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SHORT COMMUNICATION

Nitrogen inaccessibility protects spider silk from bacterial growth

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ABSTRACT

Spider silks are protein-based fibers that are incorporated into webs with the unique combination of high mechanical toughness and resistance to microbial degradation. While spiders are undoubtedly exposed to saprophytic microorganisms in their native habitats, such as the forest understory and bush, their silks have rarely been observed to decompose in either field or laboratory studies. We performed cross-streaking assays using silk from three spider species and four bacterial strains and found no inhibition zones, indicating the absence of antibacterial properties. We also cultured all bacteria directly upon silk in Luria–Bertani (LB) broth (full nutrients), phosphate-buffered saline (PBS; no nutrients) and nitrogen-free glucose broth (NFG; full nutrients, no nitrogen), and found that bacteria grew readily on silk in LB broth but not in PBS or NFG buffer. Our results indicate that spider silk's resistance to bacterial degradation is likely due to bacteriostatic rather than antibacterial mechanisms when nitrogen is inaccessible.

KEY WORDS: Bacteriostatic characteristics, *Cyrtophora moluccensis*, *Hippasa holmerae*, *Nephila pilipes*, Spider silk

INTRODUCTION

Spider silks are fascinating protein-based, multifunctional biopolymers that are simultaneously capable of: (i) achieving mechanical toughness that rivals any synthetic fiber (Gosline et al., 1999; Heim et al., 2009), (ii) transmitting vital vibratory information in a web to the nearly blind host spider without resonating (Masters and Markl, 1981; Mortimer et al., 2014), (iii) maintaining high resistance to degradation by microbial activity (Wright and Goodacre, 2012). Spider silk has, therefore, been a source of inspiration for the development of improved remote sensors and noise reducers (Miniaci et al., 2016; Osaki, 2012), tissue scaffolding (Kundu et al., 2013; Wang et al., 2006) and sutures (Hennecke et al., 2013; Kuhbier et al., 2011), among many other potential applications (Kluge et al., 2008). Of the three aforementioned characteristics of spider silk, resistance to biodegradation has received the least attention. However, understanding the underlying mechanisms protecting silk holds practical value for future bio-inspired materials, particularly in medical and agricultural industries. A first step in understanding these defenses is to closely inspect the interaction between saprophytic microorganisms and spider silk in its native state.

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Spiders use their silk to build webs and egg sacs in a variety of environments, such as underground, in forests and even under water, which undoubtedly come into contact with microbial organisms looking to consume this high energy material (Foelix, 2011; Uetz, 1991). Some spiders, such as *Cyclosa* and *Nephila* (Araneidae), even include leaf debris, previous moults or captured prey in their webs to serve as camouflage or storage for later consumption; however, these behaviors secondarily create good media for saprophytic microbes (Bjorkman-Chiswell et al., 2004). Furthermore, there are many araneopathogenic fungi known to infect the body of spiders, among which at least ten genera have been observed to germinate and could come in contact with silk (Evans, 2013; Evans and Samson, 1987). Although exposed to all kinds of saprophytic microbial organisms, spider silks have seldom been observed to decompose in nature.

Laboratory studies have shown spider silk to be resistant to degradation and inhibit microbial growth. Web silk of the common house spider *Tegenaria domestica* (Agelenidae) was reported to inhibit the growth of the Gram-positive bacterium *Bacillus subtilis* (Wright and Goodacre, 2012). Egg sac silk of another orb-weaver *Araneus diadematus* (Araneidae) displayed no decrease in fiber tenacity or elongation after 12 weeks in phosphate buffered saline (PBS) suggesting little or no degradation (Gellynck et al., 2008). However, treatment of spider silk with proteinase K, diethyl ether, 0.1% Triton-X-100 or simply washing with water (Gellynck et al., 2008; Wright and Goodacre, 2012; Yazawa et al., 2019) rendered the silk ineffective at inhibiting microbial growth or preventing degradation. Fibroblast cells have also been shown to adhere and proliferate upon silk scaffolds from *Nephila clavipes* after washing with salt solutions (Kuhbier et al., 2010; Wendt et al., 2011).

