Protocol Standardization for Total DNA Extraction of Tropical Silages

Preparing 16S rRNA Analysis for Microbial Community Characterization

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Introduction

- The conservation success of silages depends highly on the microbial activity in the forage.
- Hence, it is of great interest to characterize the microbial communities of silages of different qualities.
- Here, an approach with molecular tools is presented.
- A problem which arises when working with genetic markers in products of spontaneous fermentation is often to obtain amplifiable DNA (desoxyribonucleic acid).
- In the present study three DNA extraction methods, two traditional ones and one with a commercial kit, were evaluated.
- The silage samples consisted of the tropical forage legumes *Canavalia brasiliensis* and *Vigna unguiculata* and sweet potato tubers (*Ipomoea batatas*) solely or in mixture.
- The objective was to determine the method unifying best feasibility and efficiency to quantify and to amplify DNA using primers for community analysis by PCR (polymerase chain reaction).

Materials and Methods

- 40 g each of a total of 20 silage samples were macerated, liofilized and again macerated until obtaining a fine powder for the DNA extraction.

Figure 1. Treatment of silage samples prior to ADN extraction

Three methods for DNA extraction were compared:

Method a)

SDS (sodium dodecyl sulfate) and proteinase K

- Extraction buffer containig 1.4 M NaCl, 20 mM EDTA (ethylenediaminetetraacetic acid), 100 mM Tris-HCl (pH 8.0) and 1 % SDS, and 1.5 µl of proteinase K (10 mg/ml) was added, mixed by vortex and incubated at 65 ºC for 1 h.
- Further steps with amonio acetate 7.5 M, chloroform-isoamyl (24:1) and cold isopropanol followed.
- The next day the preparation was washed with cool ethanol (70 %) two times to finally obtain the dried pellet. The pellet was resuspended in 50 µl TE buffer 1:10 (composed of Tris and EDTA). RNAse (1 µl) was added and incubated at 37 ºC for 30-60 min.

Method b)

CTAB (cetrimonium bromide)

- Extraction buffer, containing 2 % CTAB, EDTA 20 mM (pH 8.0), Tris-HCl 100 mM (pH 8.0), NaCl 1.4 M, PVP-40 1% (polyvinylpyrolidone).
- The following steps were performed with phenole:chloroform :isoamyl alcohol (25:24:1), RNAse A and chloroform:isoamyl alcohol (24:1).
- The nucleic acid was precipitated. The pellet was washed cautiously and resuspended in TE 50:10. It was incubated and washed again. Finally, the dried pellet was resuspended in 50 µl TE buffer 10:1 and incubated at 4 ºC for one night.

Method c)

a commercial kit for the DNA extraction of soil

- samples were processed following the instructions of the producer for the total extraction of genomic DNA.

In common, DNA extracted by either of the three methods, was visualized by horizontal gel electrophoresis. DNA concentration of the samples was quantified by a fluorometer.
For the amplification of the phylogenetically conserved region of the 16S rRNA of bacteria by PCR, the universal primers Com 1 and Com 2-Ph were used.

Results and Discussion

❖The different extraction methods show that with method a) DNA could be extracted, however with some pollutions, with method b) DNA was extracted neatly and with method c) extremely little DNA if at all was recovered.

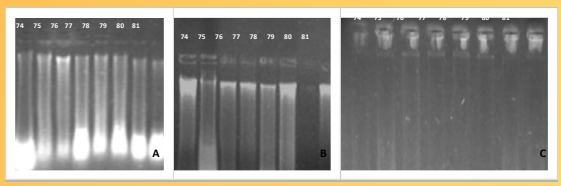

Figure 2. Total DNA extraction of tropical silage using different methods. a) SDS and Proteinase K. b) CTAB and c) Comercial kit.

❖ After running PCR it became obvious that only DNA samples of method b) could be amplified.

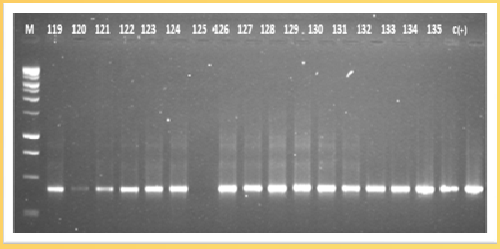

Figure 3. Amplification with Com1 and Com 2-Ph primers of DNA extracted by CTAB method. The numbers are samples and **(M)** is a 1 KB DNA Ladder by Promega®.

Conclusions

✓ The method using CTAB resulted in being the method of choice as DNA of high molecular weight could be obtained with high purity, free from inhibitors or contaminants, amplifiable for the following steps to finally molecularly characterize the bacterial community of silage samples by single strand conformation polymorphism (SSCP), besides being an economic technique.