# Characterization and identification of lactic acid bacteria isolated from silage prepared in Lao PDR

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**Keywords:** Lactic acid bacteria, 16S rRNA, silage.

## Introduction

Silage is now the most common preserved cattle feed in many countries and lactic acid bacteria (LAB) play an important role during silage fermentation process. In order to establish an animal feed production system to cover the shortage of animal feed in the dry season of Lao PDR, silage fermentation technology is needed that has potential application in poor villages. Therefore, the characterization of LAB in various forage crops and their species identification requires further study. In the present study, the characterization of LAB species isolated from forage crops in Lao PDR was examined. In order to determine their taxonomic status, these strains were also studied by 16S rRNA sequence analysis.

## Materials and methods

Silage samples were prepared in Vientiane, Lao PDR using corn (*Zea mays*), paddy rice (*Oryza sativa)* straw and sugarcane (*Saccharum officinarum*) tops. LAB were isolated on MRS agar (Difco Laboratories, Michigan, USA). Gram stain, morphology, catalase activity, spore formation, motility, nitrate reduction and gas production from glucose were determined. Carbohydrate fermentation patterns were examined using AP 50 CH strips. The isomers of lactate formed from glucose were determined enzymatically with reagents obtained from Boehringer GmbH, Mannheim, Germany. The 16S rRNA sequence was analyzed as described by Cai *et al*. (1999; 2012).

## Results and Discussion

Seven representative strains of lactic acid bacteria were isolated from silage in Vientiane, Laos (Table 1). These strains were Gram-positive, catalase-negative bacteria. Strains LA1 and LA 46 were homofermentative rods that formed DL-lactic acid. Strain LA 1 could grow in low pH condition (3.5), but Strain LA 46 did not grow below pH 4.5. Strains LA 3 and LA 8 were homoferementative cocci that formed L(+) lactic acid. Strains LA 22 and LA 37 were heterofermentative cocci that formed D(-) lactic acid. Strain LA 40 was homofermentative cocci that formed DL lactic acid, and could grow at pH 3.5. Based on 16S rRNA gene sequence analysis, these isolates were identified as *Lactobacillus plantarum, Enterocuccus faecalis, Leuconostoc mesenteroides*, *Leuconostoc mesenteroides*, *Lactococcus lactis* subsp. *Lactis, Weissella confusa, Pediococcus pentosus*, *Lactobacillus brevis,* respectively.

In the present study, the isolates were Gram-positive and catalase-negative cocci or rod that produced lactate from glucose, formed approximately equal quantities of L(+) , DL and D(-) lactic acid. These properties showed that these strains belong to the genus of LAB. However, as available phenotypic procedures are difficult to assign isolates to known species because it is difficult to differentiate easily and clearly between species of LAB (Cai 1999; Ennahar *et al*. 2003; Pang *et al*. 2011). Based on 16S rRNA gene sequence analysis, these isolates could be assigned to species level.

It is well established that LAB play an important role in silage fermentation and animal health. LAB are naturally present on forage crops, silage and animal intestines, therefore collection and identification of LAB isolated from silage is important. In the present study, *Lactobacillus plantarum* and *Weissella confusa* were the most dominant LAB in the silage. Isolation and identification of the LAB strains are key first steps for future research in which the relationship between LAB species and silage fermentation is being evaluated.

## Conclusions

Seven representative strains of lactic acid bacteria were isolated from silage in Vientiane, Lao PDR. These strains were Gram-positive, catalase-negative cocci or rod, they are homo or heterofermentative

**Table 1. Characteristics of lactic acid bacteria isolated from silage in Laos. Isolates were *Lactobacillus plantarum* LA 1 (1), *Enterococcus faecalis* LA 8 (2), *Leuconostoc pseudo-mensenteroides* LA 22 (3), *Lactococcus lactis* subsp. *lactis* LA 3 (4), *Weissella confusa* LA 37 (5), *Pediococcus pentosus* LA 40 (6), and *Lactobacillus brevis* LA 46 (7). All strains were Gram-positive and catalase-negative bacteria. +, positive; -, negative; w, weakly positive.**

| Character | Isolate 1 | Isolate 2 | Isolate 3 | Isolate 3 | Isolate 5 | Isolate 6 | Isolate 7 |
|---|---|---|---|---|---|---|---|
| Cell form | Rod | Cocci | Cocci | Cocci | Cocci | Cocci | Rod |
| Fermentation type | Homo | Homo | Hetero | Homo | Hetero | Homo | Hetero |
| Lactate isomer | DL | L(+) | D(-) | L(+) | D(-) | DL | DL |
| Growth at temperature | | | | | | | |
| 15 ° | + | + | + | + | + | + | + |
| 45 ° | + | + | w | + | + | + | + |
| Growth at pH | | | | | | | |
| 3.5 | + | - | - | - | - | + | - |
| 4.0 | + | - | - | - | - | + | - |
| 4.5 | + | + | - | + | w | + | w |
| Growth at MRS medium | | | | | | | |
| OD 620 | 2.2 | 0.8 | 0.7 | 0.7 | 0.9 | 1.7 | 1.7 |
| Lactate production (g/L) | 14.5 | 8.7 | 8.9 | 8.8 | 8.1 | 12.3 | 11.2 |
| Final pH | 3.6 | 4.7 | 4.6 | 4.7 | 4.6 | 3.8 | 3.7 |
| Similarity of 16S rRNA sequence | 99.6 | 99.7 | 99.2 | 99.5 | 99.8 | 99.7 | 99.1 |

type and formed L(+), DL or D(-) lactic acid. Based on 16S rRNA gene sequence analysis, these isolates were identified as species of *Lactobacillus, Enterocuccus, Leuconostoc, Weissella, Lactococcus and peidococcus,* respectively. *Lactobacillus plantarum* and *Weissella confusa* were the mostly dominate members of the population in these silages. The relationship between LAB species and silage fermentation is currently in progress.

## References


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