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# A lactic acid bacterium isolated from grass in native grassland in Northern China

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## Introduction

The epiphytic LAB converts sugar into lactic acid during the ensiling process. As a result, the pH is reduced, and the forage is preserved. Therefore, further study of epiphytic LAB species is required, especially the screening of excellent LAB. However, to our knowledge, limited information is available on the epiphytic microflora on grass in native grassland. The present study set out to screen, isolate and identify the LAB from grass silages made in native grassland in northern China.

## Materials and methods

Grass was harvested and silages were made in native grassland at Guyuan Grassland Research Station of China Agricultural University. Lactic acid bacteria were isolated and identified according to Y Cai (1999). The sequence was aligned with the following published sequences from NCBI: *Lactobacillus suebicus* strain CECT 5917, *Lactobacillus fabifermentans* strain : LMG 24284, *Lactobacillus paraplantarum* strain DSM 10667, *Lactobacillus plantarum* strain NRRL B-14768, *Lactobacillus pentosus* strain 124-2, *Lactobacillus plantarum* WCFS1 strain WCFS1, *Lactobacillus plantarum* subsp. *argentoratensis* strain: DK0 22, *Lactobacillus versmoldensis* strain KU-3. *Leymus chinensis* was harvested and ensiled at native grassland in northern China and fermentation characteristics of silage treated with ZC3 and PS (Lalsil PS, a commercial inoculant produced by Lallemand inc.) were determined after 30 days ensilage.

## Results and discussion

The lactic acid bacteria (ZC3) isolated from native grassland was gram-positive and catalase-negative rod bacterium. The carbohydrate fermentation patterns of ZC3 were examined and it produced acid from lactose, glucose, maltose, fructose, galactose, sucrose and did not produce acid from starch. Positive results were shown in gluconate, cellobiose, salicin, mannitol. More than 1,500 bases of 16S rRNA of ZC3 was determined. The phylogenetic tree shown in Figure 1 was constructed from evolutionary distances by the neighbor-joining method. *Lactobacillus pentosus* strain 124-2 was the specy most closely related to the ZC3, and they showed a high sequence homology value (98%) with each other. Fermentation characteristics of *Leymus chinensis* silage treated with ZC3 and PS were shown in Table 1. ZC3 significantly ( $P < 0.05$ ) decreased the pH and improved the fermentation quality.

## Conclusions

ZC3 was an excellent lactic acid bacteria isolated from native grassland of China and it was identified as *Lactobacillus pentosus* strain.

## References

Y Cai, S Kumai, M Ogawa, *et al* (1999) Characterization and identification of *Pediococcus* species isolated from forage crops and their application for silage preparation. *Applied and Environmental Microbiology*, **65**, 2901–2906.

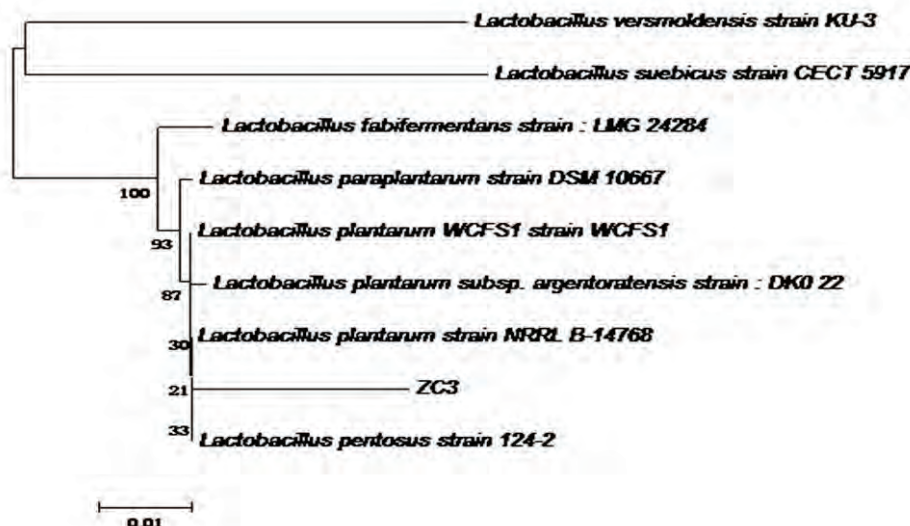


Figure 1. Phylogenetic tree derived from 16S ribosomal DNA sequence. The tree was created by the neighbor-joining method with Knuc values. The numbers indicate bootstrap values for the branch points.

**Table 1. Fermentation characteristics of *Leymus chinensis* silage treated with ZC3 and PS.**

Treatments	pH	Lactic acid	Acetic acid	Propionic acid	Lactic acid: Acetic acid
CK	4.63 ± 0.01 a	5.52 ± 0.13 b	4.22 ± 0.26 a	1.09 ± 0.07 b	1.31 ± 0.05 b
ZC3	4.36 ± 0.01 b	6.62 ± 0.18 a	4.12 ± 0.07 a	1.17 ± 0.04 b	1.61 ± 0.03 a
PS	4.63 ± 0.02 a	6.37 ± 0.20 a	4.42 ± 0.19 a	1.49 ± 0.04 a	1.51 ± 0.03 a

Values followed by different letters show significant differences among treatments  $P < 0.05$ .