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Prokaryotic expression and solubilisation of *Arabidopsis* ROOT UVB SENSITIVE 1 from inclusion bodies in *Escherichia coli*

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ABSTRACT:
The RUS (ROOT UVB SENSITIVE 1) proteins characterized by their unique DUF647 domain are widely distributed in eukaryotes. Their functional roles are largely unknown except for the possible involvement of *Arabidopsis* RUS1 and RUS2 in early seedling development. To investigate the biochemical roles of the RUS proteins, full length and truncated *Arabidopsis RUS1* were seamlessly fused with *GFP* and cloned into prokaryotic expression vector pQE-100 which allows proteins expressed with an N-terminal 6×His tag. Expression of the full length RUS1-GFP could not be detected after adding the inducer IPTG, while a truncated RUS1-GFP was expressed at high levels and formed inclusion bodies in *Escherichia coli*. The inclusion bodies were dissolved in a denaturing buffer, and then the truncated RUS1-GFP fusion protein in the supernatant was bound to a Ni-NTA slurry. The bound proteins were eluted after the non-specific binding proteins were washed away. The purified truncated proteins were detected as a single clear band of the expected size in SDS-PAGE, and were further confirmed by the Western blot test. Our results suggest that the impossible expression of the full length RUS1 protein in *E. coli* can be expressed in truncated form, and inclusion bodies can be effectively solubilized.

Keywords:
Seamless cloning, inclusion body, renaturation, protein purification, Western blot

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INTRODUCTION

RUS1 (*ROOT UVB SENSITIVE1*) genes were first genetically identified in *Arabidopsis* as essential players in early seedling development (Tong *et al.* 2008). Further studies demonstrated that RUS1 genetically and biochemically interact with another RUS member, RUS2 (Leasure *et al.* 2009). *RUS1* and *RUS2*, also named *WXR3* (*WEAK AUXIN RESPONSE 3*) and *WXR1* respectively, were independently identified for their roles in polar auxin transportation (Ge *et al.* 2010; Yu *et al.* 2013). More recently, *Arabidopsis* RUS4 was reported to play a role in male fertility through anther dehiscence by suppressing secondary thickening in the endothecium (Zhao *et al.* 2019). Bioinformatic analyses suggest that *RUS* genes are broadly distributed in almost all eukaryotes, including plants, fungi and animals (Leasure *et al.* 2009). One common feature of all *RUS* genes is that the proteins they encode contain the DUF647 domain (domain of unknown function 647). No other *RUS* genes have been experimentally characterized and very little is known about the biochemical functioning of these DUF647-containing proteins. Current research on the functions of *RUS* genes is mainly focused on *Arabidopsis* and rice (Yu *et al.* 2016), and the functions of *RUS* genes in non-plant organisms have not been reported to date. The *in vitro* expressed proteins for both RUS1 and RUS2 appeared to be rapidly degraded and the attempts to characterize them were thus unsuccessful (Leasure *et al.* 2009). The expression of RUS proteins in published reports was detected indirectly either through

GFP fluorescence or yeast two-hybrid systems (Tong *et al.* 2008; Leasure *et al.* 2009; Ge *et al.* 2010; Yu *et al.* 2013; Zhao *et al.* 2019). The biochemical characterizations of RUS proteins require a protein expression system which allows the heterologously expressed proteins to be solubilized, renatured and purified.

There are several heterologous protein expression systems including prokaryotic (Belenkaya *et al.* 2020), yeast (Baghban *et al.* 2019), mammalian and insect cell expression systems (Qu *et al.* 2020; Yi *et al.* 2020). Although prokaryotic expression systems lack the proper post-translation modifications for eukaryotic proteins, this expression system in *Escherichia coli*, for example, offers several advantages. These advantages include a clear genetic background, low costs, a high expression level, and relatively simple purification procedures. We thus selected the *E. coli* expression system as our first choice to heterologously express the RUS1 protein. The full length and truncated *Arabidopsis RUS1* fragments were seamlessly fused with *GFP* before cloning them into the prokaryotic expression vector pQE-100 which provides an N-terminal 6×His tag for expressed proteins. While the full length *RUS1-GFP* could not be expressed in *E. coli*, just as previously reported (Leasure *et al.* 2009), the truncated RUS1-GFP proteins can be expressed at high levels as aggregates in the inclusion bodies. We employed a denature-renature method which solubilized the aggregated protein and the purified truncated RUS1-GFP protein was further confirmed by Western blots using both the anti-AtRUS1 peptide antibody and anti-GFP antibody respectively.