While it is clear that spider silk demonstrates properties of degradation prevention, the mechanism protecting silk remains uncertain. Spider silk is not known to contain sericin (Vepari and Kaplan, 2007), a gum coating of the silk of the silkworm *Bombyx mori* that displays antimicrobial properties (Wu et al., 2007). Spider dragline silk does display a complex, multi-layer skin-core structure (Augsten et al., 2000; Li et al., 1994), including lipid, glycoprotein high molecular weight protein skin layers that surround the inner core silk (Sponner et al., 2007), which some authors attribute to having protective properties (Gellynck et al., 2008; Wright and Goodacre, 2012; Yazawa et al., 2019). However, it is unknown whether other silk types used in webs, e.g. minor ampullate and flagelliform, similarly display this skin-core morphology. Additionally, some antimicrobial peptides have been isolated and identified in spiders. For example, agatoxins have been found in the venom of the funnel web spider *Agenelopsis aperta* (Agclenidae) (Miller, 1993; Mintz et al., 1992) and gomesin, a small antimicrobial peptide, has been extracted and purified from the hemocytes of the tarantula *Acanthoscurria gomesiana* (Silva et al., 2000). However, to date, no antimicrobial compounds have been identified from spider silk, which may indicate that other mechanisms could also be at play.

While spider silk is an organic material with an abundance of potential nutrient content (Vollrath and Knight, 2001), we postulated that bacteriostatic rather than antibacterial agents prevent silk degradation. We hypothesized that nitrogen, an element necessary for bacterial growth and essential for protein and nucleic acid biosynthesis (Prescott et al., 2004), is not sufficiently accessible to bacteria, constraining their ability to decompose silk in nature. To test our hypotheses, we examined the ability of four bacterial strains to utilize spider silk as a source of nitrogen for growth. We selected Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) strains and isolated two separate strains (Strain A and G) directly from spider webs. We used nitrogen-free glucose (NFG) broth (full nutrients without nitrogen) as the medium to culture the bacteria on web silks from three spider species. Phosphate-buffered saline (PBS) (no nutrients) and Luria–Bertani (LB) broth (full nutrients, including nitrogen) were used as positive and negative controls. We predicted that all bacteria should not grow on the silk cultured in PBS and NFG, but should grow well on silk cultured in LB broth.

MATERIALS AND METHODS

Collection and housing of spiders

We selected silks from three spider species that build webs in different environments: an orb web builder *Nephila pilipes* Fabricius 1793 (Araneidae), a funnel web builder *Hippasa holmerae* Thorell 1895 (Lycosidae) and a tent web builder *Cyrtophora moluccensis* Doleschall 1857 (Araneidae). These spiders build webs in the forest understory, at ground level and on bushes, respectively. Chaudhary and Rizvi (2017) revealed that spider age has a significant impact on the protein content in the web silk; thus, the spiders we used in this study were within 7 days of sexual maturity. We collected five females of each species from the Tunghai University campus, and housed them in a greenhouse. The environmental conditions in the greenhouse were 26±2°C and 80±10% RH with a natural photoperiod (∼14 h:10 h light:dark). The spiders were fed three mealworms twice a week and watered once a week. Webs were destroyed before silk sample collection and after feeding, allowing spiders to build new webs without contamination from dust, prey or leaf litter.

Selection of test bacteria

In order to determine the bacteria most capable of decomposing spider silk, we isolated bacteria from detritus and decorations on the webs of *Cyclosa mulmeinensis* (Araneidae). This spider is known to put prey remains, leaf litter and previous moults in its web to construct a line of decoration for camouflage/crypsis (Tan and Li, 2009). The decoration is, however, a good medium for bacteria and fungi. All experiments were carried out under sterile conditions to avoid any kind of contamination. We placed 0.5 g of detritus in a 1.5 ml Eppendorf tube and added 1 ml distilled water to the tube, which was then centrifuged at 4500 ***g*** for 5 min to suspend the solutes. Then, 100 µl of suspension was spread on LA plates to isolate three replicates of bacterial colonies, and colonies were incubated for 24 h at 27°C. Bacterial colonies with different morphological characters (size/shape/color) were aseptically sampled with a sterile loop and inoculated onto a new LA plate using a standard four-quadrant plate-streaking method and incubated for 24 h at 27°C. After incubation, we determined a dominant bacterium, strain A. Using the aforementioned procedure, we isolated another dominant bacterial species, strain G, from the web silk of *N. pilipes*. Strain A and strain G belonged to Gram-positive and Gram-negative bacteria, respectively. To identify them, the 16S rDNA of strain A and strain G were amplified with primers BSF8 and BSR1541 and sequenced with primers BSR534, BSF1099, BSR1114 and BSR1541. Sequence data were analyzed using Bioedit and BLAST databases. Taxonomic affinities were assigned to strain A and strain G based on BLAST sequence similarity analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The16S rDNA sequences of strain A and strain G closely matched *Bacillus altitudinis* (CP040747) and *Enterobacter bugandensis* (LT992502), respectively.

