A first screening of LAB strains as inoculants for tropical legume silages

Siriwan Martens and Javier Abello

Siriwan Martens

Centro Internacional de Agricultura Tropical (CIAT), A.A. 6713, Cali, Colombia
s.martens@cgiar.org

1. INTRODUCTION

- In temperate to subtropical regions starter cultures based on lactic acid bacteria (LAB) are well established for ensiling (Fig.1) and their effectiveness is approved in many cases.
- On the contrary, the availability of biological silage additives in the tropics is very limited and the range of efficacy often unknown.
- Ensiling conditions and objectives frequently differ from those in temperate regions, regarding e.g. secondary compounds in tropical legumes, C4-grasses, roots and tubers, higher ambient temperatures and different target animals to the point of non-ruminants.
- Thus, the screening for suitable inoculants is justified.

Fig. 1: Example of diversity of biological silage additives in Europe (Photo by Limpinsel/JKI Braunschweig)

2. MATERIALS & METHODS

- LAB for screening were obtained from the Rostock Fermentation Test (RFT) or from lab scale silages (Fig. 3) respectively.
- Those were made from *Vigna unguiculata* and *Canavalia brasiliensis* (Fig. 2) harvested at different ages (6-20 weeks) solely or mixed with sweet potato tubers (*Ipomoea batatas*) and stored at 25 ºC for 3 months.
- After opening for quality evaluation LAB were cultivated on solid MRS or Rogosa medium respectively.
- Alternatively LAB were recuperated from the RFT performed with the same fresh material.
- The LAB were isolated randomly and stored at -80 ºC.
- The isolates were evaluated using the RFT with *Canavalia brasiliensis*, which is difficult to ensile:
- Prior to the in-vitro study LAB strains were grown in MRS broth for one day.
- For the RFT for each treatment and triplicate 50 g of macerated forage was weighed into a beaker, 2 % sucrose (FM-base) was added and 200 ml distilled water poured on top. A fixed volume of various LAB in MRS broth was inoculated into the medium.
- A commercially available *Pediococcus acidilactici* strain served as control.
- The samples, covered with a sterilized cover, were incubated at 35 ºC for two days (Fig. 4).
- pH as indicator for the acidic fermentation was measured after 0, 20, 26, 43 and 51 h of incubation (Fig. 5).

Figure 2: *Canavalia brasiliensis*

Figure 3: Lab scale silos (PVC tubes)

Fig. 4: *In-vitro* cultures (RFT) in incubator

Fig. 5: pH measurement

2. RESULTS & DISCUSSION

- The results from 9 out of a total of 21 tested LAB strains is shown in the Figure 6. The graph reflects the pH development starting at 10 h of incubation. The initial pH at 0h was 5.6.
- In Table 1 the OD600 (20h) of the different strains prior to inoculation is listed.
- Slow reproduction in MRS broth resulted in similar performance in the legume medium.
- Several strains rapidly fermented the forage, five out of nine were faster than the commercial product.

Table 1: OD_{600} (20h) of the different LAB strains prior to inoculation in silage medium

Isolate nº	Tiii 15	Tiii 5.2	S 94.1	Ti 5	Tiv 4.8	Tiv 17.6	Tvi 21.3	S 15.5	S 66.7
OD_{600} 20 h	2.0	2.0	1.9	0.1	2.0	2.0	0.7	2.0	2.0

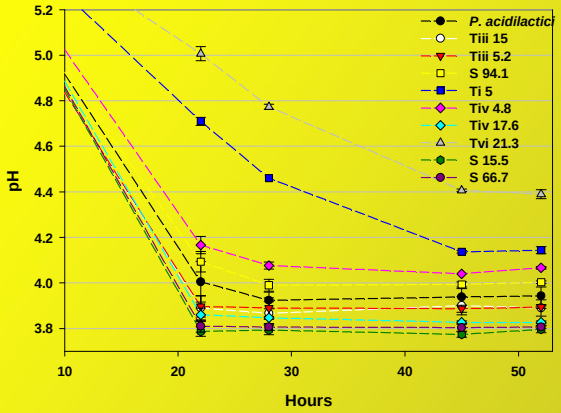

Figure 6: pH development in *Canavalia brasiliensis* medium inoculated with different LAB isolates during 2 days of incubation at 35 ºC

4. CONCLUSIONS & OUTLOOK

- Among the LAB strains isolated from tropical silages there are some promising candidates.
- Their further evaluation as potential starter culture in terms of silage quality, aerobic stability, reduction of anti-nutritive compounds, influence on amino acid pattern as important criteria for monogastric feeding is envisaged.
- As next step the LAB shall be characterized phylogenetically by molecular tools (PCR-SSCP, single strand conformation polymorphism) to avoid working with duplicates.