Fig. 2: Evaluation of nutrients that could affect the growth of recombinant *R. sulfidophilum* in artificial seawater under photoautotrophic conditions. Recombinant *R. sulfidophilum* harboring pBBR1-P_{trc}-MaSp1-(1-mer) was cultivated using 20 mL of Daigo's artificial seawater (ASW) SP for marine microalgae medium in a 20 mL glass vial with a rubber stopper cap at 30°C with continuous far-red LED light (730 nm, 20 to 30 W m^{-2}) for 7 days. Inorganic carbon was supplied as 1 g L^{-1} sodium bicarbonate, while nitrogen was supplied via daily nitrogen gas bubbling at 0.5 L d^{-1}. ASW contained 100 mg L^{-1} kanamycin.



Supplementary Fig. 3: Semiquantitative measurement of MaSp1-(6-mer) expression in recombinant *R. sulfidophilum* under photoheterotrophic and photoautotrophic growth conditions (as in Fig. 2c-d). Recombinant *R. sulfidophilum* harboring pBBR1-P_{trc}-MaSp1-(6-mer) was cultivated using 20 mL of Daigo's artificial seawater (ASW) SP for marine microalgae medium in a 20 mL glass vial with a rubber stopper cap at 30°C with continuous far-red LED light (730 nm, 20 to 30 W m^{-2}) for 7 days. Inorganic carbon was supplied as 1 g L^{-1} sodium bicarbonate, while nitrogen was supplied via daily nitrogen gas bubbling at 0.5 L d^{-1}. Marine broth (MB) and ASW contained 100 mg L^{-1} kanamycin. **a**, Western blot using a monoclonal anti-His•Tag antibody, which targeted the histidine-tagged MaSp1 protein. **b**, Purified MaSp1-(1-mer) with a known concentration was used as a standard protein to generate a calibration curve plot. **c**, MaSp1-(6-mer) concentrations (mean values ± SD, n = 3 independent biological replicates) were quantified using Fiji/ImageJ version 1.52p and calculated based on a calibration curve. (Abbreviation: std. = standard; vol.= volume; BG. = background; C = $NaHCO_3$, YE = 0.4 g L^{-1} yeast extract, N_2 = nitrogen gas and P = 0.5 g L^{-1} KH_2PO_4).



Supplementary Fig. 4: Purification of MaSp1-(6-mer) spidroin. **a**, His-Tag affinity chromatography. Soluble proteins were injected into a HisPrep™ FF 16/10 20 mL column, washed with binding buffer and eluted with elution buffer. E1 and E2 were combined and further concentrated using an Amicon Ultra-15 6 MWCO 3000 Da centrifugal filter. **b**, Gel-filtration chromatography. The concentrated eluent was further purified with an ÄKTAexplorer equipped with a Superdex®200 10/300 GL column. Fractions from tube numbers 10 to 12, which contained the appropriate size of MaSp1-(6-mer) protein, were combined and concentrated using an Amicon Ultra-15 6 MWCO 3000 Da centrifugal filter before a desalting step.

Supplementary Tables

Supplementary Table 1: List of primers used for construction of the pBBR1-P_{trc}-MaSp1 plasmid

Primer	**Nucleotide sequence (5' to 3')**
Trc-Prom_Fwd-*Xba*I	ATA TCTAGA TGC TTC TGG CGT CAG GCA G
Trc-Prom_Rev-RBS-*EcoR*I	AGC GAATTC **TCT CCT** ATT GTC TCT CTG CAC GTG C
MaSp1_Fwd-*EcoR*I	AGC GAATTC ATG CAC CAT CATCATCATCAT TCT
MaSp1_Rev-*Sal*I	AGT GTCGAC TTA ACT AGT CCC CTG AGA ACC CAG
MaSp1-Rep_Rev-*Sal*I	AGT GTCGAC TGG TGG TGG TGG TGC TGC

Restriction enzyme digestion sites are underlined.

Bold letters represent the ribosome binding site (RBS) sequence '**AGGAGA**'

Supplementary Table 2: List of components and amino acid sequences in the MaSp1 protein constructs

Protein component	**Amino acid sequence**
N-terminus	M**HHHHHH**SSG**LVPRG**SGM**KETAAAKFERQHMDS**PDLGT**DDDK**A
MaSp1-(1-mer)	MAA*SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT*S
MaSp1-(2-mer)	MAA*SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT* *SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT*S
MaSp1-(3-mer)	MAA*SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT* *SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT* *SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT*S
MaSp1-(6-mer)	MAA*SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT* *SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT* *SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT* *SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT* *SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT* *SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT*S

6X His-Tag 'HHHHHH', thrombin cleavage site '**LVPRG**', S-Tag '**KETAAAKFERQHMDS**' and enterokinase cleavage site '**DDDK**'.

Italic letters represent the repetitive amino acid residues of MaSp1