Two additional microorganisms, *B. subtilis* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria), were also used in this study. To preserve the tested bacteria, they were suspended in 5 ml of LB broth and incubated for 24 h at 27°C. We then added 20% glycerol to the tubes, and stored them at −80°C. The bacterial strains in this study were deposited in the Microbial Ecology Laboratory, Department of Life Science, Tunghai University, Taiwan.

Cross-streaking assays

The ability of spider silk to inhibit the growth of bacteria was assessed in an LA agar plate using a cross-streaking method (Hill et al., 2009). Four bacterial strains were individually inoculated in LA agar plates by streaking the bacterial suspension in three replicate parallel lines. Then we bundled spider silk (radial silk plus spiral silk) from webs onto a sterilized pipette tip. Bundles were gently pulled using forceps to form a string (about 5 cm in length, and 0.5 mm in width) composed of multiple threads. The silk string was positioned perpendicularly across the bacterial streaks. The plates were then incubated for 24 h at 27°C, and after which, we assessed the existence of an inhibition zone under a light microscope. The experiment was repeated three times to determine the potential antibacterial activity of the silk string.

Bacteria cultured directly on spider silk

We inoculated the four bacteria strains (*B. subtilis*, *E. coli*, strain A and strain G) directly on spider silk to investigate whether the nutrients of the silk can support growth. We cut a 1.5 ml Eppendorf tube into a small ring (transverse section, 0.5 cm in height, 9.0 mm in diameter) that was then sterilized by autoclave. Spider silk from a newly built web (within 12 h) of adult female was harvested directly by an open face of the ring, until the face was covered with a thin layer of silk (0.2 mm in thickness containing spiral and radial threads) (Fig. 1). One ring was used as one sample. For each of the three spider species, we collected 15 silk samples (three webs, five samples from each web), which were independently subjected to two cross factors, bacterial species (*B. subtilis*, *E. coli*, strain A, strain G and control) and nutrient level (LB, NFG and PBS).

Bacteria were inoculated in LB broth at 27°C overnight, then briefly centrifuged and washed with PBS buffer, and resuspended in culture medium (LB, NFG and PBS). For each bacterial strain, 5 µl of bacterial suspension (10^5–10^6 cells ml^{-1}) was dropped onto the silk layer of the ring. Culture fluids without bacteria were also applied using the same procedure as a control. The media used in the experiment were made from the following: LB broth (10 g peptone, 5 g yeast extract and 5 g NaCl, with 1 liter of water), PBS buffer solution (10 ml 0.5 mol l^{-1} KH_2PO_4 and 2.5 ml 0.4 mol l^{-1} $MgCl_2 \cdot 6H_2O$, with 2 liters of water) and NFG (13.6 g KH_2PO_4, 7.1 g $NaHPO_4$, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2$ and 8 g glucose, with 1 liter of water). After preparation, samples were incubated at 27°C overnight and the suspension was then collected and the concentration of bacteria calculated using a hemocytometer.

