

RECOMBINANT SPIDER SILK PROTEIN PRODUCTION, PURIFICATION AND POLYMERIZATION

Oliveira, P.E.F.^{1,3}; Verza, N.C.¹; Bittencourt, D.^{1,2}, Souto, B.M.^{1,2}; Madeira, L.M.^{1,2}; Andrade, A.C.¹; da Silva, F.R.¹; Lewis, R.V.⁴; Rech, E.L.¹

¹Núcleo de Biotecnologia, EMBRAPA Recursos Genéticos e Biotecnologia, Brasília-DF, Brazil; ²Instituto de Ciências Biológicas, Departamento de Biologia Celular, Universidade de Brasília, Brasília-DF, Brazil; ³Departamento de Ciências Genômicas e Biotecnologia Molecular, Universidade Católica de Brasília, Brasília-DF, Brazil; ⁴Department of Molecular Biology, University of Wyoming, Laramie-WY, USA.

Spiders produce a variety of silks that display extraordinary molecular and mechanical properties. Dragline silk has a tensile strength that is comparable to Kevlar associated with a reasonable elasticity, and is an extremely strong fiber. Therefore, genetic engineering approaches to generate spider silk and to process the proteins into new useful materials are actively under study. We sequenced expression sequence tags from major ampullate, minor ampullate, flagelliform and tubuliform silk glands from the Brazilian spider *Parawixia bistriata* (Araneae: Araneidae) and were able to identify a number of silk related proteins, including two distinct cDNAs which encode proteins similar to major ampullate spidroin 1 and 2 (MaSp1 and MaSp2) from *Nephila clavipes*. Using PCR approaches, we cloned the repetitive module of the *P. bistriata* MaSp2 (PbMasp2) gene in tandem into the pET19b expression vector and obtained recombinant *E. coli* BL21(DE3)pLysS expressing the engineered PbMasp2. We proceeded the purification using affinity chromatography columns and the purified protein was polymerized and produced a recombinant spider silk in vitro.