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Supplementary Information for

A Bioinspired Approach to Engineer Seed Microenvironment to Boost Germination and Mitigate Soil Salinity

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Movies S1

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Materials and Methods

MD simulation – The initial model for the $(GAGSGA)_2$ oligomer was constructed by threading the dodecapeptide sequence onto the structure of a poly-(Gly-Ala) β-sheet (Protein Data Bank identification code 2slk) using UCSF Chimera and Modeller. The system was then equilibrated in a transferable intermolecular potential 3P (TIP3P) explicit water box. Simulations were run for 40 ns with a time-step of 2 fs at constant temperature (300 K) and pressure (1 bar) using GROMACS. The force field used was GROMOS53a6. Alpha,alpha-Trehalose structure was provided by the Automated Topology Builder (ATB) and Repository available from the Molecular Dynamics Group at the University of Queensland, QLD, Australia. The stability of the β-sheet assembly was verified from hydrogen-bond dynamics and root mean squared deviation (RMSD) data obtained from the molecular dynamics' trajectory.

Materials fabrication – To investigate biopolymer preservation mechanism, films were fabricated via drop casting and spray drying. Suspensions were made of gram negative PGPRs (*Rhizobium tropici* CIAT 899 Martinez-Romero et al. - ATCC 49672) mixed with the silk fibroin, trehalose or silk fibroin – trehalose (ST) mixtures. NaCl solution was used as a negative control. Alamar Blue metabolic assay and agar streaking were used to evaluate bacteria viability upon resuscitation. Biopolymer solutions at a concentration of 1 dry wt% solution were prepared for consistency throughout the manuscript. Silk fibroin aqueous suspensions were prepared as described in "Materials fabrication of Bombxy silk". Trehalose (TCI America, Portland, OR, USA), polyvinylpyrrolidone (PVP, 30kDa) (Sigma Aldrich, St Louis, MO, USA), methyl cellulose (Sigma Aldrich, St Louis, MO) and NaCl (Sigma Aldrich, St Louis, MO) were all dissolved in H_2O. However, for methyl cellulose the H_2O had to be cooled at 4ºC for mixing to occur. For bacteria handling and culture, 50% tryptic Soy Broth (Becton Dickinson, Franklin Lakes, NJ, USA) was generated by mixing 500ml of H_2O with 7.5g BDTM Tryptic Soy broth (Soybean-Casein Digest Medium) (Becton Dickinson, Franklin Lakes, NJ, USA). The media was autoclaved for 60 min at 121ºC. CIAT 899 was sourced and cultured in a shaker incubator at 200rpm and 30ºC up to an OD600 measure of 0.7. Once bacteria reached an OD600 of 0.7, 11ml of bacteria broth solution was centrifuged at 4300 rpm for 20 min. The bacteria formed a pellet and the supernatant was discarded. Concentrated bacteria suspension was made, 1.1ml of biopolymer was pipetted into the pelleted bacteria strain and uniformly mixed by thoroughly pipetting up and down. This was carried out for all biopolymers used. These were labeled as *live* samples. For the *dead* samples, used as negative control, the bacteria pellet was mixed with 70% (w/v) isopropanol and incubated for 60 min. After the 60 min the bacteria solution was centrifuged as above and supernatant discarded. Concentrated bacteria suspension was made, 1.1ml of biopolymer was pipetted into the pelleted bacteria and uniformly mixed by thoroughly pipetting up and down. This was carried out for all biopolymers used. 5 repeats of 100 μl of *live* and *dead* samples were produced for each time point. Bacteria-biopolymer suspensions were drop cast onto 1" by 1" PDMS slab and left to air-dry. Films were then preserved at room temperature for the required period of time in petri dishes. Samples that required water annealing were placed in a vacuum chamber with H_2O, sealed for 6 hours to anneal, removed and placed in petri dishes.

