

ReadMe File

1. Instrument information

RT-PCRs (Singleplex assay) were performed using Applied Biosystems 7500 Fast system.

The housekeeping gene 16S rRNA served as an internal standard. a control reaction without added cDNA was run as a negative control

2. Data processing methods

The PCR was programmed starting with an initial activation step at 95°C for 2 min, followed by amplification for 40 cycles at 95°C for 15 s, 30 s at various annealing temperatures, depending on the melting temperature of the set of primers (Table S1), and 72°C for 11 s. The specificity of amplification for each product was determined by a melting curve analysis at 95°C for 5 s and 60°C for 15 s, followed by a progressive increase of the temperature to 95°C with a ramp rate of 0.11°C s⁻¹, with continued measurement of fluorescence, and finally cooling of the plate at 40°C for 30 s.

5. Analysis methods

Expression of target genes (prfA, sigB, rsbR, gadD, lmo0799) in the treatment group relative to that in control group, normalized to 16S rRNA housekeeping gene, were calculated using delta-delta CT (2- $\Delta\Delta$ Ct) method. Calculations were carried out following the advanced relative quantification settings of the Applied biosystem fast 7500 software program. Relative expression of each gene was calculated by comparison of its expression relative to that of the 16S rRNA gene. Results were expressed as Log₂ Fold (2- $\Delta\Delta$ CT). All transcription analyses were done at least twice. RAW data with technical replicates submitted in excel format.