Synthetic Piriform Spider Silk Protein Production

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INTRODUCTION

Silk from *Nephila clavipes* has been shown to have extraordinary mechanical properties. Native silk has been shown to have a higher toughness than Kevlar and twice the extensibility of nylon.[1-4] Of the seven types of silk produced by *N. clavipes*, Piriform remains the least characterized. Silk is produced and stored in glands upstream from the spigot from which the fiber is drawn (Figure 1). By generating cDNA libraries from dissected glandular tissue, the mRNA sequence coding for the specific silk protein type can be elucidated.[5] Because silks require no posttranslational modification, expression in a bacteria such as Escherichia coli is possible. In order to generate a synthetic silk gene, the repetitive motifs of the silk are iterated to the extent of the bacteria's metabolic capacity. Piriform silk is comprised of two repetitive motifs, PXP (P motif) and QQAS (Q motif, Figure 2) in addition to a non-repetitive region. The structural roles of these motifs are not clear.

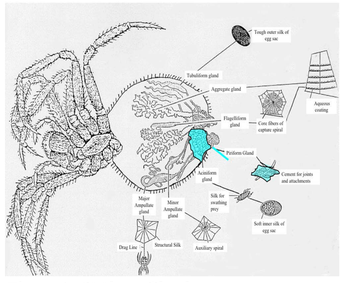

Figure 1: Piriform Gland

Figure 2: Amino acid sequences of P (blue) and Q (red) structural motifs, and full Piriform cassette.

Based on Raman Spectroscopy analysis, the Piriform silk structure has been shown to be similar to Aciniform silk. Based on this comparison, it could have similar mechanical properties.[6] Piriform also has potential as an adhesive. When naturally extruded, it forms attachment disks that the spider will use to secure the web onto a surface (Figure 3).

Piriform Silk Constructs
(QQSSVAQS)n
(RPLPAPRPLPAPLPAPRPIPAPLPRPVI)n
(VSQVQQASIQQAQSSSAQSRQSSVAQQASISQSQQASVSQSQQASVSQSQSQQASVSQSQ
QSSNAYSAASNAASSVSQASSDSSYFNSQVVQSALSSSLQSSSALSSIAYGQTSANINDVAA
AVARSVSQSLGVSQQAAQSVISQQLASAGSGASAQTLAQLISSAVSSLVQQSGTVSAGQE
QSISQSLSSSILSSLSQVVAQRPLPVPAPRLPVPAPRPLPAPLPAPRIPAPLPRPVPI)n

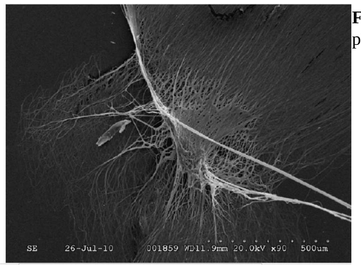

Figure 3: Scanning Electron Microscopy of native piriform attachment disc from Geurts et al.

METHODS

Expression

A synthetic Piriform gene subunit was synthesized based on the Piriform protein sequence. This subunit was iterated up to 4 times. A synthetic gene of the P motif was created by iterating the original sequence 24 times. A synthetic gene of the Q motif was created by iterating the original sequence 18 times. All of these genes were codon-usage optimized for *E. coli*. Considerable laddering of the Piriform protein under a variety of expression conditions has been observed (Figure 4). Laddering occurs when the protein is incompletely expressed, but all products contain the his-tag. This results from the his-tag being coded on the N-terminal of the protein, instead of the C-terminal. The anticipated size of the PiriformX4 construct (2884bp) is roughly 100kDa. The major expression product is roughly 75kDa, with laddered products at 25kDa increments.

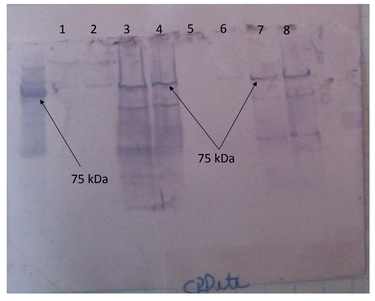

Figure 4: Western blot of PiriformX4 construct expressed in various vectors. Lanes 1-2 and Lanes 5-6 were expressed in pET19k. Lanes 3-4 and lanes 7-8 were expressed in pET_SX. Note that while pET_SX produces a greater quantity of protein, the laddering problem exists for both cases.

Purification

Because of the glue-like nature of the synthetic Piriform protein, a batch-nickel resin system was used. The eluent was dialyzed and lyophilized. Protein yields were found to be roughly 3g per 100ml of lysate. Reverse Phase Chromatography (RPC) was done in order to further purify protein for Amino Acid Analysis (AAA). A unique Formic Acid gradient was used to selectively remove his-tagged Piriform.

RESULTS CONT'D

We have generated synthetic genes coding for a full Piriform protein repeat unit, and for its motifs P and Q. In the case of the full Piriform construct, it has been shown to express when iterated 4 times (Figure 4). In order to mitigate laddering, an adapter for a C-terminal His-tag has been designed. Fully iterated synthetic proteins (Piriformx4, Px24, Qx18) have been made, but do not express a full-length major product. Amino Acid Analysis remains inconclusive at this time. A protein product of correct mass can be produced reliably, though partially expressed iterants often form the major products.

CONCLUSIONS

Piriform silk shows potential to form a strong fiber or a useful adhesive. It can be expressed in *E. coli*. By expressing unique proteins comprised of the structural motifs of native Piriform, the structural role of these motifs can be determined.

Future Work

Future work remains to be done in order to obtain a protein of the maximum potential size.

- Implement C-Terminal his-tag adapter
- Improve purification process

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