Live dead assay – To develop calibration curve of alamar Blue assay (Resazurin), the following procedure was followed: (*i*) OD600 = 0.7 bacteria solution was collected and centrifuged. (*ii*) For negative control (*dead* bacteria), centrifuged bacteria were resuspended into 1ml of 70% isopropanol (Sigma Aldrich, St Louis, MO, USA) for 60 min., then centrifuged and resuspended into 1ml of NaCl.(*iii*) The bacteria suspension obtained at point (*i*) was centrifuged and resuspended in NaCl (*live* bacteria). (*iv*) Dead and live bacteria were mixed in increasing relative concentration to form the following ratios: 100% live, 80:20 live:dead, 50:50 live:dead, 20:80 live:dead and 100% dead and the relative OD600 was measured (*v*) 100 μl of the above suspensions were added to 96 well plate and viability was measured. To measure viability an alamar Blue assay was performed following the manufacturer protocol. The excitation wavelength was 570nm and emission wavelength 585nm. Samples were prepared in the dark and kept under wrap of foil paper for 60 minutes before the analysis was performed. Alamar Blue assay microplate reader (Tecan Safire 2, Mannedorf, Switzerland) gain was kept constant for all experiments performed.

Film degradation – Biopolymer films encapsulating GFP-CIAT 899 were placed in contact with 1cm thin phytagel films (artificial soil) and time-dependent degradation was studied with the ChemiDoc MP Imager

(Bio-rad, Hercules, CA) at GFP emission and excitation. Phytagel gel (Sigma Aldrich, St Louis, MO) was made by following protocol from Sigma Aldrich by mixing 2g/l phytagel and 1.5% $CaCl_2$ in water solution. Further, film degradation was studied under fluorescence microscopy and videoed (Movie S1). Phytagel films were placed above air-dried films.

DLS – Dynamic Light Scattering (DLS) measurements were performed on a DynaPro NanoStar Light Scatterer (Wyatt Technology). Samples at 0.1 mg/ml were measured in plastic cuvettes (UVette, Eppendorf). The laser was set at 658 nm. The acquisition time for each data point was 5 seconds, and ten data points were acquired for each sample.

CD – Circular Dichroism (CD) spectra were recorded from 185 to 260nm using a JASCO J-1500 spectrometer, with each spectrum averaged from three consecutive scans. Samples of concentrations higher than 0.1mg/ml were diluted to 0.1mg/ml with the corresponding buffer and measured in a 1 mm path length quartz cuvette (Starna Cells, Inc.).

FTIR – Drop cast films were analyzed using Thermo Fisher FTIR6700 Fourier Transform Infrared Spectrometer through attenuated total reflection (ATR) germanium crystal. For each sample, 64 scans were coadded with a resolution of 4 cm^{-1}, at wave numbers between 4000 and 650 cm^{-1}. The background spectra were collected under the same conditions and subtracted from the scan for each sample.

SEM – Drop cast films were freeze cracked after being dipped in liquid nitrogen and analyzed with a Zeiss Merlin High-resolution scanning electron microscope. Samples prepared did not charge, therefore no gold plating or any preparation of samples was required. An EHT of 1.00kv was used with a 100pA probe.

Nanoindentation – Nanoindentaion measurements were performed on a Hysitron TriboIndenter with a nanoDMA transducer (Bruker). Samples were indented in load control mode with a peak force of 500 μN and a standard load-peak hold-unload function. Reduced modulus was calculated by fitting the unloading data (with upper and lower limits being 95% and 20%, respectively) using the Oliver-Pharr method and converted to Young's modulus assuming a Poisson's ratio of 0.33 for all samples. Each type of sample was prepared and indented in triplets to ensure good fabrication repeatability. For each sample, indentation was performed at a total of 49 points (7×7 grid with an increment of 20 μm in both directions) to ensure statistical reliability of the modulus measurements.

Viscosity – Rheological measurements were performed on 6 dry wt% biopolymer suspensions using a TA Instruments (New Castle, DE, USA) stress-controlled AR-G2 rheometer with a 60mm, 2° cone-and-plate fixture at 25℃. Solutions were allowed to equilibrate on the rheometer before running stepped flow from 0.1000 1/s to 1000 1/s.

