

1. Rationale

- Fermentation is one of the oldest biotechnologies utilized by man for food conservation. A huge diversity of sour traditional food has derived from it throughout the world, e.g., chuno, kenkey, uji, kimchi, miso and yoghurt.
- The microbial group dominant in lactic acid fermented food and feed belongs to the lactic acid bacteria (LAB), such as the genera *Lactobacillus* (Fig.1), *Pediococcus* (Fig.2), *Enterococcus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*.
- Many desirable attributes have been demonstrated for various LAB strains, i.e. ◊ antimicrobial effects, ◊ decomposing anti-nutritional factors, ◊ increasing ratio of essential amino acids, ◊ probiotic effects and others.
- The utilization of starter cultures has improved and homogenized product quality and safety.



Figure 1: *Lactobacillus*, electron micrograph (by Smith, Guelph, Canada)

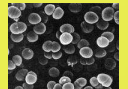


Figure 2: *Pediococcus* (by vietsciences.free.fr)

- *Tropical forages, cassava and sweet potato often have restricted value as fresh feed for non-ruminant farm animals due to their limited storage life and/or their anti-nutritive compounds. However, ensiled with selected starter cultures, they may turn into a desirable supplement at relatively low cost.*

2. Objective

- Relate distinct microbial ecosystems of different tropical silages to their chemical quality.
- Test epiphytic LAB strains isolated from tropical silages for their fermentation performance and other beneficial traits.



Figure 3: Lab scale silos (PVC tubes)

3. Materials & Methods

- Forage of *Canavalia brasiliensis* CIAT17009 and *Vigna unguiculata* 9611 and sweet potato roots var. Tainung-69 were ensiled solely or in mixture in triplicates in lab scale silos (Fig.3).
- After 3 months storage the silages were evaluated according to a scheme on organoleptic traits and chemical analyses.
- Samples were taken for microbiological investigation, serving two strategies:

Strategy A

- Total DNA-extraction of silage
- Com- and LAB specific PCR targeting bacterial 16S ribosomal RNA
- Com1-Primer (5'-3'): CAG CAG CCG CGG TAA TAC
- Com2p-Primer (5'-3'): CCG TCA ATT CCT TTG AGT TT
- Microbial community analysis by Single-Strand-Conformation-Polymorphism (SSCP)

Strategy B

- Random isolation of LAB strains from the silages grown on selective solid media
- Inoculation of LAB strains from different silages in *in-vitro* cultures
- Assessing their potential for rapid acidification
- Comparison of strains by genetic fingerprint (see A)
- Applying promising strains *in vivo* on lab-scale to screen for further desirable traits.

4. Results

- Some early results are shown below.

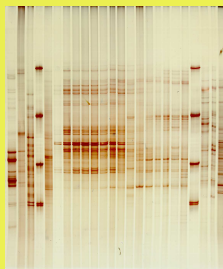


Fig. 4: Bacterial communities visualized by PCR-SSCP

A

- Total DNA of all silages has been extracted.
- Amplifiable PCR-products for community analysis have been obtained.
- Conditions for vertical gel electrophoresis (Fig. 4) are being optimized.

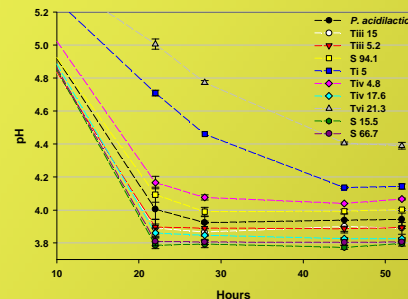


Figure 5: pH development in *Canavalia brasiliensis* medium inoculated with different LAB isolates during 2 days of incubation at 35 °C

B

- Five out of 9 selected LAB strains showed an *in-vitro* acidification rate even better than the control, a European *Pediococcus* based silage additive product (Fig.5).

5. Conclusions & Outlook

- The epiphytic bacterial stocking, once visualized by vertical electrophoresis, will be analyzed for their phylogenetic relatedness. The particular influence on silage quality by bacteria will be assessed regarding the biochemical pathways.
- Some LAB strains that have been isolated from tropical silages have revealed as promising candidates for further evaluation as potential starter cultures.