Enhancing Corn Silage Fermentation and Microbial Stability Using *L. paracasei* K−68 as a Bio−Inoculant

Ki Choon Choi*, and Soundharrajan Ilavenil*
* Forage production system division, National Institute of Animal Science, Rural Development Administration
e−mail:kais@choiwh@korea.kr

L. paracasei K−68 접종이 사료용 옥수수 사일리지의 발효 및 안정성 향상

최기춘*, 사운드라젠일라베닐*
*농촌진흥청 국립축산과학원 조사료생산시스템과

Abstract
This study investigated the use of *Lacticaseibacillus paracasei* strain K−68 as a silage inoculant to improve the fermentation quality and microbial stability of whole−crop corn silage. The isolated K−68 strain exhibited strong antibacterial and antifungal activity, particularly against spoilage organisms, and was confirmed as *L. paracasei*. When applied to corn silage, *L. paracasei* K−68 significantly enhanced fermentation by lowering pH, increasing lactic acid production, and promoting the growth of beneficial lactic acid bacteria (LAB), while simultaneously reducing yeast and mold populations. These findings demonstrate that K−68 is a promising candidate for use as a bio−inoculant in corn silage production, offering improved preservation and better feed quality for livestock.

1. Introduction

Whole crop corn silage is more nutritious for livestock, has a higher yield and provides more energy at a lower cost than other forages. Fermentation of corn silage is dominated by a variety of microbes that conserve the quality of the corn silage and improve animal performance(Muck et al., 2018). The ensiling process involves in fermentation of forage materials under anaerobic conditions which predominantly driven by lactic acid bacteria(Guo et al., 2023). Lactic acid bacteria (LAB) has been extensively used in silage production for their potential to accelerate the accumulation of lactic acid, reducing pH, preventing protein degradation, reducing dry matter loss and enhance animal performance (Arasu et al., 2014; Jung et al., 2024). In the present study, the beneficial effects of *Lacticaseibacillus paracasei*−K68 on corn silage fermentation was investigated.

2. Methods and Materials

Lactic acid bacteria were isolated from fermented corn silages and identified by Physio−chemical method and 16srRNA molecular tool. Extracellular enzyme production and fermentation of different carbohydrates were confirmed by API ZYM & APICH−50 kits respectively. Antibacterial and antifungal activity was determined according to Valan Arasu et al (Valan Arasu et al., 2013). Corn silage was developed with *L. paracasei* K−68 (10^5CFU/g,FM,) using an ensiling method and microbial counts and organic acid profiles in fermented silages were studied (Jung et al., 2024). SPSS16 software was used to analyze significant differences followed by the least significant difference test. Significant P values were less than 0.05.

3. Results and Discussion

Table 1 represents the antimicrobial activity of K68 against silage spoiling bacteria and fungi. Cell free metabolites of K68 showed a strong inhibitory activity against *E.coli* and *Klebseilla sp* followed by K56, K50, K60, K30, K23 and K28. In addition, live K68 exhibited significant anti−fungal activity against *Aspergillus sp* and

Pencillium sp compared to other isolates.

[Table 1] Antifungal and Antibacterial activity of LAB against silage spoilage.

Strains	Fungi inhibition (mm)		Bacterial inhibition (mm)	
	Aspergillus sp	*Penicillium sp*	*Klebsiella sp*	*E.coli*
K-23	14.0 ± 1.2	12.0 ± 1.0	21.3 ± 1.2	22.8 ±0.8
K-28	14.0 ± 3.6	13.3 ± 1.5	22.3 ±0.9	22.5 ±0.9
K-30	15.7 ± 3.2	15.0 ± 0.0	2.21 ±1.8	21.8 ±2.4
K-50	23.3 ± 1.4	14.7 ± 1.5	20.7 ±0.5	21.5 ±1.7
K-56	28.7 ± 1.2	14.7 ± 2.1	20.7 ±0.5	21.8 ±1.9
K-60	21.7 ± 1.4	15.7 ± 1.5	22.0 ±0.8	21.3 ±1.3
K-68	35.7 ± 2.1	28.0 ± 1.3	25.5 ±0.5	24.5 ±0.5

[Table 2] Corn silage pH and organic acids after 2 months of treatment with K-68 or cocktail LAB.