Contact angle measurement – Contact angle analysis was performed using an optical contact angle apparatus Rame-hart goniometer (Succasunna, NJ, USA) equipped with a video measuring system with a high-resolution CCD camera and a high-performance digitizing adapter. SCA 20 software (Data Physics Instruments GmbH, Filderstadt, Germany) was used for data acquisition. Soda lime glass slides were fixed and kept flat throughout the analysis. The contact angle of silk fibroin, trehalose and their mixtures was measured by the sessile drop method by gently placing a droplet of 5 μl of biopolymer suspension onto the seed surface, according to the so-called pick-up procedure. All droplets were released from a height of 1 cm above the surface to ensure consistency between each measurement. The contact angle (θ, the angle between the baseline of the drop and the tangent at the drop boundary) was monitored using a software-assisted image-processing procedure. Five droplets were examined for the different biopolymer formulations considered on both the left and right sides and the resulting mean θ values were used.

Seed coating – *Phaseolus vulgaris* seeds were surface sterilized with 50% bleach for 3 minutes, rinsed in H_2O three times and left to air dry. GFP-CIAT 899 was grown overnight to an OD600 measurement of 1. 80 ml of GFP-CIAT 899 was centrifuged at 4200 rpm in a Eppendorf (Hamburg, Germany) centrifuge 5910 R. The supernatant was discarded and 8ml of 6 dry wt% silk fibroin-trehalose (1:3) suspension was

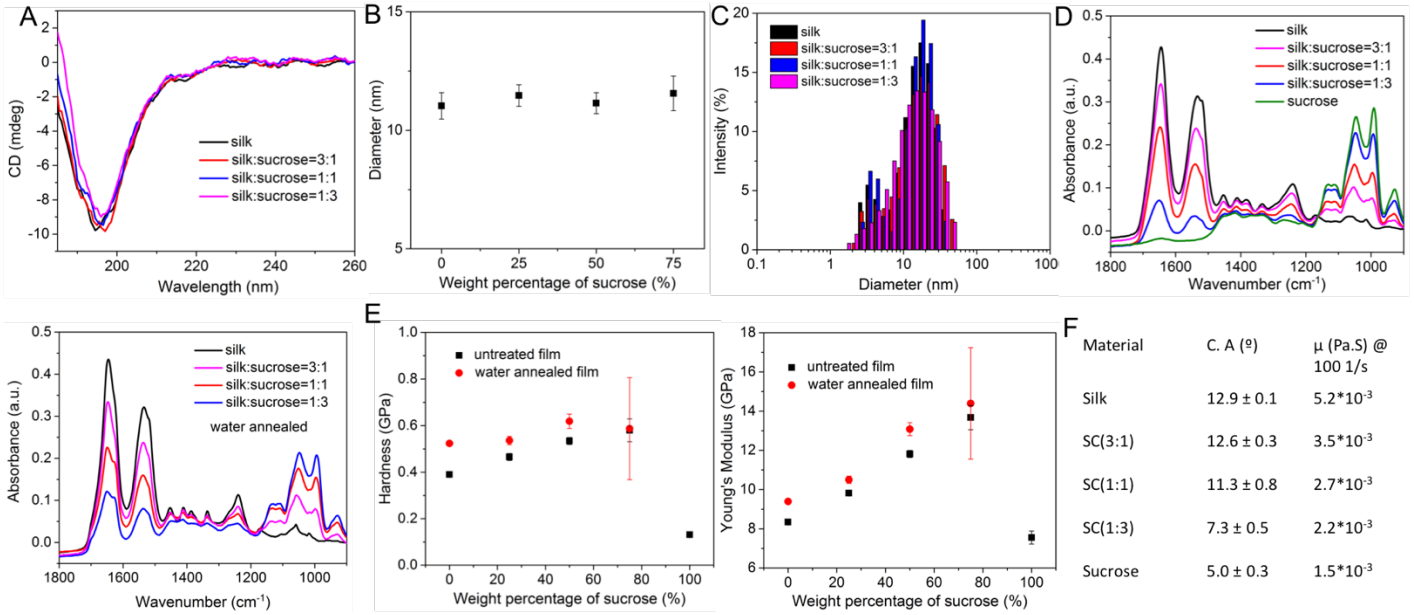
added to the spun down GFP-CIAT 899. Air-dried seeds were then dipped into this solution for 120 seconds, taken out and left to dry. After drying, the seeds were planted at the 24-hour mark. When water annealing post-processing was applied, seeds were placed in a vacuum chamber with H_2O, sealed for 6 hours to anneal and planted at the 24-hour point.

