Quantitative Evaluation of Endogenous Reference Genes for ddPCR under Salt Stress Using a Moderate Halophile

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Abstract : Droplet digital PCR (ddPCR) is being increasingly adopted for gene detection and quantification because of its higher sensitivity and specificity. According to previous observations and our lab data, it is essential to use endogenous reference genes (RGs) when investigating gene expression at the mRNA level under salt stress. This study aimed to select and validate suitable RGs for gene expression under salt stress using ddPCR. Six candidate RGs were selected based on the tandem mass tag (TMT)-labeled quantitative proteomics of Alkalicoccus halolimnae at four salinities. The expression stability of these candidate genes was evaluated using statistical algorithms (geNorm, NormFinder, BestKeeper and RefFinder). There was a small fluctuation in cycle threshold (Ct) value and copy number of the pdp gene. Its expression stability was ranked in the vanguard of all algorithms, and was the most suitable RG for quantification of expression by both qPCR and ddPCR of A. halolimnae under salt stress. Single RG pdp and RG combinations were used to normalize the expression of ectA, ectB, ectC, and ectD under four salinities. The present study constitutes the first systematic analysis of endogenous RG selection for halophiles responding to salt stress. This work provides a valuable theory and an approach reference of internal control identification for ddPCR-based stress response models.

Keywords : endogenous reference gene, salt stress, ddPCR, RT-qPCR, Alkalicoccus halolimnae
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