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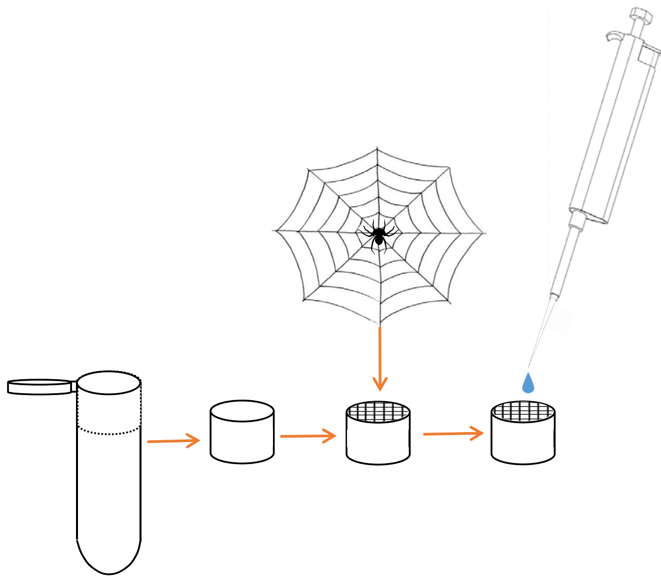

Fig. 1. Schematic diagram showing how bacteria were cultured on spider silk. Rings were cut from Eppendorf tubes, a section of web was collected atop the ring and then 5 μl bacterial suspension was dropped directly onto the silk.

Measurement of bacterial growth and statistical analysis

The exponential growth rate for each bacterial species on spider web silk was calculated as follows:

$$R = 100 \times (\exp(\ln(N_t/N_0)/t) - 1), \quad (1)$$

which is derived from the exponential growth model:

$$N_t = N_0 \times (1 + R/100)^t, \quad (2)$$

where R is the exponential growth rate (% concentration change h^{-1}), and N_0 and N_t are the initial and t hour bacteria concentration (cells ml^{-1}). To calculate the growth rate for the control, we assumed the initial concentration (N_0) of control treatment as 1 cell ml^{-1}. We then fitted the exponential growth rates by using a general linear mixed model (GLMM):

$$R_{ijkmnl} = \mu_{ijk} + (\text{web})_m + (\text{piece})_{m(n)} + (\text{error})_{mnl(ijk)}, \quad (3)$$

where i is spider species, j is bacterial species, k is broth type, m is spider web identity, n is web silk sample identity nested on spider web, and l is replicate identity. We used the Markov chain Monte Carlo (MCMC) technique to fit the model by using an R package MCMCglmm version 2.22.1. The combination of spider species, bacterial species and nutrient level was considered as a fixed factor with a non-informative prior: normal distribution (expected value=0, variance=1×10^6). Spider web identity and web piece nested on spider web were considered two random factors, in which their variances follow non-informative priors: inverse gamma distribution (shape number=0.1, rate number=0.1). We assumed that the error term followed a normal distribution (expected values=0, variances=σ_{ijk}^2), where the variances assumed a non-informative prior: inverse gamma distribution (shape number=0.1, rate number=0.1). Our model accepted heteroscedastic errors among different spider species and culture condition. We performed 5.1×10^7 MCMC iterations in total with a thinning rate of 5000, including the beginning 1×10^6 burn-in iterations. We performed multiple comparisons among spider species, bacterial strains and broth types by using Bayesian equivalence tests, so that two significant groups were determined only when their 95% credible interval of Bayesian Cohan's d was greater than 0.2 (a 'small' effect size). All analyses were conducted in R version 3.6.0 (https://www.r-project.org/).

Ethical statement

This research was conducted in accordance with the 'research ethics and animal treatment' legal requirements of Tunghai University. The spiders used in the experiment were released to their original habitat after collecting their silks.

RESULTS AND DISCUSSION

We investigated the abilities of spider silk to defend against degradation by saprophytic microbes. Our results demonstrate three important aspects of the interactions between saprophytic bacteria and spider silk. First, spider silk, in its native state, does not inhibit the growth of bacteria even when in direct contact, indicating that antibacterial compounds are not present (Fig. 2). Second, bacteria can grow on spider silk when supplemented with a full complement of nutrients (Fig. 3). Finally, bacteria grow poorly, or not at all, if nitrogen is withheld, even among an otherwise full complement of nutrients (Fig. 3). These results do not support the presence of an antibacterial substance on spider web silk, as has been previously suggested but never identified (Amaley et al., 2014; Keiser et al., 2015; Wright and Goodacre, 2012). However, our findings do