Encapsulation of bacteria on seed models – 50 seed models made by borosilicate glass beads (diameter = 5 mm, VWR, Radnor, PA, USA) were coated and air-dried. Once coatings dried, the glass beads were dropped into a measuring tube with 20ml of 1X phosphate buffer solution (Thermo Fisher scientific, Waltham, MA, USA). The solution was mixed, diluted and plated on agar plates for colony counting. For glass bead fluorescence imaging, beads were imaged under UV light and glass bead fluorescence imaged for the following conditions: uncoated, dip coated with silk fibroin, dip coated with silk fibroin and GFP-CIAT 899 and spray dried with silk fibroin and GFP-CIAT 899. The glass beads were left to air-dry.

Planting – Twelve square pots per experimental run were used and two seeds planted per pot. Four experimental runs were conducted with control (surface sterilized seeds) and GFP-CIAT 899 coated seeds. Two conditions were used, non-saline (4 ds/m) and saline (8 ds/m). Artificial salinity was created by adding 12g of NaCl into 1.2 litres of water, which was then mixed with 650g of soil. Salinity was measured with a salinity meter. The plants were then watered every other 3rd day. Plant heights and root lengths were then recorded at week 1 and week 2 after germination.

Fluorescence microscopy – Root nodules were imaged under GFP fluorescence with a Nikon Eclipse TE2000-E to confirm GFP-CIAT 899 root nodulation.

Figure S1



F

Material	C. A (º)	μ (Pa.S) @ 100 1/s
Silk	12.9 ± 0.1	$5.2*10^{-3}$
SC(3:1)	12.6 ± 0.3	$3.5*10^{-3}$
SC(1:1)	11.3 ± 0.8	$2.7*10^{-3}$
SC(1:3)	7.3 ± 0.5	$2.2*10^{-3}$
Sucrose	5.0 ± 0.3	$1.5*10^{-3}$

Figure S1. Physical analysis of dried silk film, sucrose film and their mixture films. (A) CD of air-dried films (B) DLS intensity average diameter (C) DLS size distribution (D) FTIR spectra of films (E) Mechanical properties of films using nanoindentation (F) Contact angle and viscosity table of solutions. S=silk, C=sucrose, SC(3:1)=silk:sucrose=3:1.

Figure S2

A

Material	C. A (º)	μ (Pa.s) @ 1/100 1/s	Thickness (mm)
Silk	15.9 ± 0.1	$5.2*10^{-3}$	0.118 ± 0.004
ST(3:1)	12.9 ± 0.3	$3.6*10^{-3}$	0.125 ± 0.006
ST(1:1)	10.7 ± 0.2	$3.0*10^{-3}$	0.123 ± 0.005
ST(1:3)	7.9 ± 1.0	$2.2*10^{-3}$	0.116 ± 0.003
Trehalose	4.7 ± 1.0	$1.2*10^{-3}$	N/A

