BENJAMIN T. LANHAM, Dept. of Chemistry, Shepherd University, Shepherdstown, WV, 25443, and JONATHAN GILKERSON, Dept. of Biology, Shepherd University, Shepherdstown, WV, 25443. Expression and purification of AtRALF1 from *Escherichia coli*.

Rapid Alakalinization Factors (RALFs) are a family of plant specific peptide growth factors involved in myriad plant processes including growth, development, and response to stresses. These secreted peptides are genetically encoded and processed into an active form from a larger pre-pro-peptide. The active forms are ~5 kDa and have two disulfide bridges. Here we report our attempts to express the active form of AtRALF1 (At1g02900) from the soluble fractions of two different expression strains of *E. coli*, Origami 2 (DE3) and SHuffleT7 pLysY. Both of these strains are commercially available and genetically altered for expression of proteins with disulfide bonds. Expression of these peptides is challenging because misfolded disulfides or other structural issues result in insoluble peptides. We sought to optimize expression conditions to bulk purify $HIS_{(6x)}$-AtRALF1 by cobalt column chromatography. Under most conditions the peptide was insoluble. However, at 25^{o}C around 50% of total $HIS_{(6x)}$-AtRALF1 was soluble in Origami cells. As controls, we expressed mutated versions with alterations in the conserved YISY motif which are physiologically inactive. Surprisingly, these mutant versions were 100% soluble in most conditions tested. All versions of $HIS_{(6x)}$-AtRALF1 were observed to migrate ~12 kDa in SDS-PAGE gels although their predicted molecular weights is ~8 kDa. Treatment under strong reducing conditions had no effect on this migration pattern. We conclude that AtRALF1's insolubility issues could be more related to a structure in the YISY motif than to the formation of disulfide bonds.