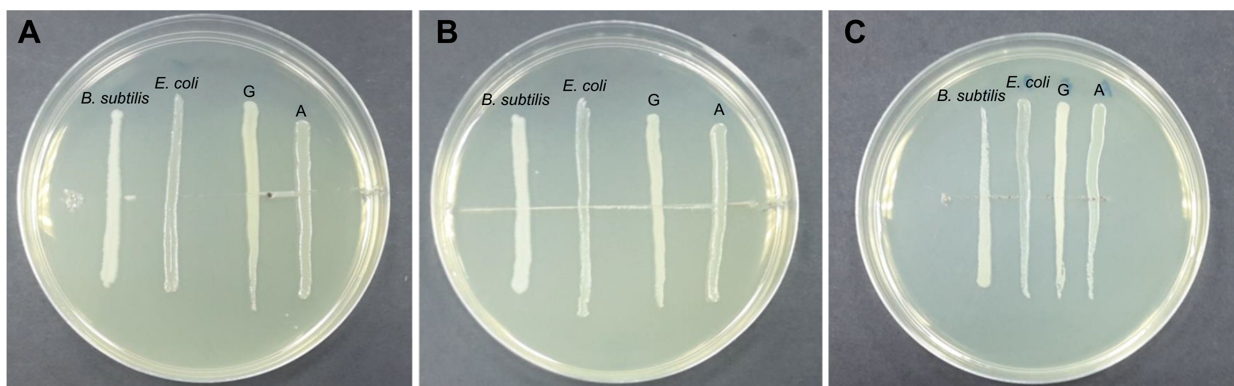

Fig. 2. Cross-streaking assays show no inhibition zones around spider silk. Three spider species, (A) *Nephila pilipes*, (B) *Cyrtophora moluccensis*, (C) *Hippasa holmerae* were tested with four bacterial strains (*B. subtilis*, *E. coli*, strain G and strain A) on LA agar plates. Strain G and A were identified as *E. bugandensis* and *B. altitudinis*, respectively. The experiment was replicated three times.

