A 16S rDNA-based quantitative assay for monitoring *Lactobacillus plantarum* in silage

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Introduction Ensilage of herbaceous biomass can be enhanced by applying pre-selected fermentative bacteria, however insufficient is known about the population dynamics of such starter cultures under a range of ensiling conditions. Classical methods for species-specific quantification of bacteria are labour intensive. An alternative approach is the detection of bacteria based on molecular markers for species-specific regions within their genomic DNA (e.g. the 16S rDNA sequence). In this study, a quantitative marker assay using the real-time PCR technique (Q-PCR) is described for *Lactobacillus plantarum*, a bacterium often used for silage starter cultures.

Materials and methods Based on a variable region in the 16S rDNA of *L. plantarum* (Chagnaud *et al.,* 2001), the following PCR-primers were developed: forward primer Lplan-vreg1-F 5'-TTACATTTGAGTGAGTGGCG AACT, reverse primer Lplan-vreg1-R 5'-AGGTGTTATCCCCCGCTTCT, TaqMan® probe Lplrh-vreg1-T 5'-VIC®-GTGAGTAACACGTGGGWAACCTGCCC-TAMRA®. Q-PCR was performed in triplicate using an ABI 7000 and the following conditions: reaction mixture 1x JumpStart™ *Taq* ReadyMix™, 900nM forward primer, 300 nM reverse primer, 200 nM TaqMan® probe, 0.25 µl internal dye ROX®, *ad* 25 µl H_2O_{dd}; cycle regime 1: 120s 94°C, 2: 15s 94°C, 3: 60s 59°C, 4: 60s 72°C, 2-4 were repeated 40x. Plasmid pATB875 containing a 1500 bp fragment of *L. plantarum* 16S rDNA was used as a standard. Grass was inoculated with equal concentrations of *L. plantarum* ATB-8 and *L. rhamnosus* ATB-14 and sampled on days 2, 13, 20 and 40 of ensilage. The genomic DNA was prepared from paddled samples (Rheims & Stackebrandt, 1999).

Results Using the pATB875 plasmid as a standard, an optimised Q-PCR protocol was developed. From a triplicate dilution series an equation for the species-specific estimation of the copy number of *L. plantarum* 16S rDNA sequences in unknown samples was calculated (Figure 1A, B). Applied to grass silage samples, the assay monitored the rise of the *L. plantarum* population during the first two weeks of ensiling. Due to the increased acidification, the population decreased during prolonged ensiling (Figure 1C).

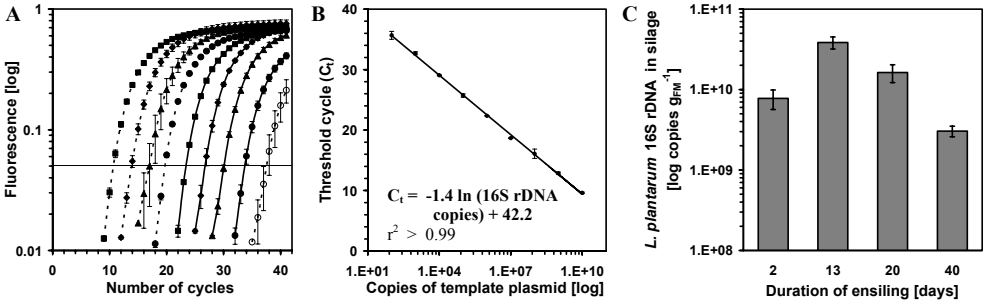

Figure 1 (A) Representative Q-PCR amplification curves of pATB875 10 fold dilution series (10^{10} (···■···) to 10^3 copies (—●—) and no-template control (○). (B) The mean C_t values of triplicates were plotted against the copy number of pATB875. (C) Determination of *L. plantarum* 16S rDNA copy number within grass silage samples.

Conclusions This Q-PCR assay is a first attempt at a direct DNA-based quantification of *L. plantarum* within silages. The Q-PCR assay enables the analyses of population dynamics of starter cultures containing *L. plantarum*. Similar approaches should also be applicable for monitoring other fermentative bacteria species.

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References

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