MATERIALS AND METHODS

Strain, plasmid, and culture growth conditions. The *E. coli* XL10-Gold strain was used for constructs cloning and the *E. coli* M15 strain was used as the host for protein expression. The pGEM-T Easy vector was used for DNA fragment cloning and the pQE-100 was used as the prokaryotic expression vector. The *E. coli* XL10-Gold strain containing pGEM-T or pQE-100 was cultured on an LB plate or liquid medium with 100 μg/mL ampicillin at 37°C, and the *E. coli* M15 strain containing pQE-100 was cultured on an LB plate or liquid medium with 100 μg/mL ampicillin and 50 μg/mL kanamycin at 37°C. If cultured in the liquid medium, the cells were grown in a shaker at 250 rpm. When the OD600 reached around 0.6, the final concentration of 0.5 mM IPTG (isopropyl-1-thio-β-D-galactoside) was added to the liquid medium to induce protein expression. At the 0, 2, 4, 6 hr of induction, the OD600 was monitored by Nanodrop 2000 (Thermo Fisher), and the1 ml liquid medium was collected for subsequent analysis.

Construction of the prokaryotic expression vector for the full length and truncated RUS1-GFP. For the *RUS1-GFP* prokaryotic expression vector construction, the overlap-PCR approach was used to seamlessly fuse *AtRUS1 ORF* with *GFP ORF* (Zhang *et al.* 2010; Hou *et al.* 2021). In order to amplify the *AtRUS1 ORF* (At3g45890), the previously cloned pQE-100-*AtRUS1 ORF* was used as a template. The primers used to amplify this 1.8 kb gene were forward primer 1: 5′-AGGATCCATGAGTTGTTCTTATCTACTTTCTGG-3' and reverse primer 1: 5'- *TTCTCCTTTACTCAT*TGAGCT GTTTGGAGAAGATAATAATAG -3'. For amplifying the *GFP ORF*, the previously cloned pPZP222-*GFP* was used as a template. The primers used to amplify this 0.7 kb gene were forward primer 2: 5'-*ATGAGTAAAGGAGAA*GAACTTTTCACTG-3' and reverse primer 2: 5'- AGAGCTCTTATTTGTATAG TTCATCCATGCCATG-3'. The nucleotides in italics are the overlapping sequences for the seamless fusion of the *RUS1* and *GFP* fragments, and the underlined nucleotides are the introduced restriction sites for cloning. The 25 μL PCR reaction consisted of 1 μL template (1 ng of plasmid), 1.25 μL of each primer (5 μM), 2.5 μL dNTP mixture (2.5 mM each), 2.5 μL 10× Easy-A buffer, 0.5 μL easy-A high-fidelity DNA polymerase (Stratagene) (1.25U) and 16 μL autoclaved dH_2O. The reaction started with 2 min at 95°C to pre-denature the template, followed by 25 cycles of 30 s at 95°C, 30 s at 58°C, and 1 min/kbp at 72°C, and 5 min elongation at 72°C at the end. The above PCR products were run on 1% agarose gel, and bands of the expected size were cut and purified by a QIAquick gel extraction kit (Qiagen). The concentration of the purified PCR products was determined by Nanodrop 2000.

For the second round of PCR, the 20 μL PCR reaction was composed of the above *AtRUS1 ORF* and *GFP* products (each 25-30 ng), 2 μL dNTP mixture (2.5 mM each), 2 μL 10× Pfu buffer, 0.4 μL Pfu DNA polymerase (Promega) (1U) and the addition of autoclaved dH_2O to 20 μL. The reaction started with 2 min at 95°C for pre-denaturation of the template, followed by 10 cycles of 30 s at 95°C, 30 s at 52°C, 1 min/kbp at 72°C, and 5 min elongation at 72°C at the end.

