

Characterization and identification of lactic acid bacteria isolated from alfalfa silages

Q. Z. Sun¹, H. J. Zhang², X. L. Wang³, X. Y. Hou¹, Y. M. Cai⁴

¹CAAS, Grassland Research Institute, Hohhot, China, ² CAAS, Graduate School, Beijing, China,

³CAAS, Institute of Lanzhou Animal Science and Veterinary Pharmaceutics, Lanzhou, China,

⁴National Institute of Livestock and Grassland Science, Nasushiobara, Japan

Email: sunqz@ 126.com

Introduction Alfalfa (*Medicago sativa*) are now widely used for silage preparation in China. Usually, Lactic acid bacteria (LAB) present on forage crops and grass play an important role during silage fermentation and silage contains many kinds of LAB, including *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Lactococcus*, *Streptococcus* and *Weissella* (Cai et al., 1998). In this study, LAB isolated from alfalfa silages was characterized.

Materials and Methods Six alfalfa (*Medicago sativa*) cultivars were obtained from Linxi, Inner Mongolia in July 2009. Alfalfa were cut into about 2-3cm after mowing and appropriate drying, and put it into polyethylene bags about 150 g per bag, then exhaust air in the bag. LAB were separated and identified after ensiling 1, 3, 45 days. Gram stain of LAB and morphological characteristics were determined after 24h of incubation on MRS agar. Catalase activity and gas production from glucose were determined. Growth at different temperatures was detected in MRS broth after incubation at 10°C and 15°C for 14 d, and at 40°C and 45°C for 7d. Growth at pH 3.0, 3.5, 4.0, 4.5, 5.0, 7.5, 8.0 was observed in MRS broth after incubation at 37°C for 7d. Salt tolerance of LAB was tested in MRS broth containing 3.0% and 6.5% NaCl. Carbohydrate assimilation and fermentation of 49 different compounds with one control were identified on API strips (Ennahar et al., 2003).

Results and Discussion 39 strains of LAB were isolated from alfalfa silage and they were all gram-positive, catalase negative and homofermentative LAB. These strains growth well at 10°C, pH 5.0 and 7.5, and in MRS broth containing 3.0% and 6.5% NaCl. All strains grew weakly or could not grow at 5°C, 10°C, 40°C, 45°C and pH 3.0~8.0 in addition that strain GI44 grew weakly at 40°C and pH 3.5, 4.5, and could not grow at 45°C, pH 3.0, 4.0, and 8.0. These LAB isolates were divided into seven groups (A-G) according to their morphological and biochemical characters. Strains of group A and B belong to the cluster of genus *Pediococcus*. Strains of group C、D and E were identified as *Enterococcus*. Group F and G strains were placed in *Lactobacillus*.

Conclusions The results confirmed that silage contains abundant kinds of LAB, and they all have different characteristics.

References

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Table 1. Alfalfa cultivars and LAB strains isolated from alfalfa silage

Samples	Cultivars	Kinds	Strain Number
1	Rangelander	<i>Medicago varia</i> Martin	GI14、 15、 16、 17
2	Algonquin	<i>Medicago sativa</i> L.	GI18、 19、 20、 21、 23、 24、 25
3	Rambler	<i>Medicago sativa</i> L.	GI46、 47、 48、 49、 62
4	Zhungeer	<i>Medicago sativa</i> L.	GI26、 27、 28、 29、 30、 31、 32、 33、 34、 35、 36
5	Gannong No.3	<i>Medicago sativa</i> L.	GI37、 38、 39、 40、 42、 44、 45、 69
6	Aohan	<i>Medicago sativa</i> L.	GI6、 7、 9、 10、 11、 12

Table 2. Characteristics of representative strain of each group from alfalfa silage

Characteristics	Group A	Group B	Group C	Group D	Group E	Group F	GroupG
	GI36	GI69	GI6	GI30	GI14	GI62	GI44
Shape	Coccus	Coccus	Coccus	Coccus	Coccus	Coccus	Rod
Gram stain	+	+	+	+	+	+	+
Catalase activity	-	-	-	-	-	-	-
Fermentation type	Homo	Homo	Homo	Homo	Homo	Homo	Homo
5°C	-	+	-	+	+	+	+
10°C	+	+	+	+	+	+	+
40°C	+	+	+	+	+	+	w
45°C	+	+	+	+	+	+	-
3.0% NaCl	+	+	+	+	+	+	+
6.5% NaCl	+	+	+	+	+	+	+
PH3.0	w	-	w	w	w	w	-
PH3.5	w	w	w	+	+	+	w
PH4.0	-	+	w	+	+	+	-
PH4.5	+	+	w	+	+	+	w
PH5.0	+	+	+	+	+	+	+
PH7.5	+	+	+	+	+	+	+
PH8.0	+	+	+	+	+	+	-

+: positive reaction, -: negative reaction, w: weakly positive reaction.