B

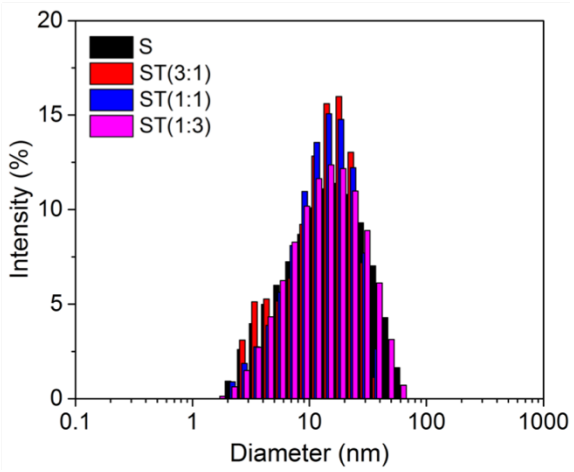


Figure S2. Physical analysis of dried silk film, trehalose film and their mixture films. (A) Viscosity and contact angle of solutions (B) DLS size distribution. S=silk, T=trehalose, ST(3:1)=silk:trehalose=3:1 relative dry weight concentration.

Figure S3

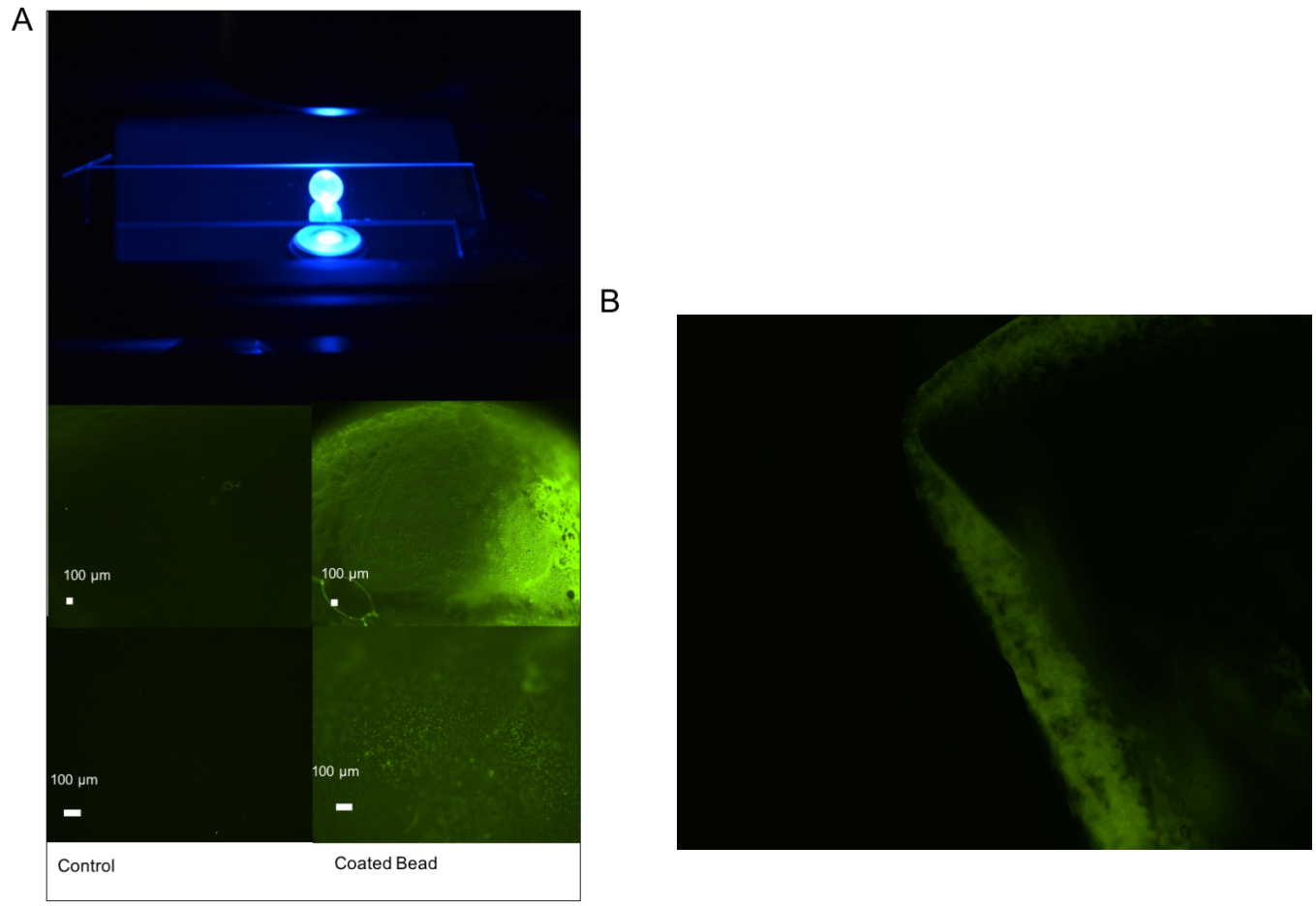