The fused gene *Bam*HI-*AtRUS1 ORF-GFP-Sac*I was amplified by using forward primer 1 and reverse primer 2 in the third round of PCR. The 25 μL PCR reaction consisted of 2 μL second round PCR products, 1.25 μL of each primer (5 μM), 2.5 μL dNTP mixture (2.5 mM each), 2.5 μL 10× Easy-A buffer, 0.25 μl Easy-A high-fidelity DNA polymerase (1.25 U) and 15.25 μL autoclaved dH_2O. The reaction started with 2 min at 95°C for pre-denaturation of the template, followed by 25 cycles of 30 s at 95°C, 30 s at 58°C, 1 min/kbp at 72°C, and 5 min elongation at 72°C at the end. This PCR product was purified as above, and cloned into the pGEM-T Easy vector. After confirmation by sequencing, the fragment was further subcloned into pQE-100 to construct pQE-100-*Bam*HI-1-*AtRUS1 ORF*-1824-*GFP-Sac*I, also called full length *RUS1-GFP*.

For cloning of the truncated *Bam*HI-1183-*AtRUS1 ORF*-1824-*GFP-Sac*I, the forward primer 3: 5'-AGGATCC

ATGCTCAAATCTTACCAGTGTATCCAGC-3' and the reverse primer 2 were used to amplify the insert of the pQE-100-*Bam*HI-1-*AtRUS1 ORF*-1824-*GFP-Sac*I plasmid. The truncated *Bam*HI-1183-*AtRUS1 ORF*-1824-*GFP-Sac*I was first cloned into the pGEM-T Easy vector, and then further subcloned into pQE-100 to construct pQE-100-*Bam*HI-1183-*AtRUS1 ORF*-1824-*GFP-Sac*I, also called truncated *RUS1-GFP*. The remaining truncated RUS1 ORF lacks N terminal 1-394 amino acids, which contains cTP (chloroplast transit peptide) and four predicted transmembrane domains.

SDS-PAGE analysis of prokaryotic protein expression. Liquid cultures of the host M15 cells containing pQE-100-*Bam*HI-1-*AtRUS1 ORF*-1824-*GFP-Sac*I, or pQE-100-*Bam*HI-1183-*AtRUS1 ORF*-1824-*GFP-Sac*I were sampled at different time points before and after adding IPTG, and the cells were pelleted by centrifugation at 10000 rpm for 5 min. Various volumes of 1× sample treatment buffer (100 mM pH6.8 Tris-HCl, 100 mM DTT, 4% SDS, 20% glycerol, 2 mg/ml bromophenol blue) were then added to make the cell concentrations approximately the same. The samples were boiled for 10 min, and centrifuged at 12000 rpm for 10 min. 20 µL of supernatant was loaded into wells of 10% SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) with ProSieve colour protein marker. After electrophoretic separation, the protein bands were visualised by the Coomassie brilliant blue method (Laemmli 1970).

Purification and renaturation of the recombinant truncated RUS1-GFP protein. The following method for the solubilisation and purification of the truncated RUS1-GFP protein was modified from Holzinger *et al.* (1996). Briefly, after 6 hr IPTG induction, the cells were pelleted and washed with 1× phosphate buffered saline solution (PBS). The cells were then resuspended in 5 mL Tris-buffered saline solution (TBS: 150 mM NaCl, 20 mM Tris-HCl, pH 7.9) and broken through ultrasonication. The soluble and insoluble fractions (separated by centrifugation at 14000 rpm for 20 min) were analysed by SDS-PAGE and Coomassie brilliant blue staining (Laemmli 1970). The target proteins were found to be in the insoluble fraction, and subsequent purification procedures were performed under denaturing conditions.