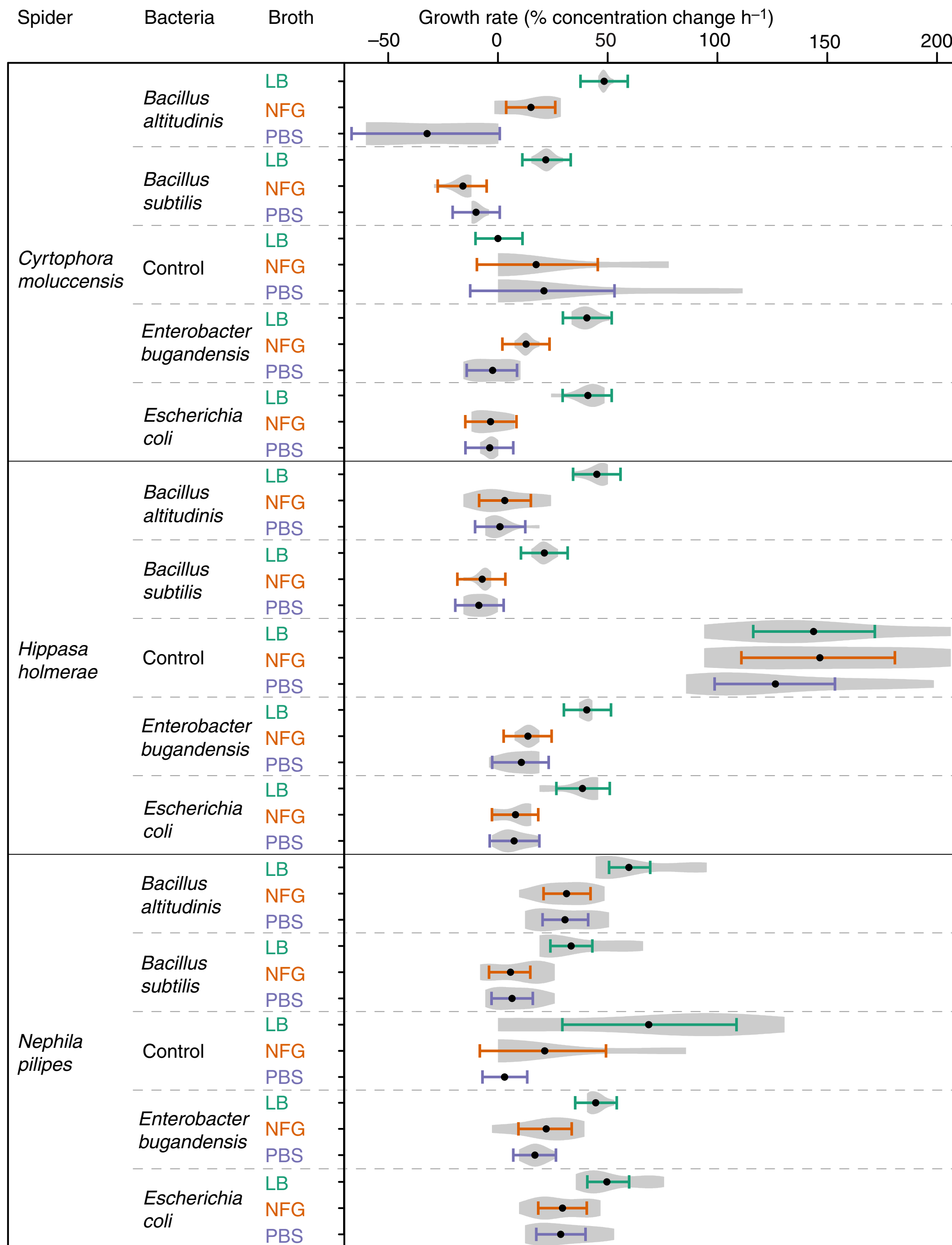

Fig. 3. Bacterial growth rates with corresponding posterior distributions among combinations of spider species, bacterial strains and broth types. Different colors of whiskers represent different broth types. Solid circle symbols and whiskers indicate the expected value and 95% highest posterior density intervals of the posterior distribution of bacteria growth rate average estimated by MCMC GLMM. Gray areas indicate the kernel density estimations for the growth rates distributions based on the empirical data. Three spiders were used in each species, and each spider was used only once. Five silk samples were collected from each web.

support an alternative bacteriostatic mechanism preventing bacterial growth: limiting nutrient accessible.

Four bacteria, including *B. subtilis*, *E. coli* and spider web inhabitants strain A and strain G, which were later identified as *B. altitudinis* and *E. bugandensis*, respectively, were cross-streaked with spider silk to determine the ability of spider silk to inhibit bacterial growth. No inhibition zones were found in the vicinity of any silk samples in the cross-streaking assays (Fig. 2). These results demonstrated that there was no antibacterial agent within the spider silk.

We used the same four aforementioned bacterial strains to investigate whether the nutrients of spider silk can support their growth. On the silk web of *C. moluccensis*, the growth rates of *B. subtilis*, *E. coli*, strain A and strain G in LB broth ranged from 25% h^{-1} to 50% h^{-1} and were significantly higher than that in PBS (Fig. 3). This demonstrated that these bacterial species grew well on

spider web silk in the presence of a full supply of nutrients and were not growth inhibited by the silk. The growth rates of the control groups ranged from 0 to 200% h^{-1} (Fig. 3) with relatively low populations (1–10%), which represented the growth of inhabitant bacteria in LB broth on the spider web. The growth rates of these four bacterial species in PBS was ∼0% h^{-1} (Fig. 3), which indicated these bacteria cannot grow on the spider web silk without a supplement of nutrients. In the NFG broth treatments, containing full nutrients without a source of nitrogen, the bacterial growth rate was close to 0% h^{-1} (Fig. 3) and was not significantly different from the PBS treatment, except in the case of *B. subtilis* (Fig. 3; Table S1). This result suggested that these bacteria did not grow on spider web silk when silk was the sole nitrogen source. The same trends were observed on the webs of the two other spider species, *N. pilipes* and *H. holmerae*, and *B. subtilis* growth in NFG broth was not significantly different to that in PBS (Fig. 3; Table S1).

Prevention of bacterial growth on spider silk

Silk used by most spiders to build webs or wrap prey is, undoubtedly, exposed to microbial decomposers from nearby environments or foreign objects such as prey or organic debris positioned within the web (Bjorkman-Chiswell et al., 2004; Foelix, 2011; Uetz, 1991). The presence of antimicrobial compounds would not only prevent bacterial growth upon the surface of the silk itself but also inhibit growth on nearby substrates (Gomes et al., 2011), which we explicitly tested for and did not find. Genetically engineered spider silk proteins fused with antibacterial peptides did, on the other hand, demonstrate inhibition zones preventing growth of *E. coli* and *Staphylococcus aureus* (Gomes et al., 2011). Furthermore, our finding that four bacterial strains were able to grow on web silk of three spider species in the presence of supplemental nutrition without any manipulation of the silk (Fig. 3) also supports the absence of antibacterial compounds. We contend that bacterial growth and decomposition of silk is prevented via bacteriostatic activity rather than inhibited by antibacterial means.