Figure S3. (A) GFP-CIAT 899 coated glass beads setup (B) Framework of the video depicting diffusion of CIAT 899 embedded in ST(1:3) films in artificial soil (phytagel).

Figure S4

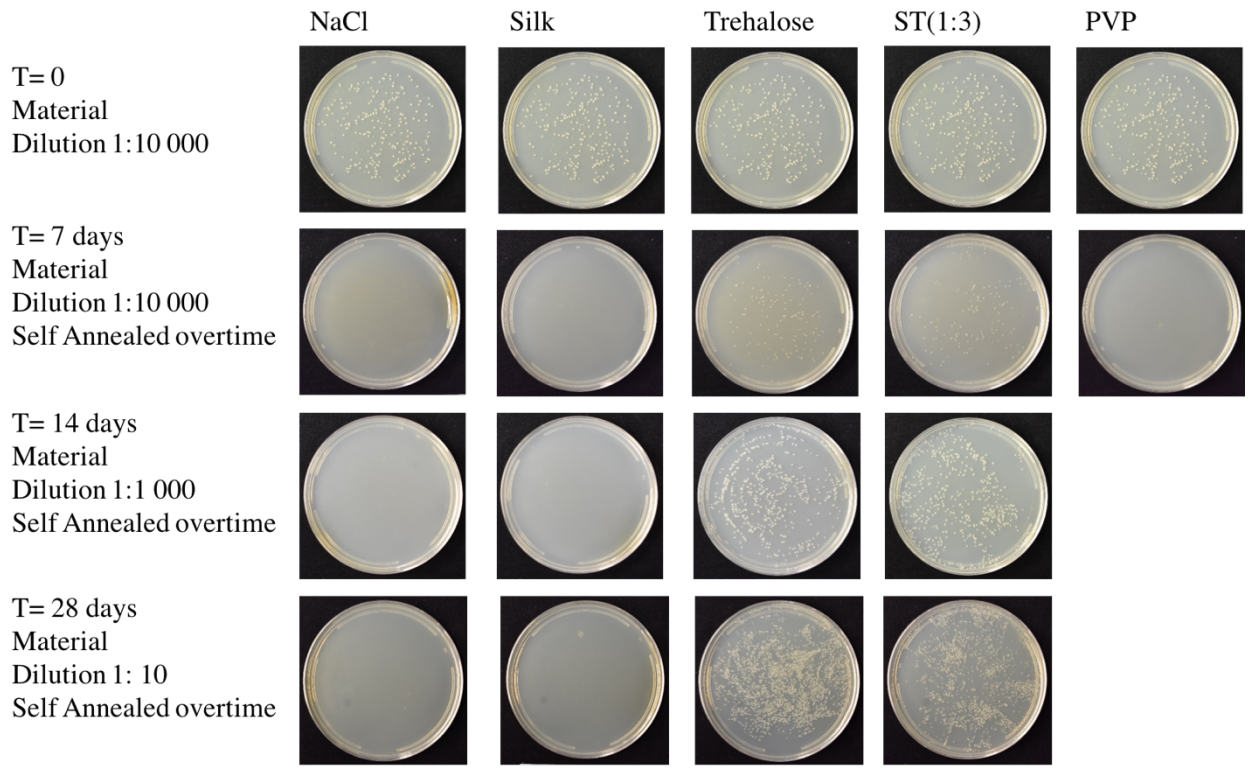


Figure S4. Selected agar colony counts for stability of CIAT 899 in silk film, trehalose film and their mixture films after storage at 23 ℃ for 4 weeks at 25% humidity.