The insoluble fraction was washed with 1×PBS 2 times, then dissolved in buffer 1 (6 M guanidine hydrochloride, 500 mM NaCl, 20 mM Tris-HCl, pH 7.9), by shaking for 30 min at room temperature. The supernatant was collected by centrifugation at 14000 rpm for 5 min, and then mixed with an equal volume of Ni-NTA slurry (Thermo Fisher) equilibrated with buffer 1 for 30 min at room temperature. The Ni-NTA slurry bound with the target proteins was washed 3 times with an equal volume of buffer 2 (6 M urea, 500 mM NaCl, 20 mM Tris-HCl, 20 mM imidazole, pH 7.9). The denatured protein bound with Ni-NTA was refolded by shaking with an equal volume of buffer 3 (150 mM NaCl, 20 mM Tris-HCl, pH 7.9) three times. Finally, the soluble target recombinant proteins were eluted with buffer 4 (150 mM NaCl, 20 mM Tris-HCl, 50 mM EDTA, pH 7.9). The purified protein was measured by Nanodrop 2000, and further verified by SDS-PAGE and Western blot.

Western blot of the truncated RUS1-GFP. The Western blot was performed according to Hou *et al.* (2005) with certain modifications. Different amounts of purified truncated RUS1-GFP hybrid proteins were loaded on 10% SDS-PAGE. After electrophoresis, the proteins on SDS-PAGE gel were electroblotted to a nitrocellulose membrane using the semi-dry method in the transfer buffer (25 mM Tris, 192 mM glycine, and 20% methanol) at 120 mA for 90 min. After blocking the membrane with 5% non-fat milk in PBS 2 hr at 4°C, the blot was probed with an anti-AtRUS1 peptide polyclonal antibody or anti-GFP polyclonal antibody as the primary antibody in PBS overnight at 4°C with shaking, washed three times (PBS with 0.025% IGEPAL CA-630), and incubated with horseradish peroxidase conjugated rabbit anti-goat IgG (Sigma-Aldrich) as the secondary antibody in PBS for 3 hr at 4°C by shaking. Visualisation was performed using SuperSignal West Dura (Thermo Fisher) following the manufacturer's protocol.

RESULTS

Full length and truncated *RUS1* seamlessly fused to *GFP* in the prokaryotic expression vector. The full length *Arabidopsis RUS1 ORF* without a stop codon was seamlessly fused with *GFP* by overlap-PCR (Figs. 1A & D). The truncated *RUS1-GFP* was amplified directly using the above full length *RUS1-GFP* as the template (Fig. 1B). These two fragments were cloned into the pGEM-T vector and confirmed by Sanger-sequencing. The confirmed gene fragments were subcloned into the prokaryotic expression vector pQE-100 (Qiagen) by the traditional digestion-ligation method. The positive clones were verified by digestion and sequencing (Fig. 1C). The confirmed pQE-100-*Bam*HI-1-*AtRUS1 ORF*-1824-*GFP-Sac*I and pQE-100-*Bam*HI-1183-*AtRUS1 ORF*-1824-*GFP-Sac*I were transformed into the *E. coli* host M15 for protein expression.

Inefficient expression of the full length RUS1-GFP in *E. coli*. The expression cassette of the full length RUS1-GFP is depicted in Fig. 2A. The length of this fused protein is 858 amino acids, and its predicted molecular weight is approximately 94.61 kD. The protein expression patterns before and after IPTG induction appeared to be the same, and the expected 94.61 kD band was not induced (Fig.