The spider species tested in this study are genetically distant (Garrison et al., 2016; Wheeler et al., 2016), inhabit different foraging environments (Uetz, 1991), construct dissimilar web types and recycle/relocate their webs at different rates (Eberhard, 1990), yet we find the same trends in bacterial inhibition and nutritional requirements for growth across species (Fig. 3). It is possible that our observation of nitrogen limitation restricting bacterial growth may itself be a conserved feature of silk used by web building spiders, although, deeper and broader phylogenetic analysis is necessary for confirmation. However, several other aspects of spider silk are highly conserved, such as the N- and C-terminal domains of the major ampullate silk protein (Ittah et al., 2007; Motriuk-Smith et al., 2005), and several amino acid motifs (Hayashi et al., 1999; Gatesy et al., 2001). Webs of the most primitive web-builders are not recycled regularly (Eberhard, 1990) like those of derived orb web spiders (Opell, 1998), such as *N. pilipes*. Defense against biological degradation was likely an important feature of silk used in webs exposed to the environment for up to weeks at a time.

Potential mechanisms limiting nitrogen accessibility

While we are able to reasonably conclude that bacteriostatic rather than antibacterial mechanisms protect spider silk from degradation in our study, we are unable to explain how spider web silk, an organic material predominantly composed of protein (Vollrath and Knight, 2001), makes its abundant nitrogen content inaccessible to the bacteria we tested. One solution may lie in the skin-core morphology demonstrated in dragline silk from *N. clavipes* (Augsten et al., 2000; Li et al., 1994; Sponner et al., 2007). The outer layers contain lipids and glycoproteins that surround the inner protein core. This outer skin was found to be highly resistant to chaotropic agents, such as lithium thiocyanate, hexafluorisopropanol etc., while the inner core reacted quickly once exposed (Sponner et al., 2007). Additionally, when the outer skin of silk is removed by washing with water, diethyl ether or 0.1% Triton X-100, the inner core becomes very vulnerable to degradation by bacteria (Gellynck et al., 2008; Wright and Goodacre, 2012; Yazawa et al., 2019). It is possible that the skin serves as a protective barrier shielding the inner core (Sponner et al., 2007; Wright and Goodacre, 2012). However, it is not known if this skin-core morphology is a common trait in dragline silk among web building spiders and if it is present in other web silk types, such as flagelliform silk and aggregate glue, which will require further testing and broader sampling.

Another possibility for how spider silk makes nitrogen inaccessible lies in its complex network of interconnected crystalline and non-crystalline structures (Termonia, 1994), which may be difficult for some bacteria to consume. A recent study (Dinjaski et al., 2018) showed that increased crystallinity and protein chain length reduced *in vivo* (mice) degradation time of recombinant silk based on consensus domains of *N. clavipes* dragline silk. Other studies involving biofilms from recombinant spider silks (Lu et al., 2011; Müller-Herrmann and Scheibel, 2015) and silkworm silks demonstrated that β-sheet (crystalline) structures are highly resistant to enzymatic activity and degrade slower than non-crystal structures. While it may be unlikely that the internal molecular structures of spider silk are solely responsible for protecting against its own biodegradation, as degradation does still occur, these structures likely do play some role that requires further investigation.

Conclusions

Silk excreted by spiders to build webs is a multifunctional biopolymer that demonstrates impressive tensile, vibratory and anti-degradation properties (Gosline et al., 1999; Mortimer et al., 2014; Wright and Goodacre, 2012). We examined why spider silk can persist in its natural environment for weeks at a time with very little degradation, and found that the spider silk does not show anti-bacterial properties but prevents bacterial growth by limiting nitrogen accessibility. While other biopolymers display the combined properties of strength and resistance to biodegradation, such as plant lignin (Jeffries et al., 1981), there are few protein-based examples outside of silk. Improved understanding of how spider silk prevents bacterial growth have applications in medical and agricultural industries, such as improved sutures and nerve/tissue scaffolding, and protection of vulnerable biological tissue from infection (Kluge et al., 2008).