Treatment	pH	LA (DM%)	AA (DM%)	PA (DM%)	IBA (DM%)
Control	3.7 ± 0.01a	4.10 ± 0.06c	0.73 ± 0.12a	0.10 ± 0.06a	0.04 ± 0.04a
K68	3.5 ± 0.02c	6.10 ± 0.25a	0.42 ± 0.28a	0.04 ± 0.00a	0.03 ± 0.02a

LA: Lactic acid; AA: Acetic acid; PA: Propionic acid; IBA: Isobutyric acid; DM: Dry matter content. The data are presented as the mean ± STD of three replicates (n=3). Different alphabets (a,b,c) within a column indicates significant differences between the experimental groups.

[Table 3] Corn silage pH and organic acids after 2 months of treatment with K-68 or cocktail LAB.

Treatment	LAB ($X10^5$CFU/g)	Yeast ($X10^4$CFU/g)	Mold ($X10^3$CFU/g)
Control	17.0 ± 2.0c	529.3± 47.4a	97 .0 ± 28a
K68	30.0 ± 4.6b	150.0± 10.0c	23.3 ± 1.7c

CFU: Colony Forming Unit. The data are presented as the mean ± STD of three replicates (n=3). Different alphabets (a,b,c) within a column indicates significant differences between the experimental groups ($p<0.05$).

Table 2 shows a the impact of *L. paracasei*-K68 and cocktail LAB (CLAB) on pH and organic acid levels in corn silage after two months. *L. paracasei* -K68 treatment significantly reduced the pH to 3.5 ± 0.02 and increased lactic acid content to 6.10 ± 0.25% compared to control silage. Levels of acetic acid, propionic acid, and isobutyric acid did not show significant differences between the groups

The microbial composition such as LAB, yeast and mold in fermented corn silage was evaluated after 2 months (Table 3). The data suggest that LAB treated group had significantly higher LAB counts and lower yeast/ mold populations compared to control group silage ($p<0.05$). *L. paracasei*-K68 treatment had the highest LAB population (30.0 ± 4.6 x 10^5CFU/g), while the control had the lowest LAB population (17.0 ± 2.0 x 10^5CFU/g). Total yeast and mold counts were higher in control silages (529.3 ± 47.4 × 10^4 CFU/g for yeast and 97 .0 ± 28 × 10^3 CFU/g for mold) compared to LAB treated groups. In contrast, *L. paracasei*-K68 treated silage had the lowest yeast counts (150.0 ± 10.0 × 10^4 CFU/g) and mold (23.3 ± 1.7 × 10^3 CFU/g) compared to control.

4. Conclusions

Lacticaseibacillus paracasei strain showed significant antimicrobial activity and the potential to ferment whole crop corn silage by reducing pH, increasing lactic acid levels, and promoting LAB populations, making *L. paracasei* K-68 an excellent candidate for use as a bio-inoculant in corn silage production, which could offer better preservation for livestock and better feed quality.

References

[1] Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C., Kung, L., 2018. Silage review: Recent advances and future uses of silage additives. Journal of Dairy Science 101(5), 3980-4000.

[2] Guo, X., Xu, D., Li, F., Bai, J., Su, R., 2023. Current approaches on the roles of lactic acid bacteria in crop silage. Microbial Biotechnology 16(1), 67-87.

[3] Arasu, M.V., Jung, M.W., Kim, D.H., Ilavenil, S., Jane, M., Park, H.S., Al-Dhabi, N.A., Jeon, B.T., Choi, K.C., 2014. Enhancing Nutritional Quality of Silage by Fermentation with Lactobacillus plantarum. Indian J Microbiol 54(4), 396-402.

[4] Arasu, M.V., Jung, M.W,. Ilavenil, S., Jane M., Kim, D.H., Lee, K.D., 2013. Isolation and characterization of antifungal compound from Lactobacillus plantarum KCC-10 from forage silage with potential beneficial properties. Journal of Applied Microbiology. 115, 1172-85.

[5] Jung, J.S., Wong, J.W.C., Soundharrajan, I., Lee, K.-W., Park, H.S., Kim, D., Choi, K.C., Chang, S.W., Ravindran, B., 2024. Changes in microbial dynamics and fermentation characteristics of alfalfa silage: A potent approach to mitigate greenhouse gas emission through high-quality forage silage. Chemosphere 362, 142920.