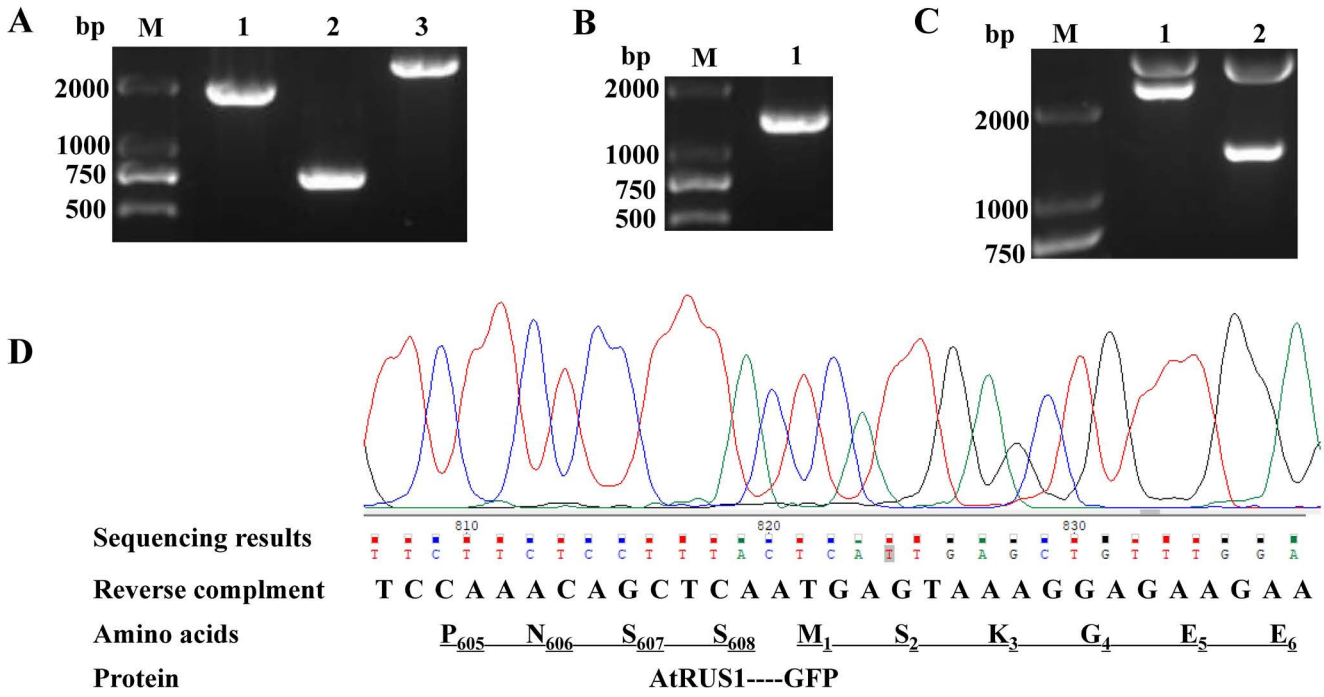

Fig. 1. Cloning of the full length and truncated *RUS1-GFP* into pQE-100. M is the DL2000 marker. **A**. Seamless fusion of the full length *RUS1* and *GFP* through overlap-PCR. Lanes 1 and 2 are the first round PCR, lane 1: PCR product of *1-AtRUS1 ORF*-1824, lane 2: PCR product 1-GFP-717-*Sac*I, lane 3 is the third round PCR, the PCR product of *Bam*HI-*1-AtRUS1 ORF*-1824-*GFP-Sac*I; **B**. Amplification of the truncated *RUS1-GFP*. Lane1: PCR product of *1183-AtRUS1 ORF*-1824-*GFP-Sac*I; **C**. Verification of the constructed prokaryotic expression vector by restriction enzyme digestion. Lane 1: pQE-100-*Bam*HI-*1-AtRUS1 ORF*-1824-*GFP-Sac*I digested by *Bam*HI and *Sac*I, lane 2: pQE-100-*Bam*HI-*1183-AtRUS1 ORF*-1824-*GFP-Sac*I digested by *Bam*HI and *Sac*I; **D**. The sequencing result confirms the seamless fusion of AtRUS1 and GFP. The number next to the amino acid is the original position of the amino acid in the AtRUS1 and GFP proteins.

2B). These results indicate that the full length RUS1-GFP could not be expressed in *E. coli*.

Truncated RUS1-GFP can be expressed as inclusion bodies in *E. coli* and purified through denaturation-renaturation treatment. The schematic expression cassette of the truncated RUS1-GFP is shown in Fig. 3A. The length of this fused protein is 464 amino acids, and its predicted molecular weight is approximately 52.64 kD. After induction by IPTG, the truncated RUS1-GFP was expressed at a high level (Fig. 3B). As shown in Fig. 3C (lanes 3 and 4), the induced truncated RUS1-GFP was found in the form of aggregates in the inclusion bodies in *E. coli*, and no soluble truncated RUS1-GFP protein was detected.