Figure S5

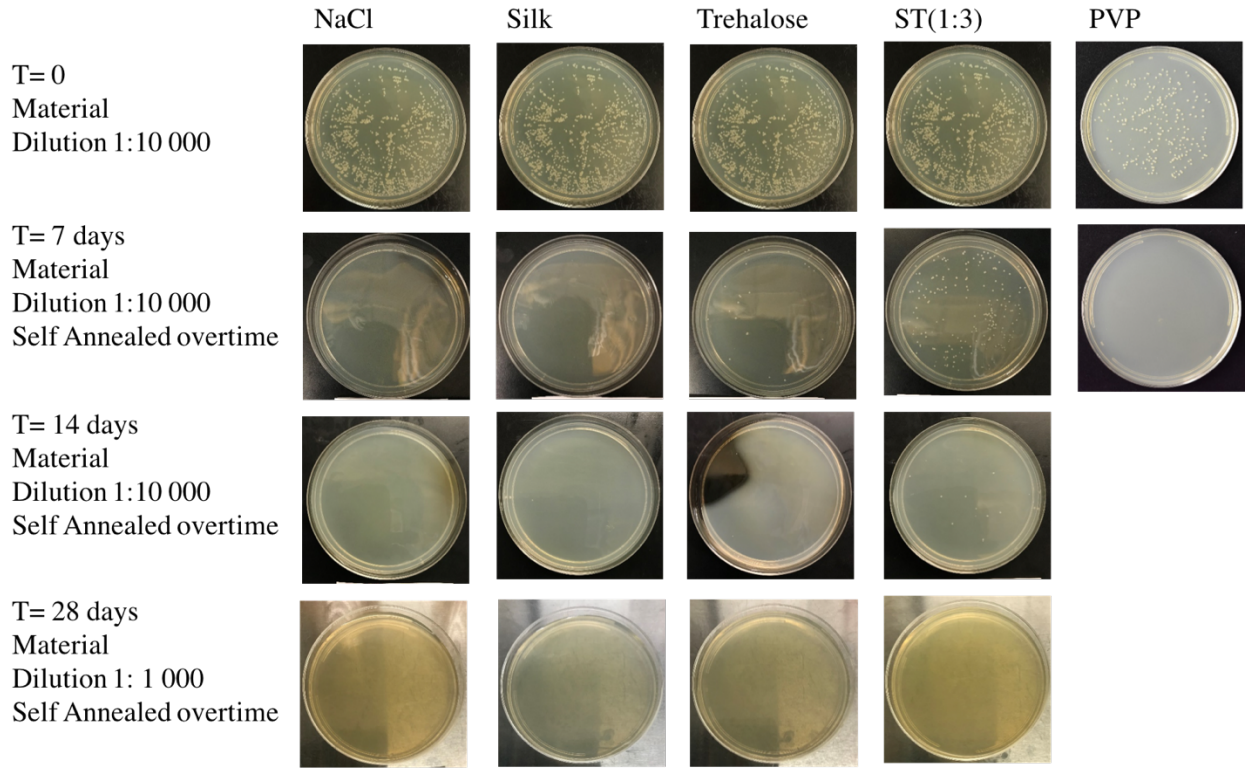


Figure S5. Selected agar colony counts for stability of CIAT 899 in silk film, trehalose film and their mixture films after storage at 23 ℃ for 4 weeks at 50% humidity.

Legend for Movies S1

Movie S1. Biodegradation of ST(1:3) coating on phytagel and release of GFP-CIAT 899.