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: S.Z., I-M.T.; Methodology: S.Z., D.P., P.-H.W., Y.-R.L., I-M.T.; Formal analysis: D.P., C.-P.L.; Investigation: S.Z., W.-R.L., Y.-R.L., I-M.T.; Data curation: S.Z., D.P., W.-R.L., Y.-R.L., C.-P.L., I-M.T.; Writing - original draft: S.Z.; Writing - review & editing: S.Z., D.P., W.-R.L., P.-H.W., C.-P.L., I-M.T.; Visualization: S.Z., P.-H.W., Y.-R.L.; Supervision: P.-H.W., I-M.T.; Project administration: P.-H.W., I-M.T.; Funding acquisition: I-M.T.

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Table S1. Statistical summary of growth rate among spider species, bacterial strains and broth treatments. Two treatments that do not share any alphabet letter in the multiple comparison columns are considered significantly different as the 95% highest posterior density of the Bayesian Cohan's *d* between their respective posterior distributions fall outside the range of −0.2 to 0.2, which indicated a 'small' effect size between two groups.

Spider	Bacteria	Broth	*N*	Growth rate percentage		Multiple comparison
				Average	SD	
Cyrtophora moluccensis	*Bacillus altitudinis*	LB	10	48.47	1.947	abc
		NFG	9	15.04	12.23	fghijklm
		PBS	6	−32.67	30.69	nopqr
	Bacillus subtilis	LB	10	21.95	3.862	ghi klm st
		NFG	9	−16.09	5.616	n
		PBS	9	−15.40	17.01	nop
	Control	LB	9	8.648	25.94	f opqr
		NFG	9	17.30	34.32	bcdefghijklmnopqrstu
		PBS	9	21.03	42.58	abcdefghijklmnopqrstu
	Enterobacter bugandensis	LB	9	40.47	5.083	bcde u
		NFG	9	12.78	3.147	fg ij lm r
		PBS	9	−2.382	9.798	nopqr
	Escherichia coli	LB	9	40.91	7.139	bcde u
		NFG	9	−3.314	7.754	nopqr
		PBS	9	−3.634	3.063	nopq
Hippasa holmerae	*Bacillus altitudinis*	LB	9	45.00	5.474	abcde
		NFG	9	3.07	13.31	fg j nopqr
		PBS	9	0.918	7.287	f j nopqr
	Bacillus subtilis	LB	9	21.17	3.546	hi klm s
		NFG	9	−7.128	3.677	nop
		PBS	9	−8.637	5.634	no
	Control	LB	9	143.6	39.70	v
		NFG	9	131.2	64.83	v
		PBS	9	126.4	39.22	vw
	Enterobacter bugandensis	LB	8	40.23	2.420	bcde u
		NFG	9	13.65	3.497	fghijklm r
		PBS	8	10.41	8.585	fg jklm pqr
	Escherichia coli	LB	9	38.51	10.50	bcde tu
		NFG	9	8.020	8.005	fg j qr
		PBS	9	7.352	6.858	fg j m pqr
Nephila pilipes	*Bacillus altitudinis*	LB	13	62.01	20.25	a
		NFG	14	31.67	11.67	b de hi kl stu
		PBS	12	30.54	13.78	d h k stu
	Bacillus subtilis	LB	15	35.86	18.09	b de stu
		NFG	14	4.056	22.16	fg j opqr
		PBS	14	8.117	10.23	fg j m opqr
	Control	LB	9	65.59	51.13	abcde hi stu w
		NFG	9	18.17	36.11	abcdefghijklmnopqrstu
		PBS	9	14.51	43.54	f j opqr
	Enterobacter bugandensis	LB	13	44.84	3.832	bc e
		NFG	13	22.23	13.83	ghijklm stu
		PBS	13	17.00	4.902	fghijklm
	Escherichia coli	LB	16	52.85	13.75	a c
		NFG	13	29.15	12.25	d hi kl stu
		PBS	13	29.74	14.19	de hi kl stu