As there is a 6×His tag at the N-terminal of the truncated RUS1-GFP (Fig. 3A), Ni-NTA slurry was used to purify this protein through denaturation-renaturation treatment. The truncated RUS1-GFP was displayed as a clear band on SDS-PAGE (Fig. 3C, lane 5), indicating that this protein can be efficiently purified through the above process. The size of this purified protein is consistent with the predicted theoretical molecular weight of 52.64 kD of the truncated RUS1-GFP. To confirm that this band was indeed the truncated RUS1-GFP protein, Western blots using either an AtRUS1 peptide antibody or GFP antibody were conducted. As shown in Figs. 3D & E, both Western blots displayed a strong and clear band. These results confirmed that this purified protein was the truncated RUS1-GFP.

DISCUSSION

Prokaryotic protein expression systems are usually the first choice for protein heterologous expression because of their obvious advantages. For example, the envelope protein VP62 of the white spot syndrome virus, a protein with vaccine potential, was expressed and purified in *E. coli* (Lu *et al.* 2020). The novel antibacterial fusion protein cecropin B-human lysozyme was also expressed in *E. coli* (Zhang *et al.* 2019). Lin *et al.* (2017) reported that the functional SUMO-LsGRP1^{C} recombinant fusion protein was successfully expressed in the *E. coli* expression system as well. In this study, the full length *RUS1-GFP* was constructed into the prokaryotic expression vector pQE-100 in-frame with the N-terminal 6×His tag (Fig. 1). Our experiment demonstrated that the full length RUS1-GFP failed to be expressed in *E. coli* (Fig. 2). This is not

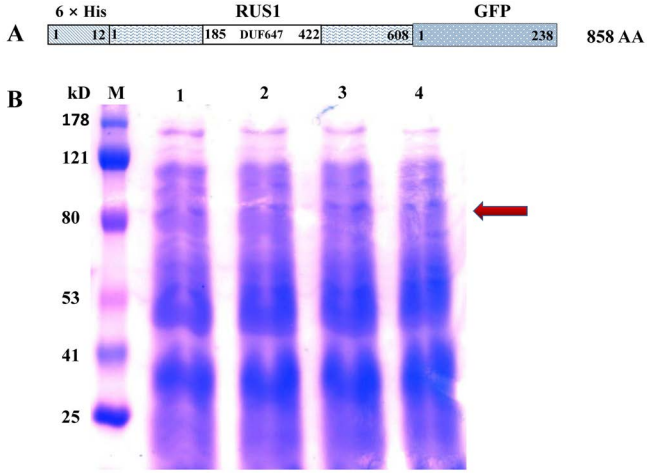

Fig. 2. Prokaryotic expression of the full length RUS1-GFP. **A**. Schematic structure of the full length RUS1-GFP hybrid protein. The names on the frame are the source proteins. The numbers in the frame are the start and end positions of the source proteins. The predicted DUF647 domain (185 to 422 amino acids) of RUS1 is indicated. 858 is the number of amino acids of the full length RUS1-GFP hybrid protein; **B**. Prokaryotic expression of the full length RUS1-GFP hybrid protein. The arrow shows the expected full length RUS1-GFP hybrid protein band. M: ProSieve colour protein marker; Lane 1: Proteins extracted from the *E. coli* cells right before adding IPTG(CK); Lanes 2-4: Proteins extracted from the *E. coli* cells after adding IPTG at 2 hr, 4 hr and 6 hr respectively.

