

Identification of Forage Ensiling Lactic Acid Bacteria from Silage

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Lactic acid bacteria (LAB) inoculants used to accelerate forage ensiling are not currently produced in Sri Lanka. This study aims to extract genomic DNA from LAB present in silage to precisely identify the species with the goal of producing silage inoculants locally. Silage samples were collected from the Central, North Central and Northern provinces and stored at -4° C. Each sample (10 g) was aseptically homogenized with sterile distill water (90 mL) in plastic bag using a stomacher (3 min, high speed). Supernatants were serially diluted with peptone water, surface-plated on MRS agar, and incubated (37° C, 48 h). The colonies presenting typical LAB morphology (white, round, entire margins) were streaked on MRS agar. Further, Gram staining, mobility, and catalase activity of the pure-cultures were assessed. All the isolates were Gram positive, non-mobile, and negative for catalase, confirming them as LAB. The isolates were frozen (-20° C) in 20% (v/v) MRS glycerol broth. Genomic DNA of the isolates was extracted using the Presto™ Mini gDNA Bacteria Kit. The quality of genomic DNA was determined using 1% agarose gel electrophoresis and a NanoDrop spectrophotometer. The DNA concentration of the extracts ranged between 12.20 and 34.60 ng/μL with a mean value of 20.80±2.20 ng/μL. Additionally, the ratio of absorbance at 260/280 nm ranged between 1.31 and 1.99 with a mean value of 1.70±0.08. The PCR success rate was 100%. The PCR products representing forage species and province were selected for the DNA sequence analysis. The 16S rRNA gene was performed using the 27f (forward) and 1492r (reverse) universal primers. The NCBI database and BLAST search were used to identify the species of the isolates. The DNA sequencing analysis resulted identification of *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Lactobacillus paracasei* with 100% query cover and percentage identity. Meanwhile, the query cover and percentage identity for *Lactobacillus oris* were 99% and 94.81%, respectively. The identified LAB species can be used to produce inoculants for forage ensiling.

Keywords: Concentration, DNA Extraction, Gel electrophoresis, NanoDrop Spectrophotometer

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