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Construction and expression of prokaryotic expression vectors fused with genes of *Magnaporthe oryzae* effector proteins and mCherry

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ABSTRACT. The aim of the current study was to investigate the prokaryotic expression of the *Magnaporthe oryzae* effector genes *BAS1* and *BAS4* fused to the fluorescent protein mCherry. Based on previous polymorphic analysis of *BAS1* and *BAS4* in rice blast strains using PCR, blast strains containing the PCR products of *BAS1* and *BAS4* were selected for liquid culture for total RNA extraction. For PCR analysis, cDNA was selected as a template to amplify the coding region of *BAS1* and *BAS4*, the plasmid pXY201 was selected as template to amplify the mCherry sequence, and the three sequences were cloned into pMD®19-T vectors. Positive recombinant plasmids were digested using two restriction enzymes and the cleaved fragments of *BAS1* and mCherry and *BAS4* and mCherry were ligated to pGEX-4T-1 vectors and expression was induced using IPTG. The PCR results

showed that the sequence sizes of *BAS1*, *BAS4,* and mCherry were 348, 309, and 711 bp, respectively, and these were cloned into pMD®19-T vectors. After digestion and gel purification, the fragments of *BAS1* and mCherry, *BAS4* and mCherry were ligated into pGEX-4T-1 vectors and expressed in *Escherichia coli* BL21 competent cells. The expressed proteins were approximately 60 kDa, corresponding to their theoretical size. Prokaryotic expression products of *BAS1* and *BAS4* fused to mCherry were presented in this study, providing a base for constructing prokaryotic expression vectors of pathogen effector genes fused to mCherry, which will contribute to further study of the subcellular localization, function, and protein interactions of these effectors.

Key words: *Magnaporthe oryzae*; Prokaryotic expression; Effector