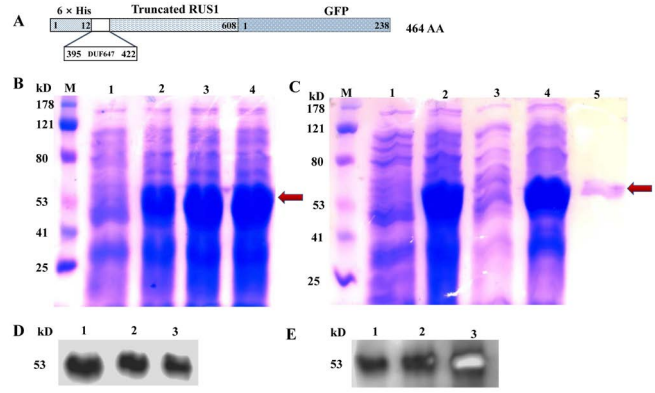

Fig. 3. Prokaryotic expression of the truncated RUS1-GFP. **A**. Schematic structure of the truncated RUS1-GFP hybrid protein. The names on the frame are the source proteins. The numbers in the frame are the start and end positions of the source proteins. The remaining predicted DUF647 (395 to 422 amino acids) of RUS1 is indicated. 464 is the number of amino acids of the truncated RUS1-GFP hybrid protein; **B**. Prokaryotic expression of the truncated RUS1-GFP hybrid protein. The arrow shows the truncated RUS1-GFP hybrid protein band. M: ProSieve colour protein marker; Lane 1: Proteins extracted from the *E. coli* cells right before adding IPTG (CK); Lanes 2-4: Proteins extracted from the *E. coli* cells after adding IPTG at 2 hr, 4 hr and 6 hr respectively; **C**. Truncated RUS1-GFP hybrid protein expressed as inclusion bodies and purification. M: ProSieve colour protein marker; Lane 1: Proteins extracted from the *E. coli* cells right before adding IPTG (CK); Lane 2: Proteins extracted from the *E. coli* cells after adding IPTG at 6 hr; Lane 3: Soluble proteins extracted from the ultrasonication of *E. coli* cells after adding IPTG at 6 hr; Lane 4: Proteins extracted from pellets after the ultrasonication of *E. coli* cells after adding IPTG at 6 hr; Lane 5: The purified truncated RUS1-GFP hybrid protein; **D**. Western blot using a GFP antibody as the first antibody. Lane 1: 75 ng purified truncated RUS1-GFP hybrid protein; Lane 2: 50 ng purified truncated RUS1-GFP hybrid protein; Lane 3: 25 ng purified truncated RUS1-GFP hybrid protein; **E**. Western blot using a RUS1 peptide antibody as the first antibody. Lane 1: 25 ng purified truncated RUS1-GFP hybrid protein; Lane 2: 50 ng purified truncated RUS1-GFP hybrid protein; Lane 3: 75 ng purified truncated RUS1-GFP hybrid protein.

surprising as the transmembrane domains in proteins are known to hinder protein expression in prokaryotic cells. For example, glycoprotein 5 from the porcine reproductive and respiratory syndrome virus and UL45 from the duck enteritis virus could not be successfully expressed until their transmembrane domains were deleted (Ren *et al*. 2010; Shen *et al*. 2010). We used two common transmembrane domain prediction software packages to predict the presence of transmembrane domains in the RUS1 protein. Four distinct potential transmembrane domains of RUS1 were predicted using the TMpred software (https://embnet.vital-it.ch/software/TMPRED_form.html, Hofmann & Stoffel 1993). The regions between amino acid positions 107 to 128, 219 to 242, 299 to 318, and 360 to 392 are predicted to be transmembrane regions. Five potential transmembrane domains of RUS1 were predicted using the HMMTOP software (http://www.enzim.hu/hmmtop/server/hmmtop.cgi, Tusnády & Simon 2001). The following regions between amino acid positions 236 to 254, 285 to 303, 308 to 325, 356 to 373, and 378 to 395 were also predicted by the HMMTOP software. Thus, when viewed together, it is highly likely that RUS1 is a transmembrane protein. We reasoned that the predicted transmembrane domains might be the main cause for the unsuccessful prokaryotic expression of the whole RUS1 protein. We thus applied strategies to express truncated RUS1-GFP without potential transmembrane domains. Our experiments demonstrated that the truncated RUS1 without the predicted transmembrane domains can be highly expressed in *E. coli* (Fig. 3).

E. coli-expressed proteins can usually be found in either of two forms: a soluble form and an insoluble form in the inclusion bodies (Vu *et al*. 2016). While soluble proteins usually have their natural conformation and maintain biological activity, proteins expressed as inclusion bodies are in their denatured form exhibit a loss of biological activity. Various factors are responsible for the formation of inclusion bodies (Fahnert *et al*. 2004). Some inclusion bodies can become soluble proteins

by altering the expression vectors or by changing the culture conditions such as culture temperature, IPTG concentration and induction times. However, other inclusion bodies remain insoluble proteins regardless of alterations to the expression vectors or culture conditions (Makrides 1996; Rabhi-Essafi *et al*. 2017; Lu *et al*. 2020). It is highly likely that the insolubility may be related to the lack of a post-translational modification mechanism and/or the lack of respective chaperon proteins for the target protein in the heterologous prokaryotic expression host *E. coli* (Sui *et al*. 2018). In this study, although various cultural conditions such as culturing temperature and final IPTG concentration were tested, the truncated RUS1-GFP could only be expressed as inclusion bodies. Various methods have been developed to solubilize and renature the denatured proteins in inclusion bodies. Mouse midkine was expressed in *E. coli* as inclusion bodies, and the highly purified recombinant mouse midkine with bioactivity was generated following denaturing, refolding, ion exchange chromatography and affinity chromatography (Gao & Wang 2015). Palm tree peroxidases were highly expressed in *E. coli* as inclusion bodies, and their enzyme activities were shown to be restored after renaturation (Yuan *et al*. 2021). Holzinger *et al*. (1996) reported a single-step purification/solubilisation method of recombinant proteins in inclusion bodies. In this study, the truncated RUS1-GFP expressed in the inclusion bodies was successfully solubilized and purified through an *in vitro* denature-renature approach using Ni-NTA as the affinity medium (Fig. 3). Our approach appears to be effective and the purified protein was confirmed as the truncated RUS1-GFP protein by Western blots (Figs. 3D & E).

CONCLUSION

To sum up, we compared the expressions of both a full length and a truncated RUS1-GFP in a prokaryotic expression vector. Whereas the full length RUS1-GFP could not be expressed, the truncated RUS1-GFP could be expressed as inclusion bodies in *E. coli*. Further, the truncated RUS1-GFP in the inclusion bodies could be solubilised, purified and renatured through an *in vitro* denature-renature approach. The availability of this truncated RUS1-GFP protein can be an important source for future experiments to dissect the biochemical roles of RUS1.

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Abbreviations: cTP (chloroplast transit peptide); DUF647 (domain of unknown function 647); GFP (green fluorescent protein); IPTG (isopropyl-1-thio-β-D-galactoside); *Ls*GRP1 (*Lilium* Stargazer glycine-rich protein 1); ORF (open reading frame); PBS (phosphate buffered saline); RUS (ROOT UVB SENSITIVE); SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis); SUMO (small ubiquitin-like modifier); *WXR* (*WEAK AUXIN RESPONSE*).

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REZIME

Botanica SERBICA

Prokariotska ekspresija i solubilizacija *Arabidopsis* ROOT UVB SENSITIVE 1 iz inkluzijskih tela u *Escherichia coli*

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RUS (ROOT UVB SENSITIVE 1) proteini koji se karakterišu unikatnim DUF647 domenom, široko su prisutni kod eukariota. Njihove funkcionalne uloge su uglavnom nepoznate osim mogućeg učešća *Arabidopsis* RUS1 i RUS2 u ranom razvoju klijanaca. Da bi se istražile biohemijske uloge RUS proteina, puna dužina i skraćeni *Arabidopsis RUS1* su besprekorno spojeni sa *GFP*-om i klonirani u prokariotski ekspresioni vektor pKE-100 koji omogućava proteine eksprimirane sa N-terminalnom oznakom 6×His. Ekspresija RUS1-GFP pune dužine nije mogla biti otkrivena nakon dodavanja induktora IPTG, dok je skraćeni RUS1-GFP eksprimiran na visokim nivoima i formirao inkluziona tela u *Escherichia coli*. Inkluziona tela su rastvorena u puferu za denaturaciju, a zatim je skraćeni RUS1-GFP fuzioni protein u supernatantu vezan za Ni-NTA suspenziju. Vezani proteini su eluirani nakon što su isprani nespecifični vezujući proteini. Prečišćeni skraćeni proteini su detektovani kao jedna čista traka očekivane veličine na SDS-PAGE, i dalje su potvrđeni Western blot metodom. Naši rezultati sugerišu da se nemoguća ekspresija RUS1 proteina pune dužine u *E. coli* može izraziti u skraćenom obliku, a inkluziona tela se mogu efikasno rastvoriti.

Ključne reči: Besprekorno kloniranje, inkluzivno telo, renaturacija, prečišćavanje proteina, Western blot